UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

Programa de Pós-Graduação em Ciências Biológicas (Zoologia)

MIOLOGIA COMPARADA DE REPRESENTANTES DO GÊNERO *AMPHISBAENA* LINNAEUS, 1758 (SQUAMATA, AMPHISBAENIDAE)

INGRID LIMA E LIMA

Botucatu – SP
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Dissertação apresentada ao Instituto de Biociências, Câmpus de Botucatu, UNESP, para obtenção do título de Mestre no Programa de Pós-graduação em Zoologia.

Orientador: Prof. Dr. Ivan Sergio Nunes da Silva Filho

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Botucatu – SP

2024

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP

BIBLIOTECÁRIA RESPONSÁVEL: ROSANGELA APARECIDA LOBO-CRB 8/7500

Lima e Lima, Ingrid.
Miologia comparada de representantes do gênero *Amphisbaena Linnaeus*, 1758 (Squamata, *Amphisbaenidae*) / Ingrid Lima e Lima. - Botucatu, 2024

Dissertação (mestrado) - Universidade Estadual Paulista (UNESP), Instituto de Biociências, Botucatu
Orientador: Ivan Sergio Nunes Silva Filho
Coorientador: Angele dos Reis Martins
Capes: 20604009

1. Anatomia comparada. 2. Cobras. 3. Glândulas. 4. Músculos.

Palavras-chave: Anatomia; Anfisbênias; Caracteres miológicos; Descrição muscular; Glândulas cefálicas.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: Miologia comparada de representantes do gênero Amphisbaena Linnaeus, 1758 (Squamata, Amphisbaenidae)

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Botucatu, 30 de agosto de 2024

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AGRADECIMENTOS

Gostaria de agradecer primeiramente aos meus pais, que sempre me inspiraram e me apoiaram a buscar e a realizar meus maiores sonhos, por mais loucos que fossem. Em especial minha mãe, que topa qualquer aventura, e sempre me socorreu nos piores momentos. Obrigada por estarem sempre presente quando precisei, sem vocês nada disso seria possível, e eu jamais teria chegado até aqui. E ao meu irmão, que me tira sempre do sério, mas é a pessoa mais companheira que eu poderia ter na vida. Vocês são luz!

Agradeço à espiritualidade, pelos momentos de reflexão que me ajudam a buscar minhas melhores versões, e me guiam em direção ao autoconhecimento.

Não poderia deixar de agradecer, também, à toda minha família que está no Pará, e mesmo em longas distâncias, estiveram comigo comemorando cada vitória e cada passo dado em minha vida, desde que saí tão pequena da cidade. Esse apoio sempre me deu forças para continuar e com vocês eu aprendi o verdadeiro significado de ter sempre um lar para retornar.

Ao meu orientador Ivan Nunes, que me recebeu de volta no LHERP após a graduação e me deu a oportunidade de realizar este trabalho. À minha coorientadora Angele Martins, que abriu as portas do seu laboratório na UnB para que eu pudesse investigar a morfologia dessas quase “minhocas”, e se dispôs a compartilhar informações e tirar dúvidas a respeito do complexo mundo dos músculos desses minis queridos animais.

Agradeço aos meus amigos do laboratório, Juan Aquino (Cabron), Bel, Vitany, Camila, Manu e Lívia (Dis), que compartilharam tanto os melhores momentos, quanto momentos não tão bons assim. Muito obrigada pelas horas do café, pelas risadas e descontração, pelas reflexões, ideias compartilhadas, momentos de desabafo, saídas de campo, enfim, por estarem ali dia após dia. Nos apoiamos uns nos outros até aqui e podem continuar contando

comigo para qualquer coisa. E aos parceiros Gabriel, Enzo, e Ed, pelas conversas do dia a dia, à técnica de laboratório da UNESP Cláudia, que me ensinou diversas metodologias de extração de DNA, PCR entre outras técnicas moleculares, certamente me será muito útil durante minha jornada acadêmica.

À minha namorada e companheira para todos os momentos, que compartilha a vida comigo e tem tanta urgência de viver quanto eu. Muito obrigada por acreditar em mim, pela compreensão, pela paciência quando eu quero falar sobre coisas da biologia, que mesmo sem entender nada, me escuta com todo amor do mundo. Amo você!

Agradecimento especial aos meus quatro bebês de quatro patas, que são um momento de conforto em meio ao caos. Sem eles não seria possível aguentar tantos momentos de estresse e ansiedade, acariciar um animal de fato acalma a mente!

Agradeço a Caroline e a Carla que lá atrás coletaram essa espécie que hoje estou tendo o privilégio de estudar. A todos os curadores de museus que emprestaram e me permitiram analisar os espécimes de suas coleções.

Ao CNPq pela bolsa concedida que me permitiu dedicação integral a este projeto.

E a todos que de alguma forma contribuíram para que hoje eu pudesse estar aqui realizando mais uma etapa rumo ao futuro!

“All that is gold does not glitter,
Not all those who wander are lost;
The old that is strong does not wither,
Deep roots are not reached by the frost.

From the ashes a fire shall be woken,
A light from the shadows shall spring;
Renewed shall be blade that was broken,
The crownless again shall be king.”

