



Renata Perez Maciel

Revisão taxonômica e sistemática filogenética do complexo de espécies associadas à *Amphisbaena darwini* (Amphisbaenia: Amphisbaenidae) a partir de dados morfológicos e moleculares

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Tese apresentada ao Programa de Pós Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito à obtenção do título de Doutor em Biologia Animal

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Orientador: Prof. Dr. Márcio Borges-Martins

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*Closing time, open all the doors
And let you out into the world
Dan Wilson*

Deixando o Pago

Vitor Ramil

*Alcei a perna no pingo
E saí sem rumo certo
Olhei o pampa deserto
E o céu fincado no chão
Troquei as rédeas de mão
Mudei o pala de braço
E vi a lua no espaço
Clareando todo o rincão*

*E a trotezito no mais
Fui aumentando a distância
Deixar o rancho da infância
Coberto pela neblina
Nunca pensei que minha sina
Fosse andar longe do pago
E trago na boca o amargo
Dum doce beijo de china*

...
*Cruzo a última cancela
Do campo pro corredor
E sinto um perfume de flor
Que brotou na primavera.
À noite, linda que era,
Banhada pelo luar
Tive ganas de chorar
Ao ver meu rancho tapera*

*Como é linda a liberdade
Sobre o lombo do cavalo
E ouvir o canto do galo
Anunciando a madrugada
Dormir na beira da estrada
Num sono largo e sereno
E ver que o mundo é pequeno
E que a vida não vale nada*

...
*Falam muito no destino
Até nem sei se acredito
Eu fui criado solito
Mas sempre bem prevenido
Índio do queixo torcido
Que se amansou na experiência
Eu vou voltar pra querência
Lugar onde fui parido*

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Resumo

Amphisbaenidae é a mais diversa das seis famílias de Amphisbaenia, com 12 gêneros e 176 espécies distribuídas no Caribe, África e América do Sul. Mesmo com uma melhor descrição da diversidade atual do grupo, há uma necessidade clara de revisões taxonômicas e de descrições morfológicas detalhadas que auxiliem nas identificações dos táxons e seus limites. São ainda comuns identificações equivocadas em coleções científicas, em parte pela má delimitação de muitos táxons ao longo de suas distribuições geográficas. Até a década de 1960, não existiam trabalhos de revisão taxonômica que incluíssem anfisbenas Sul-Americanas. Alguns grupos de espécies foram propostos, tendo por base similaridades morfológicas. Apesar de várias espécies já estarem formalmente descritas na época, a maioria dos exemplares da região sul da América do Sul, depositados em coleções científicas, eram identificados como *Amphisbaena darwinii* Duméril & Bribon 1839. Gans (1966) realizou uma extensa revisão e identificou uma complexa diversidade de formas amplamente distribuídas nas regiões sudeste e sul do Brasil, Uruguai e Argentina, reconhecendo um grupo de espécies relacionadas à *Amphisbaena darwinii*. Esse complexo, composto inicialmente por oito espécies (*A. albocingulata*, *A. darwinii*, *A. heterozonata*, *A. hogei*, *A. munoai*, *A. prunicolor* e *A. trachura*), era associado por apresentar similaridades morfológicas e de distribuição geografia. Vários destes táxons apresentam sobreposição em suas características (número de anéis do corpo, cauda e segmentos dorsais e ventrais), o que vinha dificultando a sua identificação. Com o acúmulo de dados oriundos de coletas realizadas na última década e através de revisões prévias, observamos a existência de variações morfológicas não descritas e limites mal definidos entre essas espécies. Essas observações reforçaram a necessidade de uma revisão taxonômica detalhada dos táxons associados à *Amphisbaena darwinii*, além de testar o relacionamento filogenético destes táxons. Revisando as espécies de *Amphisbaena* associadas à *A. munoai* observamos existência de uma diversidade maior que a formalmente descrita, com pelo menos cinco linhagens principais, que apresentam distribuição geográfica disjunta e distâncias genéticas compatíveis com o status de espécies plenas. Das cinco linhagens, apenas duas estão associadas a nomes disponíveis, sendo as outras três descritas no presente estudo. Em relação às espécies mais associadas à *Amphisbaena darwinii*, nossos resultados identificaram a presença de três táxons, um destes novo, e um complexo de espécies com pelo menos duas linhagens uma ao sul do Rio Grande do Sul e outra no Uruguai. Esses resultados aumentam para nove as espécies, deste grupo, conhecidas de *Amphisbaena* para a região sul do Brasil, Uruguai e Argentina. A relação filogenética dos táxons do grupo *darwinii* foi testada utilizamos uma matriz com 4323 caracteres, sendo um total de 4271 pares de bases (quatro genes mitocondriais e quatro genes nucleares) e 51 caracteres de morfologia interna e externa. Nosso resultado corrobora a hipótese de monofilia do grupo, identificando, contudo, poucas sinapomorfias morfológicas. Além disso, reforça que a utilização da nomenclatura genérica atual torna *Amphisbaena* um agrupamento parafilético. Estudos com a inclusão de um maior número de espécies são necessários para a tomada de decisões quanto as considerações taxonômicas ao nível genérico entre as Amphisbaenidae Neotropicais.

Introdução Geral

Amphisbaenia Gray 1844 é um grupo monofilético de répteis Squamata (Lee 1998; Kearney 2003, Kearney & Stuart 2004, Macey et al. 2004, Conrad 2008, Pyron et al 2013, Longrich et al 2015) totalmente adaptados ao modo de vida fossorial (Gans 1974, 1978, Kearney 2003). Possuem diversas características consideradas vantajosas para esse modo de vida como corpo alongado, cauda curta, além de modificações no ouvido interno e um crânio fortemente ossificado e modificações dependendo do modo de escavação que utilizam (Gans 1974, Kearney 2003). Embora a origem do clado permaneça em discussão (Gauthier et al 2012), diversos estudos as relacionam com Lacertidae, indicando que sua similaridades com serpentes e outros Squamata ‘serpenteformes’ pode ser convergente (Vidal & Hedges 2005, Conrad 2008, Gauthier et al 2012, Pyron et al 2013, Longrich et al 2015).

São reconhecidas atualmente seis famílias, sendo Amphisbaenidae Gray 1865 a mais diversa, com 12 gêneros e 176 espécies distribuídas no Caribe, América do Sul e África subsaariana (Gans 2005, Measey & Tolley 2013, Costa & Bérnils 2015, Uetz & Hosek 2016). Todas as hipóteses de relacionamento filogenético disponíveis consideram Amphisbaenidae grupo irmão de Trogonophidae 1865, formando o clado Afrobaenia Gauthier et al 2012 que provavelmente teve sua origem na África (Gauthier et al 2012, Measey & Tolley 2013, Longrich et al 2015). Estudos recentes sugerem a origem de Amphisbaenidae no Paleoceno, através de eventos de dispersão a longas distâncias da América do Norte para a África (64,7–56 milhões de anos atrás), com posterior dispersão para América do Sul (43,4–35,8 milhões de anos atrás) e Caribe (34,7–30 milhões de anos atrás) (Longrich et al 2015). Os Amphisbaenidae atuais, apresentam fortes indicativos de radiação adaptativa e diversidade paralela entre África e América do Sul, apresentando formatos de cabeça similares nos dois continentes, provavelmente devido a evolução convergentes de estratégias especializadas para escavação (Longrich et al 2015).

As hipóteses de relacionamento filogenético dentro de Amphisbaenidae são escassas e instáveis, variando conforme os táxons ou caracteres incluídos (Mott & Vieites 2009; Measey & Tolley 2013; Longrich et al 2015). Nas hipóteses moleculares, as espécies Africanas não formam um agrupamento monofilético, porém o clado com os gêneros *Geocalamus* Günther 1880, *Dalophia* Gray 1865 e *Monopeltis* Smith 1848 é recuperado como grupo irmão das espécies Neotropicais (Measey & Tolley 2013, Longrich et al 2015). As espécies Caribenhas são recuperadas em um clado monofilético entre os demais clados de Amphisbaenidae sul-americanas (Mott & Vieites 2009; Longrich et al 2015), o que, segundo Longrich et al

(2015), indica uma dispersão secundária de Amphisbaenidae sul-americanas para a América Central.

Em relação a morfologia, as sinapomorfias são disponíveis apenas para o reconhecimento entre as famílias (Kearney 2003, Conrad 2008, Gauthier et al 2012), sendo o reconhecimento de gêneros e demais agrupamentos infra-genéricos dependentes de características morfológicas externas, sem teste de significância filogenética. Nas espécies Sul-americanas, os gêneros tradicionalmente reconhecidos e diagnosticáveis principalmente pelas modificações no formato da cabeça e cauda (*Anops* Bell 1833, *Aulura* Barbour 1914, *Bronia* Gray 1865, *Cercolophia* Vanzolini 1992 e *Leposternon* Wagler 1824), não se mostraram monofiléticos, o que levou à proposta de sinonimização de todos a *Amphisbaena* (Mott & Vieites 2009). Ribeiro et al (2012) argumentarem contra a proposta de Mott & Vieites (2009) para manter *Leposternon*, sendo este o único gênero monofilético obtido através das análises (ver Mott & Vieites 2009, Pyron et al 2013, Longrich et al 2015). Esse arranjo tornou *Amphisbaena* parafilético, mas é utilizado para evitar novas mudanças taxonômicas neste grupo que apresenta relacionamento filogenético ainda instável (Costa & Bérnils 2015). Portanto, a delimitação de muitos grupos é tentativa e tem como base associações taxonômicas tradicionais entre as espécies morfológicamente mais similares (ver Vanzolini 1951; Gans 1962, 1966, 1971). Caracteres merísticos ou de escutelação cefálica, amplamente empregados no reconhecimento de grupos, têm significância filogenética incerta.

O Brasil possui a maior diversidade de espécies de Amphisbaenidae da América do Sul, com três gêneros e 73 espécies reconhecidas (Costa & Bérnils 2015, Uetz & Hosek 2016), algumas delas, descritas nos últimos anos, baseadas principalmente em novas coletas (ver Pinna et al 2010; Gomes & Maciel 2012; Pinna et al 2014; Roberto et al 2014; Teixeira et al 2014). Mesmo com uma melhor descrição da diversidade atual do grupo no país, ainda são escassos os estudos com caracterizações morfológicas e que auxiliem nas identificações dos táxons e seus limites (Vanzolini 2002, Ribeiro et al 2009, Perez et al 2012; Pinna et al 2010), sendo ainda comum as identificações equivocadas dos exemplares nas coleções científicas e a má delimitação dos táxons ao longo de suas distribuições geográficas.

Ao revisar as espécies de *Amphisbaena* do sul da América do Sul, Gans (1966) reconheceu um complexo de espécies comumente associadas à *Amphisbaena darwinii* Duméril & Bibron 1839. Essas espécies, *Amphisbaena albocingulata* Boettger 1885, *A. heterozonata* Burmeister 1861, *A. hogei* Vanzolini 1950, *A. munoai* Klappenbach 1960, *A. nigricauda* Gans 1966, *A. prunicolor* (Cope 1885) e *A. trachura* Cope 1885, em algum momento foram identificadas ou descritas a partir de *A. darwinii*. A associação inicial entre

essas espécies, no entanto, não é clara, os exemplares compartilham algumas características como cerca de 200 anéis do corpo, menos de 40 segmentos no anel do meio do corpo e quatro poros pré-cloacais que são observadas em outras espécies de *Amphisbaena*. Além disso, Gans (1966) reconheceu *A. heterozonata* e *A. trachura*, como subespécies de *A. darwinii*; *A. albocingulata* como subespécie de *A. prunicolor*; invalidou a determinação feita por Vanzolini (1950) identificando *A. hogei* como uma espécie plena e não como uma subespécie de *A. darwinii*; descreveu *A. nigricauda*; e fez comentários sobre a variação de *A. munoai*. Anos mais tarde, Vanzolini (2002) elevou ao nível específico essas táxons indicados como subespécies, sem comentários acerca de das diagnoses. A identificação destas espécies permaneceu baseada nos caracteres subespecíficos indicados em Gans (1966) tornando-se complexa devido à grande sobreposição de caracteres morfológicos. No entanto, *Amphisbaena darwinii* e *A. munoai* têm sido hipotetizadas como espécies irmãs em todas hipóteses filogenéticas disponíveis (ver Mott & Vieites 2009, Measey & Tolley 2013, Longrich et al 2015).

Considerando o acúmulo de dados oriundos de coletas realizadas na última década e revisões prévias (ver Perez et al 2012) observamos a existência de variações morfológicas não descritas e limites mal definidos entre esses táxons. Essas observações reforçaram a necessidade de uma revisão taxonômica detalhada dos táxons associados à *Amphisbaena darwinii*, além de apresentar uma hipótese de relacionamento filogenético testando se formam um agrupamento monofilético e discutindo sua relação com demais *Amphisbaena*.

O presente projeto foi vinculado ao projeto ‘Taxonomia e Sistemática de Lagartos e Serpentes (Lepidosauria, Squamata) no Bioma Pampa no sul do Brasil e Uruguai’, aprovado pelo Edital MCT/CNPq/MEC/CAPES nº 52/2010 do Programa de Capacitação em Taxonomia (PROTAX).

Objetivos

- I. Revisar e (re)descrever as unidades taxonômicas associadas à *Amphisbaena munoai* no sul do Brasil e Uruguai.
- II. Revisar e (re)descrever as unidades taxonômicas associadas à *Amphisbaena darwinii* no sul do Brasil, Uruguai e Argentina.
- III. Apresentar uma hipótese de relacionamento filogenético para as espécies associadas à *Amphisbaena darwinii*, testando se formam um grupo monofilético e discutindo sua relação com os demais grupos de *Amphisbaena*.

Estrutura da Tese

A tese está organizada em três capítulos que seguem a formatação das revistas a que serão submetidos, menos em relação a disposição das figuras, incluídas no meio do texto para facilitar a interpretação.

- **Capítulo I** – Revisão taxonômica das pequenas anfisbenas relacionadas à *Amphisbaena munoai* Klappenbach 1960 do extremo sul do Brasil e Uruguai (Amphisbaenia, Amphisbaenidae)
 - Neste capítulo avaliamos a existência de unidades taxonômicas entre espécimes recentemente coletadas e morfologicamente similares à *Amphisbaena munoai* e *A. prunicolor*. Nossos resultados indicaram uma diversidade maior que a conhecida com pelo menos cinco linhagens principais, distribuídas de forma disjunta na região sul do Brasil e Uruguai. Essas linhagens apresentam forte estrutura filogenética e divergências morfológicas compatíveis com espécies plenas.

- **Capítulo II** – Revisão taxonômica das cobras-cegas associadas à *Amphisbaena darwinii* Duméril & Bibron 1839 (Amphisbaenia: Amphisbaenidae).
 - Neste capítulo utilizamos caracteres morfológicos (qualitativos e quantitativos), análises filogenéticas moleculares (genes mitocondriais e nuclear) e limites geográficos para reavaliação o status taxonômico das espécies associadas à *Amphisbaena darwinii*. Nossos resultados indicam a existência de um novo táxon, confundido com *A. trachura* e *A. darwinii*, e dificultado a delimitação destas espécies. Além disso, restringe o complexo *Amphisbaena darwinii* a duas linhagens, uma com população no sul do Rio Grande do Sul, Brasil e outra com população em Montevideo. E redefine a variação de *Amphisbaena trachura* e *A. heterozonata*.

