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Câmpus de São José do Rio Preto

Diego Gomiero Cavalheri

**Revisão taxonômica de *Phalotris matogrossensis* (Squamata: Dipsadidae),  
com comentários sobre o grupo *Phalotris tricolor***

São José do Rio Preto

2023

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biodiversidade, junto ao Programa de Pós-Graduação em Biodiversidade, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

Financiadora: CAPES – Processo: 001

Orientador: Prof. Dr. Diego José Santana Silva

São José do Rio Preto

2023

C376r Cavalheri, Diego Gomiero  
Revisão taxonômica de *Phalotris matogrossensis* (Squamata:  
Dipsadidae), com comentários sobre o grupo *Phalotris tricolor* / Diego  
G. Cavalheri. -- , 2023  
50 p. : il., tabs., fotos, mapas

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp),  
Instituto de Biociências Letras e Ciências Exatas, São José do Rio  
Preto,  
Orientador: Diego José Santana

1. Biology Classification. 2. Taxonomy. 3. Zoologia. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto  
de Biociências Letras e Ciências Exatas, São José do Rio Preto. Dados fornecidos pelo  
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13 de janeiro de 2023

## AGRADECIMENTOS

Ao meu orientador, Diego José Santana, por toda paciência com problemas pessoas e psicológicos que passei durante o processo de mestrado. O suporte e a humanidade que lidera o laboratório Mapinguari não só garante que ótimos profissionais sejam formados, mas que estes o sejam de forma saudável e gentil.

Agradeço aos colegas de laboratório e de “Campão” que fizeram da minha estadia nessa cidade muito mais leve e amigável, além de proporcionar muitos momentos incríveis.

À Priscila Carvalho, pelos conselhos, suporte, por ter me incentivado a adentrar no mundo acadêmico mais uma vez e mostrar que quem dita o rumo que um laboratório pode tomar são as pessoas que nele existem. Agradeço-lhe por ter dado o empurrão que precisava para passar por tudo isso.

À Sarah Mângia, pelos puxões de orelha sobre igualdade na academia, por ter me ensinando tanto ao longo da minha vida em Campo Grande e por compartilhar um amor inexplicável pelo nosso mundo Caatinga.

À “Bia”, por ser a pessoa mais alto-astral que conheci ao longo dessa jornada, ter tato sobre assuntos que exigem empatia.

À Carlinha, por ter me dado muita tranquilidade quando cheguei, deixando claro que quando todos estão distantes das famílias originais, novas famílias se criam e um ajuda o outro.

Aos amigos latinos, “Huguito” e Juan, pelos longos papos, trocas de informações sobre culturas de cada um, e pelas parcerias nos trabalhos desenvolvidos. Ao Huguito, um agradecimento especial pela revisão bastante criteriosa feita para esta dissertação.

Aos “garotos da Campina” Ibrahim e “Nuno” pela amizade, papos e bons momentos de descontração.

E aos demais colegas de laboratório que compartilhei ótimos momentos, Sean, Márcia, “Leo”, Matheus, Kelvin, Lauany, Ana e todos que passaram pelo Mapinguari.

Agradeço a Caroline Tie, por ter sido um grande suporte, apoio e parte fundamental na reta final do mestrado.

Ao Ibilce pelo suporte burocrático, rápida resposta às dúvidas que surgiram ao longo deste período e pela organização e empatia com os discentes durante o período de pandemia.

Agradeço à minha família, meu suporte mais forte para minha formação para chegar até aqui e minha base para suportar as dificuldades que foram vividas nos últimos anos.

À banca, por ter aceitado fazer parte deste processo e contribuir com importantes considerações.

Por fim, agradeço e dedico este trabalho ao meu avô, João Aparecido Gomiero, que infelizmente não poderá vibrar comigo mais essa vitória por não ter tido a chance de receber a vacina a tempo, dado o governo ineficaz que tivemos durante o período pandêmico. Agradeço-lhe por ter me inspirado por todos os anos que esteve aqui, a fazer o que ama, a fazer com paixão, e a fazer com vontade. O Senhor foi e sempre será inspiração na minha vida, exemplo de um homem íntegro, honesto e de coração que nunca coube no peito. Este trabalho é inteiramente dedicado ao senhor.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001\*, à qual agradeço.

## RESUMO

O gênero *Phalotris*, composto atualmente por 20 espécies, comprehende serpentes de médio porte que se alimentam de répteis serpentiformes, possuem hábitos fossoriais e são tipicamente encontradas na diagonal de formações abertas da América do Sul. Muitas espécies do gênero carecem de revisões sistemáticas que usem da taxonomia integrativa para compreender as variações morfológicas, a presença de espécies crípticas e suas relações filogenéticas. Neste estudo utilizamos de dados de morfologia externa e hemipeniana, dados morfométricos, coloração e dados moleculares a fim de verificar o status taxonômico de *P. matogrossensis*. Analisamos os limites de sua variação ao longo da distribuição, a existência de espécies crípticas dentro da entidade taxonômica, e avaliamos os caracteres utilizados para diagnosticar esta espécie. Nossos resultados moleculares mostraram que, apesar de possuir alta diversidade genética, *P. matogrossensis* representa uma única entidade taxonômica e válida, e o grupo de espécies *P. tricolor* é parafilético, com *P. mertensi* como grupo irmão do grupo *P. bilineatus*, e *P. matogrossensis* como irmão das demais espécies do gênero. Os dados morfológicos mostram que *P. matogrossensis* possui caracteres que a diferenciam das demais espécies do gênero, com exceção de *P. sansebastiani*, não sendo possível separar as duas espécies com nenhum dos caracteres analisados neste estudo. Por conta da ausência de diferenças entre *P. matogrossensis* e *P. sansebastiani*, decidimos considerar *P. sansebastiani* como sinônimo junior de *P. matogrossensis*. Dado a divergências da osteologia craniana, hemipeniana e dos padrões dos colares nucais, optamos por retirar *P. mertensi* do grupo *P. tricolor*, e apresentamos uma redescrição da espécie. *Phalotris matogrossensis* então inclui espécimes com ocorrência na região centro-oeste, sudeste e sul do Brasil, leste do Paraguai, norte da Argentina, e oeste da Bolívia, possui dimorfismo sexual, e apresenta bons caracteres diagnósticos com relação as demais espécies do gênero.

**Palavras-chave:** Elapomorphini. Hemipênis. Neotropical. Sistemática. Taxonomia. Xenodontinae.

## ABSTRACT

The genus *Phalotris*, currently composed by 20 species, comprise medium sized snakes, which feed snake like reptiles, have fossorial habits and are typically from the diagonal of open formation in South America. Many species of the genus lack a systematic revision which use integrative taxonomy to understand the morphological variation, the presence of cryptic species and their phylogenetic relationship. Herein we used external morphology, and hemipenial, morphometric data, coloration pattern, and molecular data for the purpose to check the taxonomic status of the *P. matogrossensis*. We analyzed the limits of the variation along the distribution, the presence of the cryptic species within the taxonomic entity, and evaluate the characters used to diagnose this species. Our molecular results show a high genetic diversity in *P. matogrossensis*, which represent a valid species without cryptic diversity, and the group *P. tricolor* appears as paraphyletic, with *P. mertensi* as sister group of *P. bilineatus* group, and *P. matogrossensis* as sister for all other species of the genus. The morphological data show that *P. matogrossensis* has diagnosed characters in relation with other species of the genus, but *P. sansebastiani*, which was not possible to separate these two species with any of the characters analyzed in this study. Given the lack of differences between *P. matogrossensis* and *P. sansebastiani*, we decided consider *P. sansebastiani* as a junior synonym of *P. matogrossensis*, and we present a redescription of the species. Due to divergences in cranial osteology, hemipenis morphology and nuchal collars pattern, we opt to remove *P. mertensi* from the *P. tricolor* group. *Phalotris matogrossensis* includes specimens with occurrence in the midwest, southeast and south of Brazil, east of Paraguay, north of Argentina, and west of Bolivia, has sexual dimorphism, and presents good diagnostic characters in relation to the other species of the genus.

**Keywords:** Elapomorphini. Hemipenis. Neotropical. Systematic. Taxonomy. Xenodontinae.

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## INTRODUÇÃO GERAL

As Serpentes, atualmente com cerca de 4038 espécies, representam uma linhagem com representativa diversidade dentre os répteis (UETZ et al., 2022; VITT; CALDWELL, 2014). Atualmente essa diversidade é dividida em 29 famílias (FIGUEROA et al., 2016; ZAHER et al., 2019), sendo que 12 destas possuem ocorrência na região Neotropical, contando com uma diversidade de mais de 886 espécies (GUEDES et al., 2017). Grande parte dessa riqueza encontra-se na família Dipsadidae, que, apesar de não existir um consenso quanto a hierarquia taxonômica dessa linhagem, com alguns autores optando pelo uso de Colubridae *sensu lato*, mantendo essa linhagem como subfamília (=Dipsadinae), enquanto outros optaram por elevar este grupo como família (=Dipsadidae), o monofiletismo desta linhagem têm sido extensivamente corroborado (FIGUEROA et al., 2016; PYRON; BURBRINK; WIENS, 2013; ZAHER et al., 2009, 2019). A subfamília Xenodontinae, que representa um importante ramo deste grupo para novo mundo, atualmente com 342 espécies, e 14 tribos (UETZ et al., 2022; ZAHER et al., 2009). Uma de suas tribos é a Elapomorphini, composta pelos gêneros *Apostolepis* Cope, 1862, *Coronelaps* Lema e Deiques, 2010, *Elapomorphus* Wiegmann, 1843, *Parapostolepis* Amaral, 1922, e *Phalotris* Cope, 1861, compreendendo atualmente 50 espécies, todas com hábitos fossoriais e criptozoicas com ocorrência principalmente cisandina pela América do Sul (ENTIAUSPE-NETO et al., 2021a, 2021b; UETZ et al., 2022; ZAHER et al., 2019). O gênero *Phalotris* é composto por 20 espécies de médio porte que se alimentam de répteis serpentiformes e são encontradas nas áreas abertas de Cerrado e Mata Atlântica (CARVALHO et al. 2018; SAWAYA et al. 2008). O gênero foi separado de *Apostolepis* por apresentar uma placa pré-frontal única (COPE, 1861). Posteriormente, *Phalotris* foi rebaixado a subgênero por Laurent (1974) até sua revalidação por Ferrarezi (1994). O gênero *Phalotris* é caracterizado por possuir escamas pré-frontais fundidas em uma placa transversal única, cauda curta e romba, glândula de Duvernoy ligada ao músculo *levator anguli oris*, e por detalhes da osteologia craniana (FERRAREZZI, 1994). O monofiletismo do gênero foi corroborado *a posteriori* em filogenias utilizando dados moleculares (e.g. ZAHER et al., 2019).