— J.R.R. Tolkien

RESUMO

Dentre os Squamata, a subordem Amphisbaenia possui indivíduos que apresentam diversas adaptações para o modo de vida fossorial, atualmente sendo representada por 202 espécies divididos em seis famílias. Apesar do recente aumento no interesse e progresso acerca do grupo, ainda nos deparamos com a falta de diversos tipos de dados. Sendo assim, devido ao número limitado de descrições miológicas conduzidas neste grupo taxonômico, o presente estudo visa fornecer uma descrição da anatomia muscular cefálica de quatro espécies de *Amphisbaena* – *Amphisbaena dubia*, *Amphisbaena darwinii*, *Amphisbaena munoai* e *Amphisbaena microcephala*. Além disso, incorporamos dados de espécies com descrições musculares previamente documentadas na literatura para fornecer uma comparação mais abrangente. Foram descritas variações na musculatura associada ao complexo mandibular e alterações na estrutura dos tendões que sustentam esses músculos. Além disso, foram identificadas variações na forma, origem e inserções do *m. adductor mandibulae externus superficialis*, *medialis* e *profundus*, na disposição das fibras do *m. retractor anguli oris* e do *m. depressor mandibulae*. Ademais, novas percepções sobre o sistema glandular das espécies, com a descrição de uma glândula rictal ainda não registrada para este grupo, juntamente com a criação de 24 de caracteres baseados nessas variações, foram fornecidas. Tais resultados traduzidos em caracteres podem ser utilizados para refinar e aprimorar futuras investigações filogenéticas, esclarecendo a evolução do grupo e suas adaptações morfológicas.

Palavras-chave: anfisbênias, anatomia, caracteres miológicos, descrição muscular, glândulas cefálicas

ABSTRACT

Among Squamata, the suborder Amphisbaenia includes individuals that exhibit various adaptations for fossorial lifestyle, currently represented by 202 species divided into six families. Despite the recent surge in interest and progress concerning the morphology of the group, most studies are mainly focused on their skull and hemipenial shape/morphology, with little data on other morphofunctional complexes such as postcranial data, muscles, cephalic glands and viscera. Herein we aim to describe in detail the cephalic myology of four *Amphisbaena* species – *Amphisbaena dubia*, *Amphisbaena darwinii*, *Amphisbaena munoai* and *Amphisbaena microcephala*. Furthermore, we incorporate data from species with previously documented muscular descriptions available in the literature to provide a broader comparative framework. Variations in the musculature associated with the mandibular complex and alterations in the structure of tendons supporting these muscles were described. Additionally, variations in shape, origin and insertions of the *m. adductor mandibulae externus superficialis, medialis and profundus*, iber arrangement of *m. retractor anguli oris* and *m. depressor mandibulae* were identified. Moreover, novel insights into the glandular system of the species, with the observation of a rictal gland not yet recorded for this group, along with the creation of a set of characters based on these variations, were provided. The obtained results may supply valuable insights and interesting character information to be used in future phylogenetic investigations of the group.

Keywords: amphisbaenians, anatomy, cephalic glands, muscular descriptions, myological characters

SUMÁRIO

Comparative myology of four species of Amphisbaena (Linnaeus, 1758) (Squamata, Amphisbaenidae) with descriptions on the cephalic glands

Short running title: Comparative myology of four *Amphisbaena* Linnaeus, 1758

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1. INTRODUCTION

The order Squamata encompasses over 12,000 extant species of lizards, snakes, and amphisbaenians (Uetz et al., 2024), and represent the most diverse clade of terrestrial vertebrates worldwide (Simões & Pyron, 2021), occurring in nearly all habitats (Vitt & Caldwell, 2013). Amphisbaenia is a suborder of Squamata, comprised by fossorial animals commonly known as worm-lizards, currently represented by 202 species divided into six families (Uetz et al., 2024). The geographic range of the group includes North America in eastern USA (Rhineuridae); Central America (Cadeidae and Bipedidae); Europe and North Africa (Blanidae); North Africa and Asia (Trogonophidae; Gans, 2005). The family Amphisbaenidae is the most speciose and has a wide distribution in South America, Central America, and Africa (Costa & Garcia, 2019), even though many species exhibit endemic or restricted distributions and remain poorly understood (Gans, 2005; Costa, 2020).

These fossorial Squamata display significant adaptations for subterranean life, including the construction of their own tunnel systems by compressing the soil within the galleries, with horizontal or vertical movements of the head (Gans, 1968). This head-first burrowing behavior is a primary tool for the fossorial lifestyle of Amphisbaenia (Navas et al., 2004), being supported by a suite of morphological features, including cranial adaptations (head shape: rounded, shovel-shaped, spade-shaped, and keel-shaped; Kearney 2003), tegumentary characteristics, and muscular arrangements (Gans, 1986). Round-headed species use a generalized mechanism, while shovel-headed, spade-headed, or keel-headed species employ specialized mechanisms (Gans, 1969; Hohl et al., 2014), this strongly ossified skull enhances resistance to excavation impacts (Gans & Montero, 2008), requiring enduring muscle contractions, especially in dorsal, pectoral and cephalic muscles (Gans, 1968; Navas et al., 2004). Additionally, the tegumentary musculature exhibits a

unique configuration, wherein muscles are loosely interconnected with the skin, thereby reducing frictional resistance against the tunnel walls during locomotion (Gans, 1962).