- **Capítulo III** – Sistemática filogenética do grupo de *Amphisbaena darwinii* Duméril & Bibron 1839 (Amphisbaenia: Amphisbaenidae), baseado em caracteres morfológicos e moleculares.
 - Neste capítulo a monofilia do grupo *darwinii* é investigada através de caracteres morfológicos e moleculares. A matriz com 39 táxons e 4323 caracteres (4227 moleculares e 51 morfológicos) foi analisada através de Inferência Bayesiana. Os

resultados sugerem que o grupo *darwinii* é monofilético e é fortemente relacionado à *Amphisbaena kingii*, seu grupo irmão.

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Capítulo I

Revisão taxonômica das pequenas anfisbenas relacionadas à *Amphisbaena munoai* Klappenbach 1960 do extremo sul do Brasil e Uruguai (Amphisbaenia, Amphisbaenidae)

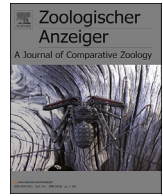
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Review

Integrative taxonomy of small worm lizards from Southern South America, with description of three new species (Amphisbaenia: Amphisbaenidae)

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ABSTRACT

Fossorial vertebrates such as amphisbaenians suffer from morphological constraints as a result of their burrowing strategies. The morphology is conserved in many groups and taxonomic arrangements may be confusing within closely related species. *Amphisbaena munoai* and *A. prunicolor* slender bodied worm lizards associated with the *Amphisbaena darwini* species group and distributed in southern South America (southern Brazil and Uruguay) were traditionally considered allopatric and distinguished from each other mainly by their coloration pattern and cephalic shields. However, the reassessment of morphological variation including specimens from new localities indicated the need for a taxonomic reappraisal. We used morphological, molecular and distributional data in an integrative approach to reassess taxonomic diversity and propose a phylogenetic hypothesis for the evolution of the group. Our results indicated a hidden diversity, allowing the description of three new species from Coastal and Grasslands formations in southern Brazil. *Amphisbaena tiaraju* sp. nov. occurs in the northwestern region of Rio Grande do Sul State; *Amphisbaena arenicola* sp. nov. is restricted to Restinga formations in the coastal plain of Santa Catarina State and *Amphisbaena nana* sp. nov. is restricted to the northern portion of the Sul-rio-grandense Shield in Rio Grande do Sul State.

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1. Introduction

In fossorial vertebrates, the environment imposes strong restrictions on morphology, causing the evolution of convergent and conservative morphology in several groups, including amphisbaenian squamates (e.g., Kearney & Stuart 2004; Measey 2006; Measey & Tolley 2013). In amphisbaenians, the limblessness, body elongation and cranial modifications, are characters related to different burrowing strategies (Kearney 2003; Gans 1974, 1978; Longrich et al. 2015). Morphology is allegedly conserved among several species groups (Gans 1977) and taxonomic arrangements are traditionally based on morphological characters with uncertain phylogenetic significance and lack of intra or interspecific variation, resulting in a confusing taxonomy (Kearney 2003; Gans 2005). Consequently any hypothesis of phylogenetic relationships within amphisbaenians remains scarce and unstable (see Mott & Vieites

2009; Measey & Tolley 2013; Longrich et al. 2015). *Amphisbaena Linnaeus 1758* is the most speciose genus in the family Amphisbaenidae Gray 1865, including a paraphyletic arrangement of 97 species distributed in the Caribbean and South America (Costa & Bérnils 2018; Uetz & Hosek 2018). The diversity, correct identification of species and their boundaries remains an open matter in many groups of *Amphisbaena* (e.g., Vanzolini, 1951; Gans, 1962, 1963a; Gans & Diefenbach, 1972; Vanzolini, 1997, 2002). For a lineage to acquire distinct properties, such as become morphological diagnosable, reciprocally monophyletic, ecological distinct or reproductive incompatible depends on various factors and occur in a different timeframe (Templeton 1981; De Queiroz 1998, 2005, 2007).

This forms a large gray zone within species present only some of these properties and represents a conflict for species delimitation (De Queiroz 1998, 2005, 2007). Recently DNA markers has been used to recognize divergent lineages, hidden in a single species with widespread geographical distribution (Fouquet et al., 2007; Geurgas & Rodrigues 2010; Recoder et al. 2014; Guarnizo et al. 2016). When applying an integrative taxonomical approach it is

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also possible to delimit species as a hypothesis (Dayrat 2005; Padial et al. 2010) considering species as a single set of metapopulations evolving separately and forming an ancestor-descendant lineage (De Queiroz 1998, 2005, 2007). Different data sets i.e., geographical distribution, ecological and physiological differences, morphological and molecular data, are relevant evidences for lineages separations and may be used in an integrative way to recognize different species (Padial et al. 2010). Usually, the congruence between more lines of evidence is better to delimit species and their boundaries (Dayrat 2005; De Queiroz 2005; Padial et al. 2010).

When revising South American *Amphisbaena*, Gans (1966) recognized eight species related to *Amphisbaena darwinii* Duméril & Bibron, 1839. These species overlapped in meristic characters (i.e., number of segments and body annulus) and geographical distribution, being sympatric in southern Brazil, eastern Argentina and Uruguay (Gans 1966; Perez et al. 2012). Out of these *Amphisbaena munoai* Klappenbach, 1960 and *Amphisbaena prunicolor* (Cope, 1885) are distinguished from the other congeners by having a slender body, higher number of body annuli (close to 200), the presence of post-malar scales row, a uniform tail coloration and parietal shields enlarged and differentiated from other body shields (Perez et al. 2012). Interspecifically they are conventionally distinguished by the shape of the frontal shields (angular in *A. munoai* and quadrangular in *A. prunicolor*) and their coloration pattern (uniform light brown in *A. munoai* and purplish brown with checkered ventral pattern in *A. prunicolor*) (Perez et al. 2012). In addition they have allopatric distributions i.e., *A. munoai* is found under stones in rocky outcrops in the Uruguayan Savannah in central and southern Rio Grande do Sul state, Brazil and Uruguay and *A. prunicolor* in Atlantic Forest remnants in northern Rio Grande do Sul, western Santa Catarina and Paraná, with historical records in the States of Rio de Janeiro, São Paulo and Espírito Santo, in Brazil; Corrientes and Misiones provinces, in Argentina and Itapuá department, in Paraguay (Perez et al. 2012). During field expeditions into open areas in northwestern Rio Grande do Sul (Missões region in the Araucaria Plateau) and Restingas areas in eastern Santa Catarina (surrounding Santa Catarina Island, Florianópolis municipality) in Southern Brazil specimens morphologically similar to *A. munoai* and *A. prunicolor* were found. Despite the overall similarity, these specimens show pronounced differences in cephalic and body proportions and coloration pattern making a taxonomic allocation impossible. Besides, these specimens were collected in areas where both species were previously unknown to occur. These new findings including the morphological variation previously recorded in *A. munoai* (Perez et al., 2012) reinforced the need for a taxonomic reassessment of amphisbaenids found in southern Brazil and Uruguay.

Herein we applied an integrative taxonomic approach through phylogenetic analysis of molecular markers (mitochondrial and nuclear), jointly with morphology (meristic and morphometric characters), ecology (habitat use), and geographic distribution, which allowed the recognition of a hidden diversity and the need for description of three new species of *Amphisbaena*.

2. Material and methods

2.1. Taxon sampling

Our sample included 293 specimens, all examined for morphological characters. For 67 of these specimens we also obtained DNA sequences for four markers. We included specimens from different localities of the two recognized species, *Amphisbaena munoai* and *A. prunicolor* of southern Brazil and Uruguay, both holotypes were examined and original descriptions revised (i.e. Klappenbach 1960 and Cope 1885) to confirm the identifications.

Besides additional specimens from the new site records, which included at least two disjunct and distinctive populations, one from the surroundings of Florianópolis, State of Santa Catarina, Brazil and the other from the northwest State of Rio Grande do Sul, Brazil. We have sampled specimens to encompass the entire geographic distribution and morphological variation (see Gans 1966; Perez et al. 2012). Specimens were obtained from scientific collections throughout extensive field effort in the region to obtain new records and allow for tissue sampling. The material examined is housed in the following institutions: Academy of Natural Sciences (ANSP), Philadelphia, United States of America; Coleção Herpetológica da Universidade Federal de Santa Catarina (CHUFSC), Florianópolis, Brazil; Coleção Herpetológica da Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; Museo Nacional de Historia Natural, Montevideo, Uruguay (MNHN); Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul (MCN), Porto Alegre, Brazil; Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Porto Alegre, Brazil; Museu de História Natural Capão da Imbuia (MHNCL), Curitiba, Brazil; Museu Nacional do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil; Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil and Sección de Zoología Vertebrados Facultad de Ciencias UDELAR (ZVCR), Montevideo, Uruguay.

We selected ten species as outgroup for phylogenetic analysis based on the phylogeny of Mott & Vieites (2009), including sequences of *Amphisbaena anaemariae* Vanzolini, 1997, *Amphisbaena angustifrons* Cope, 1861, *Amphisbaena anomala* (Barbour, 1914), *Amphisbaena leeseri* Gans, 1964a, *Amphisbaena silvestrii* Boulenger, 1902 and *Leposternon infraorbitale* (Berthold, 1859) available on Genbank (Accession FJ441668; FJ441689; FJ441694; FJ441707; FJ441712; FJ441723; FJ441911; FJ441932; FJ441937; FJ441950; FJ441955; FJ441966) and sequences of *Leposternon microcephalum* (Wagler, 1824), *A. munoai*, *Amphisbaena darwinii*, *Amphisbaena trachura* Cope, 1885 and *Amphisbaena kingii* (Bell, 1833) generated in the current study. Being aware of the current taxonomic panorama of *Amphisbaena* we also extended our interspecific morphological comparisons to all known taxa, besides the outgroup and the *A. darwinii* complex recognized by Gans (1966) that included *Amphisbaena albocingulata* Boettger, 1885, *Amphisbaena heterozonata* Burmeister, 1861, *Amphisbaena hoguei* Vanzolini, 1950 and *Amphisbaena nigricauda* Gans, 1966 (see also Perez et al., 2012).

2.2. Molecular analyses

DNA was extracted from tissue samples previously preserved in 99% ethanol using the method of Medrano et al., 1990 with modifications. We used primers described previously (see Palumbi 1996; Pook et al. 2000; Macey et al. 2004; Noonan & Chippindale 2006) to obtain partial mitochondrial DNA (mtDNA) sequences from 16S ribosomal (16S), cytochrome b (CYTB) and NADH dehydrogenase subunit 2 (ND2) and partial nuclear sequence from neurotrophin-3 (NT3) genes to infer phylogenetic relationships within the species and to check for concordance between morphological patterns.

Amplifications were performed by polymerase chain reaction (PCR) in a 20 µl volume containing 10–50 ng of DNA, 1X Buffer, 1.5 µM of MgCl₂, 0.2 µM of dNTP, 0.2 µM of each primer, 1U of Taq DNA polymerase and 1 µl Triton 4%. The PCR reactions to 16S and ND2 genes were performed with an initial denaturation at 94 °C for 3 min, followed by 35 cycles (94 °C for 45 s, 54 °C for 45 s, 72 °C for 1 min and 30 s) and a final elongation at 72 °C for 5 min. For CTYB gene an initial denaturation at 94 °C for 4 min, followed by 35 cycles (94 °C for 60 s, 58 °C for 60 s, 72 °C for 2 min) and a final elongation at 72 °C for 5 min. For NT3 an initial denaturation at 95 °C for 2 min, followed by 30 cycles (94 °C for 45 s, 49 °C for 45 s, 72 °C for 1 min and 30 s) and a final elongation at 72 °C for 5 min.

The software Geneious version 6.1.6 (Kearse et al. 2012) was used to visualize and align sequences as well as a platform to export different formats. The sequences were aligned independently for each gene with Mafft Multiple Alignment plug-in on Geneious (version 1.3, Biomatters Ltd, Katoh, 2013), following the algorithm G–INS–I with standard definitions (gap opening = 1.53, gap extension = 0.123). We performed Bayesian Inference analyses on mitochondrial DNA (1551 bp), nuclear DNA (420 bp NT3 gene) and concatenated data set (1971 bp in total) with MrBayes 3.2 (Ronquist et al. 2012) to characterize the phylogenetic structure. The analysis was performed with 100 million Markov Chain Monte Carlo (MCMC), sampled every 1000 steps and the first 10% of steps were discarded as burn-in. We implemented the analyses with the GTR + Inv + Gamma model in 16S, SYM + Inv + Gamma in CYTB first partition, GTR + Inv model in CYTB second partition, GTR + Gamma model in CYTB and ND2 third partition, GTR + Gamma model in ND2 first and second partition and HKY + Inv in NT3, based on Partition Finder v1.1.0 previous analysis (Lanfear et al. 2012). We calculated mean distances between groups with software Mega 7 (Kumar et al. 2016), using Tamura–Nei corrected distances (Tamura & Nei 1993), and 1000 bootstrap replicates to estimate standard errors.

2.3. Morphological analyses

The terminology of cephalic shields followed Gans & Alexander (1962) and the arrangement of scales, counts and measures followed Perez et al. (2012). The measurements were taken with a digital caliper of 0.01 mm accuracy, except snout-vent length, measured with a ruler. Sex determination was performed by direct dissection and gonad analysis. To evaluate maturity and sexual dimorphism we followed Shine (1988), Colli & Zamboni (1999) and Balestrin & Cappellari (2011).

For initial comparative analysis, we considered the clades as groups recovered through molecular analysis (see Fig. 1). An exploratory descriptive analysis was performed to summarize the character variation among groups (i.e., means, medians, ranges and standard deviations). Normality and homoscedasticity assumptions were tested using Kolmogorov–Smirnov and Levene, respectively (Zar 2010). ANOVA or Kruskal–Wallis tests were performed to verify significant differences between characters (mean/median) on certain groups. We conducted an exploratory discriminant function analysis in the software Statistica10 (SystatSoftware Inc., 2006) to access the degree of distinction between taxa and possible diagnostic taxonomic characters (Manly 2000; StatSoft Inc., 2011). We used the ratios of morphometric characters and head length or snout-vent length to remove the effect of size in the samples.

3. Results

3.1. Molecular analyses

The phylogenetic analysis recovered a large monophyletic clade with five sub-clades representing distinct taxonomic units. These five monophyletic sub-clades were consistent with the geographic samples from southern Brazil and Uruguay. Based on the distribution and correlation with the holotype (see Perez et al., 2012) the Southern Atlantic Forest (AF) clade corresponded to *Amphisbaena prunicolor* and was identified as the sister group to all other taxa (Fig. 1). The northwestern Rio Grande do Sul, Araucaria Plateau (NW) clade was determined as the sister group to the other three clades. The clade of specimens of the northern portion of the sul-riograndense Shield of Rio Grande do Sul state (NSR) was recognized as sister taxa to the Coastal Plain of Santa Catarina (CP) clade

and the southern region of the sul-riograndense shield in Brazil and Uruguay (SSR) clade. The SSR clade from various localities of southeast Rio Grande do Sul state and northeast Uruguay, including the type locality, corresponded to *A. munoai* specimens. The specimens from the NSR clade corresponded to specimens usually identified as *A. munoai* but considering a morphological variation already noticed by Gans (1966) and reinforced by Perez et al. (2012). The NW and CP clades had no taxonomic allocations.