*Phalotris* foi subdividido em três grupos de espécies: grupo *P. nasutus*, *P. bilineatus* e *P. tricolor* (FERRAREZZI, 1993). O grupo *P. nasutus* é diagnosticado, principalmente, pela escama rostral proeminente em formato pontudo ou cônico, com sua porção visível dorsalmente muito maior do que sua distância da pré-frontal; colar nucal amarelo com até três escamas dorsais de largura; coloração dorsal vermelho uniforme ou com faixa lateral longitudinal de cor escura; hemipênis bilobado, semicapitado e ornamentado por duas fileiras longitudinais, com cerca de seis espinhos bem desenvolvidos (FERRAREZZI, 1994). Atualmente o grupo *P.*

*nasutus* é composto por seis espécies: *Phalotris concolor* Ferrarezzi, 1994, *Phalotris labiomaculatus* Lema, 2002, *Phalotris lativittatus* Ferrarezzi, 1994, *Phalotris nasutus* (Gomes, 1915), *Phalotris nigrilatus* Ferrarezzi, 1994, e *Phalotris shawnella* Smith, Brouard e Cacciali, 2022 (SMITH; BROUARD; CACCIALI, 2022).

O grupo *P. bilineatus* apresenta coloração dorsal com faixas escuras; ventre preto ou com manchas; colar nucal estreito, com menos de três escamas de largura; hemipênis com sulco espermático bifurcado no ápice (FERRAREZI, 1994). Atualmente é composto por oito espécies: *Phalotris bilineatus* (Duméril, Bibron e Duméril, 1854), *P. illustrator* Scrocchi, Giraudo e Nenda, 2022, *P. lemniscatus* (Duméril, Bibron e Duméril, 1854), *P. multipunctatus* Puerto e Ferrarezzi, 1994, *P. normanscotti* Cabral e Cacciali, 2015, *P. reticulatus* (Peters, 1860), *P. spegazzinii* (Boulenger 1913) e *P. suspectus* (Amaral, 1924) (SCROCCHI; GIRAUDO; NENDA, 2022).

Por fim, o grupo *P. tricolor* apresenta a escama rostral arredondada e pouco proeminente, padrão dorsal avermelhado uniforme, sem faixas ou linhas longitudinais, ventre branco, hemipênis bilobado, ou ligeiramente bilobado, espinhos pouco desenvolvidos e sulco espermático bifurcado proximalmente (FERRAREZI, 1994). Este grupo possui ocorrência na Argentina, Brasil, Paraguai e Bolívia e é composto por seis espécies, *P. cuyanus* (Cei, 1984), *P. matogrossensis* Lema, D'Agostini e Cappellari, 2005, *P. mertensi* (Hoge, 1955), *P. punctatus* (Lema, 1979), *P. sansebastiani* Jansen e Köhler, 2008 e *Phalotris tricolor* (Duméril, Bibron e Duméril 1854) (FERRAREZI, 1993; JANSEN; KÖHLER, 2008; LEMA; D'AGOSTINI; CAPPELLARI, 2005; MARTINS; DE LEMA, 2017; ZAHER, 1999).

*P. tricolor* foi descrita inicialmente como *Elapomorphus*, sendo então a única espécie do gênero que possuía escama pré-frontal fundida, extremidade da cauda sem a mancha preta, corpo sem faixas e bandas nas regiões dorsal e ventral, e com colares nucais longos ( $> 3$  escamas de largura) (DUMÉRIL; BIBRON; DUMÉRIL, 1854). Posteriormente foi descrita *P. mertensi*, endêmica do Brasil, para separar os espécimes que possuem a borda das escamas dorsais pretas (HOGE, 1955). *Phalotris punctatus* posteriormente descrita para separar as espécies com ocorrência mais ao sul da distribuição, no norte e centro da Argentina; a principal característica diagnóstica apresentada em sua descrição é a coloração, com a lateral do dorso de cor ocre e o ventre amarelado (LEMA, 1979; MARTINS; DE LEMA, 2017). Ao sudoeste da distribuição do grupo foi descrita *P. cuyanus*, com ocorrência a oeste da Argentina, na Província de Córdoba, com indivíduos de coloração ocre uniforme (CEI, 1984). Lema, D'Agostini e Cappellari (2005) descreveram *P. matogrossensis* para separar os espécimes da região centro-oeste, sul e sudeste do Brasil, e a região oriental do Paraguai, que possuíam o

colar nucal branco muito maior que o colar nucal preto, ventre da cabeça branco imaculado, e colares que não atingem as escamas ventrais. Neste mesmo trabalho definiram *P. punctatus* como sinônimo júnior de *P. tricolor*; contudo, análises e discussões sobre o novo rearranjo não foram apresentadas, sendo apenas mencionado que “*A análise dos tipos e de séries de exemplares de P. tricolor e P. punctatus produziram subsídios para a proposição de um novo arranjo taxonômico[...]*” (LEMA; D’AGOSTINI; CAPPELLARI, 2005).

Em uma revisão dos caracteres diagnósticos de *P. cuyanus* ficou constatado que, se por um lado existe a sobreposição com relação ao tamanho e razões dos colares nucais, por outro o número de escamas ventrais nos machos pode ser usado como bom caráter diagnóstico com relação a *P. tricolor*, não possuindo sobreposição (LEYNAUD; CABRERA; CARRASCO, 2005). Posteriormente, algumas incongruências sobre os caracteres diagnósticos empregados na descrição de *P. matogrossensis* foram apresentadas, como a variação quanto ao tamanho dos colares nucais, que possui grande sobreposição entre *P. tricolor* e *P. matogrossensis*, e o uso por parte de Lema et al. (2005) de um crânio de *P. matogrossensis* para descrever a osteologia de *P. tricolor* (CACCIALI; MOTTE, 2007). Cacciali e Motte (2007) sugerem o uso da razão entre o colar nucal preto e o comprimento total dos indivíduos, mostrando que, para este caráter, não existe sobreposição entre as duas espécies.

Em 2008 foi descrita *P. sansebastiani*, proveniente da Bolívia, cujo principal caráter diagnóstico é um triângulo vermelho na face dorsal da cabeça, além de características do colar nucal e coloração da cabeça (JANSEN; KÖHLER, 2008). É importante destacar que Jansen e Köhler analisaram apenas um indivíduo de *P. matogrossensis*, tendo como principal base comparativa o trabalho de descrição dessa espécie. Scrocchi e Giraudo (2012) registraram pela primeira vez *P. sansebastiani* para Argentina, e comentaram algumas diferenças quanto aos caracteres encontrados, como a ausência do triângulo vermelho no topo da cabeça e sobreposições com caracteres relacionados ao colar nucal de *P. tricolor*. Martins e Lema (2017) revalidaram *P. punctatus*, apontando que o motivo da sinonimização (veja LEMA; D’AGOSTINI; CAPPELLARI, 2005) se deu pela utilização de espécimes fixados com coloração perdida, e, com base na fotografia de espécimes vivos, a diferença entre *P. tricolor* e *P. punctatus* fica clara. *Phalotris punctatus* então ficaria com a distribuição mais austral do grupo *P. tricolor*, junto com *P. cuyanus*. Contudo, há algumas incongruências no texto de Martins e Lema (2007), como o indivíduo IBSP 21655 citado como material examinado tanto para *P. punctatus* e quanto para *P. tricolor*. Este mesmo exemplar fora usado para descrição da osteologia craniana de *P. matogrossensis* (LEMA; D’AGOSTINI; CAPPELLARI, 2005).

Além disso, o mapa apresentado é apontado por autores subsequentes por possuir diversos problemas quanto a distribuição da *P. punctatus* (SCROCCHI; STAZZONELLI; CABRERA, 2019). Não obstante, *P. mertensi* teve seu posicionamento no grupo *P. tricolor* questionado por Martins e Lema (2017), por apresentar caracteres cranianos e hemipenianos divergentes das demais espécies do grupo; contudo, nenhuma decisão taxonômica foi tomada.

É notado que os padrões de coloração e os colares nucais, apesar de terem sido extensivamente utilizados na taxonomia do grupo *P. tricolor*, apresentam grande variação quanto ao tamanho, formato, contato com as escamas ventrais, e a razão entre os colares (e.g. CACCIALI; MOTTE, 2007; SCROCCHI; GIRAUDO, 2012). Os dados de folidose e morfométricos, apesar de possuírem algumas exceções (e.g. LEYNAUD; CABRERA; CARRASCO, 2005), dificilmente têm a capacidade de serem utilizados como caracteres diagnósticos dada sua sobreposição em relação a quase todas as espécies do grupo *P. tricolor*. De acordo com o cenário apresentado, o limite específico das espécies do grupo *Phalotris tricolor* necessita de uma revisão detalhada, utilizando uma taxonomia integrativa (análises moleculares, dados morfológicos, hemipênis, padrões de coloração, e dados morfométricos), com uma amostragem significativa ao longo de sua distribuição, com representantes jovens e adultos para anotação de possíveis variações ontogenéticas, além de possíveis caracteres sexualmente dimórficos. Essa ampla abordagem integrativa trará uma melhor resolução taxonômica, definição do limite de distribuição das espécies e, consequentemente, sua história biogeográfica. Assim, será possível definir suas variações morfológicas, identificação de possíveis espécies crípticas e a validade das espécies do grupo *P. tricolor* evitando identificações errôneas, que podem atrapalhar projetos de conservação para o grupo.

## REFERÊNCIAS

- CACCIALI, P.; MOTTE, M. Variación intraespecífica en *Phalotris matogrossensis* y *P. tricolor*: una evaluación de sus caracteres diagnósticos (Squamata, Colubridae). **Cuadernos de Herpetología**, v. 21, n. 2, p. 75–82, 2007.
- CARVALHO, P. S. et al. Snakes of campo Grande municipality, Mato Grosso Do Sul state, Central Brazil. **Herpetology Notes**, v. 11, p. 321–328, 2018.
- CEI, J. M. Una nueva especie de *Elapomorphus* de la region de cuyo (Colubridae, Serpentes). **Boletín del Museo de Ciencias Naturales y Antropológicas Moyano**, n. 4, p. 47–51, 1984.
- COPE, E. D. On *Elapomorphus*, *Sypholis*, and *Coniophanes*. **Proceedings of the Academy of Natural Science of Philadelphia**, v. 13, p. 524, 1862.
- DUMÉRIL, A. M. C.; BIBRON, G.; DUMÉRIL, A. H. A. **Erpétologie générale ou histoire naturelle complète des reptiles. Tome septième. Deuxième partie, comprenant l'histoire des serpents venimeux.** Paris: Librairie Encyclopédique de Roret, 1854.
- ENTIAUSPE-NETO, O. M. et al. Redescription of *Apostolepis ambiniger* (peters, 1869) (Serpentes: Dipsadidae: Elapomorphini). **Vertebrate Zoology**, v. 71, p. 231–251, 2021a.
- ENTIAUSPE-NETO, O. M. et al. Taxonomic status of *Apostolepis tertulianobeui* Lema, 2004 based on an integrative revision of *Apostolepis assimilis* (Reinhardt, 1861) (Serpentes: Dipsadidae). **Zoologischer Anzeiger**, v. 291, p. 123–138, 2021b.
- FERRAREZZI, H. Nota sobre o gênero *Phalotris* com revisão d grupo nasutus e descrição de três novas espécies (Serpentes, Colubridae, Xenodontinae). **Mem. Inst. Butantan**, v. 55, n. 1, p. 21–38, 1994.
- FIGUEROA, A. et al. A species-level phylogeny of extant snakes with description of a new colubrid subfamily and genus. **PLoS ONE**, v. 11, n. 9, 2016.
- GUEDES, T. B. et al. Patterns, biases and prospects in the distribution and diversity of Neotropical snakes. **Global Ecology and Biogeography**, v. 27, n. 1, p. 14–21, 2017.
- HOFSTADLER-DEIQUES, C.; LEMA, T. DE. On the cranial morphology of *Elapomorphus*, *Phalotris* and *Apostolepis* (Serpentes: Colubridae), and its phylogenetic significance. **Zootaxa**, v. 1042, p. 1–26, 2005.