Multiple aspects of amphisbaenian biology remain underexplored, with many species having been described only recently, with their natural history receiving limited attention (Colli et al., 2016). This phenomenon is partly attributed to the fossorial behavior of these animals, which can restrict field observations and complicate specimen collection for laboratory research (Navega-Gonçalves & Benites, 2019; Paiva et al., 2024). Consequently, amphisbaenians remain as the group of Squamata less studied and poorly represented in biological collections (Gans, 1967; Kearney, 2003; Colli et al., 2016), which delays the execution of descriptive or comparative studies (Gans, 2005; Pinna et al., 2014; Roberto et al., 2014). This creates a gap in the knowledge of their morphological, anatomical, functional, and phylogenetic characteristics. Which is concerning, as morphological data, especially those related to myology, have proven effective in studies of functional biology and phylogenetic analyses among vertebrates (O'Reilly et al., 2000; Diaz et al., 2022; Lowie et al., 2023). Addressing this gap can provide more detailed answers regarding the musculature involved in burrowing, the influence of mandibular musculature on food acquisition (Cundall, 1983), and the similarity between amphisbaenians and other highly fossorial squamate species (Daza et al., 2011), among other evolutionary questions concerning this group.

Some studies in recent decades have focused on the myology analysis of reptiles in the order Squamata (Abdala & Moro, 2006; Bhullar, 2009; Martins et al., 2019; Westphal et al., 2019). However, both older and contemporary studies primarily concentrate on snakes and lizards (e.g., Jayne, 1982; Cundall, 1986; De Souza Leite et al., 2021). One extensively investigated factor, for example, is the limb loss in groups exhibiting this characteristic (Greer, 1991; Abdala et al., 2015; Camaiti et al., 2021). These investigations

have demonstrated that osteology and myology provide better insights into the evolutionary patterns of limb loss compared to studies solely focused on external characteristics (Camaiti et al., 2021).

Although there are descriptive myology studies on amphisbaenians, they are scarce and mostly outdated (Renous, 1977; Bonin, 1965), leaving a wide-open niche for studies. For example, earlier studies described part of the jaw adductor musculature of *Amphisbaena alba* Linnaeus, 1758 (Lakjer, 1926), and some observations and descriptions were added for the same species, as well for *Amphisbaena microcephala* Wagler, 1824 and *Trogonophis wiegmanni* Kaup, 1830 (Haas et al., 1973). Later, Rieppel (1979) partially described the jaw adductor musculature of *A. alba* and *Amphisbaena fuliginosa* Linnaeus 1758. More recently, we have descriptions of muscles located around the neck-trunk region for *A. alba* (Tsuihiji et al., 2012) and descriptions of pectoral muscles through diceCT visualization for five species of Amphisbaenia (Westphal et al., 2019). This indicates that the descriptions for this group are sporadic and further investigations are needed for better correlations between the homologies of this muscular system.

The cranial muscles of amphisbaenians are of special interest due to the complete loss of the upper temporal arch (jugal) and the closure of the cranial box, modifications similar to those seen in snakes (Haas et al., 1973). This cranial structure, associated with powerful temporal musculature, also facilitates food capture and crushing (Gans, 1969). Therefore, descriptive myology studies allow correlating these and other specializations with the different muscular characteristics of these species (Gans & Montero, 2008), providing important data on the functional morphology of amphisbaenians. Furthermore, we must not overlook the knowledge regarding their cephalic glands, as information about their macro and microstructure remain unknow (Foureaux et al., 2010), the conception and

integration of these internal characters for a phylogenetic analysis has proven valuable for other Squamata groups (Abdala et al., 2009), yet it has not been applied to amphisbaenians.

Among the South American worm-lizards, we highlight four species, *Amphisbaena dubia* (Müller, 1924), *Amphisbaena darwinii* (Duméril & Bribon, 1839), *Amphisbaena munoai* (Klappenbach, 1960) and *Amphisbaena microcephala* (Wagler, 1824). *A. dubia* is endemic from Brazil (Evers et al., 2006), but its population density is unknown, and its phylogenetic placement remains unaddressed in extant evolutionary studies (Mott & Vieites, 2009; Gauthier et al., 2012; Pyron et al., 2013). *A. munoai* and *A. darwinii* are found under stones in rocky outcrops in the Uruguay and southern Brazil (Perez & Borges-Martins, 2019). All those three share the round-headed pattern, differing only in size (Gans & Montero, 2008). Conversely, *A. microcephala* is shovel-headed, and has a widespread distribution (Hohl et al., 2017), occurring in different regions of South America (Ribeiro et al., 2011).

The diversity, precise identification of species and their boundaries, remain unresolved in many *Amphisbaena* groups (Perez & Borges-Martins, 2019), being crucial to employ diverse datasets, including myological descriptions, to advance knowledge of the group's evolutionary history. For that reason, herein, we described the cranial myology and its variations in these four species of amphisbaenians. This research involves comparing these anatomical features and discuss with respective cranial shapes, alongside a review of existing literature. Beyond that, we also described the cephalic glands observed and propose a list of myological characters.

2. MATERIAL AND METHODS

2.1 Methods of dissection and muscle descriptions

We analyzed eight preserved specimens from four different species: three specimens of *A. dubia*, deposited in the Laboratório de Herpetologia (LHERP) da Universidade Estadual Paulista (UNESP), Brazil; two specimens of *A. microcephala* deposited at Instituto Nacional da Mata Atlântica (INMA-MCTI), Brazil; one of *A. darwinii*, and two of *A. munoai*, from Museu de Ciências e Tecnologia (MCT-PUCRS), Brazil. *A. darwinii* and *A. munoai* were available only for partial dissections, due to the protocols of the scientific collection, this means that only one side of the specimen was dissected for observations.