Corrected mitochondrial DNA distances (genes concatenated) varied between 3.3% and 8.5% within ingroup clades and between 8.6% and 58.3% with the outgroup (Table 1). Most distant *A. prunicolor* specimens varied between 6.7% and 8.5% within the ingroup. The NW and CP clades were the least distant with a variation of only 3.3%. In relation to *A. darwinii* and *A. trachura*, the phylogenetically closest species, the ingroup clades varied between 8.6% and 14.4%. A greater distance observed in *Leposternon microcephalum* and *L. infraorbilata* represented a monophyletic and separate clade in Mott & Vieites 2009 previous analysis.

Considering only the 16S gene (missing data excluded) the corrected distance ranged from 2.4% to 6.9% between the ingroup clades (Table 1) and from 4.1% to 19.9% with the outgroup. The NW clade showed higher distances, varying between 5.1% and 6.9% within the ingroup. *A. munoai*, CP and NSR clades had smaller distances, varying between each other from 2.4% to 3.6%. The distance of the *A. darwinii* complex varied between 4.1% and 7.9%. Greater distances observed in ingroup clades and between *A. anaemariae* and *A. silvestrii*, varying between 18.2% to 19.8% and 18.1%–20.7% respectively.

3.2. Morphological analyses

All meristic characters, except the autotomic annuli, varied significantly (Kruskal–Wallis, $P < 0.01$) between molecular clades (reassembled sample). The exploratory discriminant analysis indicated a greater differentiation between *Amphisbaena prunicolor* than in other groups. The first discriminant function represented 89% of variation and was positively correlated with the number of body annuli (Fig. 2).

Regarding morphometric characters, we observed significant variations between means/medians in almost all characters (Kruskal–Wallis or ANOVA, $P < 0.01$). The first discriminant function represented 71% of variation and was positively correlated to pre-frontal width and negatively correlated to jaw length (Fig. 3A–C) indicating differentiation between *A. prunicolor* and the NSR specimens. The second discriminant function represented only 14.9% of variation and was positively correlated to snout length and negatively to mental width (Fig. 3A, D–E). The discriminant function indicated differentiation between NW and CP specimens.

In our attempt to detect morphological characters in order to differentiate the closest molecular clades (NSR, CP and *A. munoai*), we performed an exploratory discriminant function analysis only with these samples. In our analysis, the first discriminant function represented 57.2% of variation and was positively correlated to nasal suture length and negatively to pre-frontal length, allowing the distinction of CP specimens from the *A. munoai* sample (Fig. 3F–H). The second discriminant function represented 42.8% of variation and was positively correlated to parietal length and negatively to rostral width separating the CP from the NSR sample (Fig. 3F, I–J). The *A. munoai* and SR sample overlapped but had a good tendency to discriminate in both discriminant functions.

The classification matrix for the NW and CP sample of *A. prunicolor* indicated an accuracy of identification of more than 90%. *A. munoai* and the NSR sample had lower values of 86.9% and 83.3% of correspondence respectively (Table 2).

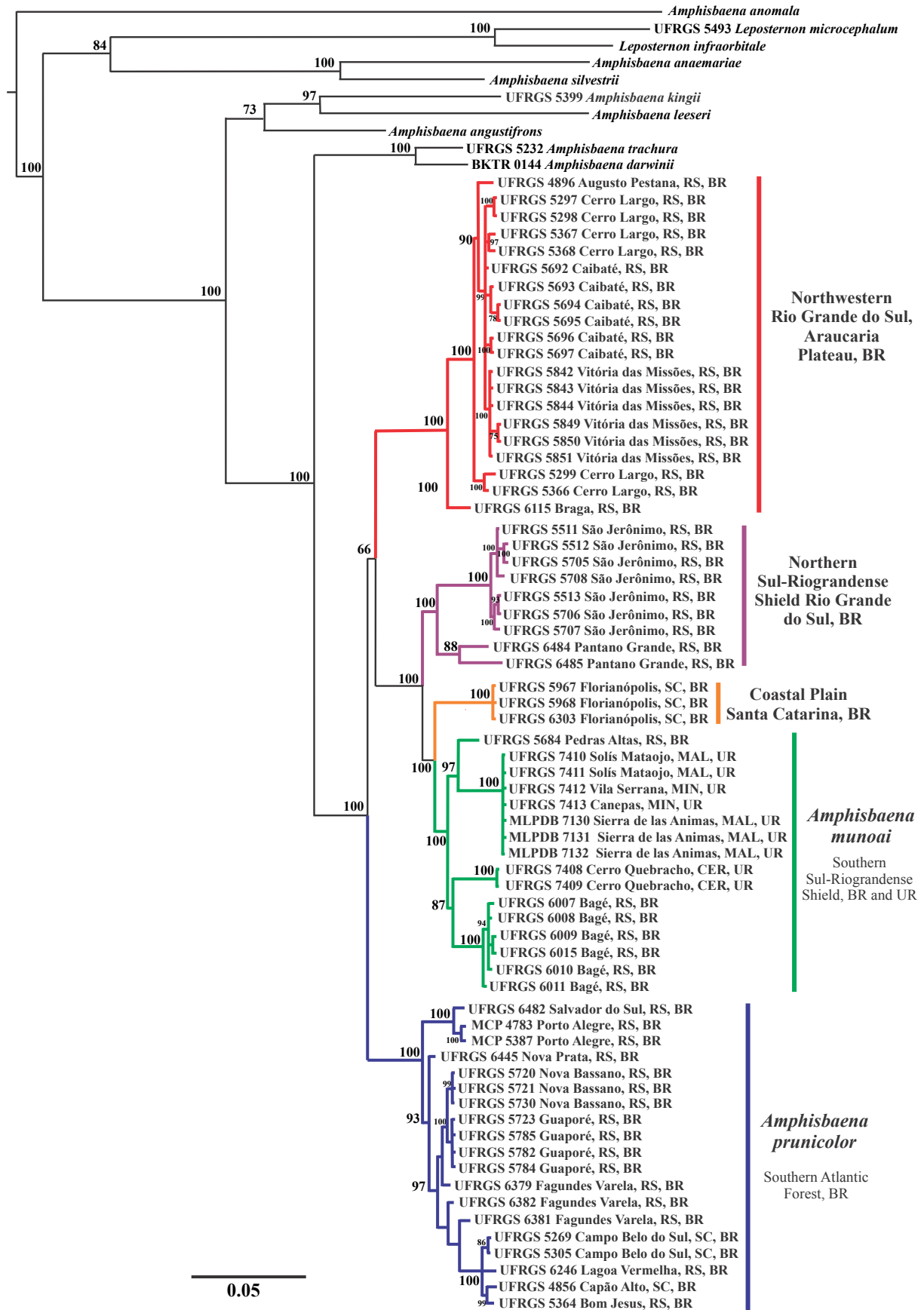


Fig. 1. Bayesian Inference tree based on mitochondrial (16S, CYTB and ND2) and nuclear (NT3) genes concatenated of *Amphisbaena* spp. related to *Amphisbaena munoai* from southern Brazil and Uruguay. Node values represent posterior probability in percentage.

Table 1
Net among group mean distance between *Amphisbaena* spp. Values below the diagonal are Tamura Nei corrected p-distance of mtDNA concatenated plus respective standard error, calculated using 1000 bootstrap replicates. Values above diagonal are Tamura-Nei corrected p-distance of 16S rDNA plus respective standard error, calculated using 1000 bootstrap replicates. Aa: *Amphisbaena anaemariae*; Ad: *A. darwinii*; AF: Southern Atlantic Forest (*Amphisbaena prunicolor*); Ag: *A. angustifrons*; Ak: *A. kingii*; Al: *A. leeseri*; Ao: *A. anomala*; As: *A. silvestrii*; At: *A. trachura*; CP: coastal plain of Santa Catarina state; Lm: *Leposternon microcephalum*; Li: *L. infraorbitale*; NSR: northern region of sul-riograndense Shield Rio Grande do Sul state; NW: northwestern of Rio Grande do Sul state; SSR: southern region of sul-riograndense shield in Brazil and Uruguay (*Amphisbaena munoai*).

	AF	NW	NSR	CP	SSR	At	Ad	Ag	Ak	Aa	Al	As	Lm	Li	Ao
AF		0.051 (0.01)	0.049 (0.01)	0.045 (0.01)	0.050 (0.01)	0.041 (0.01)	0.067 (0.01)	0.089 (0.01)	0.099 (0.02)	0.194 (0.03)	0.139 (0.02)	0.181 (0.02)	0.165 (0.02)	0.172 (0.02)	0.172 (0.02)
NW	0.085 (0.02)		0.069 (0.01)	0.061 (0.01)	0.064 (0.01)	0.050 (0.01)	0.063 (0.01)	0.087 (0.01)	0.125 (0.02)	0.182 (0.02)	0.142 (0.02)	0.207 (0.03)	0.150 (0.02)	0.173 (0.02)	0.164 (0.02)
NSR	0.067 (0.02)	0.062 (0.01)		0.034 (0.01)	0.036 (0.00)	0.066 (0.01)	0.079 (0.01)	0.095 (0.01)	0.112 (0.02)	0.190 (0.02)	0.159 (0.02)	0.199 (0.02)	0.182 (0.02)	0.172 (0.02)	0.173 (0.02)
CP	0.070 (0.02)	0.033 (0.01)	0.047 (0.01)		0.024 (0.00)	0.046 (0.01)	0.058 (0.01)	0.080 (0.01)	0.085 (0.01)	0.198 (0.03)	0.142 (0.02)	0.196 (0.03)	0.166 (0.02)	0.157 (0.02)	0.162 (0.02)
SSR	0.080 (0.02)	0.066 (0.01)	0.071 (0.02)	0.053 (0.01)		0.060 (0.01)	0.074 (0.01)	0.082 (0.01)	0.100 (0.02)	0.195 (0.02)	0.147 (0.02)	0.195 (0.02)	0.179 (0.02)	0.167 (0.02)	0.165 (0.02)
At	0.144 (0.04)	0.126 (0.03)	0.141 (0.03)	0.086 (0.02)	0.139 (0.03)		0.028 (0.01)	0.070 (0.01)	0.101 (0.02)	0.191 (0.03)	0.119 (0.02)	0.189 (0.03)	0.150 (0.02)	0.160 (0.02)	0.146 (0.02)
Ad	0.114 (0.03)	0.127 (0.03)	0.120 (0.03)	0.088 (0.03)	0.136 (0.03)	0.096 (0.03)		0.083 (0.01)	0.111 (0.02)	0.180 (0.02)	0.125 (0.02)	0.194 (0.03)	0.165 (0.02)	0.186 (0.03)	0.151 (0.02)
Ag	0.261 (0.06)	0.251 (0.06)	0.253 (0.06)	0.220 (0.05)	0.265 (0.06)	0.199 (0.04)	0.212 (0.05)		0.110 (0.02)	0.202 (0.03)	0.143 (0.02)	0.188 (0.03)	0.159 (0.02)	0.174 (0.02)	0.171 (0.02)
Ak	0.256 (0.05)	0.256 (0.05)	0.237 (0.05)	0.216 (0.05)	0.242 (0.05)	0.227 (0.05)	0.255 (0.06)	0.262 (0.05)		0.207 (0.03)	0.153 (0.02)	0.180 (0.02)	0.204 (0.03)	0.199 (0.03)	0.200 (0.03)
Aa	0.436 (0.10)	0.388 (0.08)	0.362 (0.08)	0.342 (0.07)	0.397 (0.10)	0.367 (0.08)	0.365 (0.07)	0.377 (0.08)	0.419 (0.08)		0.239 (0.03)	0.230 (0.03)	0.226 (0.03)	0.199 (0.03)	0.175 (0.02)
Al	0.289 (0.07)	0.277 (0.06)	0.297 (0.07)	0.232 (0.05)	0.291 (0.07)	0.263 (0.05)	0.220 (0.05)	0.242 (0.05)	0.299 (0.06)	0.429 (0.09)		0.116 (0.02)	0.220 (0.03)	0.230 (0.03)	0.242 (0.03)
As	0.337 (0.07)	0.348 (0.07)	0.340 (0.07)	0.322 (0.07)	0.354 (0.08)	0.305 (0.06)	0.279 (0.05)	0.371 (0.08)	0.283 (0.06)	0.343 (0.07)	0.366 (0.07)		0.235 (0.03)	0.246 (0.03)	0.239 (0.03)
Lm	0.605 (0.22)	0.553 (0.15)	0.601 (0.22)	0.544 (0.21)	0.583 (0.18)	0.482 (0.11)	0.513 (0.17)	0.515 (0.12)	0.477 (0.10)	0.424 (0.11)	0.516 (0.16)	0.370 (0.09)		0.100 (0.02)	0.216 (0.03)
Lf	0.402 (0.09)	0.385 (0.08)	0.380 (0.08)	0.340 (0.08)	0.396 (0.08)	0.365 (0.07)	0.373 (0.08)	0.392 (0.08)	0.399 (0.08)	0.372 (0.10)	0.350 (0.07)	0.287 (0.06)	0.192 (0.05)		0.232 (0.03)
Ao	0.329 (0.06)	0.267 (0.05)	0.299 (0.05)	0.273 (0.05)	0.277 (0.05)	0.368 (0.08)	0.359 (0.07)	0.381 (0.08)	0.378 (0.08)	0.418 (0.08)	0.446 (0.11)	0.389 (0.08)	0.512 (0.17)	0.378 (0.08)	

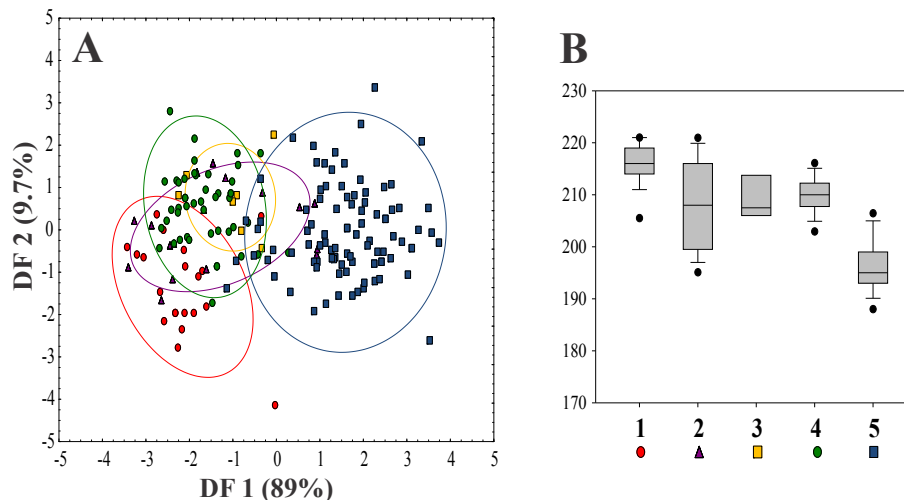


Fig. 2. Descriptive statistics of *Amphisbaena* spp. related to *Amphisbaena munoai* from southern Brazil and Uruguay. Exploratory discriminant function analysis with meristic characters (A). Boxplot from body annuli (B). Taxonomic Units: 1 and red circle: northwestern Rio Grande do Sul (NW); 2 and purple triangle: northern sul-riograndense Shield of Rio Grande do Sul (NSR); 3 and yellow square: Coastal Plain of Santa Catarina (CP); 4 and green circle: *Amphisbaena munoai* (southern sul-siograndense shield, BR and UR); 5 and blue square: *Amphisbaena prunicolor* (southern Atlantic Forest). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

We also observed that *A. prunicolor* can be distinguished from all other samples by its coloration pattern i.e., the specimens showed a dark purplish color with dorsal and ventral segments uniformly pigmented in a checkered pattern (Fig. 4). The other samples had a uniformly light brown color on the dorsum and were gradually fainter on the venter, with no contrasting pattern.