- HOGÉ, V. A. R. Eine neue Schlange der Gattung *Elapomorphus* aus Brasilien. **Senckenbergiana biologica**, v. 36, n. 5/6, p. 301–304, 1955.
- JANSEN, M.; KÖHLER, G. A new species of *Phalotris* from the eastern lowlands of Bolivia (Reptilia, Squamata, Colubridae). **Senckenbergiana biologica**, v. 88, p. 103–110, 2008.
- LAURENT, R. F. Sobre la existência de *Elapomorphus nasutus*, Gomes en el Paraguay. **Acta Zoologica**, v. 31, n. 7, p. 65–67, 1974.
- LEMA, T. DE. *Elapomorphus punctatus*, nova especie de Colubridae para a Argentina (Ophidia). **Revista Brasileira de Biologia**, v. 39, p. 835–853, 1979.
- LEMA, T. DE; D'AGOSTINI, F. M.; CAPPELLARI, L. H. Nova espécie de *Phalotris*, redescrição de *P. tricolor* e osteologia craniana (Serpentes, Elapomorphinae). **Iheringia. Série Zoologia**, v. 95, n. 1, p. 65–78, 2005.
- LEYNAUD, G. C.; CABRERA, M. R.; CARRASCO, P. A survey of the southernmost representatives of the tricolor species group, genus *Phalotris* (Serpentes, Colubridae). **Phyllomedusa**, v. 4, n. 2, p. 103–110, 2005.
- MARTINS, L. A.; DE LEMA, T. The distribution of *Phalotris tricolor* group in Argentina and Paraguay, with notes on its taxonomy (Serpentes, Elapomorphini). **Neotropical Biology and Conservation**, v. 12, n. 2, p. 100–108, 2017.
- PYRON, R. A.; BURBRINK, F. T.; WIENS, J. J. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. **BMC evolutionary biology**, v. 13, p. 13–93, 2013.
- SAWAYA, R. J.; MARQUES, O. A. V.; MARTINS, M. Composição e história natural das serpentes de Cerrado de Itirapina, São Paulo, sudeste do Brasil Composição e história natural das serpentes de Cerrado de Itirapina. **Biota Neotropica**, v. 8, n. 2, p. 127–149, 2008.
- SCROCCHI, G. J.; GIRAUDET, A. R. First records of *Phalotris sansebastiani* Jansen and Kohler, 2008 (Serpentes: Dipsadidae) from Argentina. **Check List**, v. 8, n. 5, p. 900–902, 2012.
- SCROCCHI, G. J.; GIRAUDET, A. R.; NENDA, S. J. Taxonomic notes on the *Phalotris bilineatus* group (Serpentes: Dipsadidae: Elapomorphini), with the description of a new species from northwestern Argentina. **Cuad. herpetol**, v. 36, n. 1, p. 47–63, 2022.

SCROCCHI, G. J.; STAZZONELLI, J. C.; CABRERA, P. Nuevas citas de Squamata (Gekkonidae, Phyllodactylidae y Dipsadidae) para la provincia de Tucumán, Argentina. **Cuadernos de Herpetología**, v. 33, n. 2, p. 75–78, 2019.

SMITH, P.; BROUARD, J. P.; CACCIALI, P. A new species of *Phalotris* (Serpentes, Colubridae, Elapomorphini) from Paraguay. **Zoosystematics and Evolution**, v. 98, n. 1, p. 77–85, 2022.

UETZ, P. et al. **The Reptile Database**. Disponível em: <<http://www.reptile-database.org>>. Acesso em: 13 set. 2022.

VITT, L. J.; CALDWELL, J. P. **Herpetology: An Introductory Biology of Amphibians and Reptiles**. 4th ed. ed. [s.l.] Academic Press, 2014.

ZAHER, H. Hemipenial morphology of the South American Xenodontine Snakes, with a proposal for a monophyletic Xenodontinae and a reappraisal of Colubroid hemipenes. **Bulletin of the American Museum of Natural History**, v. 240, n. 240, p. 168 pages, 95 figures, 1 table, 2 appendices, 1999.

ZAHER, H. et al. Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South American Xenodontines: a revised classification and descriptions of new taxa. **Papéis Avulsos de Zoologia (São Paulo)**, v. 49, n. 11, p. 115–153, 2009.

ZAHER, H. et al. Large-scale molecular phylogeny, morphology, divergence-time estimation, and the fossil record of advanced caenophidian snakes (Squamata: Serpentes). **Plos ONE**, v. 14, n. 5, p. 1–82, 2019.

**Reassessment of *Phalotris matogrossensis* Lema, Agostini and Cappellari, 2005**

**(Squamata: Dipsadidae), with comments on *Phalotris tricolor* group**

(to be submitted to Zootaxa ISSN: 1175-5334)

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## Abstract

The genus *Phalotris*, currently composed of 20 species, comprise medium-sized snakes which feed on snake-like reptiles, have fossorial habits and occur mostly along the diagonal of open formations in South America. Herein we reassess the taxonomy of *P. matogrossensis*, one of the six species of the *P. tricolor* group, to investigate the limits of the variation along its distribution, the presence of the cryptic species, and its diagnostic characters. Our molecular results reveal a high genetic diversity in *P. matogrossensis*, which represents a single taxonomic entity, but the *P. tricolor* group is paraphyletic, with *P. mertensi* being the sister of *P. bilineatus* group, while *P. matogrossensis* is the sister taxon of all remaining *Phalotris*. Due to divergences in cranial osteology, hemipenis morphology and nuchal collars patterns, we remove *P. mertensi* from the *P. tricolor* group. Given the lack of morphological differences, we consider *P. sansebastiani* a junior synonym of *P. matogrossensis*, and we present a redescription of the latter species.

## Introduction

*Phalotris* Cope, 1861 is a South American genus composed by 20 species of medium colorful snakes distributed in open formations from Northeastern Brazil to Southern Argentina (Martins & de Lema 2017; Nogueira *et al.* 2019; Uetz *et al.* 2022). Ferrarezi (1994) revalidated the genus to allocate species of Elapomorphini Jan, 1862 which have their prefrontal scale fused in a single plate. The genus was divided into three group of species: *P. nasusuts* group, (*P. concolor* Ferrarezi, 1994, *P. labiomaculatus* Lema, 2002, *P. lativittatus* Ferrarezi, 1994, *P. nasutus* (Gomes, 1915), *P. nigrilatus* Ferrarezi, 1994, and *P. shawnella* Smith, Brouard &

Cacciali, 2022) with six species; *P. bilineatus* group (*P. bilineatus* (Duméril, Bibron & Duméril, 1854), *P. illustrator* Scrocchi, Giraudo & Nenda, 2022, *P. lemniscatus* (Duméril, Bibron & Duméril, 1854), *P. multipunctatus* Puerto & Ferrarezzi, 1994, *P. normanscotti* Cabral & Cacciali, 2015, *P. reticulatus* (Peters, 1860), *P. spegazzinii* (Boulenger 1913), and *P. suspectus* (Amaral, 1924)), composed by eight species; and *P. tricolor* group, (*P. cuyanus* (Cei, 1984), *P. matogrossensis* Lema, D'Agostini & Cappellari, 2005, *P. mertensi* (Hoge, 1955), *P. punctatus* (Lema, 1979), *P. sansebastiani* Jansen & Köhler, 2008, *Phalotris tricolor* (Duméril, Bibron & Duméril 1854)) with six species (Puerto & Ferrarezzi 1994; Scrocchi *et al.* 2022).

The *Phalotris tricolor* group includes species with a rounded rostral scale, dorsal pattern reddish, without stripes, ventral region immaculate whitish, and nuchal collars (white and black) more than three dorsal scales wide (Ferrarezzi 1994). The taxonomy of some species of this group has been pointed to as instable (e.g. *P. matogrossensis* – Cacciali and Motte, 2007; *P. sansebastiani* – Scrocchi and Giraudo, 2012; *P. punctatus* – Lema *et al.*, 2005, Martins and Lema, 2017). Although these species have been subject of taxonomy studies, they have never been revised under an integrative approach.

*Phalotris matogrossensis* is a species of the *P. tricolor* group occurring in Brazilian midwest, southeast and south, and in eastern Paraguay (Cacciali and Motte 2007; Lema *et al.* 2005; Nogueira *et al.* 2019). After its description some inconsistencies was pointed, as the skull of a *P. matogrossensis* that was used as *P. tricolor* to diagnosed it, and characters related to the size of the white collar in relation to the black collar that was pointed as an ineffective to differentiate *P. matogrossensis* from other species of the group, due to its variation (Cacciali & Motte 2007; Lema *et al.* 2005). Additionally, the description *P. sansebastiani* is based on a small sample of specimens from the *P. tricolor* group, which not cover the entire variation of the species, used these character to differentiate both species (Jansen & Köhler 2008).

Given such incongruences and the lack of a deep taxonomic revision by using an integrative approach on this species, in this research we aim to evaluate the external morphology, coloration, hemipenis morphology, measurements and molecular data of *Phalotris matogrossensis*.

## Material and methods

### Morphological analysis

We examined 88 specimens of *P. matogrossensis* and 75 individuals from other species, including *P. labiomaculatus* (n=2), *P. mertensi* (n=43), *P. nasutus* (n=8), *P. sansebastiani* (n=3), and *P. tricolor* (n=12) (Appendix A) from six collections: BRAZIL–Coleção

Herpetológica "Alphonse Richard Hoge", Instituto Butanta (IBSP); Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP); Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (ZUFMS); Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso" (ZUEC); PARAGUAY– Museo Nacional de Historia Natural del Paraguay (MNHNP); ARGENTINA– Museo Noel Kempff Mercado (MNKR).

We examined 14 meristic and six morphometric characters (Table 1), in addition to the coloration pattern and morphology of the hemipenis. Sex was determined by the presence or absence of hemipenes through a ventral incision at the base of the tail. A digital caliper (0,01 mm) and a flexible ruler (1 mm) were used for measured individuals. We followed the terminology of Dowling (1951) for counting the ventral scales, and Peters (1964) and Vanzolini et al. (1980) for pholidosis. For lower jaw length we followed (Cundall *et al.* 2016) for the length/wide of the nuchal collar we count the numbers dorsal scales at the vertebral line and measured with a pachymeter. We surveyed the geographic coordinates of the data catalogs of zoological collections using Google Earth software and the map was made in QGIS 3.12.3.

As there is no data about reproductive information of *Phalotris matogrossensis*, we considered adults the individuals which has at least 60% of the size of the longest individual of that sex, following Parker and Plummer (1987), who indicate that most snakes reach sexual maturity at 60 to 75% of their maximum size, information is corroborated by Braz et al. (2014) for *P. lativittatus*. For coloration and morphometric characters we included only adults, considering a possible ontogenetic variation; for scale counts we included all individuals, since there is no ontogenetic variation in this type of character (Thorpe 1975).