All specimens were studied regarding their myology using a stereomicroscope, we also described the position and morphology of the cephalic glands, as well as their presence or absence, since data on this structure for amphisbaenians are rare in the literature. The dissections were performed according to a systematic procedure, starting with the removal of superficial layers, initially by skin removal from the supralabial and infralabial scales, progressing towards the medial region of the body, until the entire cephalic musculature was exposed. Topical applications of potassium iodide/iodine solution (Bock & Shear, 1972) were employed to enhance the visualization of muscle fiber details. To visually show the variation observed, specimens were photographed using the ZEISS ZEN Digital Imaging Software and a ZEISS Axiocam ERc 5s (5 MP) digital camera coupled to the stereomicroscope. Schematic drawings of muscle fibers based on the photographs were digitized and prepared through digital illustration using the Ibis Paint X software (version 12.1.5).

The terminologies used for the cephalic muscles follows Haas (1973) and the osteological terminologies follows Gans & Montero (2008). To facilitate the understanding

of the muscular descriptions and the respective bones associated with the described muscles, illustrations were constructed with distinct cranial representations for the species *A. dubia,* representing the model for round-headed shape (Fig. 1) and *A. microcephala* representing the model for shovel-headed shape (Fig. 2), both obtained from Gans & Montero (2008), being the two different cranial morphotypes here analyzed. The skulls of the other two species (*A. darwinii* and *A. munoai*) were not schematized due to the lack of clear diagrams in the literature and their overall similarity to the rounded skull of *A. dubia*.

2.2 Construction of the proposed characters

The methodology for constructing the proposed phenotypic characters followed the "contingent" model, not the "multistate" model (see Simões et al., 2017 for review), as proposed by Sereno (2007). In this model, characters are defined as heritable traits of an organism and expressed as independent variables and character states are defined as mutually exclusive conditions of a character. Additionally, it is established that characters can be either neomorphic, when they consist of the appearance or disappearance of traits that do not have comparable transformational states with other organisms, or transformational, when they consist of variations that have undergone state transformations comparable with other organisms (Sereno, 2007). Subsequently, a synthesis of the myological data present in the scientific literature was conducted, thus enabling a combined analysis to ensure that all available evidence was considered for the construction of characters.

In this study, we follow the taxonomy nomenclature proposed by Mott & Vieites (2009), which considered all South American and West Indian amphisbaenid species to be included in the genus *Amphisbaena*. Supported by observations in some phylogenies

(Longrich et al., 2015; Graboski et al., 2022), where it is noted that the genus *Amphisbaena* is not monophyletic if the genera *Leposternon*, *Bronia*, *Cercolophia*, and *Aulura* are considered valid.

3. RESULTS

A total of 21 muscles were described for the cephalic region. The musculature exhibited a high level of conservatism across the species studied. We did not identify intraspecific variation in the examined structures related to musculature. Variability among the three round-headed *Amphisbaena* (*A. darwinii*; *A. dubia* and *A. munoai*) was minimal; any observed variation will be noted alongside the muscle descriptions for clarity. The descriptions are organized according to the different muscle groups, each followed by a list of the specific muscles within that group. Beginning with the description of the muscles specifically in round-headed *Amphisbaena* species, followed by a section detailing the differences found in the muscles of the shovel-headed *A. microcephala*.

3.1 Description of the cephalic musculature of round-headed species (*A. dubia, A. darwinii e A. munoai*)

The muscles discussed in this section will encompass those located in both the cranial and mandibular regions, covering both superficial and deeper layers.

M. adductor mandibulae externus complex (sensu Haas, 1973)

(m. levator anguli oris; m. adductor mandibulae externus superficialis; m. retractor anguli oris)

***M. levator anguli oris (mlao)*:** It originates from the most dorsal part in parietal, immediately posterior to the eye orbit, and inserts into the rictal area (rictal plate, at the angle of the mouth). A small portion is partially covered by another muscle, the *M. adductor mandibulae externus superficialis* (mames), particularly the region that passes almost behind the orbit, lying adjacent to the Harderian gland. In general, this muscle is nearly indistinguishable from the most external muscle of the mandibular adductors, both forming a continuous superficial layer of muscles, which could only be differentiated based on a slight change in fiber arrangement (just after the eye orbit, the fibers are slightly more verticalized) (Fig. 4, 13), different insertion angles into the rictal plate (some more external angles and others more internal and deep passing through the coronoid process), and comparison with previous descriptions of this muscle for the group.

***M. adductor mandibulae externus superficialis* (mames):** This muscle is the first to be observed upon removal of the skin from the cephalic region, it is a significantly expanded and robust muscle observed in dorsal view of the entire specimen, it comprises a large muscular mass (Fig. 3, 4, 5) in which most posterior fibers and ventralmost part are delimited by the trigeminal nerve, also part of this nerve and a thin bifurcate tendon divide the left side from the right side of this muscle. It originates along the entire length of the parietal bone, extending to the crest on the supraoccipital and a portion of the lateral crest of the exoccipital. It inserts into a region that deepens along the lateral surface of the coronoid process and along the entire extent of the region between the palatine and the quadrate.

***M. retractor anguli oris (mrao)*:** a small horizontal band of muscle (Fig. 4, 12, 13). It occurs anteroventrally to the larger *M. adductor mandibulae externus superficialis*

*(*mames*)*, originating from the lateral crest of the quadrate and passing laterally to the extracolumella and the quadrato-maxillary ligament, some fibers insert into the rictal angle, in the middle of the rictal plate, and some fibers extend to the mandibular region attaching close to the fascia associated with the supralabial glands.