3.3. Taxonomic accounts

Our phylogenetic analysis (monophyletic clades and genetic distance) identified the existence of two known species and three more candidate species associated to various geographical areas in southern Brazil and Uruguay. The morphological characters

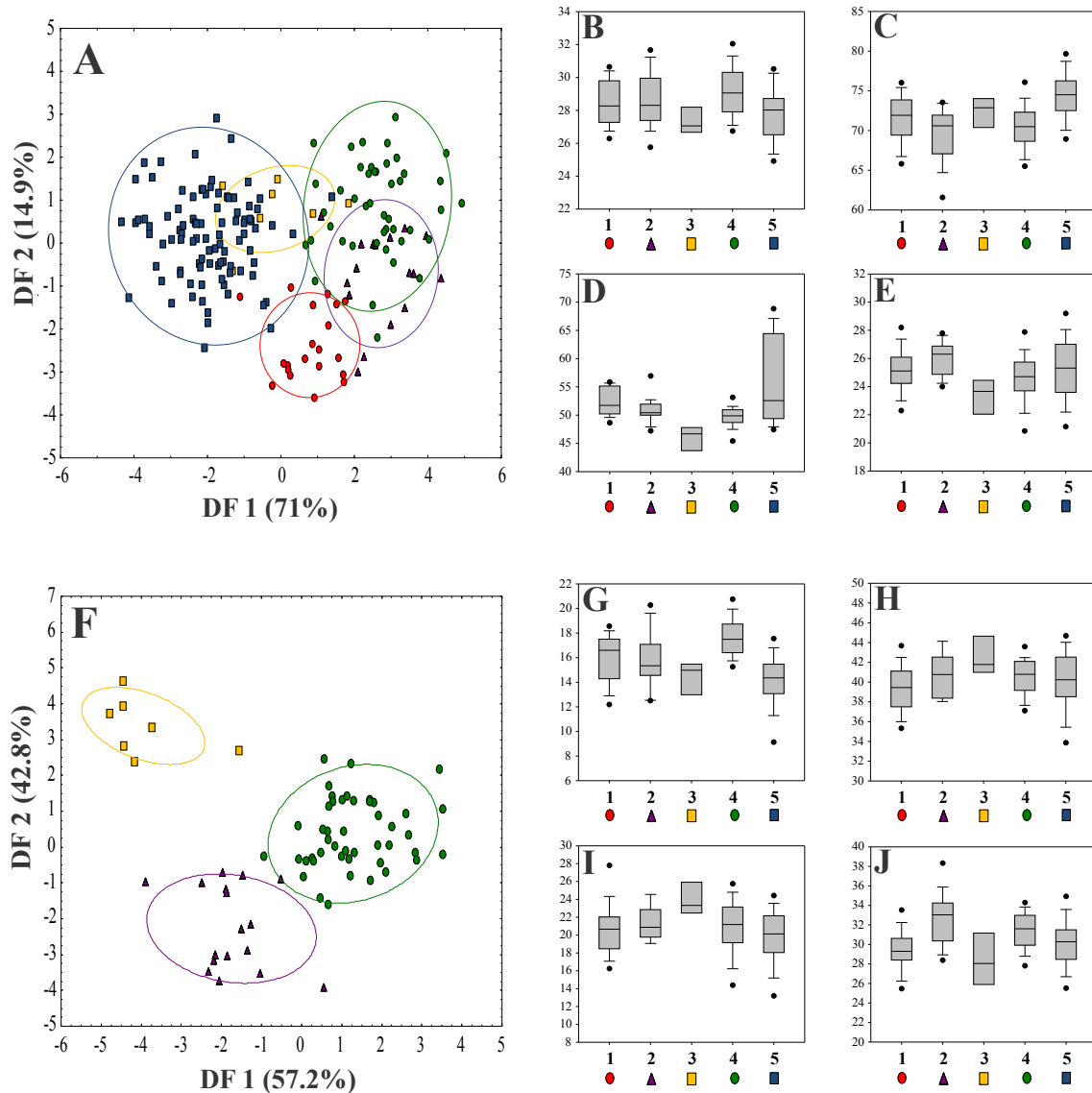


Fig. 3. Descriptive statistics of *Amphisbaena* spp. related to *Amphisbaena munoai* from southern Brazil and Uruguay. Exploratory discriminant function analysis with morphometric characters (A). Boxplot from prefrontal length (B), jaw length (C), snout length (D) and mental width (E). Exploratory discriminant function analysis of morphometric characters for clades CP, SR and *Amphisbaena munoai* (SSR). Boxplot from nasal suture length (G), prefrontal length (H), parietal length (I) and rostral width (J). Taxonomic Units: 1 and red circle: northwestern Rio Grande do Sul (NW); 2 and purple triangle: northern sul-riograndense Shield of Rio Grande do Sul (NSR); 3 and yellow square: Coastal Plain of Santa Catarina (CP); 4 and green circle: *Amphisbaena munoai* (southern sul-riograndense Shield, BR and UR); 5 and blue square: *Amphisbaena prunicolor* (southern Atlantic Forest). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Classification matrix for *Amphisbaena* spp. related to *Amphisbaena munoai* from Southern Brazil and Uruguay.

	Percent correct (%)	NW	NSR	CP	SSR	AF
		P = 0.10	P = 0.09	P = 0.03	P = 0.24	P = 0.50
Northwestern Rio Grande do Sul, BR (NW)	90.0	18	0	0	1	1
Northern Sul-Riograndense Shield, BR (SR)	83.3	0	15	0	3	0
Coastal Plain Santa Catarina (CP)	100.0	0	0	7	0	0
<i>Amphisbaena munoai</i> Southern Sul-Riograndense Shield, BR and UR (SSR)	86.9	2	4	0	40	0
<i>Amphisbaena prunicolor</i> Southern Atlantic Forest (AF)	96.8	2	0	0	1	91
Total	92.4	22	19	7	45	92

(meristic and morphometric data) supported the existence of these species. Habitat use (rock outcrops in savannah areas) was similar for *Amphisbaena munoai* and the northern sul-riograndense Shield of the Rio Grande do Sul clade. However, the use of rocks as shelter

is also known for *A. prunicolor*, *A. darwini* and *A. kingii* (personal observation). As demonstrated previously different independent lines of evidence supported the existence of candidate species we will describe of the three new taxa, redescribe *A. munoai* and



Fig. 4. Coloration pattern of *Amphisbaena* spp. related to *Amphisbaena munoai* from southern Brazil and Uruguay. A: *Amphisbaena prunicolor*; B: northwestern Rio Grande do Sul (NW); C: Coastal Plain Santa Catarina (CP); D: northern sul-riograndense Shield Rio Grande do Sul State (NSR). Photos A, C and D by Márcio Borges-Martins; Photo B by Tobias S. Kunz.

comment further on *A. prunicolor* (recently redescribed in Perez et al., 2012).

Aware of the current taxonomic panorama of South American Amphisbaenidae and the lack of a stable phylogeny, we herein present a previous comparison of the new or redescribed taxa and all currently recognized species. Closely related the comparison between the *Amphisbaena darwinii* complex, sensu Gans 1966, including *A. albocingulata*, *A. darwinii*, *A. hoguei*, *A. heterozonata*, *A. nigricauda* and *A. trachura* will be given in each description (see

Table 3 for standard comparison). Because of our ongoing studies, we decided to keep *A. heterozonata* and *A. trachura* as separate species for comparison and not as *A. darwinii* synonyms following Montero (2016).

Comparison: *Amphisbaena munoai*, *A. prunicolor* and the three candidate species are distinguishable from *Amphisbaena anomala* and *Leposternon* species by presenting a round-shaped head (vs. shovel-shaped head). It differs from *Amphisbaena acrobelsi* (Ribeiro, Castro-Mello & Nogueira 2009), *Amphisbaena bilabialata* (Stimson, 1972), *A. kingii* and *Mesobaena* species also by presenting a round-shaped head (vs. keel-shaped head). It differs from *Amphisbaena absaberi* (Strussman & Carvalho, 2001), *Amphisbaena bahiana* Vanzolini, 1964, *Amphisbaena borelli* Peracca, 1897, *Amphisbaena cuiabana* (Strussman & Carvalho, 2001), *Amphisbaena filiformes* Ribeiro, Gomes, Rodrigues, Cintra & Silva, 2016, *Amphisbaena maranhensis* Gomes & Maciel, 2012, *Amphisbaena robertii* Gans, 1964b and *Amphisbaena steindachneri* Strauch, 1881 by presenting a rounded tip of the tail (vs. tip of tail with a vertical keel). It differs from *Amphisbaena caetitisensis* Almeida, Freitas, Silva, Valverde, Rodrigues, Pires & Mott, 2018, *Amphisbaena rozei* Lancini, 1963, *Amphisbaena spurelli* Boulenger, 1915 and *Amphisbaena uroxena* Mott, Rodrigues & Freitas e Silva, 2008 by presenting smooth scales in the tip of the tail (vs. tuberculate scales in the tip of the tail).

It differs from *Amphisbaena anaemariae*, *Amphisbaena brevis* Strussman & Mott, 2009, *Amphisbaena caiari* Teixeira-Jr, DalVechio, Neto & Rodrigues, 2014, *Amphisbaena carli* Pinna, mendonça, Bocchigliera & Fernandes, 2010, *Amphisbaena crisae* Vanzolini, 1997, *Amphisbaena dubia* Müller, 1924, *Amphisbaena fuliginosa* Linnaeus, 1758, *Amphisbaena hiata* Monteiro & Céspedes, 2002, *Amphisbaena ignatiana* Vanzolini, 1991a, *Amphisbaena kiriri* Ribeiro, Gomides & Costa, 2018, *Amphisbaena kraoh* (Vanzolini, 1971), *A. leeseri*, *Amphisbaena leucocephala* Peters, 1878, *Amphisbaena litoralis* Roberto, Brito & Ávila, 2014, *Amphisbaena metallurga* Costa, Resende, Teixeira, DalVechio & Clemente, 2015, *Amphisbaena miringoera* Vanzolini, 1971, *Amphisbaena michelli* Procter, 1923, *Amphisbaena neglecta* Dunn & Piatt, 1936, *Amphisbaena persephone* Pinna, Mendonça, Bocchigliera, Fernandes, 2014, *A. silvestrii* and *Amphisbaena stejnegeri* Strauch, 1881 by presenting four pre-cloacal pores (vs. two, two or three, six, six to ten or 12 pre-cloacal pores).

It differs from *Amphisbaena arenaria* Vanzolini, 1991b, *Amphisbaena carvalhoi* Gans, 1965, *Amphisbaena cegei* Montero, Sáfadez, Álvares, 1997, *Amphisbaena frontalis* Vanzolini, 1991b, *Amphisbaena hastata* Vanzolini, 1991a, *Amphisbaena heathi* Schmidt, 1936, *Amphisbaena hoogmoedi* Oliveira, Vaz-Silva, Santos-Jr, Graboski, Teixeira, DalVechio & Ribeiro, 2018, *Amphisbaena ibijara* Rodrigues, Andrade & Dias-Lima, 2003, *Amphisbaena lumbricalis* Vanzolini,

Table 3

Morphological characters for standard comparison between *Amphisbaena munoai* and related species from *Amphisbaena darwinii* species group (sensu Gans 1966). A: absent; AUT: autonomy scale; BDA: body annuli; CDA: caudal annuli; DMB: dorsal midbody annulus; IL: number of infralabials; P: present; PML: posmalar row; POR: precloacal pores; S: smooth shaped; SL: number of supralabials; SVL: snout vent length; T: tuberculate shaped; TL: tail length; TS: tip of tail shape; VMB: ventral midbody annulus. * holotype information from Vanzolini 1950; ** holotype MNRJ 3305 analyzed by RPerez.

Species	POR	BDA	CDA	AUT	DMB	VMB	SL	IL	PML	TS	SVL + TL (mm)
<i>Amphisbaena albocingulata</i>	4	190–204	24–27	8–9	12–14	16–18	3	3	A	L	121–138 + 15–20
<i>Amphisbaena arenicola</i> sp. nov.	4	199–216	20–22	8–9	12–14	16–18	3 or 4	3	P	L	117–168 + 14–19
<i>Amphisbaena darwinii</i>	2–5	174–195	19–25	7–10	13–19	16–23	3	3	P	L	150–304 + 23–39
<i>Amphisbaena heterozonata</i>	2–6	189–206	15–18	6–8	15–18	18–20	3	3	P	L	150–277 + 14–24
<i>Amphisbaena hoguei</i>	4	177–191	15–19	4–7	10–13	14–18	3	3	P	L	134 + 14 *
<i>Amphisbaena munoai</i>	4	202–218	18–23	5–9	10–14	13–18	3	3	P	L	102–123 + 11–17
<i>Amphisbaena nana</i> sp. nov.	4	195–216	18–22	7–10	12–14	14–17	3	3	P	L	103–152 + 11–18
<i>Amphisbaena nigricauda</i>	4	222–226	19–24	6–9	10	16	3	3	A	L	120 + 16 **
<i>Amphisbaena prunicolor</i>	4	181–215	18–27	7–11	10–17	14–20	3	3	P	L	141–238 + 15–35
<i>Amphisbaena tiaraju</i> sp. nov.	4	204–221	18–25	7–9	10–14	13–16	3	3	P	L	127–183 + 14–20
<i>Amphisbaena trachura</i>	3–4	186–203	18–25	6–9	14–18	16–24	3	3	P	T	150–322 + 18–38

1996, *Amphisbaena medemi* Gans & Mathers, 1977, *Amphisbaena mensae* Castro-Mello, 2000, *Amphisbaena pericensis* Noble, 1921, *Amphisbaena slevini* Schmidt, 1936, *Amphisbaena supernumeraria* Mott, Rodrigues & Santos, 2009 and *Amphisbaena talisiae* Vanzolini, 1995 by the presence of a post-malar row (vs. post-malar row absent). It differs from *Amphisbaena alba* Linnaeus, 1758, *A. angustifrons*, *Amphisbaena cunhai* Hoogmoed & Ávila-Pires, 1991, *Amphisbaena occidentalis* Cope, 1875 and *Amphisbaena ridleyi* Boulenger, 1890 by presenting an autotomy scale visible externally (vs. autotomy scale not visible externally).