### **Hemipenial morphology**

We prepared eight hemipenes, one from *P. mertensi* and seven from *P. matogrossensis*. We followed the technique described by Pesantes (1994), with modifications by Zaher (1999), and Zaher and Prudente (2003). We stained the external calcareous structures with alizarin red, as suggested by Nunes et al. (2012), for a better visualization of microstructures in the surface of the organ. Terminology follows Dowling & Savage (1960), Zaher (1999) and Myers & Cadle (2003).

### **Molecular Analysis**

We sequenced fragments of the Cytochrome-b mitochondrial gene (Cytb) from 10 specimens of *P. matogrossensis*. We extracted genomic DNA from liver samples using the

phenol-chloroform protocol of Sambrook and Russell (2001). We used the Cytb primer pair of Pook et al. (2000), following PCR conditions described by Carvalho et al. (2020). PCR reactions consisted of 1× buffer, dNTPs at 0.2 mM, each primer at 0.2 µM, MgCl<sub>2</sub> at two mM, one U Taq polymerase, and two µl of template DNA, in a total reaction volume of 25 µl. We used the following PCR cycling program: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 59 °C for 1 min, and 72 °C for 1 min, and a final 5 min extension at 72 °C. We purified PCR products with Ethanol/Sodium Acetate and sequenced them on an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Resulting sequences were edited and aligned using Geneious v9.1.2 with the MUSCLE algorithm using default parameters (Edgar 2004). We aligned our Cytb sequences with Cytb sequences of other species of *Phalotris* and with *Apostolepis flavotorquata* (GQ895854.1) as outgroup, which are available in GenBank. The final aligned dataset used in all analyses comprised 582 base pairs (bp) of Cytb.

We implemented a Maximum Likelihood tree inferred in RAxML (Stamatakis 2014) via raxmlGUI 2.0 (Edler et al. 2021). We run the analysis using a ML + rapid bootstrap setting with HKT+G substitution model and 1,000 bootstrap replicates. Substitution model was tested with Modeltest (Darriba et al. 2020) in raxmlGUI 2.0. In addition, we ran a PTP species delimitation analysis (Zhang et al. 2013) using the ML Tree, which the calculations were performed on PTP websever (<http://species.h-its.org/ptp/>), with 500,000 MCMC generations, thinning set at 100 and burn-in at 10%. We also ran a Bayesian Inference (BI) performed in BEAST v.2.6.3 (Bouckaert et al. 2019) for 10 million generations, sampling every 1,000 steps using a Yule Process tree prior. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample size were above 200 in Tracer v1.7.1 (Rambaut et al. 2018). The first 10% of sampled genealogies were discarded as burn-in, and the maximum clade credibility tree with median node ages was calculated with TreeAnnotator v.2.6.3 (Bouckaert et al., 2019). With this tree, we ran a Generalized Mixed Yule Coalescent (GMYC) for species delimitation (Fujisawa & Barraclough 2013; Pons et al. 2006) in the R v 4.1.1 (R Core Team, 2021) by using the package splits (Ezard, Fujisawa & Barraclough, 2017). We calculated sequence divergences (uncorrected p-distances) among species/individuals using MEGA v10.1.1 (Kumar et al. 2018).

## Results

### Molecular results

In this study 6 of the 20 species were included. This is the first study that includes *P. matogrossensis* in a phylogeny (Figure 1). Our phylogeny recovered three major clades within *Phalotris* with high support. The first clade is represented only by *P. matogrossensis*, a member of *P. tricolor* group, sister of the remaining clades. The second clade is composed of *P. nasutus* and *P. lativittatus*, members of the *P. nasutus* group. The third clade is composed of *P. bilineatus*, *P. lemniscatus* (members of the *P. bilineatus* group) and *Phalotris mertensi*. *Phalotris matogrossensis* has high genetic diversity, forming three subclades with high support (pp=1); however, our topology did not recover any morphological or geographical pattern. On the other hand, *P. mertensi* shows a low genetic diversity, demonstrated by the branches size. Out of *P. tricolor* group we found *P. nasutus* as paraphyletic, due to an individual from Mato Grosso state.

### Morphological results

*Phalotris matogrossensis* presents noticeable difference from the other species of *P. tricolor* group, except for *P. sansebastiani*, which we were not able to find any diagnostic characteristic to separate both species. The black and white collars show a wide variation in size and shape in *P. matogrossensis*, which included the intervals find in *P. sansebastiani*. Additionally, we observed gradient levels of the presence of the red triangle on top of the head. We found differences between male and female in size, which males are smaller (maximum SVL 549mm) than females (maximum SVL 885mm), the ratio between SVL and TL, and the number of subcaudals scale (Table 1).

After a review of part of type series, and a topotype of *P. sansebastiani* and several specimens of *P. matogrossensis*, and since there are no diagnostic characters that split *P. matogrossensis* and *P. sansebastiani*, thus, we considered *P. sansebastiani* as a junior synonym of *P. matogrossensis*.

### *Phalotris matogrossensis* Lema, D'Agostini & Cappellari (Figure 2)

Type locality: Cuiabá, Mato Grosso, Brazil; Type Specimen: MCP 12801

*Phalotris matogrossensis* Lema, D'Agostini & Cappellari, 2005. Iheringia. Série Zoologia vol. 95: 65-78.

*Phalotris sansebastiani* Jansen & Köhler, 2008. Senckenbergiana biológica vol. 88: 103-110  
**(New Synonymy).**

*Definition*—*Phalotris matogrossensis* belong to the *P. tricolor* group by rounded and no prominent rostral; dorsal coloration reddish or ocher, without lines or bands; venter cream and

immaculate; and, wide white and black nuchal collars (3 or more scales of width) it differs from all other species of the genus, but *P. tricolor* group. Diagnosed from the other species of *P. tricolor* group by the following combination of characters: 1) pattern of the coloration of the ventral portion of the head (immaculate or with black spot, but never predominantly black); 2) snout red, immaculate, or with tiny black spots; 3) color of infralabials (usually immaculate or with black spot, but predominantly red); 4) pattern of black collar (well defined at the posterior margin of dorsal region; reaches the first dorsal scale row and usually the extreme tips of the ventral scale); 5) the size of the black collar in relation to the white nuchal collar (usually subequal), 6) all dorsal, with exception to the nuchal portion, are red and immaculate; 7) hemipenis deeply bilobed, with long lobes (twice as long as wide), semicalyculate, semicapitate, with small spines at the body of the organ, and sulcus spermaticus bifurcating at the first half of the body.

*Diagnosis* (conditions for *P. matogrossensis* in parentheses)—Differs from *P. mertensi* by its black tips on dorsal scales (absent); number of ventrals in males, 219–230 (193–218 in *P. matogrossensis*); format of the distal border of the black nuchal collar in “M” shape and undefined (distal border of the black nuchal collar well defined and without “M” shape); and the maximum size, 700mm for males and 1200mm for females (600 for males and 900mm for females). From *P. cuyanus* by its dorsal color greyish yellow to ochre (uniform red); and a tendency of more ventrals scale in male, 216–224, mean 220.3 (193–218 [205,3 ± 6,9]). From *P. tricolor* by the ratio of the black nuchal collar in relation to the SVL in males, 0,027–0,039, mean 0,034 (0,012–0,020 [0,016 ± 0,002]); the ratio of the black nuchal collar in relation to the HL, 0,9 and 2,1 in males, and 1–1,6 in females (0,33–0,67[0,48 ± 0,09] in males and 0,25–0,55 [0,41 ± 0,08] in females); the black nuchal collar usually reach the ventrals (usually reach the paraventrals of the extreme tip of ventrals); ventral face of the head usually entirely black, or with more black than white in adults (ventral face of the head is white immaculate or with few black spots). From *P. punctatus* by its dorsal coloration reddish on vertebral zone, becoming ocher at lower sides (dorsal region fully reddish).

*Description* (Table 1, Figure 3 and Figure 4)—*Phalotris matogrossensis* is medium-size snake (♂ SVL 142–549mm and TL 15,1–57,8 mm, and ♀ SVL 165–885 mm and TL 10,7–55,7mm) with short tail in relation with the SVL (♂ 7,9% to 11,3% [9,9% ± 0,6], and ♀ 5,8% and 8% [6,9% ± 0,4%]). The head is slightly distinct from neck, with an oval shape in dorsal view, and a round and not prominent rostral. There are two internasal, a pre-frontal scale with a trapezoid format, more wide than high, one supra-ocular, a frontal with a pyramidal shape, and two parietals. The lateral view of the head shows six supralabials, with the second and the

third in contact with the orbit, seven infralabials, rarely six, with the first to the fourth (rarely third) in contact with the anterior gular, and the fourth and five (rarely third and fourth) in contact with the posterior gular; one pre-ocular, two post-ocular and temporals in 1+1 composition. The ventral view of the head has two pairs of genials, the first pair in contact with the first four infralabial scale (rarely 1–3), and the second in contact with the fourth and fifth (rarely third and fourth); number of gular scales vary between four and five, rarely three. Dorsal region of the body has 15 rows of dorsal scale along the entire dorsum (15-15-15); the ventral region of the body has numerous ventrals (194–218♂ and 204–235♀). The tail is short and has a subtle constriction at the cloacal region, the cloacal scale is simple and the number of subcaudals varied between 26–33 for males and 19–25 for females.

*Coloration* –The coloration in life and in preservative follow a similar pattern, with differences on thered which in preservative becomes whitish. The dorsal view of the head has a black hood, with a reddish blotch on the snout which may vary from a reddish snout spotted by black dots to a red triangle with sharp edges. At the nuchal region there is a white collar, with sharp edges that starts ate the end of the parietals, and the size vary from three to seven scales width (Figure 3). The white nuchal collar is followed by a black collar with a sharp edge at the proximal edge and a soft border at the distal margin, with two to nine scales width (Figure 3). Supralabials are red, usually forming a gradient from immaculate at the first, some black spots starting at the second and in the fourth and fifth completely black at the top of the scale, until the medial portion of the sixth with a sharp edge and white at the distal part of the scale. Variation in this pattern occurs, with some individuals with almost no black spots or the with collar takes all sixth scale; infralabials are usually red and immaculate, but some individuals have black spot, or even blotches; the ventral portion of the head is immaculate white or with black spots; white nuchal collar may starts at the middle of sixth until the distal portion of fifth supralabial; black collar has a sharp edge at both limits and finish at paraventrals, rarely in the second row of dorsals or in the border of ventrals. The dorsal color of the body is uniform red, and the color of the venter is immaculate white, the color of the tail maintains the same of the body for both faces, dorsal and ventral region.

*Sexual dimorphism* (Table 1 and Figure 5)– The ratio between SVL and TL show some differences between male and female with almost no overlap, the tail of the male in relation with the SVL varied from 7,9% to 11,3% ( $9,9\% \pm 0,6$ ), and in female this ratio varied between 5,8% and 8% ( $6,9\% \pm 0,4\%$ ). The number of ventral scales is bigger in female (♂ 194–218 [205,6 ± 6,7]; and ♀ 204–235 [220,5 ± 7,3]), and, on the other hand, the number of subcaudals is bigger in males (♂ 26–33 [30,2 ± 1,5]; and ♀ 19–25 [23,2 ± 1,3]) with no overlap.

*Hemipenial morphology* (n=7)– When fully everted and expanded hemipenis of *P. matogrossensis* is deeply bilobed, caliculate and semicapitate. The sulcate face present small spines in the proximal region, distributed in three or four diagonal lines, forming a “V” shape, which finished where the sulcus bifurcated. The sulcus bifurcated in the proximal region of the hemipenis body, with a centrolineal condition to the branches. The branches in the proximal region present lateral flounces which became calyces in the distal portion. The assulcate face present a nude region in the proximal portion of the body following by small spines, follows by body calyces in the capitulum, calycular pockets in the point of bifurcation, and calyces in the branches.