M. depressor mandibulae (mdm): A relatively elongated muscle that inserts along the posteroventral region of the mandible (Fig. 4). In *A. dubia*, this muscle is associated with a mandibular ligament, with some fibers extending more anteriorly on the mandible and partially covering the infralabial glands. In *A. darwinii* and *A. munoai*, this muscle is located more posteriorly and presents a right and left band at the posterior end of the mandible.

M. adductor mandibulae externus medialis (mamem): a muscle located beneath the entire *m. adductor mandibulae externus superficialis*. It also originates in the parietal region and is divided into a system of fibers separated by thin layers of connective tissue. The more dorsal fibers merge with a thin upper layer of fascia, while the more lateral fibers converge to form a thin tendinous sheet that attaches to the narrow basal aponeurosis (Fig. 7) and dorsal end of the coronoid process. Most fibers of this muscle insert through this tendinous sheet. The medial portion consists of more horizontally oriented fibers that extend anteriorly to their insertion on the coronoid process; this part lies deep to most of the rest of the muscle. In *A. darwinii* and *A. munoai*, this muscle does not exhibit the system of fiber division through the tendinous plate, being continuous from origin to insertion.

M. adductor mandibulae externus profundus (mamep): the muscle originates from the descending process of the parietal bone, the supraoccipital, and the prootic along a small portion of the lateral skull (Fig. 8). Most fibers converge to the medial surface of a very thin fibrous tissue layer, while the deeper fibers extend from the ventral part of the parietal and prootic towards the medial region of the surangular. Apparently, they do not insert into the quadrate.

M. constrictor internus dorsalis **complex**

(*M. levator pterygoidei*; *M. protractor pterygoidei; M. retractor pterygoidei)*

M. levator pterygoidei (mlpt): a relatively long muscle, originating from the anterior ventral edge of the parietal bone and extending ventrally to the posterior region to insert into the posterior end of the pterygoid bone.

M. protractor pterygoidei (mppt): originates medially to the foramen of the trigeminal nerve in the cranium, running vertically to insert into the posterior third of the pterygoid bone. It is partially overlapped by the *M. levator pterygoidei* and the *M. retractor pterygoidei.*

M. retractor pterygoidei (mrpt): a very slender muscle, arises from a relatively elongated region on the braincase wall and extends to the more anterior ventral region, running to the lower jaw.

M. pseudotemporalis superficialis (mpsts)**:** originates just after the orbital region, and its fibers run vertically until insertion into the coronoid process. This muscle is covered

by the mlao. For visualizing this muscle, it is necessary to gently open the mouth, expanding the mandibular opening.

M. pseudotemporalis profundus (mpstp): originates from the ventral part of the parietal and prootic bones, anterior to the maxillary and mandibular branch of the trigeminal nerve, inserting on the surface of the medial region of the mandible, from the posterior coronoid process to the mandibular joint.

M. pterygoideus profundus (mptp): this muscle originates from the pterygoid and inserts near the posterior end of the mandible, being observed only after gently opening the mouth of the specimen, not being visible in lateral view. It is not a very robust muscle, being composed of a thin arrangement of fibers.

M. cervico-mandibularis (mcm): a relatively well-developed muscle, some fibers insert into the anterior medial region of the lower mandible (Fig. 4, 10, 13, 14). The fibers run horizontally and expand towards the *m. sphincter colli* (msc), where it is partially covered in the posterior region by the same muscle. In *A. dubia* this muscle extends laterally along the body, being both broad and long. In *A. darwinii*, it is a relatively shorter muscle, not extending laterally along the body and confined to the more anterior region near the mandible.

M. sphincter colli (msc): this muscle originates more dorsally in a thin layer of connective tissue, the fibers run vertically covering parts of the *m. cervico-mandibularis* (mcm) and inserting into the fascia of the ventral region. This muscle is extremely thin and

is intimately associated with a superficial layer of fascia. During the process of skin removal from the specimen, these fibers can be easily removed together and damaged.

M. intermandibularis posterior (mimdp): this muscle exhibits a relatively triangular shape, or fan-shaped configuration (Fig. 9), with fibers originating from the posterior edge of the lower jaw and inserting into a layer of connective tissue between the *mcm*. It is covered by this same muscle and, in the posterior region, by the *msc*. In. *A. darwinii*, this muscle is not covered by the mcm. Apparently its positioned in a more superficial layer.

M. intermandibularis anterior (mimda): a thin layer of muscle consisting of vertical fibers that run from the medial edge of the mandibular bone to the opposite medial edge, partially covering the *mggl* (Fig. 10).

M. genioglossus (mggl): originates from the most anterior and central region of the mandibular bone (Fig. 10), being one of the deepest in this region, covered by all other muscles. The fibers run horizontally until they insert where the posterior intermandibularis begins.

M. rectus capitis anterior (mrca): muscle originates between the occipital crest and the most posterior region of the quadrate, with fibers running ventrally beneath the vertebrae horizontally until they insert into the more distal vertebrae.

M. longissimus (mld): a very extensive dorsal muscle that originates near the occipital complex, in the proximal region, and runs along almost the entire dorsal part of the body to the distal region.

M. spinalis capitis (mscap): exhibits fibers oriented towards the vertebrae, completely horizontally along the body, expanding in a double layer. Inserting into the most dorsal part of the skull near the parietal crest to insert through a double a tendon. The fibers run along all the body of the specimen.