It differs from *Amphisbaena arda* Rodrigues, 2003, *Amphisbaena bedai* (Vanzolini, 1991c), *Amphisbaena gracilis* Strauch, 1881, *Amphisbaena plumbea* Gray, 1872, *Amphisbaena polygrammica* Werner, 1901, *Amphisbaena pretei* Duméril & Bibron, 1839, *Amphisbaena sanctaeritae* Vanzolini, 1994, *Amphisbaena saxosa* (Castro-Mello, 2003), *Amphisbaena townsendi* Stejneger, 1911 and *Amphisbaena vanzolini* Gans, 1963b by presenting 221 or less body annuli (vs. 224 or more body annuli). It differs from *Amphisbaena myersi* Hoogmoed, 1989 and *Amphisbaena tragorrhectes* Vanzolini, 1971 by presenting 27 or less caudal annuli (vs. 28 or more caudal annuli). It differs from *Amphisbaena bolivica* Mertens, 1929, *Amphisbaena brasiliiana* (Gray, 1865), *Amphisbaena camura* Cope, 1862, and *Amphisbaena vermicularis* Wagler, 1824 by presenting 17 or less dorsal segments in the midbody annulus (vs. 18 or more dorsal segments in the midbody annulus). It differs from *Amphisbaena slateri* Boulenger, 1907 by presenting two rows with a variable number of post-genial shields (vs. one row with two elongated post-genial shields). It differs from *Amphisbaena mertensii* Strauch, 1881 by presenting parietal shields quadrangular and smaller than frontal shields (vs. parietal shields enlarged laterally and with similar size of the frontal shields).

3.3.1. Description of *Amphisbaena tiaraju* sp. nov.

Amphisbaena munoai—Perez et al., 2012: 5 (in part).

Holotype: UFRGS 5370 from Cerro Largo, State of Rio Grande do Sul, Brazil (28° 8' 30"S, 54° 49' 59.9"O). Collected by Rafael L. Balestrin, Simone B. Leonardi and Luis Fernando M. da Fonte on 30 August 2009 (Fig. 5).

Paratypes. UFRGS 4896* Augusto Pestana, Rio Grande do Sul, Brazil; UFRGS 5037, 5039, 5040, 5041, 5297*, 5298*, 5299*, 5366*, 5367*, 5368*, 5371, 5999 Cerro Largo, Rio Grande do Sul, Brazil; UFRGS 5692*, 5693*, 5694*, 5695*, 5696*, 5697* Caibaté, Rio Grande do Sul, Brazil; UFRGS 5842*, 5843*, 5844*, 5849*, 5850*, 5851* Vitória das Missões, Rio Grande do Sul, Brazil.

Referred material: UFRGS 6115* Braga, Rio Grande do Sul, Brazil.

Specimens indicated with an asterisk where included on genetic analysis.

Etymology: The species is named after the South-American Indigenous warrior Sepé Tiaraju, supposedly born around 1723, leader of the “Sete Povos das Missões”, a set of seven indigenous settlements founded by the Spanish Jesuits in northwest Rio Grande do Sul, Brazil. Sepé Tiaraju is a Guarani hero, symbol of indigenous sentiment of liberty, but he was also part of the gaucho folklore traditions, immortalized in many regional literary works. He died in the Caiboaté battle against the Portuguese and Spanish army in 1756, together with other 1500 Guaranis. He now is officially recognized as a hero at regional (State Law 12.366, Rio Grande do Sul, Brazil) and federal level (Brazilian Federal Law 12.032/09) by its importance in the formation of the southern Brazilian frontiers. The specific epithet is a noun in apposition of the official Portuguese spelling. The tribute was a reference to the restricted distribution of the species to the “Missões” region.

Diagnosis: *Amphisbaena tiaraju* sp. nov. is distinguished from other species of *Amphisbaena* by the following character combination: (1) rounded head; (2) smooth segments at the tip of the tail;



Fig. 5. Holotype of *Amphisbaena tiaraju* sp. nov., UFRGS 5370, Cerro Largo, State of Rio Grande do Sul, Brazil. Dorsal, lateral and ventral view of the head. Scale = 1 mm.

(3) four pre-cloacal pores; (4) 204–221 body annuli; (5) 18–25 caudal annuli; (6) caudal autotomy seen externally from 7–9 caudal annuli; (7) presence of postmalar row; (8) 10–14 dorsal segments on the mid-body annulus; (9) distance between ocular shields representing 35%–43% of the head width; (10) dark brown colouration with uniform shield pigmentation.

Comparison: *Amphisbaena tiaraju* sp. nov. is distinguished from *A. albocingulata* by presenting the post-malar row (vs. post-malar row absent). It differs from *A. darwinii* and *A. hoguei* by presenting 204–221 body annuli (vs. 178–199 and 177–191 body annuli, respectively). It differs from *A. heterozonata* by presenting 18–25 caudal annuli (vs. 13–17 caudal annuli). It differs from *A. trachura* by presenting smooth scales in the tip of the tail (vs. tuberculate scales). Differs from *A. nigricauda* by presenting a uniform coloration in the tip of the tail (vs. a darker coloration in the tip of the tail). Differs from *A. prunicolor* by presenting a dark brown colouration with uniform shield pigmentation (vs. checkered pattern colouration). Differs from *A. munoai* by presenting a larger distance between ocular shields, varying between 35.3% and 42.7% (\bar{x} =

39.5 ± 2.3) of head length (HL) (vs. 28.7–37.5, \bar{x} = 32.7 ± 0.9), a dark brown uniform coloration (vs. light brown uniform coloration) and by 6.4% of 16S rDNA mean genetic distance.

Holotype description: Adult male, 158.9 mm total length [snout-vent-length (SVL) 142 mm and caudal length 16.9 mm (11.9% SVL)]. Slender body, diameter 3.5 mm (2.4% SVL). Head length (HL) 4.5 mm (3.2% SVL), head width (HW) 3.2 mm (2.3% SVL). Snout 2.28 mm (50.2% HL). Triangular rostral shield, barely visible dorsally, with height representing 21.6% HL. One pair of quadrangular nasal shields, nasal suture 0.8 mm (18.3% HL). Nostrils arranged in the lateral portion of the nasal shields. Rounded suture between nasals and prefrontals shields. One pair of quadrangular prefrontal shields relatively large with 1.6 mm length (36.3% HL) and 1.23 mm width (27.1% HL). Frontal shields longer than wide, smaller than prefrontal, with 1.4 mm length (31.5% HL) and 0.9 mm width (20.7% HL). Posterior suture of frontals forming an open 'V'. A row of parietal shields well differentiated from other body shields, representing the first ventral body annulus. Quadrangular central parietals with 1 mm length (22% HL) and 0.9 mm width (19.8% HL). A row of occipital shields present. Small and rhomboid ocular shield with 0.9 mm length (20.5% HL) and 0.8 mm width (18.7% HL); distance between ocular shields 1.83 mm (56.6% HW). Widened post-ocular shield with 1.3 mm length (27.7% HL) and 0.9 mm width (20% HL). One temporal shield and one post-supralabial shield, both quadrangular. Three supralabial shields of equivalent size. Three infralabial shields of distinct forms: first smaller and quadrangular, second wider and deeper, third elongated and rectangular; the three together occupy 2.9 mm of the side of the mouth. Mental shield with an inverted trapezoidal shape of 0.9 mm length (19.6% HL) and 1.1 mm width (24.2% HL). Postmental shield heptagonal of 1.2 mm length (25.8% HL) and 1 mm width (22.9% HL). One row with two postgenital shields. One pair of triangular malar shields of 1 mm inner length (22% HL), 0.5 mm outer length (10% HL) and 1.1 mm width (24% HL). Row with nine postmalar shields. Body annuli 220. Caudal annuli 22, caudal autotomy present on the eighth annulus, 22 segments on the fourth caudal annulus, tip of the tail smooth. Twelve dorsal segments and 14 ventral segments on the midbody annulus. Four precloacal pores arranged in sequence on the posterior portion of the segments of the last ventral annulus. Cloacal plate with six precloacal and ten post-cloacal shields. Dark brown color, pale in the venter, uniform pigmentation on the shields. On the side of the head, specimen shows no color on supralabial, temporal and post-supralabial shields. The color of the venter is apparent near the lateral sulcus, and pale on the center. The cloacal plate is pigmented and the colors on the tail becomes darker.

Variation: Total length varies between 142.7 and 201.2 mm (\bar{x} = 173.7 mm ± 15.6), snout-vent length (SVL) varies between 127 and 183 mm (\bar{x} = 157.3 mm ± 13.7), tail length varies between 14 and 19.9 mm (\bar{x} = 17.4 mm ± 15.7; N = 18). Specimens with slender body, diameter varying between 2.0 and 3.2% of SVL (\bar{x} = 2.6 ± 0.3). Head length varies between 2.5 and 3.5% of SVL (\bar{x} = 3 ± 0.2%), head width varies between 1.8 and 2.5% of SVL (\bar{x} = 2 ± 0.2%). Variation in width between ocular shields 1.5–2.1 mm (\bar{x} = 58.7 ± 2.8% HW). Body annuli vary between 204 and 221 (\bar{x} = 215.8 ± 3.9). Caudal annuli vary between 18 and 25 (\bar{x} = 20.7 ± 1.5; N = 19), caudal autotomy between 7 and 9 annulus (\bar{x} = 8.2 ± 0.6; N = 19), segments on the fourth caudal annulus varying between 19 and 25 (\bar{x} = 21.8 ± 1.5). Dorsal segments of the midbody annulus vary between 10 and 14 (\bar{x} = 11.8 ± 0.9) and ventral segments vary between 13 and 16 (\bar{x} = 14.7 ± 0.9).

Distribution: *Amphisbaena tiaraju* sp. nov. is known only from five municipalities in the State of Rio Grande do Sul, Brazil at altitudes ranging between 50 and 250 m (Fig. 6). These localities are in the northwestern portion of the State, in the “Missões”

physiographic region, an area of ecological tension between the Atlantic Forest and the Subtropical Grasslands. The region was covered originally with seasonal deciduous forest and natural grasslands, but is now severely impacted by human occupation mainly due to agriculture and cattle raising.

3.3.2. Description of *Amphisbaena nana* sp. nov.

Amphisbaena munoai— Gans, 1966: 243 (in part).

Amphisbaena munoai— Perez et al., 2012: 5 (in part).

Holotype: UFRGS 5705* from São Jerônimo, State of Rio Grande do Sul, Brazil (29° 57'S, 51° 43'W). Collected by Márcio Borges-Martins and team, on 3 July 2010 (Fig. 7).

Paratypes: UFRGS 5511*, 5512*, 5513*, 5614, 5616, 5704 and 5706* São Jerônimo, Rio Grande do Sul state, Brazil.

Referred material: MCP 11790, 12006 Dom Feliciano, Rio Grande do Sul, Brazil; MCP 14572, 14573, 14574, 14576 Encruzilhada do Sul, Rio Grande do Sul, Brazil; MCP 13317, 13318 Mariana Pimental, Rio Grande do Sul, Brazil; UFRGS 6248* Nova Santa Rita, Rio Grande do Sul, Brazil; UFRGS 6484*, 6485* Pantano Grande, Rio Grande do Sul, Brazil; UFRGS 5707*, 5708*, 6125, MCP 15118, 15351, 15788, 16787 São Jerônimo, Rio Grande do Sul, Brazil; photographic record Viamão, Rio Grande do Sul, Brazil.

Specimens indicated with an asterisk where included on genetic analysis.

Etymology: The specific epithet *nana* is an adjective derived from the Latin word *nanus*, meaning ‘dwarf’, and is a reference to the small size of this species, the smallest within the *Amphisbaena darwini*-Group.

Diagnosis: *Amphisbaena nana* sp. nov. is distinguished from other species of *Amphisbaena* by the following character combination: (1) rounded head; (2) smooth segments at the tip of the tail; (3) four pre-cloacal pores; (4) 195–216 body annuli; (5) caudal autotomy seen externally from 7–10 caudal annuli; (6) presence of postmalar shields; (7) parental shields differentiated from body shields; (8) light brown coloration with uniform shield pigmentation.

Comparison: *Amphisbaena nana* sp. nov. is distinguished from *A. albocingulata* by presenting the post-malar row (vs. post-malar row absent). Differs from *A. hoguei* by presenting 195–216 body annuli (vs. 177–191 body annuli). It differs from *A. heterozonata* by bearing 18–22 caudal annuli (vs. 13–17 caudal annuli). It differs from *A. nigricauda* by having a uniform coloration on the tail tip (vs. a darker coloration on tail tip). Differs from *A. trachura* by presenting smooth scales on tail tip (vs. tuberculate scales). It differs from *A. darwini* by presenting a slender body and small SVL in adults, 103–152 mm (vs. sturdy body with long SVL in adults, 150–304 mm). Differs from *A. prunicolor* by presenting a uniform coloration pattern (vs. checkered coloration pattern). It differs from *A. munoai* by presenting a short frontal shield, with similar size of parietal shields, varying between 18.7 and 28.7% (\bar{x} = 23.9 ± 2.6) of head length (HL) (vs. 25.9–35.8; \bar{x} = 27.7 ± 1.0, with larger size than parietal shields) and 3.6% of 16S rDNA mean genetic distance. Differs from *A. tiaraju* sp. nov. by presenting a smaller frontal shield, varying between 18.7 and 28.7% (\bar{x} = 24.5 ± 2.8) of (HL) (vs. 28.5–34.2%; \bar{x} = 30.6 ± 1.9) and by 6.9% of 16S rDNA mean genetic distance.