*Distribution* (Figure 6): *Phalotris matogrossensis* occurs in Cerrado Ecoregion, in Midwest, Southeast, and South region of Brazil, and there is a record in South of Brazil within Uruguayan Savanna. In Paraguay, the species occur only on oriental region, in Humid Chaco; In Bolivia there are records in Chiquitano Dry Forest, in east part of the country; and in Argentina, the species occurs in the northern part of the country, the only know locality which the species occurs in sympatry with *P. tricolor*. The altitude where the species occurs varied from 78m to 1200m above the sea level when considered literature and our analyzed data.

## Discussion

### On *P. matogrossensis* and *P. sansebastiani*

Specimens of *P. matogrossensis* analyzed herein showed characters which differs from almost all other members of the *P. tricolor* group, with exception of *P. sansebastiani*. Jansen and Köhler (2007) used information from the description paper of *P. matogrossensis* to determine the differences of both species, with only one individual of *P. matogrossensis*, from Paraguay was analyzed. Herein we found many incongruences between our results and the description paper of *P. matogrossensis* (see Lema *et al.* 2005), mainly in coloration aspects of head, nuchal collar and black collar.

The description of *P. sansebastiani* indicated some characteristics of nuchal and black collar, coloration of the head, with a red triangle in the dorsal view of the head, and the shape of snout which differ from *P. matogrossensis* (Jansen & Köhler 2008). Individuals of *P. sansebastiani* found in northern Argentina did not have the red triangle in the head (see Scrocchi & Giraudo 2012), and all other characteristics are sharing with our results for *P. matogrossensis* including the ratio between the Black Collar and the Snout–vent length pointed by Cacciali and Mote (2007) to separated *P. matogrossensis* and *P. tricolor*. In the description paper of *P.*

*sansebastiani* the diagnose for separated both species are the red triangle in the head, the subequal size of the nuchal and black collar, the black collar which usually reach the first scale row and sometimes in the tips of the ventral, gular and infralabial region with black dots or mottled with black, and the shape of the head and snout (Jansen & Köhler 2008). Our results demonstrate that the main differences between both species are questionable, which show overlap in all diagnosed characters. We found different gradients about the red triangle pattern in the dorsal view of the head, gular coloration, and the reaches of the black collar in the same locality (Fig. 4), additionally the size of the black and nuchal collar varied considerably, with many specimens show collars in subequal size (Table 1).

### Comments on *P. tricolor* group

The *Phalotris tricolor* group appears in our analyses as paraphyletic, which *P. mertensi* take a position as sister of *P. bilineatus* group (with low support), and *P. matogrossensis* as sister of all other *Phalotris*. The position of *P. mertensi* in the *P. tricolor* group was already questionable due to its skull characteristics (see Hofstadler-Deiques & Lema 2005). Also differences in hemipenis was already noticed (Fig. 63 in Zaher 1999), showing a different shape than other species of *P. tricolor* group, with a slightly bilobed organ. Additionally, the black collar is not well defined at the distal margin (Figure 7), different than all other species of the group. Therefore, to maintain the monophyly of the groups/clade we opt to keep *P. mertensi* as *incertae sedis* and considered *P. matogrossensis* as a representative of *P. tricolor* group, due to its morphological and hemipenial characteristics.

*Phalotris punctatus* was described by Lema (1979), synonymized as a junior of *P. tricolor* by Lema (2005), and recently revalidate (Martins & de Lema 2017). The new distribution of *P. punctatus* is allopatric with *P. tricolor*, occurring in Bolivia and in region occidental of Paraguay, while *P. tricolor* occurs in the south part of Paraguay and Argentina (Martins & de Lema 2017). On its distribution, Scrocchi et al. (2019) pointed out some incongruences in the revalidation paper. Herein we found two individuals from Paraguay, Department of Boqueron, Filadelfia, in *P. tricolor* distribution range, with the presence of black dots on dorsal region (Figure 8), a characteristic diagnostic for *P. punctatus* (Martins & de Lema 2017). Furthermore, there are several errors along the revalidate paper, such as the individual IBSP.21655 which appears as *P. tricolor* and *P. punctatus* in the “specimens examined” section in Martins and de Lema (2017), and the same individual was used to describe the cranial osteology and pholidosis of *P. matogrossensis* and is included as material examined for *P. tricolor* in Lema et al. (2005). Beyond that, *P. sansebastiani* was not included in the material analyzed, nor in the comparison during diagnoses, even after its presence was recorded

close to the type locality of *P. punctatus* (see Scrocchi & Giraudo 2012). *Phalotris punctatus* lacks a systematic taxonomic review to better defined its taxonomic status, variation boundaries, and its validity as a valid species. Herein our sample is limited to few localities, due to that we opt to do not take any taxonomy consideration.

## Conclusions

Our results did not separate *P. matogrossensis* and *P. sansebastiani*, according to pholidosis, morphometric, hemipenial and coloration data. Therefore, the characteristics found in the population from Bolivia represents a regional variation, found in some individuals from other localities, but in lower rate. However, a molecular approach using data from populations from Bolivia, Argentina and Paraguay is urgent to corroborate, the data present at this work. The position of *P. mertensi* in *P. tricolor* group was questioned and we opt to maintain this species as *icertae sedis*, and *P. punctatus* present several incongruences about its taxonomy status and lacks a revision to determine the validity of the species and the taxonomic limits.

## References

- Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F.K., Müller, N.F., Ogilvie, H.A., Du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., Suchard, M.A., Wu, C.H., Xie, D., Zhang, C., Stadler, T. & Drummond, A.J. (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 15, 1–28. <https://doi.org/10.1371/journal.pcbi.1006650>
- Braz, H.B., Kasperoviczus, K.N. & Almeida-Santos, S.M. (2014) Reproductive ecology and diet of the fossorial snake *Phalotris lativittatus* in the Brazilian Cerrado. *Herpetological Journal* 24, 49–57.
- Cacciali, P. & Motte, M. (2007) Variación intraespecífica en *Phalotris matogrossensis* y *P. tricolor*: una evaluación de sus caracteres diagnósticos (Squamata, Colubridae). *Cuadernos de Herpetología* 21, 75–82.
- Carvalho, P.S., Zaher, H., Da Silva, N.J. & Santana, D.J. (2020) A morphological and molecular study of *Hydrodynastes gigas* (Serpentes, Dipsadidae), a widespread species from South America. *PeerJ* 8, 1–25. <https://doi.org/10.7717/peerj.10073>

- Cundall, D., Deufel, A., MacGregor, G., Pattishall, A. & Richter, M. (2016) Effects of Size, Condition, Measurer, and Time on Measurements of Snakes. *Herpetologica* 72, 227–234. <https://doi.org/10.1655/Herpetologica-D-16-00006.1>
- Darriba, Di., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B. & Flouri, T. (2020) ModelTest-NG: A New and Scalable Tool for the Selection of DNA and Protein Evolutionary Models. *Molecular Biology and Evolution* 37, 291–294. <https://doi.org/10.1093/molbev/msz189>
- Dowling, H.G. (1951) A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1, 97–99. <https://doi.org/10.1016/j.chabu.2013.06.011>
- Dowling, H.G. & Savage, J.M. (1960) A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. *Zoologica : scientific contributions of the New York Zoological Society*. 45, 17–28.
- Edgar, R.C. (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32, 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Edler, D., Klein, J., Antonelli, A. & Silvestro, D. (2021) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12, 373–377. <https://doi.org/10.1111/2041-210X.13512>
- Ferrarezzi, H. (1993) Nota sobre o gênero *Phalotris* com revisão do grupo nasutus e descrição de três novas espécies (Serpentes, Colubridae, Xenodontinae). *Mem. Inst. Butantan* 55, 21–38.
- Fujisawa, T. & Barraclough, T.G. (2013) Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. *Systematic Biology* 62, 707–724. <https://doi.org/10.1093/sysbio/syt033>
- Hofstadler-Deiques, C. & Lema, T. De (2005) On the cranial morphology of *Elapomorphus*, *Phalotris* and *Apostolepis* (Serpentes: Colubridae), and its phylogenetic significance. *Zootaxa* 1042, 1–26.
- Jansen, M. & Köhler, G. (2008) A new species of *Phalotris* from the eastern lowlands of Bolivia (Reptilia, Squamata, Colubridae). *Senckenbergiana biologica* 88, 103–110.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547–1549. <https://doi.org/10.1093/molbev/msy096>

Lema, T. De (1979) *Elapomorphus punctatus*, nova especie de Colubridae para a Argentina (Ophidia). *Revista Brasileira de Biologia* 39, 835–853.

Lema, T. De, D'Agostini, F.M. & Cappellari, L.H. (2005) Nova espécie de *Phalotris*, redescrição de *P. tricolor* e osteologia craniana (Serpentes, Elapomorphinae). *Iheringia. Série Zoologia* 95, 65–78. <https://doi.org/10.1590/S0073-47212005000100010>

Martins, L.A. & de Lema, T. (2017) The distribution of *Phalotris tricolor* group in Argentina and Paraguay, with notes on its taxonomy (Serpentes, Elapomorphini). *Neotropical Biology and Conservation* 12, 100–108. <https://doi.org/10.4013/nbc.2017.122.03>

Myers, C.W. & Cadle, J.E. (2003) On the Snake Hemipenis, with Notes on *Psomophis* and Techniques of Eversion: A Response to Dowling. *Herpetological Review* 34, 295–302.

Nogueira, C.C., Argôlo, A.J.S., Arzamendia, V., Azevedo, J.A., Barbo, F.E., Bérnuls, R.S., Bolochio, B.E., Borges-Martins, M., Brasil-Godinho, M., Braz, H., Buononato, M.A., Cisneros-Heredia, D.F., Colli, G.R., Costa, H.C., Franco, F.L., Giraudo, A., Gonzalez, R.C., Guedes, T., Hoogmoed, M.S., Marques, O.A.V., Montingelli, G.G., Passos, P., Prudente, A.L.C., Rivas, G.A., Sanchez, P.M., Serrano, F.C., Silva, N.J., Strüssmann, C., Vieira-Alencar, J.P.S., Zaher, H., Sawaya, R.J. & Martins, M. (2019) Atlas of Brazilian Snakes: Verified Point-Locality Maps to Mitigate the Wallacean Shortfall in a Megadiverse Snake Fauna. *South American Journal of Herpetology* 14, 1–274. <https://doi.org/10.2994/sajh-d-19-00120.1>

Nunes, P.M.S., Fouquet, A., Curcio, F.F., Kok, P.J.R. & Rodrigues, M.T. (2012) Cryptic species in *Iphisa elegans* Gray, 1851 (Squamata: Gymnophthalmidae) revealed by hemipenial morphology and molecular data. *Zoological Journal of the Linnean Society* 166, 361–376. <https://doi.org/10.1111/j.1096-3642.2012.00846.x>

Pesantes, O.S. (1994) A method for preparing the hemipenis of preserved snakes. *Journal of Herpetology* 28, 93–95.