M. semispinalis capitis (mspcap): exhibits fibers oriented towards the vertebrae, completely horizontally along the body, expanding in a double layer. Inserting into the most dorsal part of the skull near the parietal crest to insert through a double a tendon. The fibers run along all the body of the specimen. this muscle exhibits fibers oriented towards the vertebrae, more horizontally than vertically, expanding in a fan-shaped configuration (Fig. 3, 11). It inserts into the dorsal part of the skull near the parietal crest extending to the superficial layers of fascia associated with the skin and the *mld.*

3.2 Description and variations in cephalic musculature of shovel-headed specie (*A. microcephala*)

M. adductor mandibulae externus superficialis: this muscle occurs entirely beneath another layer of the *m. spinalis capitis* muscle, a configuration valid for the entire complex of the *m. adductor mandibulae externus* (Fig. 15), except for the *m. retractor anguli oris*, which is only partially covered. This muscle encompasses a smaller area in proportion to the body size of the species and has a trapezoidal shape; and it is as robust as in *A. dubia*.

Its fiber arrangement runs in a more vertical position (Fig. 16), likely due to the orientation of the skull shape. The most posterior fibers and ventralmost part are also delimited by the trigeminal nerve, but here the bifurcate tendon does not divide the left side from the right side, instead, the large and robust bifurcate parietal tendon overlap this muscle without contacting it.

M. levator anguli oris: this muscle occurs entirely beneath another layer of the *m. spinalis capitis* muscle, a configuration valid for the entire complex of the *m. adductor mandibulae externus*, except for the *m. retractor anguli oris*, which is only partially covered. Through the removal of fibers, it is possible to observe better separation between the different muscle layers of the complex (Fig. 16), characterized by the presence of a large aponeurosis and a more horizontal fiber arrangement. Some fibers superficially merge with the fibers of the *m. retractor anguli oris*, while deeper fibers pass entirely beneath the eye orbit and beneath the harderian gland, likely also contributing to their contraction.

M. retractor anguli oris: for *A. microcephala*, it is also a lateral band of muscle that inserts at the mouth angle (Fig. 16, 17), though not as elongated, terminating its insertion at the rictal, with its ending more posterior than that found in the other three species of *Amphisbaena*. Despite not being as elongated, it is wider and more robust; furthermore, this muscle layer covers a set of rictal glands found in the more posterior supralabial region (Fig. 17) but does not cover the supralabial glands.

M. adductor mandibulae externus medialis: in *A. microcephala* this complex of three muscles forming the *M. adductor mandibulae* is notably more robust than that found in *Amphisbaena dubia*. Besides that, the fibers of this muscle merge with a large basal

aponeurosis (Fig. 18), which is observed upon the removal of the *M. adductor mandibulae externus* superficialis and the *M. levator anguli oris.*

M. depressor mandibulae: in *A. microcephala* the muscle encountered was not exactly at the posterior end of the mandible; it was more pronounced and occupied a larger region slightly posterior to that of the other species, near the quadrate (Fig. 16). With the fibers almost in contact with a well-developed pectoral musculature.

M. pterygoideus profundus: in *A. microcephala* this muscle can be observed splitting into two distinct layers, thus having greater contact with the points of origin and insertion (Fig. 19).

M. cervico-mandibularis: in *A. microcephala* the fibers of this muscle merge slightly with a large tendon that originates from the lateral edge of the lower jaw (Fig. 21A).

M. intermandibularis anterior: The fibers of this muscle run beneath a thin tendon (Fig. 21B). This tendon extends from the anterior region of the mandibular bone until it meets with the muscles of the pectoral region. Apparently, the tendon also serves as an insertion point for the *m. cervico-mandibularis*.

M. rectus capitis anterior: the muscle originates from a very robust tendon that extends from the ventral region of the quadrate and extends up to the level of the vertebrae.

M. longissimus dorsi: the muscle is bilateral, with well-defined right and left sides that are separated by a tendon (two tendons are visible, one on the right side and another on

the left side of the body). Its origin also appears to be related to the tendon, rather than at the base of the skull. This tendon inserts into the "*canthus-rostralis*" region (transversal crest), the most anterior region of the parietal bone, and is associated with a large amount of fibrous tissue.

M. semispinalis capitis: the muscle in *A. microcephala* is not as long as seem in *A. dubia, A. munoai* and *A. darwinii*, having its origin more proximal than in those species and insertion into the cephalic tendons of the transversal crest (Fig. 15).

3.3 Cephalic glands

Here we report the descriptions and illustrations of cephalic glands in four representants of Amphisbaenidae family. We found a supralabial, infralabial, rictal and harderian gland (See table 1 for summary information about presence or absence in a determinate species). Indications of the presence of a nasal gland were observed; however, visualization was challenging, and it was not possible to confirm whether it was indeed a gland.

Harderian Gland: across all species, the ocular structure exhibits an absence of a discernible fibrous layer internally. Besides, we observe the Harderian gland engulfing the eyeball as it fills the orbit exhibiting an ellipsoidal shape and being covered by a layer of connective tissue that protects the eye. In some species (*A. microcephala*; *A. dubia* and *A. darwinii*) the gland extends beyond the boundaries of the ocular cavity, projecting outward and being significantly hypertrophied (Fig. 5, 13, 16, 17). In *A. microcephala* the gland is laterally and posteroventrally in contact with the fibers of *m. levator anguli oris,* ventrally with some fibers of the *m. retractor anguli oris*, and dorsally limited by prefrontal, this species shows this gland covered by the thickest layer of connective tissue. In *A. dubia* and

A. darwinii this gland is in contact with the most superior fibers of *m. levator anguli oris*, being limited by a contact with the supralabial glands ventrally and anteriorly restricted to the limits of the prefrontal. In *A. munoai* this gland is limited to the ocular cavity (Fig. 6; 7), exhibiting the smallest size, and slightly contacting the most superficial fibers of the *m. levator anguli oris*.