Holotype description: Adult male, 130.1 mm total length [snout-vent-length (SVL) 118 mm and caudal length 15 mm (12.7% SVL)]. Slender body, diameter 3.1 mm (2.7% SVL). Head length (HL) 3.8 mm (3.2% SVL), head width (HW) 1.1 mm (0.4% SVL). Snout 2 mm (52.7% HL). Triangular rostral shield, barely visible dorsally, with height representing 32.7% HL. One pair of quadrangular nasal shields, nasal suture 0.6 mm (16.5% HL). Nostrils arranged in the lateral portion of the nasal shields. One pair of relatively small prefrontal shields with 1.4 mm length (36.1% HL) and 1.1 mm width

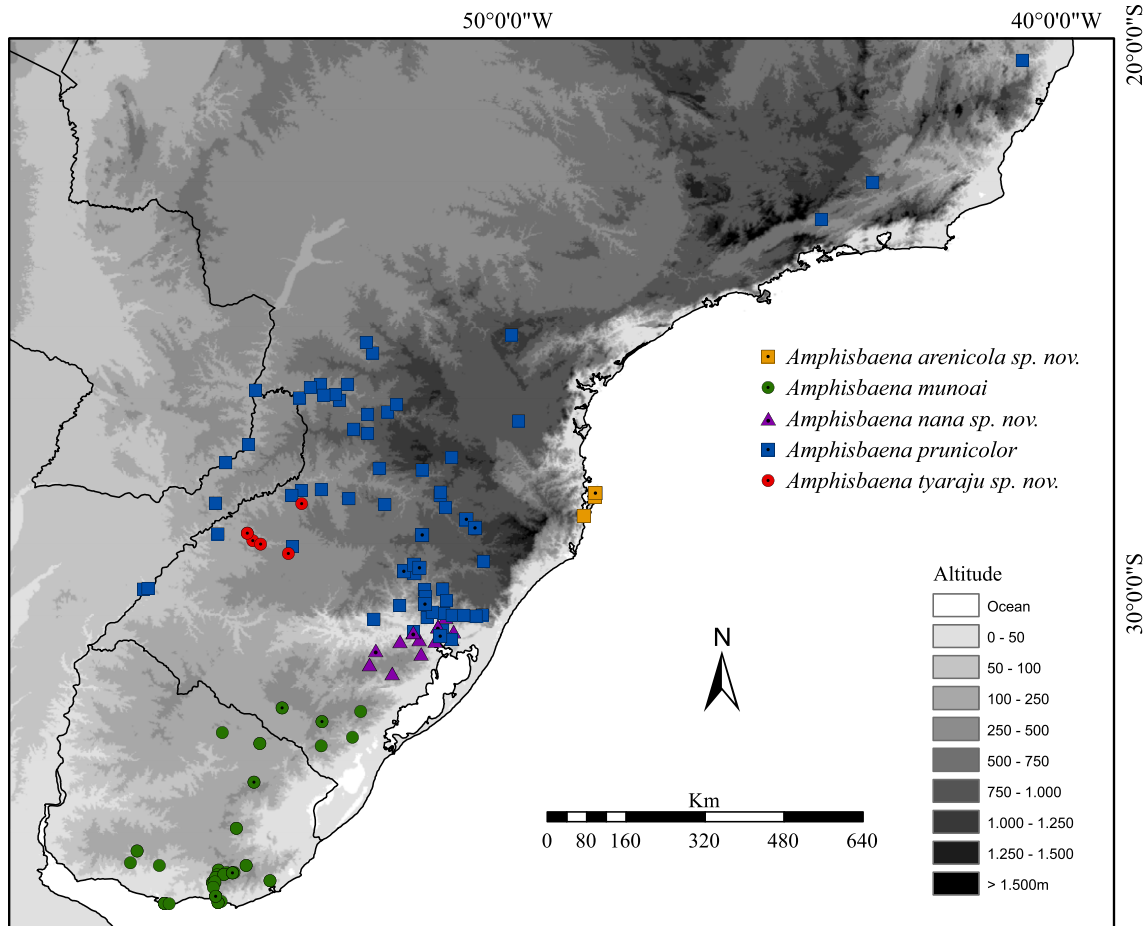


Fig. 6. Geographical distribution of *Amphisbaena* spp. related to *Amphisbaena munoai* from Southern Brazil and Uruguay.

(29.8% HL). Frontal shields longer than wide, with 0.9 mm length (23.1% HL) and 1.3 mm width (34.8% HL). A row of parietal shields well differentiated from other body shields, representing the first ventral body annulus. Quadrangular central parietals with 0.9 mm length (24.2% HL) and 0.8 mm width (20.7% HL). A row of occipital shields presents. Ocular shield small, longer than wide with 1 mm length (25.5% HL) and 0.9 mm height (22.9% HL); distance between ocular shields of 1.25 mm (33.2% HW). Widened post-ocular shield with 1 mm length (26.6% HL) and 0.7 mm width (18.6% HL). One temporal shield and three supralabial shields of equivalent size. Three infralabial shields of distinct forms: first smaller and quadrangular, second wider and deeper, third elongated and rectangular; the three together occupy 2.6 mm of the side of the mouth. Mental shield with an inverted trapezoidal shape, longer than wide with 0.8 mm length (21% HL) and 1 mm width (26.9% HL). Postmental shield larger than mental shield, with 1.2 mm length (30.9% HL) and 0.9 mm width (23.7% HL). Two rows postgenial shields, first with two large shields and second with two small shields. One pair of malar shields of 0.9 mm inner length (23.1% HL), 0.4 mm outer lengths (10.4% HL) and 0.8 mm width (20.5% HL). Row with eight postmalar shields. Body annuli 203. Caudal annuli 21, caudal autotomy present on the ninth annulus, 23 segments on the fourth caudal annulus, tip of the tail smooth. Twelve dorsal segments and 16 ventral segments on the midbody annulus. Four precloacal pores arranged in sequence on the posterior portion of the segments of the last ventral annulus. Cloacal plate with six precloacal and thirteen postcloacal shields. Shields uniformly

pigmented, light brown color with a pinkish tone at the dorsal and ventral regions.

Variation: Total length varies between 142.7 and 201.2 mm ($\bar{x} = 173.7 \text{ mm} \pm 15.6$), snout-vent length (SVL) varies between 103 and 152 mm ($\bar{x} = 124 \text{ mm} \pm 12.2$), tail length varies between 11.7 and 17.9 mm ($\bar{x} = 14.3 \text{ mm} \pm 1.8$). Specimens with slender body, diameter varying between 2.3 and 3.3% of SVL ($\bar{x} = 2.8 \pm 0.3$). Head length varies between 2.6 and 3.3% of SVL ($\bar{x} = 2.8 \pm 0.3$), head width varies between 1.8 and 2.5% of SVL ($\bar{x} = 2.2 \pm 0.2$). Body annuli vary between 195 and 216 ($\bar{x} = 208.1 \pm 8.7$). Caudal annuli vary between 18 and 22 ($\bar{x} = 20.4 \pm 1.0$), caudal autotomy between 7 and 10 annulus ($\bar{x} = 8.3 \pm 0.7$), segments on the fourth caudal annulus varying between 19 and 26 ($\bar{x} = 22.5 \pm 1.6$). Dorsal segments of the mid-body annulus vary between 12 and 14 ($\bar{x} = 12.2 \pm 0.6$) and ventral segments vary between 14 and 17 ($\bar{x} = 15.7 \pm 0.7$).

Distribution: *Amphisbaena nana* sp. nov. is distributed in the central depression of Rio Grande do Sul state, along the drainages of Taquari and Jacuí Rivers in altitudes of ca. 100 m including anthropogenic habitats near Porto Alegre and adjacent municipalities. It is also found in the eastern Sul-riograndense shield, north to the Camaquã River, in altitudes of ca. 500 m (Fig. 6). Most of its distribution is associated to the northern areas of the Pelotas Batholith in the “Cinturão Dom Feliciano” geotectonic unit. The region is originally covered by a mosaic of Seasonal Semideciduous Forest and natural grasslands in a contact zone between the Atlantic Forest and the Pampa.

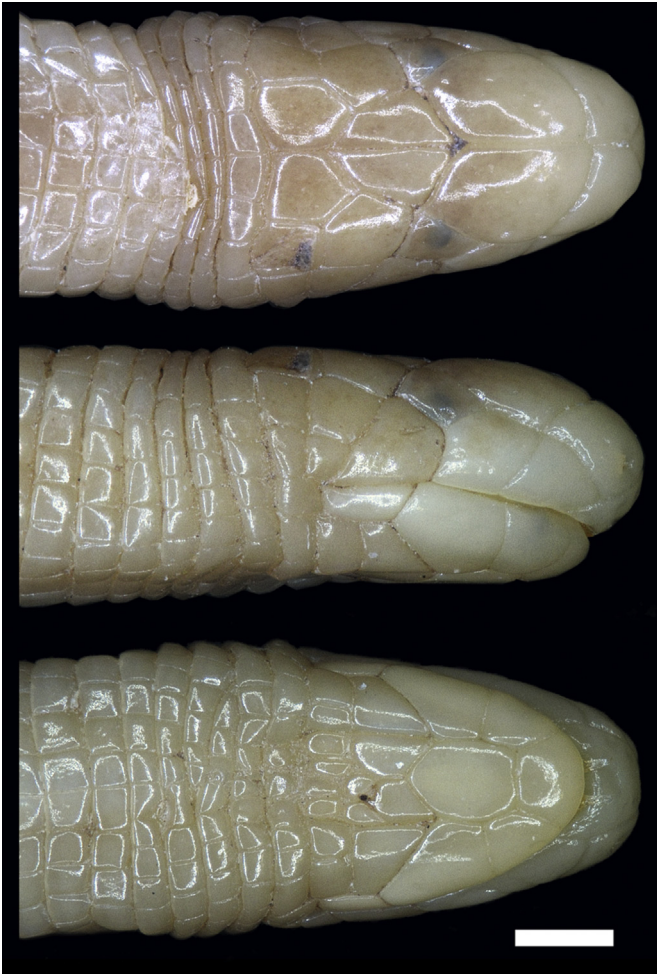


Fig. 7. Holotype of *Amphisbaena nana* sp. nov., UFRGS 5705, São Jerônimo, State of Rio Grande do Sul, Brazil. Dorsal, lateral and ventral view of the head. Scale = 1 mm.



Fig. 8. Holotype of *Amphisbaena arenicola* sp. nov., UFRGS 5892, Florianópolis, State of Santa Catarina, Brazil. Dorsal, lateral and ventral view of the head. Scale = 1 mm.

3.3.3. Description of *Amphisbaena arenicola* sp. nov.

Holotype: UFRGS 5892 from Praia dos Ingleses, municipality of Florianópolis, State of Santa Catarina, Brazil (27° 26'S, 48° 23'O). Collected by Tobias S. Kunz (Fig. 8).

Paratypes: UFRGS 5893, 5894, 5967* Ingleses, Florianópolis, Santa Catarina, Brazil; **Referred material:** CHUFSC 668, 683 Rio Vermelho, Florianópolis, Santa Catarina, Brazil; CHUFSC 696; UFRGS 5968*, 6303*, 6615*, 6616*, 6702* Ingleses, Florianópolis, Santa Catarina, Brazil; CHUFSC 856 Baixada do Macaímbu, Pinheira, Santa Catarina, Brazil.

Specimens indicated with an asterisk where included on genetic analysis.

Etymology: The specific epithet *arenicola* is an adjective derived from the Latin word for living in the sand. This name was chosen because this is the only taxon of the species-group inhabiting mainly sandy habitats (Restinga areas).

Diagnosis: *Amphisbaena arenicola* sp. nov. is distinguished from other *Amphisbaena* by the following character combination: (1) rounded head; (2) smooth segments at the tip of the tail; (3) four pre-cloacal pores; (4) 199–216 body annuli; (5) caudal autotomy seen externally from 8–9 caudal annuli; (6) 3/3 or 4/4 supralabial shields; (7) short snout, representing less than 50% of head length.

Comparison: *Amphisbaena arenicola* sp. nov. is distinguished from *A. albocingulata* by presenting the post-malar row (vs. post-

malar row absent). Differs from *A. hogei* by presenting 199–216 body annuli (vs. 177–191 body annuli). It differs from *A. heterozonata* by presenting 20–22 caudal annuli (vs. 13–17 caudal annuli). It differs from *A. nigricauda* by having a uniformly colored tail tip (vs. a darkly colored tail tip). Differs from *A. trachura* by presenting smooth scales on tail tip (vs. tuberculate scales). It differs from *A. darwinii* by presenting a slender body and smaller adult SVL ranging between 117 and 178 mm (vs. sturdy body with long SVL in adults, 150–304 mm). Differs from *A. prunicolor* by presenting a uniform coloration pattern (vs. checkered coloration pattern). Differs from *A. tiaraju* sp. nov. by presenting a larger number of ventral segments in the midbody annulus, varying between 16 and 18 (vs. 13–16) and a smaller snout, varying between 42.6 and 47.9% ($\bar{x} = 45.9 \pm 1.9$) of head length (HL) (vs. 48.5–55.8%, $\bar{x} = 52.4 \pm 3.1$ of HL). It differs from *A. munoai* by presenting 3/3 or 4/4 supralabial shields (vs. always 3/3 supralabial shields) and smaller nasal suture varying between 12.5 and 17.2% ($\bar{x} = 14.7 \pm 1.5$) of HL (vs. 14.1–20.8, $\bar{x} = 17.6 \pm 1.5$ of HL). It differs from *A. nana* sp. nov. by presenting a widened ocular shield, with larger size than ocular shield, varying between 22.0 and 24.4% ($\bar{x} = 23.1 \pm 0.9$) of

head length (HL) (vs. 17.7–22.8; \bar{x} = 21.3 ± 1.3; with similar size of the ocular shield) and by 3.4% of 16S rDNA mean genetic distance.

Holotype description: Adult male, 147.6 mm total length [snout-vent-length (SVL) 130 mm and caudal length 17.6 mm (13.5% SVL)]. Slender body, diameter 4.1 mm (3.1% SVL). Head length (HL) 4.9 mm (3.8% SVL), head width (HW) 3.2 mm (2.5% SVL). Snout 2.4 (47.8% HL). Triangular rostral shield, barely visible dorsally, with height representing 21.1% HL. One pair of quadrangular nasal shields, nasal suture 0.7 mm (15% HL). Nostrils arranged in the lateral portion of the nasal shields. Rounded suture between nasals and prefrontal shields. One pair of quadrangular prefrontal shields relatively large with 2.2 mm length (45.1% HL) and 1.4 mm width (28% HL). Frontal shields longer than wide, smaller than prefrontal, with 1.4 mm length (27.3% HL) and 1 mm width (19.2% HL). Posterior suture of frontals forming an open 'V' shape. A row of parietal shields well differentiated from other body shields, representing the first ventral body annulus. Quadrangular central parietals with 1.1 mm length (22.5% HL) and 0.9 mm width (18.4% HL). A row of occipital shields presents. Small and rhomboid ocular shield with 1.3 mm length (27.1% HL) and 1.1 mm height (23.1% HL). Post-ocular shield with size similar to ocular shield, with 1.2 mm length (25.1% HL) and 0.9 mm width (18.8% HL). One temporal shield quadrangular. Three supralabial shields of equivalent size and fourth quadrangular and smaller than others. Three infralabial shields of distinct forms: first smaller and quadrangular, second wider and deeper, third elongated and rectangular; the three together occupy 3.4 mm of the side of the mouth. Mental shield with an inverted trapezoidal shape, short with 0.9 mm length (19.9% HL) and 1.1 mm width (23.5% HL). Post-mental shield heptagonal, longer than wide, with 1.2 mm length (28.2% HL) and 1 mm width (23.3% HL). Two rows of postgenial shields, first with two and second with three smaller shields. One pair of triangular malar shields of 1 mm inner length (22% HL), 0.5 mm outer lengths (10% HL) and 1.1 mm width (24% HL). A row with six postmalar shields. Body annuli 206. Caudal annuli 21, caudal autotomy present on the ninth annulus, 22 segments on the fourth caudal annulus, tip of the tail smooth. Fourteen dorsal segments and 16 ventral segments on the midbody annulus. Four preloacal pores arranged in sequence on the posterior portion of the segments of the last ventral annulus. Cloacal plate with six preloacal and eleven postloacal shields. Light brown color, pale in the venter, uniform pigmentation on the shields.