Peters, J.A. (1964) *Dictionary of herpetology*. Hafner Publishing Company, New York, 392

pp.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D. & Vogler, A.P. (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55, 595–609.  
<https://doi.org/10.1080/10635150600852011>

Pook, C.E., Wüster, W. & Thorpe, R.S. (2000) Historical biogeography of the Western Rattlesnake (Serpentes: Viperidae: *Crotalus viridis*), inferred from mitochondrial DNA sequence information. *Molecular Phylogenetics and Evolution* 15, 269–282.  
<https://doi.org/10.1006/mpev.1999.0756>

Puerto, G. & Ferrarezzi, H. (1993) Uma nova espécie de *Phalotris* Cope, 1862, com comentários sobre o grupo bilineatus (Serpentes: Colubridae: Xenodontinae). *Mem. Inst. Butantan* 55, 39–46.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67, 901–904.  
<https://doi.org/10.1093/sysbio/syy032>

Scrocchi, G.J. & Giraudo, A.R. (2012) First records of *Phalotris sansebastiani* Jansen and Kohler, 2008 (Serpentes: Dipsadidae) from Argentina. *Check List* 8, 900–902.  
<https://doi.org/10.15560/8.5.900>

Scrocchi, G.J., Giraudo, A.R. & Nenda, S.J. (2022) Taxonomic notes on the *Phalotris bilineatus* group (Serpentes: Dipsadidae: Elapomorphini), with the description of a new species from northwestern Argentina. *Cuad. herpetol* 36, 47–63.  
[https://doi.org/10.31017/CdH.2022.\(2022-002\)urn](https://doi.org/10.31017/CdH.2022.(2022-002)urn)

Stamatakis, A. (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313.  
<https://doi.org/10.1093/bioinformatics/btu033>

Thorpe, R.S. (1975) snake systematics with particular reference to intraspecific variation in the Ringed Snake *Natrix natrix* ( L .). *Biological Journal of the Linnean Society* 7, 27–43.

Uetz, P., Freed, P., Aguilar, R. & Hošek, J. (2022) The Reptile Database. Available from:

<http://www.reptile-database.org> (September 13, 2022)

Vanzolini, P.E., Ramos-Costa, A.M.M. & Vitt, L.J. (1980) *Repteis das caatingas*. Academia Brasileira de Ciências, Texas, 161 pp.

Zaher, H. (1999) Hemipenial morphology of the South American Xenodontine Snakes, with a proposal for a monophyletic Xenodontinae and a reappraisal of Colubroid hemipenes. *Bulletin of the American Museum of Natural History* 240, 168 pages, 95 figures, 1 table, 2 appendices.

Zaher, H. & Prudente, A.L.C. (2003) Hemipenes of *Siphlophis* (Serpentes, Xenodontinae) and Techniques of Hemipenial Preparation in Snakes: A Response to Dowling. *Herpetological Review* 34, 302–307.

Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. (2013) A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29, 2869–2876.  
<https://doi.org/10.1093/bioinformatics/btt499>

## APPENDIX A – Material examined

*Phalotris labiomaculatus* (n=2) **BRAZIL:** MARANHÃO: Estreito (MPEG-24156); TOCANTINS: Filadelfia (MPEG-24378).

*Phalotris matogrossensis* (n=91): **BOLIVIA:** SANTA CRUZ: San Matias (UFMT-95), SANTA CRUZ DE LA SIERRA: Guarayos (MNKR 4811), Nuflo de Chavez (MNKR 1004 [paratype of *P. sansebastiani*]). **BRAZIL:** MATO GROSSO: Cáceres (UFMT-9000), Cuiabá (UFMT- 298, 1481, 1712, 2075, 2870, 3653, 4124, 5147, 5806, 5895, 5922, 6768, 7203, 7215, 7378, 7696, 8400, 9517, 9589, 9860, 10511, 10524, 11238, 11318, 11514, 11555, 11837, 11905, 11908, 11963, ZUEC-792), Nossa Senhora do Livramento (UFMT-11791), Poconé (UFMT-279, 1482, 1483, 11234), Pontes e Lacerda (UFMT-11049), Santo Antônio do Leverger (MCP-1747, UFMT-6591), Várzea Grande (UFMT-3187, 10494); MATO GROSSO DO SUL: Anastácio (ZUFMS-REP00185-186), Aquidauana (ZUFMS-REP00183-184, 187-190, 964, 1663, 1671, 1675, 2026, 2028, 2132, 2958, 2969-2970, 2972, 3313), Campo Grande (ZUFMS-REP00260, 1426-1427, 2027, 2110, 2169, 2386, 2483, 2487-2488, 2532, 3228, 3233, 3336), Corumbá (UFMT-1220-1222), Ladário (ZUFMS-REP00965), Porto Murtinho (ZUFMS-REP00182, 3223), Selvíria (ZUFMS-REP02682), Terenos (ZUFMS-REP03278). **PARAGUAY:** CENTRAL: Aregua, (MNHNP2627), Asunción (MNHNP9279), Mariano Roque Alonso (MNHNP10085); CORDILLERA: Caacupe (MNHNP2628).

*Phalotris mertensi* (n=43): **BRAZIL:** ESPIRITO SANTO: Cachoeira do Itapemirim (IB-11040); GOIAS: Anápolis (IB-2631); MATO GROSSO DO SUL: Campo Grande (ZUFMS-REP00191, 1132); SÃO PAULO: Altinopolis (IB-1715), Américo Brasiliense (ZUEC-1675), Araraquara (IB-89290, MCP-878), Avaré (IB-2469, 82702), Bento de Abreu (IB-8523), Botucatu (IB-89277, 89313), Brotas (MCP-7315), Buri (IB-8434), Campinas (ZUEC-3530), Candido Mota (IB-9094), Cosmopolis (IB-1466), Ibira (IB-81508), Igarapava (IB-8993), Inúbia Paulista (IB-85300), Itapetininga (IB-91517, 92240), Itapolis (ZUEC-1688), Itú (IB-79799), Jarinu (IB-5159), Matão (ZUEC-4035), Mogi Guaçú (ZUEC-1469), Nova Europa (IB-7829), Novo Horizonte (MCP-811), Paderneiras (IB-86748), Pardinho (IB-84240), Penápolis (IB-8363), Pirassunuga (IB-2676), Pontal (IB-9653), Praia Grande (MCP-867), Rio Claro (IB-89655), Sagres (IB-84172, 85301), Sales de Oliveira (MCP-16754), Sumaré (IB-82259, ZUEC-173), Tambau (IB-7512).

*Phalotris nasutus* (n=8): **BRAZIL:** MATO GROSSO: Cláudia (UFMT-3773, 3779), Guarantã do norte (UFMT-7545), Nova Maringa (UFMT-11233), Querência (MPEG-21970),

Tangará da Serra (UFMT-11951), Vale de São Domingos (UFMT-1471); MATO GROSSO DO SUL: Nova Andradina (ZUFMS-REP02190).

*Phalotris tricolor* (n=10): **BOLIVIA:** SANTA CRUZ DE LA SIERRA: Andres Ibañez (MNKR 3009), Angel Sandoval (MNKR 1393), Cordillera (MNKR 1884, 3312, 4171), Villa Olimpo (MNKR 2516). **PARAGUAY:** BOQUERÓN: Filadelfia (MNHNP5158-5159, 5161, 10029, 10099, 10574).

### Table Caption

Table 1. Meristic and morphometric characters in *Phalotris matogrossensis*. Variables: SVL, snout-vent length; TL, tail length; HL, head length; BC, Black Cap; WnL, white nuchal collar; BnL, black nuchal collar; SL, supralabials; IL, infralabials; Il+AC, infralabials in contact with the chin shield anterior; Il+PC infralabials in contact with the chin shield posterior; PE, preoculars; PO, postoculars; Te, temporal.

<i>Phalotris matogrossensis</i>		
<b>Variables</b>	<b>Male (n=47)</b>	<b>Female (n=42)</b>
SVL	142–549	165–885
TL	15,1–57,8	10,7–55,7
TL/SVL	0,079–0,111 (0,100 ± 0,006)	0,059–0,074 (0,069 ± 0,003)
HL	6,8–17,7	10,3–26,2
Suprapalbial Length/ HL	0,75–0,87 (0,8 ± 0,02)	0,65–0,87 (0,78 ± 0,04)
BC/HL	0,63–0,78 (0,72 ± 0,03)	0,64–0,77 (0,71 ± 0,03)
WnL/HL	0,37–0,64 (0,51 ± 0,06)	0,36–0,71 (0,49 ± 0,07)
WnL/SVL	0,012–0,020 (0,016 ± 0,002)	0,011–0,022 (0,015 ± 0,002)
BnL/HL	0,33–0,67 (0,48 ± 0,09)	0,25–0,55 (0,41 ± 0,08)
BnL/SVL	0,010–0,033 (0,016 ± 0,004)	0,008–0,017 (0,012 ± 0,002)
Ventrals	193–218 (205,3 ± 6,9)	204–235 (220,4 ± 7,2)
Subcaudals	26–33 (29,9 ± 1,6)	19–25 (23,1 ± 1,3)
Total segments	223–251 (236,3 ± 7,3)	230–261 (244 ± 7,5)
Gular	3–6 (4,3 ± 0,7)	3–6 (4,3 ± 0,6)
Dorsais	15–15–15	15–15–15
SL	6	6
IL	6 (n=6), 7 (n=40)	6 (n=1), 7 (n=37), 8 (n=1)
Il+AG	1–3 (n=6), 1–4 (n=40)	1–3 (n=1), 1–4 (n=38)
Il+PG	3–4 (n=6), 4–5 (n=40)	3–4 (n=1), 4–5 (n=38)
PreO	1 (n=46), 2 (n=1)	1
PO	2	2
Te	1+1	1+1
WnL (scales)	3–6 (4,6 ± 0,79)	3–7 (4,7 ± 0,9)
BnL (scales)	3–9 (4,5 ± 1)	2–6 (3,9 ± 0,8)