Supralabial Gland: the supralabial glands are situated along the lateral margin of the maxilla, subjacent to the supralabial scales (Fig. 6, 13, 16). They are strongly developed extending from the nostrils to eyes in all species (larger in the region below the eye and decreases in size as it extends anteriorly along the maxilla) and covered by a thin connective tissue that merge with some fibers of the *m. retractor anguli oris*, being dorsally in contact with the harderian gland, ventrally limited by the quadratomaxillary ligament and posteriorly by the *m. retractor anguli oris*. There are variability concerning their posterior limit in relation to the nostril, in *A. darwinii*, *A. dubia* and *A. munoai*, the gland begins immediately posterior to the nostril, positioned more anteriorly in the maxilla, whereas in *A. microcephala*, the gland is situated more posteriorly in the maxilla.

Infralabial Gland: the infralabial glands are robust, especially posteriorly, extending over the ventral and lateral lamina of the mandible (Fig. 5, 6, 13), they are paired and are all round (the more ventral portion shows an irregular shape), they are similar to but not as robust as the supralabial glands. In *A. microcephala* this gland is covered and limited by a huge cartilaginous structure denominated as "extracolumella cushion", which extends across the entire region of the lower maxilla (Fig. 16, 20). In *A. darwinii*, *A. munoai* and *A. dubia* this cartilaginous structure has a distinct and irregular fan shape and is directly connected to the infralabial glands of these species. In *A. munoai* the glands are limited to a

more posterior region at the lower maxilla, while in *A. dubia* and *A. darwinii*, the glands extend from the anterior region of the lower maxilla to near the quadrate.

Rictal Gland or Supralabial accessory: a well-developed arrangement of glands near the rictal area, posterior to the maxilla (Fig. 17), being present exclusively in *A. microcephala*. It may not be the rictal gland, as some studies suggest that the rictal gland, in snakes, has a different coloration (Underwood, 2002). However, nothing is described for amphisbaenians, and this set of glands is not connected to the set of supralabial glands; they are separated by a thin layer of fibers from *m. retractor anguli oris* and connective tissue overlapping it. This gland is limited dorsally by fibers of the *m. retractor anguli oris*, anteroventrally by the extracolumella cushion and posteriorly by some fibers of the *m. adductor mandibulae externus superficialis.*

3.4. Proposed myological characters

From the previously presented results and information on amphisbaenian musculature available in the literature, the following characters were proposed:

1. ***M. levator anguli oris,* arrangement.** (0) muscle fiber posterior to the orbit; (1) fibers run beneath the eye orbit cavity.
2. ***M. levator anguli oris,* relationship with the *M. retractor mandibulae***. (0) superficial fibers fused; (1) no contact between the fibers.
3. ***Adductor aponeurosis*.** (0) absent; (1) present.
4. ***Adductor aponeurosis*, condition.** (0) partially developed; (1) well-developed.

5. ***M. adductor mandibulae externus profundus***, relationship with ***M. adductor mandibulae externus medialis***. (0) in contact; (1) separated by the basal aponeurosis.
6. ***M. adductor mandibulae externus superficialis***
7. **Rictal gland.** (0) absent; (1) present.
8. **Harderian gland, position.** (0) filling the orbit, but not exceeding it; (0) more developed outside the orbit.
9. ***M. pterygoideus profundus,* layers.** (0) single layer; (1) double layer
10. ***M. depressor mandibulae*, condition.** (0) attached totally to end of the mandible; (1) half attached to the end of the mandible e half to the superficial musculature.
11. ***M. adductor mandibulae externus, form.*** (0) not divided; (1) partially divided; (2) completely divided.
12. ***M. levator anguli oris, insertion.*** (0) with aponeurosis; (1) without aponeurosis
13. ***M. retractor anguli oris.*** (0) absent; (1) present.
14. ***M. retractor anguli oris*, arrangement.** (0) not covering supralabial glands; (1) covering supralabial glands.
15. ***M. retractor anguli oris,* origin.** (0) more anterior to the lateral crest of the quadrate; (1) posterior to the lateral crest of the quadrate.
16. ***M. adductor mandibulae externus medialis,* insertion.** (0) just on the basal aponeurosis; (1) basal aponeurosis and coronoid.
17. ***M. adductor mandibulae externus profundus,* origin includes.** (0) quadrate; (1) parietal and prootic.
18. ***M. adductor mandibulae externus profundus,* insertion.** (0) basal aponeurosis; (1) lateral of the mandible.
19. **Parietal tendon.** (0) absent; (1) present.
20. **Parietal tendon, present.** (0) single; (1) double.

21. ***M. levator pterygoidei,* length.** (0) short; (1) long.
22. ***M. spinalis capitis,* arrangement.** (0) overlapping the entire *m. adductor complex.* (1) overlapping only partially the *m. adductor complex*.
23. **Mandibular double tendon.** (0) absent; (1) present.
24. ***M. adductor mandibulae externus medialis,* fibers arrangement.** (0) more horizontally in the most ventral region. (1) verticalized in the most ventral region.