Variation: Total length varies between 142.7 and 201.2 mm (\bar{x} = 173.7 mm ± 15.6), SVL varies between 117 and 168 mm (\bar{x} = 143 mm ± 18.9), tail length varies between 14.6 and 18.5 mm (\bar{x} = 17.4 mm ± 1.9). Specimens with slender body, diameter varying between 2.4 and 3.1% of SVL (\bar{x} = 2.6 ± 0.3). Head elongated, length varying between 3.0 and 3.8% of SVL (\bar{x} = 3.4 ± 0.3%), head width varies between 2.0 and 2.5% of SVL (\bar{x} = 2.2 ± 0.2%). Short snout, representing 42.6–47.8% of head length (\bar{x} = 45.7 ± 2.3). Body annuli vary between 199 and 216 (\bar{x} = 208.8 ± 5.9). Caudal annuli vary between 20 and 22 (\bar{x} = 21 ± 0.6), caudal autotomy between 8 and 9 annulus (\bar{x} = 8.7 ± 0.5), segments on the fourth caudal annulus varying between 24 and 26 (\bar{x} = 24.7 ± 0.7). Dorsal segments of the mid-body annulus vary between 12 and 14 (\bar{x} = 13.7 ± 0.5) and ventral segments vary between 16 and 18 (\bar{x} = 16.7 ± 0.7).

Distribution: *Amphisbaena arenicola* sp. nov. is known only from two localities in the State of Santa Catarina, Brazil, in the Island of Santa Catarina (Florianópolis) and vicinities (Pinheira) associated with sandy soils of coastal Restingas (Fig. 6). It is the only species from the group distributed in coastal areas. Its distribution is disjunct about 300 km straight-line from the nearest record of *A. nana* sp. nov., and about 550 km from the nearest record of its sister species, *A. munoai*.

3.3.4. Redescription of *Amphisbaena munoai* Klappenbach, 1960

Amphisbaena munoai Klappenbach, 1960: 3. Localidade tipo: Cerro de Animas, Departamento de Maldonado, Uruguay.

Amphisbaena munoai – Gans, 1966: 243.

Amphisbaena munoai – Perez et al., 2012: 5 (in part).

Holotype: MNHN 587 Cerro de Animas, Maldonado Department, Uruguay (34° 42' S 55° 19' W).

Paratypes: MNHN 173C, 181A, 181C, 583A–583E, 586, 588, 589, 590, 591; 718A, 862A–862C, 863, 865A, 865C, 3059 (ex 173D), 3060 (ex 181E), 3061 (ex 181D), 3062 (ex 181F); 3063 (ex 718B), 3065 (ex 865B); MNRJ 3307 (ex MNHN 181B); USNM 145301 (ex MNHN 173A), 145302 (ex MNHN 173B) Cerro de Animas, Maldonado Department, Uruguay; MNHN 178A, 182, 3058 (ex 178B) Cerro San Antonio, Piriápolis, Maldonado Department, Uruguay; MNHN 716 Aguas Blancas, Lavalleja Department, Uruguay; MNHN 861A, 3064 (ex 861B) Zapicán, Lavalleja Department, Uruguay; ZVC-R 37-1 Cerro de Arequita, Lavalleja Department, Uruguay; MNHN 714 Carpintería, Rivera Department, Uruguay; MNHN 864 Sierras de Aceguá, Cerro Largo Department, Uruguay; ZVC-R 38, 142 Cerro, Montevideo Department, Uruguay.

Referred material: UFRGS 6007*, 6008*, 6009*, 6010*, 6011*, 6014, 6015* Bagé, Rio Grande do Sul, Brazil; UFRGS 5943 Canguçu, Rio Grande do Sul, Brazil; UFRGS 5684* Pedras Altas, Rio Grande do Sul, Brazil; UFRGS 7408*, 7409*, Cerro Quebracho, Cerro Largo, Uruguay; MNHN 864 Sierras de Aceguá, Cerro Largo, Uruguay; ZVC-R 4101 Ruta 8 km 128, Lavalleja, Uruguay; MNHN 3067, 3069 Abra de la Coronilla, Lavalleja, Uruguay; MNHN 3169 Abra de Zabaleta, Lavalleja, Uruguay; MNHN, 3076, 3077, 3078, 3080 Aguas Blancas, Lavalleja, Uruguay; MNHN 5666 Arroio Cerro Blanco, Lavalleja, Uruguay; ZVC-R 37–1, 1532 Cerro de Arequita, Lavalleja, Uruguay; MNHN 5699 Cerros Blancos, Lavalleja, Uruguay; ZVC-R 2417; MNHN 6144 Cerro Verdún, Lavalleja, Uruguay; MNHN 3161 Cerro Penitente, Lavalleja, Uruguay; MNHN 934, 1437, 3088 Parque Salus, Lavalleja, Uruguay; MNHN 1438, 3070 Sierra de Minas, Lavalleja, Uruguay; ZVC-R 3878 Cerro Barboza, Maldonado, Uruguay; MNHN 1100 Cerro Sencioni, Maldonado, Uruguay; MNHN 779, 933, 1651, 3074, 3089, 3095, 3104; MCP 14865; ZVC-R 1511 Cerro de Animas, Maldonado, Uruguay; MNHN 5974 Cerro de las Espinas, Maldonado, Uruguay; MNHN 1101 Cerro San Antonio, Piriápolis, Maldonado, Uruguay; MNHN 1100, 3095, 3096, 3097, 3098, 3099, 3100, 3101 Cerro Pan de Azucar, Maldonado, Uruguay; MLPDB 7130*, 7131*, 7132*; ZVC-R 3802, 3840, 4356 Sierra de las Animas, Maldonado, Uruguay; UFRGS 7410*, 7411* Solis Mataojo, Maldonado, Uruguay; UFRGS 7412* Vila Serrana, Minas, Uruguay; UFRGS 7413* Canepas, Minas, Uruguay; ZVC-R 3798, 3835 Cerro de Montevideo, Montevideo, Uruguay; MNHN 1619; ZVC-R 308 San Gregorio, San José, Uruguay (Fig. 9).

Specimens indicated with an asterisk where included on genetic analysis.

Diagnosis: *Amphisbaena munoai* distinguishes from other species of *Amphisbaena* by the following combination of characters: (1) rounded head; (2) smooth segments on the tip of the tail; (3) four preloacal pores; (4) 202–218 body annuli; (5) row of postmalar shields present; (6) 18–25 caudal annuli; (7) caudal autotomy visible between 5 and 9 tail annuli; (8) 10–14 segments on the midbody annulus.

Comparison: *Amphisbaena munoai* is distinguished from *A. albocingulata* by presenting the post-malar row (vs. post-malar row absent). Differs from *A. hoguei* by presenting 202–218 body annuli (vs. 177–191 body annuli). It differs from *A. heterozonata* by presenting 18–23 caudal annuli (vs. 13–17 caudal annuli). It differs from *A. nigricauda* by presenting a uniform coloration in the tip of the tail (vs. a darker coloration in the tip of the tail). It differs from *A. trachura* by presenting smooth scales in the tip of the tail (vs.



Fig. 9. *Amphisbaena munoai*, ZVC-R 3840, Sierra de las Animas, Maldonado, Uruguay. Dorsal, lateral and ventral view of the head. Scale = 1 mm.

tuberculate scales). It differs from *A. darwini* by presenting a slender body and small SVL in adults, 102–151 mm (vs. sturdy body with long SVL in adults, 150–304 mm). Differs from *A. prunicolor* by presenting a uniform coloration pattern (vs. checkered coloration pattern). Differs from *A. tiaraju* sp. nov. by presenting a smaller distance between ocular shields, varying between vs. 28.7–37.5, $\bar{x} = 32.7 \pm 0.9$ of head length (HL) (vs. 35.3–42.7%, $\bar{x} = 39.5 \pm 2.3$) and 6.4% of 16S rDNA mean genetic distance. It differs from *A. arenicola* sp. nov. by presenting 3/3 supralabial shields (vs. 3/3 or 4/4 supralabial shields) and a larger nasal suture varying between 14.1 and 20.8, $\bar{x} = 17.6 \pm 1.5$ (vs. 12.5–17.2, $\bar{x} = 14.7 \pm 1.5$). Differs from *A. nana* sp. nov. by presenting a large frontal shield, with larger size

than parietal shield, varying between 25.9 and 35.8; $\bar{x} = 27.7 \pm 1.0$ of HL (vs. 18.7–28.7% $\bar{x} = 23.9 \pm 2.6$; with similar size than parietal shield).

Variation: A small species with snout-vent length (SVL) varying from 102 to 151 mm ($\bar{x} = 123.1 \text{ mm} \pm 12.1$) and tail length between 11.2 and 17.1 mm ($\bar{x} = 14.4 \pm 1.3$). Slender body with diameter 2.4–3.6% of SVL ($\bar{x} = 2.9 \pm 0.3$). Head longer than wide, with length 2.7–4% of SVL ($\bar{x} = 3.4 \pm 0.3$) and width 1.8–2.7% of SVL ($\bar{x} = 2.3 \pm 0.2$). Elongated snout varying from 44.9–54.3% of head length (HL) ($\bar{x} = 49.8 \pm 1.8$). Rostral shield triangular, barely visible dorsally, with height about 20.9–29.9% of HL ($\bar{x} = 24.0 \pm 1.9$). A pair of quadrangular nasal shields with lateral nostrils. Suture between nasals relatively long, varying from 14.1–20.9% of HL ($\bar{x} = 17.6 \pm 1.6$). A pair of enlarged prefrontal shields with length 32.2–44.1% of HL ($\bar{x} = 40.4 \pm 2.1$) and width 26.2–32.6% of HL ($\bar{x} = 29.1 \pm 1.6$). A pair of frontal shields smaller than prefrontals, varying from 21.5–35.8% of HL ($\bar{x} = 27.7 \pm 2.8$ CCB) and width 15.2–22.1% of HL ($\bar{x} = 17.8 \pm 1.5$). Ocular shield rhomboid, with length 20.9–32.9% of HL ($\bar{x} = 25.3 \pm 2.3$) and width 18.1–25.9% of HL ($\bar{x} = 22.3 \pm 1.5$). Enlarged postocular shield varying in length 20.9–41.9% of HL ($\bar{x} = 26.6 \pm 2.8$) and width 10.8–22.3% of HL ($\bar{x} = 18.3 \pm 2.2$ CCB). Relatively small rectangular temporal shield and a quadrangular and diminutive post-supralabial shield when present. Three supralabial shields with equivalent sizes. Three infra-labial shields: the first smaller and quadrangular; Second largest, being wider and deeper; and the third rectangular. Relatively small anterior mental shield, with length 17.6–24.4% of HL ($\bar{x} = 21.2 \pm 1.6$) and width 20.3–29.9% of HL ($\bar{x} = 24.5 \pm 1.8$). Posterior mental shield heptagonal, larger than anterior mental shield, with length 24.4–31.3% of HL ($\bar{x} = 28.1 \pm 1.8$) and width 20.1–31.1% of HL ($\bar{x} = 24.1 \pm 1.9$). Presence of one or two rows of postgenial shields with 2 + 3 segments. One pair of trapezoid malar shields, with length ranging from 18.4–26.4% of HL ($\bar{x} = 22.2 \pm 1.7$), and width 18.4–26.4% of HL ($\bar{x} = 8.7 \pm 2.1$). Row of postmalars present with 6–12 shields. Body annuli 202–218 ($\bar{x} = 210.1 \pm 3.7$). Caudal annuli 18–23 ($\bar{x} = 20.0 \pm 0.9$), caudal autotomy between 5 and 9 annulus ($\bar{x} = 8.3 \pm 0.7$); segments of the fourth caudal annulus 20–26 ($\bar{x} = 22.8 \pm 1.4$). Dorsal segments 10–14 ($\bar{x} = 12.6 \pm 0.9$) and ventral segments 13–18 ($\bar{x} = 16.1 \pm 1.0$) on the midbody annulus. Four precloacal pores arranged sequentially in the posterior portion of the segments of the last ventral annulus. Cloacal plate with 6–8 precloacal and 7–14 postcloacal shields. Specimens present light brown color pattern, more intense dorsally. On venter, the brown coloration extends laterally and fades in the centre.

Distribution: *Amphisbaena munoai* is restrictedly distributed in the Uruguayan Savannah areas of southern Brazil and Uruguay. It is widely distributed in Uruguay, from rock outcrops in eastern San Jose Department including Montevideo, Cerro Animas (type locality) to north in Cerro Largo Department. In Brazil, it is found only in Rio Grande do Sul state, Southern to the Camaquã River at the eastern portion of the Sul-riograndense Shield. The species is associated with rock outcrop habitats located in the southern areas of the Pelotas Batholith in the “Cinturão Dom Feliciano” geotectonic unit and other formations from the Rio de La Plata Craton (Fig. 6).

3.3.5. Comments on *Amphisbaena prunicolor* (Cope, 1885)

Aporarchus prunicolor Cope, 1885: 189. Type-locality: São João do Monte Negro, Rio Grande do Sul, Brazil.

Amphisbaena darwini—Boulenger, 1885: 297 (in part).

Amphisbaena prunicolor prunicolor—Gans, 1966: 246.

Amphisbaena prunicolor—Vanzolini, 2002: 358.

Amphisbaena prunicolor—Perez et al., 2012: 17.

Holotype: ANSP 12969 from São João do Monte Negro, currently Montenegro municipality, Rio Grande do Sul, collected by Herbert H. Smith.