### Figure caption

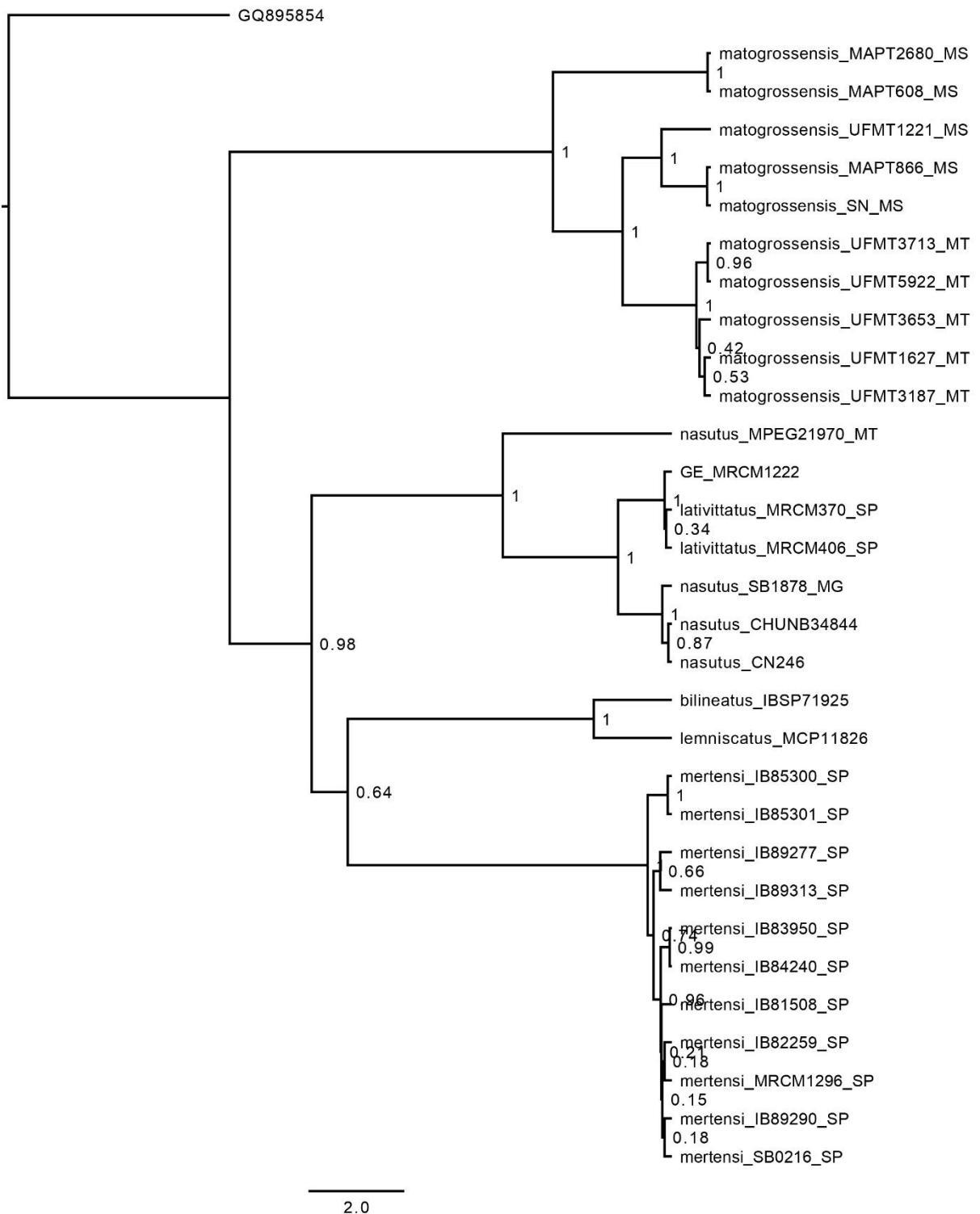


Figure 1. Phylogenetic tree using Cyt-b for species of the genus *Phalotris* recovered by Bayesian analysis in MrBayes.



Figure 2. *Phalotris matogrossensis* in life from Selviria, Mato Grosso do Sul state, Brazil. (ZUFMS-REP02682). Photo by Diego J. Santana.

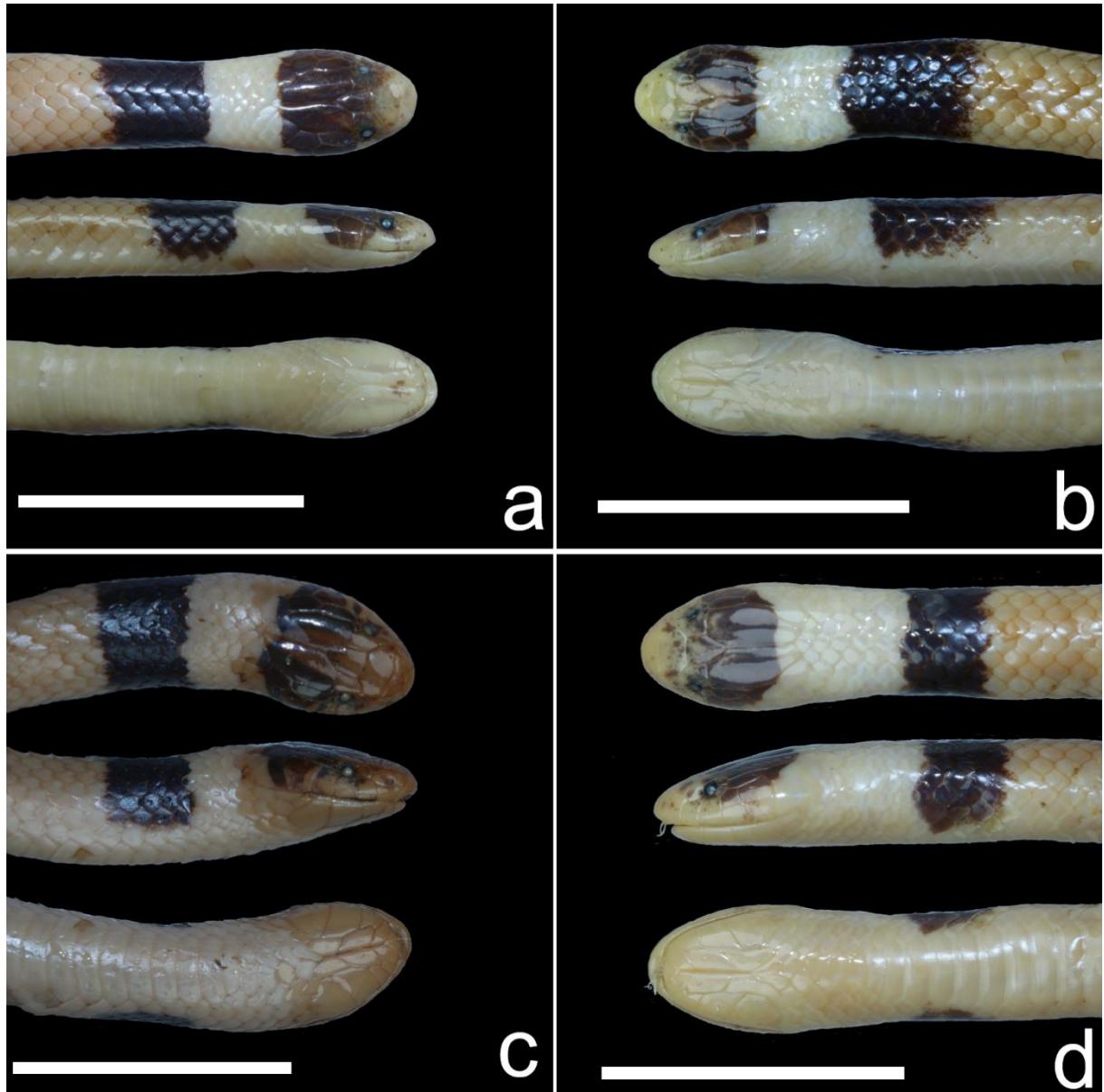


Figure 3. Patterns of coloration of the head and variation in white and black collar in dorsal, lateral, and ventral view. a) individual (UFMT-1222) from Corumbá, Mato Grosso do Sul, Brazil; b) individual (UFMT-1220) from Corumbá, Mato Grosso do Sul Brazil; c) individual (UFMT-95) from San Matias, Santa Cruz, Bolivia; and d) individual (UFMT-298) from Cuiabá (type locality), Mato Grosso, Brazil. Scale bar: 2cm.

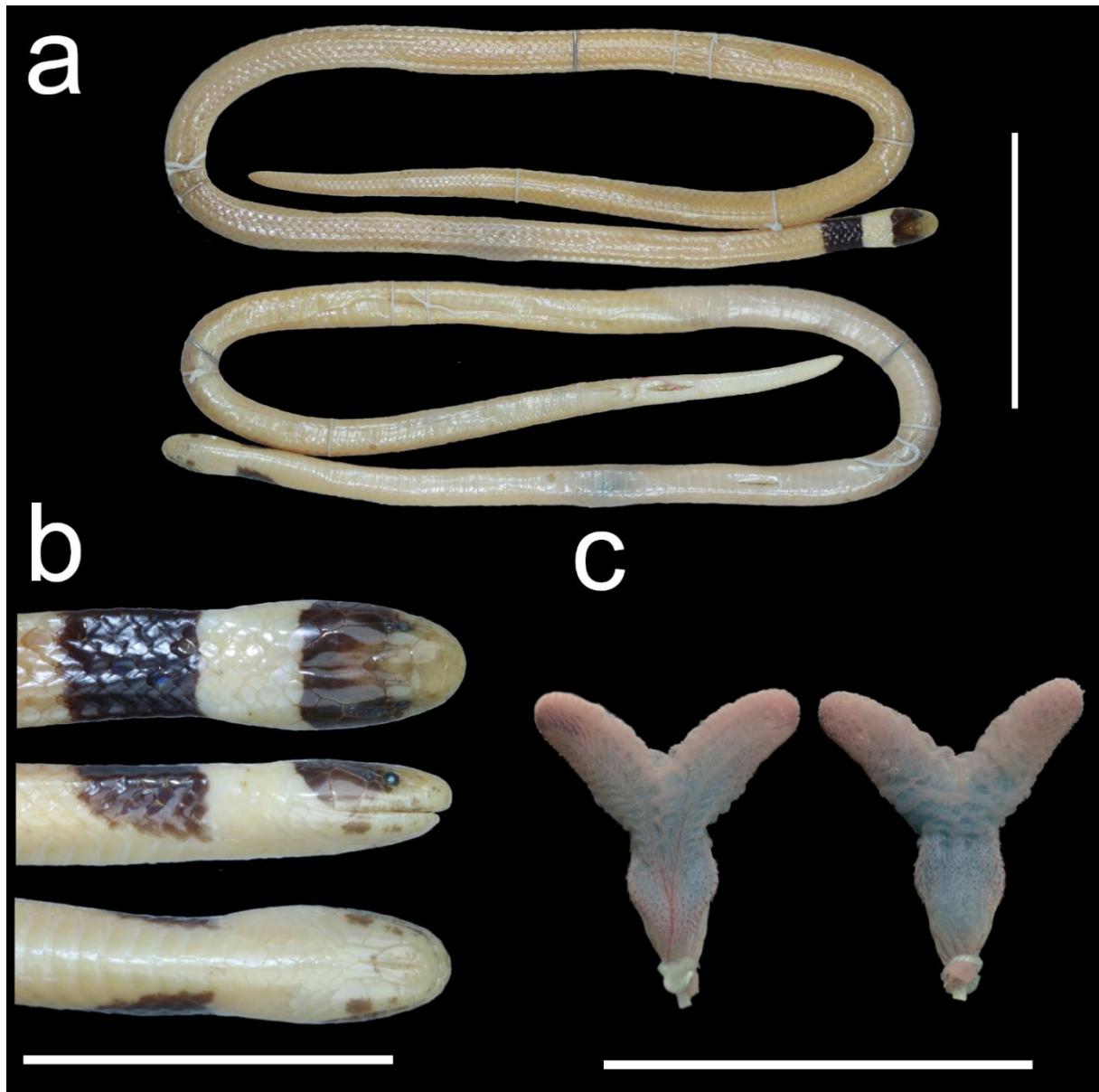


Figure 4. Coloration pattern, scalation and hemipenial morphology of *Phalotris matogrossensis*. a) dorsal and ventral view of *P. matogrossensis* (UFMT-1221); b) dorsal, lateral, and ventral view of the head and neck of *P. matogrossensis* (UFMT-1221); c) sulcate and asulcate view of the hemipenis (UFMT-298). Scale bar: 5cm (a), and 2cm (b, c).