Referred material: MZUSP 3507 Colatina, Espírito Santo, Brazil; MHNCI 8647 Boa Vista da Aparecida, Paraná, Brazil; MHNCI 8644 Capitão Leônidas Marques, Paraná, Brazil; MHNCI 10079 Coronel Vivida, Paraná, Brazil; MHNCI 8560, 8561, 8564; MCP 10903, 10904, 10905 Cruzeiro do Iguaçu, Paraná, Brazil; MHNCI 5944 Dois Vizinhos, Paraná, Brazil; MZUSP 87736, 87738, 87741 Foz do Chopim, Paraná, Brazil; MHNCI 10732 Laranjal, Paraná, Brazil; MHNCI 11912, 11913 Mangueirinha, Paraná, Brazil; MHNCI 10084 Mariópolis, Paraná, Brazil; UFRGS 5280, 5281 Nova Cantu, Paraná, Brazil; MHNCI 8648 Nova Prata do Iguaçu, Paraná, Brazil; MHNCI 8494 Quedas do Iguaçu, Paraná, Brazil; MHNCI 5163 Reserva do Iguaçu, Paraná, Brazil; UFRGS 5364* Bom Jesus, Rio Grande do Sul, Brazil; MCP 332, 333 Canoas, Rio Grande do Sul, Brazil; MCN 4357; MCP 12543 Caxias do Sul, Rio Grande do Sul, Brazil; MCN 8910, 8911, 8912 Erechim, Rio Grande do Sul, Brazil; UFRGS 6379*, 6381*, 6382* Fagundes Varela, Rio Grande do Sul, Brazil; MCN 9005 Farrroupilha, Rio Grande do Sul, Brazil; MCP 4336 Frederico Westphalen; Rio Grande do Sul, Brazil; UFRGS 5723*, 5782*, 5784*, 5785* Guaporé, Rio Grande do Sul, Brazil; MCP 3616, 3617 Ijuí, Rio Grande do Sul, Brazil; UFRGS 5322 Ivoti, Rio Grande do Sul, Brazil; MCN 4283, 4284 Lajeado, Rio Grande do Sul, Brazil; UFRGS 6246* Lagoa Vermelha, Rio Grande do Sul, Brazil; MCN 6529, 6594 Montenegro, Rio Grande do Sul, Brazil; UFRGS 5720*, 5721*, 5730* Nova Bassano, Rio Grande do Sul, Brazil; UFRGS 1980 Nova Petrópolis, Rio Grande do Sul, Brazil; UFRGS 6445* Nova Prata, Rio Grande do Sul, Brazil; MCP 5929, 6095 Planalto, Rio Grande do Sul, Brazil; UFRGS 1943, 1944, 2322; MCN 2715, 2716, 2717, 2718, 3130, 3132, 3133, 3592, 4467; MCP 341, 342, 1551, 4316, 4783*, 4784, 4786, 4787, 4837, 4838, 4839, 5379, 5380, 5381, 5384, 5386, 5387*, 5434, 9778, 13800, 15071 Porto Alegre, Rio Grande do Sul, Brazil; MCP 8448 Riozinho, Rio Grande do Sul, Brazil; MCN 2649 Rolante, Rio Grande do Sul, Brazil; UFRGS 6482* Salvador do Sul, Rio Grande do Sul, Brazil; MCN 2777 São Sebastião do Caí, Rio Grande do Sul, Brazil; MCN 7644 Sapiranga, Rio Grande do Sul, Brazil; MCP 6125 Tenente Portela, Rio Grande do Sul, Brazil; MCP 1456 Três Passos, Rio Grande do Sul, Brazil; UFRGS 5317, 5318 Trindade do Sul, Rio Grande do Sul, Brazil; MCN 15284, 15285 Triunfo, Rio Grande do Sul, Brazil; UFRGS 2366 Viamão, Rio Grande do Sul, Brazil; CHUFSC 731 Água Doce, Santa Catarina, Brazil; CHUFSC 750, UFRGS 4536 Anita Garibaldi, Santa Catarina, Brazil; MHNCI 2376 Caçador, Santa Catarina, Brazil; UFRGS 5269*, 5305* Campo Belo do Sul, Santa Catarina, Brazil; MCN 16337 Campos Novos, Santa Catarina, Brazil; UFRGS 4856* Capão Alto, Santa Catarina, Brazil; MHNCI 4076 Catanduvas, Santa Catarina, Brazil; MCP 2875 Concórdia, Santa Catarina, Brazil; MCP 3080, 3081 Itá, Santa Catarina, Brazil; MZUSP 12310 Nova Teutônia, Santa Catarina, Brazil; MCP 2905 Piratuba, Santa Catarina, Brazil; CHUFSC 604, 605, 606, 607, 608 São Domingos, Santa Catarina, Brazil; MZUSP 43026 São Miguel d'Oeste, Santa Catarina, Brazil; MHNCI 4090 Vargem Bonita, Santa Catarina, Brazil; CHUFSC 943, 944, 945 Xanxerê, Santa Catarina, Brazil; CHUFSC 1222 Xaxim, Santa Catarina, Brazil (see Fig. 10).

Specimens indicated with an asterisk where included on genetic analysis.

Diagnosis: *Amphisbaena prunicolor* is characterized by the following characters: (1) rounded head; (2) tail tip rounded, with smooth segments; (3) four precloacal pores; (4) dark brown, purplish coloration, with uniformly pigmented segments on dorsum and belly with checkered pattern.

Comparison: *Amphisbaena prunicolor* is distinguished from all species of the *A. darwini* complex, mainly by having a dorsal brown-purplish coloration and a ventral pale checkerboard pattern (vs. ventral uniformly colored). It also differs from *A. trachura* by presenting smooth segments the tail tip (vs. tuberculate segments on tail tip). Differs from *A. nigricauda* by presenting 181–215 body annuli (vs. 222–226 body annuli). It differs from *A. hogei* and *A.*



Fig. 10. *Amphisbaena prunicolor*, UFRGS 4536 from Anita Garibaldi, State of Santa Catarina, Brazil. Dorsal, lateral and ventral view of the head. Scale = 1 mm.

heterozonata by presenting a higher number of caudal annuli, 18–27 (vs. 15–19 and 13–17, respectively). Differs from *A. darwini* by its quadrangular parietal shields different from others body shields (vs. parietal region with variable shaped shields non-differentiable from body shields). It differs from *A. albocingulata* by presenting a post-malar row (vs. post-malar row absent). Differs from *A. munoai* by presenting an elongated body, 141–238 mm (SVL) with similar number of body annuli, 181–215 (vs. smaller body, 102–151 mm SVL, with similar number of body annuli 202–218 body annuli) and by 5% of 16S rDNA mean genetic distance. It differs from *A. arenicola* sp. nov. by presenting a longer snout, varying between 44 and 73.5% ($\bar{x} = 55.5 \pm 7.4\%$) of head length (HL) (vs. 42.6–47.9%, ($\bar{x} = 45.9 \pm 1.9\%$ of HL) and 4.5% of 16S

rDNA mean genetic distance. Differs from *Amphisbaena tyaraju* sp. nov. by presenting a lower distance between ocular shields, varying between 26.8% and 31.9% ($\bar{x} = 29.4 \pm 3.6$) of HL (vs. larger distance between ocular shields varying between 35.3% and 42.7%, $\bar{x} = 39.5 \pm 2.3$ of HL) and 5.1% of 16S rDNA mean genetic distance. It differs from *A. nana* sp. nov. by its similar-sized pre-frontal and frontal shields (vs. reduced frontal shields, smaller than pre-frontal shields) and 4.9% of 16S rDNA mean genetic distance.

Distribution: *Amphisbaena prunicolor* is distributed in Atlantic Forest remnants in Argentina (Corrientes and Misiones provinces), Paraguay (Itapúa department) and in northern Rio Grande do Sul, western Santa Catarina and Paraná, with historical records in the States of Rio de Janeiro, São Paulo and Espírito Santo, in Brazil.

For a recent and complete description of the morphology of *A. prunicolor* see Perez et al. (2012).

4. Discussion

Even with the recent advances in the knowledge of *Amphisbaena* including the description of 23 novel species in the last 20 years (Colli et al. 2016; Uetz & Hošek 2018), there are still many information gaps for most species. Fossorial squamates usually are not prioritized in samplings of amphibians and reptiles. Therefore, amphisbaenians remain as the group of Squamata less studied and poorly represented in biological collections (Gans 1967; Kearney 2003; Colli et al. 2016). Species boundaries have been hard to define among the *darwini*-Group mainly due to the lack of information regarding morphological variation and geographic distribution and still little is known about the barriers that can isolate amphisbaenians. Despite a rare record of water dispersion (Maschio et al. 2009) there is no concrete information about amphisbaenian dispersion ability. It has been suggested that fossorial habits would limit the ability to disperse across greater distances (Hembree 2006). Apparently, there is a correlation between the range of distribution and the snout-vent length of species, with larger species (~250 mm) occupying wider ranges (Colli et al. 2016). However, there is much uncertainty if some species have small ranges of distribution due to incomplete sampling or because it has a low vagility and a truly restricted distribution. In the other extreme, some widely distributed species may represent complex of cryptic or poorly studied species. In either case, it is clear that most *Amphisbaena* species are poorly sampled and even most of the collected material lacks a correct identification.

Fossoriality requires adaptations that might restrict colonization patterns and differentiation and population isolation processes (Albert et al. 2007). Due to the extreme underground habitat, amphisbaenians may exhibit morphological stability (Bickford et al. 2007), suggesting that speciation and molecular evolution may be uncoupled from morphological changes (Sampaio et al. 2014; Macey et al. 2004; Kearney & Stuart 2004). We would expect a gray zone in speciation that would not fit all criterions to delimit different species (De Queiroz 2007).

The *Amphisbaena*-species analyzed herein have a strong phylogenetic signal with a divergence into the mitochondrial lineages, corroborating other previous studies of *Amphisbaena* (e.g., Mulvaney et al. 2005; Albert et al. 2007; Albert & Fernandes 2009). As expected the reduction of gene flow due to limited dispersal ability would lead to a strong genetic structure (Poulson & White 1969; Albert et al., 2007). Herein, the genetic distances observed for the 16S rDNA gene (variation between 2.4% and 6.9%) were similar to those observed in the genus *Blanus* Wagler, 1830 (variation between 2.3% and 8.9%) (Sampaio et al. 2014). These values were also similar to those observed in other South American

reptiles and amphibians, which present distances ranging from 4% to 10% (Vences et al. 2010; Guarnizo et al. 2016; Gamble et al. 2012; Recoder et al. 2014; Werneck et al. 2015).

Despite their morphological similarity, some qualitative or quantitative characters allowed the interspecific distinction, such as coloration pattern, that at least distinguished *A. prunicolor* from other *Amphisbaena*. The smaller snout length observed in *A. arenicola* sp. nov. could be associate to the restinga areas with sandy soils that the specie inhabits. The closest similarities between *A. munoai* and *A. nana* sp. nov. are probably related to a similar habitat choice, rock outcrops, along the Uruguayan Savannas. Individuals of these species were not found in sympatry, however, besides the Camaquã River, we did not observe another barrier to delimit their geographical distributions.

Our results reinforce that integrative approaches are much necessary to describe the vastly underestimated diversity in *Amphisbaena* (Padial et al., 2009; Fouquet et al., 2007; Hung et al., 2004; Recoder et al., 2014). The description of three new species herein, endemic to southern Brazil, increases the known diversity to eight *Amphisbaena* distributed in the region (Lema 1994; Carreira et al. 2005; Perez et al. 2012).

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Capítulo II

Revisão taxonômica das cobras-cegas associadas à *Amphisbaena darwinii* Duméril & Bibron
1839 (Amphisbaenia: Amphisbaenidae)

(Manuscrito a ser submetido à *Zoologica Scripta*)

Revisão taxonômica das cobras-cegas associadas à *Amphisbaena darwini* Duméril & Bibron 1839 (Amphisbaenia: Amphisbaenidae)

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Título curto: Reavaliação taxonômica das espécies associadas à *Amphisbaena darwini*

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Resumo

Revisamos o status taxonômico de *Amphisbaena darwini*, *A. heterozonata* e *A. trachura*, táxons tradicionalmente considerados subespécies de *A. darwini*. Apesar de autores elevarem esses táxons a nível específico, as decisões taxonômicas foram feitas sem comentários. Os limites entre esses táxons permaneceram incertos e identificações equivocadas continuam ocorrendo ao longo da distribuição das espécies. Para reavaliarmos esse complexo de espécies, aplicamos a abordagem da taxonomia integrativa, através do uso de análises filogenéticas (genes mitocondriais e nucleares), morfológicas (caracteres quantitativos e qualitativos) e limites geográficos. Nossos resultados identificaram a presença de um novo táxon, *Amphisbaena grupoE* sp. nov., identificado principalmente pelo padrão de coloração com anéis escuros e claros ao longo do corpo. Além disso, observamos a presença de pelo menos duas linhagens dentro de *Amphisbaena darwini*, uma ao sul do Rio Grande do Sul e outra no Uruguai. *Amphisbaena trachura* e *A. heterozonata* são redescritas no presente trabalho. Esses resultados aumentam para nove as espécies conhecidas de *Amphisbaena* para a região sul do Brasil, Uruguai e Argentina.

Palavras chave: Cobra-cega, América do Sul, Taxonomia, Genética, Morfologia.

Capítulo III

Sistemática filogenética do grupo de *Amphisbaena darwini* Duméril & Bibron 1839
(Amphisbaenia: Amphisbaenidae), baseado em caracteres morfológicos e moleculares

(Manuscrito a ser submetido à *Zootaxa*)

**Sistemática filogenética do grupo de *Amphisbaena darwini* Duméril & Bibron 1839
(Amphisbaenia: Amphisbaenidae), baseado em caracteres morfológicos e moleculares**

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Título curto: Sistemática filogenética do grupo *Amphisbaena darwini*

Manuscrito a ser submetido a *Zootaxa*

Resumo

Amphisbaenidae é a mais diversa das seis famílias de Amphisbaenia, com 12 gêneros e 176 espécies reconhecidas, distribuídas no Caribe, América do Sul e África subsaariana. As hipóteses de relacionamento filogenético entre os táxons de Amphisbaenidae são escassas e instáveis, mas indicam que pelo menos um dos clados Africanos é o grupo irmão das espécies Sul-americanas e Caribenhas (Amphisbaeninae). Em relação às espécies de Amphisbaeninae, os gêneros tradicionalmente reconhecidos não se mostraram monofiléticos e foram sinonimizados à *Amphisbaena*. Essa decisão, seguida do uso do gênero *Leposternon*, tornou *Amphisbaena* um agrupamento parafilético. Através de uma revisão taxonômica, um complexo de espécies associados à *Amphisbaena darwini*, foi reconhecido. Essas espécies possuem características morfológicas similares e as hipóteses de relacionamento filogenético que em parte foram incluídas, parecem indicar um agrupamento natural, no entanto, com poucas evidências de sinapomorfias morfológicas. Buscando testar a monofilia do grupo de *Amphisbaena darwini* e sua relação com as demais espécies de *Amphisbaena*, utilizamos uma matriz com 4323 caracteres, sendo um total de 4271 pares de bases (quatro genes mitocondriais e quatro genes nucleares) e 51 caracteres de morfologia interna e externa. Nosso resultado corrobora a hipótese de monofilia do grupo. Além disso, reforça que a utilização da nomenclatura atual torna *Amphisbaena* um agrupamento parafilético. Estudos com a inclusão de um maior número de espécies são necessários para a tomada de decisões quanto as considerações taxonômicas a nível genérico entre as Amphisbaenidae Neotropicais.

Palavras chave: Amphisbaeninae, filogenia, América do Sul.

Considerações finais

Nossos resultados mostraram a existência de uma diversidade maior que a conhecida entre as espécies de Amphisbaenidae da região sul do Brasil, Uruguai e Argentina, aumentando para nove as espécies do grupo de *Amphisbaena darwinii* reconhecidas na região. Além disso, reforçam que as revisões taxonômicas detalhadas são essenciais para a delimitação destas espécies de difícil identificação e que apresentam históricos taxonômicos complexos. O uso de amostras moleculares possibilitou a identificação de diferentes linhagens entre esses táxons, reiterando a necessidade da integração de diferentes linhas de evidências para a delimitação de espécies. O conhecimento da variação morfológica e molecular para as espécies do grupo foi ampliado, no entanto, alguns táxons necessitam de uma melhor amostragem (principalmente molecular) para avaliação.

Nossos resultados, sugerem que as espécies tradicionalmente associadas à *Amphisbaena darwinii* representam um agrupamento natural monofilético. No entanto, o posicionamento de algumas espécies não incluídas no presente estudo (*Amphisbaena albocingulata*, *A. hogei* e *A. nigricauda*) ainda é incerto, sendo necessária a inclusão de suas amostras. Além disso, demonstram a existência de diferentes linhagens em *Amphisbaena* que poderiam ser elevadas à categoria de gênero. A nomenclatura atual, que reconhece os gêneros *Amphisbaena* (incluindo *Anops*, *Aulura*, *Bronia* e *Cercolophia*) e *Leposternon*, é um arranjo artificial que torna *Amphisbaena* parafilético. Portanto, estudos com a inclusão de uma diversidade mais representativa de Amphisbaenidae são necessários para a tomada de decisões quanto às considerações taxonômicas, em níveis genéricos do grupo.