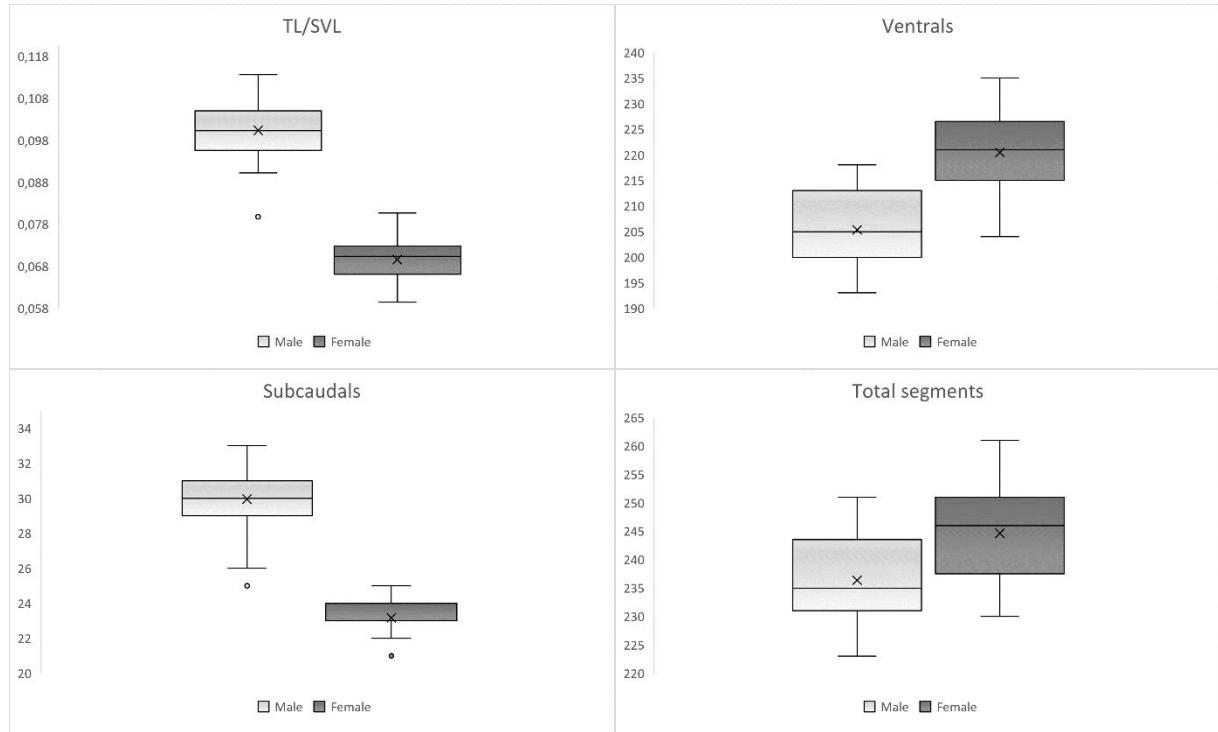


Figure 5. Box plot showing sexual dimorphism of tail length in relation with the snout-vent length (upper left), number of ventral scales (upper right), number of subcaudals scales (lower left), and number of total segments (lower right).

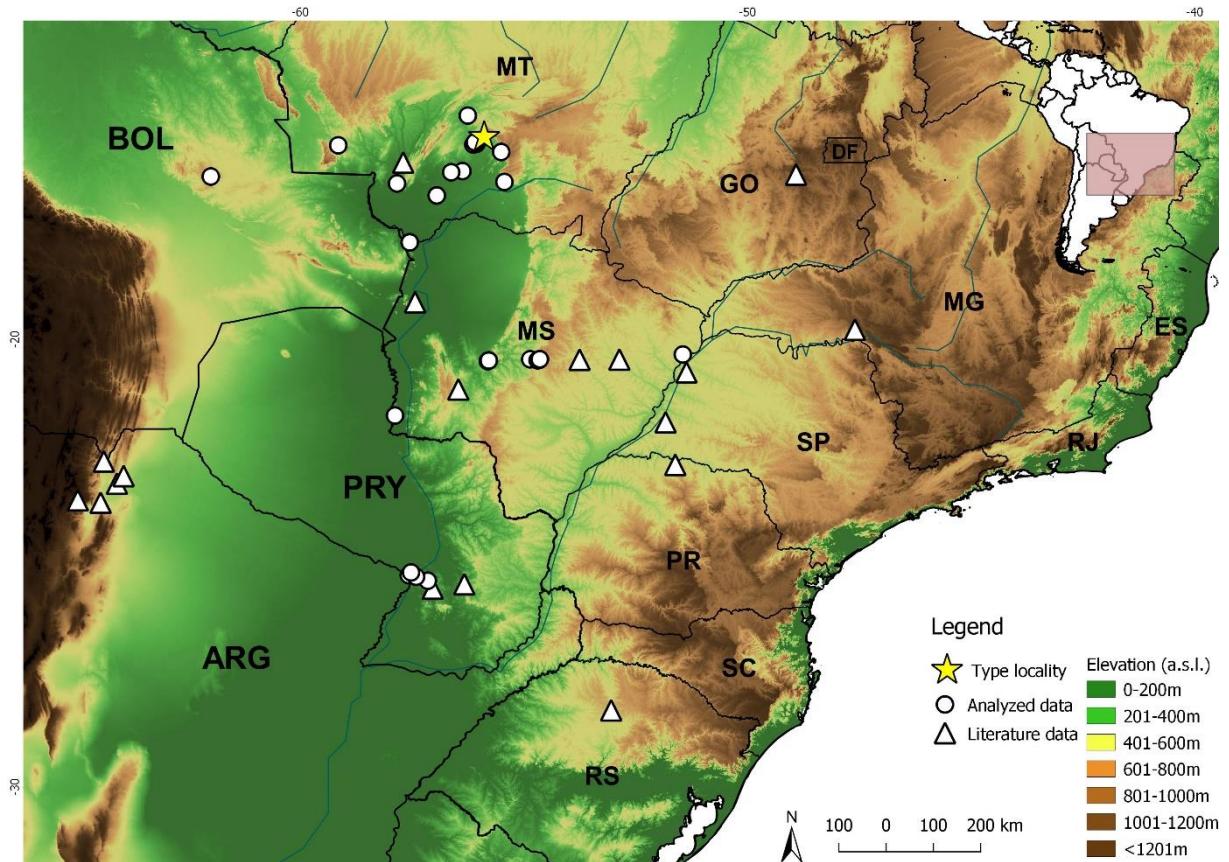


Figure 6. Distribution map based of *Phalotris matogrossensis*. Abbreviations: ES = Espírito Santo; GO = Goiânia; MG = Minas Gerais; MS = Mato Grosso do Sul; MT = Mato Grosso; PR = Paraná; RJ = Rio de Janeiro; RS: Rio Grande do Sul; SP = São Paulo; ARG= Argentina; BOL: Bolivia; PRY = Paraguay.

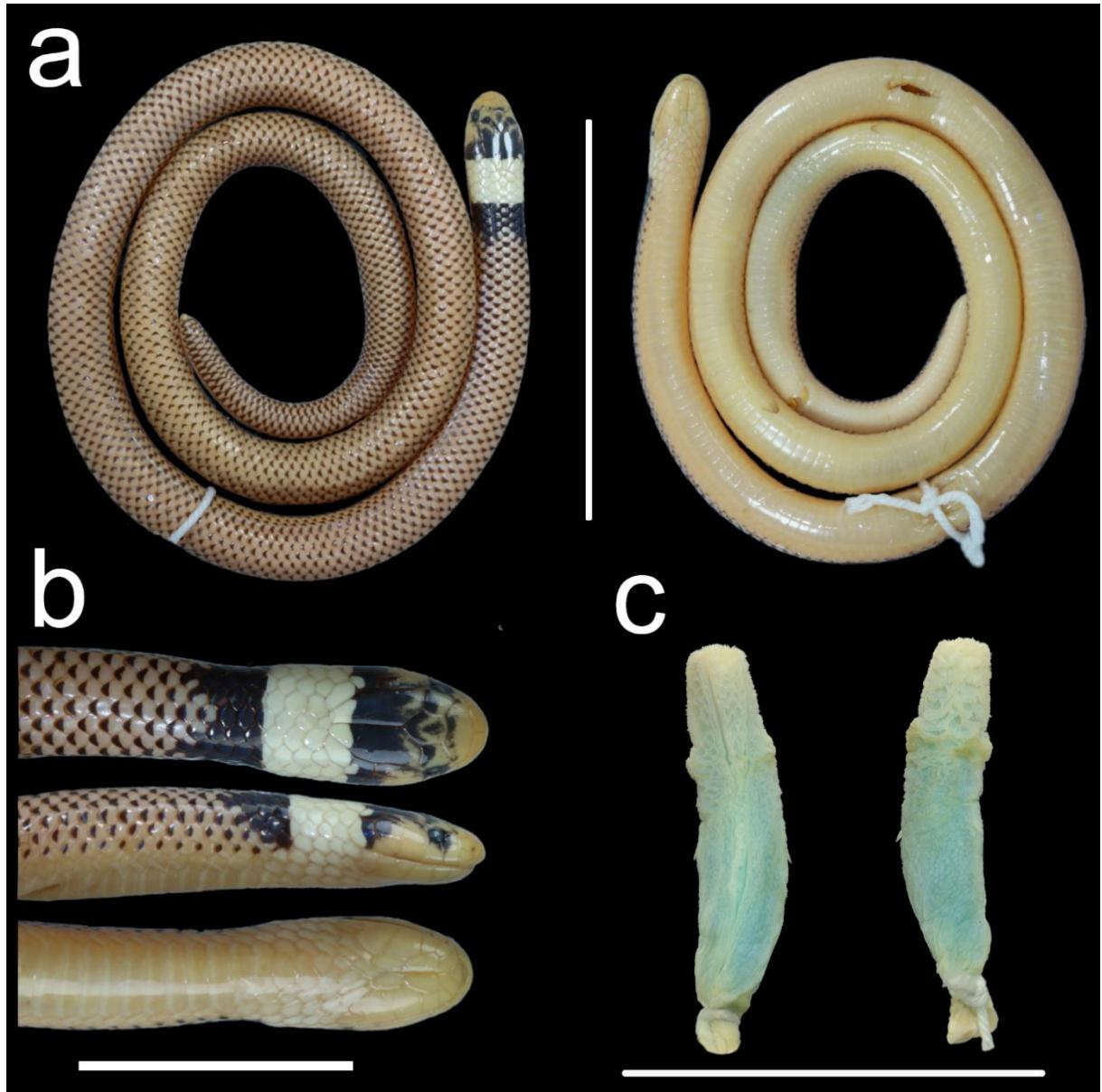


Figure 7. Coloration pattern, scalation and hemipenial morphology of *Phalotris mertensi*. a) dorsal and ventral view of *P. mertensi* (IB-89313); b) dorsal, lateral, and ventral view of the head and neck of *P. mertensi* (IB-89313); c) sulcate and asulcate view of the hemipenis (ZUEC-4035). Scale bar: 5cm (a), and 2cm (b, c)



Figure 8. *Phalotris tricolor* in dorsal and ventral view showing black spots along de dorsal region of the body. Voucher MHNHNP-10574 from Paraguay, Department of Boqueron, Filadelfia, CRC: 526mm and TL: 56mm.

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