4. DISCUSSION

Using standard dissection methods, we described the cephalic musculature and glandular structures of four *Amphisbaena* species, since few and rare studies make deeper investigations into these characteristics within this group (Haas et al., 1973; Rieppel, 1979; Westphal et al., 2019). Although some muscular complexes were previously described for *A. microcephala*, our results reveal different variations of topographic fibers arrangement and novel information on structures not represented in the literature, and details about the musculature and glandular anatomy of *A. dubia*, *A. darwinii* and *A. munoai*.

In comparison to snakes and lizards, the overall muscular organization of the jaw adductor in amphisbaenians forms vertical layers in relation to the skull, creating a nearly indistinguishable arrangement (Diaz & Trainor, 2019). Conversely, in other fossorial squamates, while the demarcation between the *m. levator anguli oris* and the *m. adductor mandibulae externus* may be challenging to discern, the musculature tends to exhibit a topographical pattern almost with longitudinal distribution relative to the skull (Abdala et al., 2009). Although defining muscle homologies in Squamata has been puzzling (Zaher, 1994; Tsuihiji, 2007; Johnston, 2014), in the species here observed we expected that the position and architecture of homologous muscles would differ among different skull types,

given their varying burrowing behaviors and overall cranial morphology. Indeed, the only species with shovel-headed shape exhibited unique and distinct modifications compared to others in this investigation.

As expected, there were no significant differences regarding muscle conformation nor in the origin and insertion of muscles observed among the three species with similar skull types, characterized by round-headed (*A. dubia, A. munoai and A. darwinii*). The minimal variation observed may be related to the species size, potentially resulting in smaller areas at the points of muscle origin and insertion (Gans & Montero, 2008), leading to changes in the arrangement and elongation/shortening of the muscle fibers. Morphological differences were primarily noted in the jaw adductor muscles between such species and *A. microcephala*, which in turn, represents the only species with shovel head analyzed (necessitating the sampling of additional species within this cranial shape). Specifically, the *M. adductor mandibulae externus superficialis* complex exhibited similar points of origin and insertion, however, in the shovel-shaped head specie (*A. microcephala*), the oblique skull shape resulted in arrangements and modifications of the adductor muscles, promoting the extension of a muscle layer covering all this muscular complex, a feature not documented in the available literature descriptions for this specie (Haas et al., 1973). An illustration of the cephalic muscles in *A. microcephala* directly highlights the presence of the *M. adductor mandibulae externus superficialis* without any associated muscle covering it (Haas et al., 1973). Also, in the mandibular adductors, the presence of a *basal aponeurosis* (fibrous tissue) was absent in *A. munoai* and *A. darwinii*. Conversely, this structure was observed in *A. microcephala* and to a lesser extent in *A. dubia*. Information regarding this structure can be found in literature for *A. alba*, a round-headed species (Haas et al., 1973), where it facilitates the differentiation between the *M. adductor mandibulae externus medialis* and the *M. adductor mandibulae externus*

profundus when present and serves as insertion point for the *M. adductor mandibulae externus medialis.*

In the shovel-headed *A. microcephala*, large tendons located dorsally, laterally, and ventrally, serves as insertion points for muscles such as *M. spinalis*, *M. semispinalis*, *M. longissimus dorsi*, *M. latissimus dorsi*, *M. cervico-mandibularis*, among others. This configuration contrasts with the species with round-headed shape here analyzed, where such associations are absent. However, similar muscular arrangements are documented in studies of the round-headed specie *A. alba* (Haas, 1973; Tsuihiji et al., 2012). The shape of the skull is associated with the method of soil excavation, with the rounded shape considered less specialized (Gans, 1974) compared to species with shovel-shaped heads. The latter are associated with a specific and complex digging technique (Hohl et al., 2017) suited for compact soils (Navas et al., 2004). Consequently, it is expected that the musculature supporting complex excavation would exhibit grater robustness. It is necessary to expand the number of species studied encompassing different cranial types to understand how these variations influence the muscular structure of the group and how they are associated with their lifestyle.

No ocular muscles were observed in any of the species studied here, despite their presence in certain fossorial snake species (Foureaux et al., 2010; Martins et al., 2019), in contrast to this, the ocular cavity in all species was occupied by a Harderian gland. In Amphisbaenia, ocular muscles were only documented in the species of *Agamodon anguliceps*, Peters 1882 one of the most specialized amphisbaenians of the family Trogonophidae, as reported by Bonin (1965), who also noted some morphological similarities of the eye of *Agamodon* with other genera, being more similar to the genus *Blanus* and *Amphisbaena*, from the families Blanidae and Amphisbaenidae, respectively. It is important to highlight the relevance of future investigations into the morphological

characteristics of the eyes of animals adapted to underground life, as these may provide crucial information about their visual ability (Yovanovich et al., 2019).

Herein, we found four cephalic glands (supralabial, infralabial, rictal and harderian gland) present or absent across different species, while, based on a survey of the published studies to date, six glands have been identified in the cephalic region for different species: supralabial, infralabial, sublingual, harderian, premaxillary, and nasal glands (Gans, 1978; Kearney et al., 2005). However, despite these studies noting the presence of these glands, no specific description has been provided yet. Considering this, the descriptions of cephalic glands observed here provides interesting insights in this anatomical feature for the group, since this knowledge remain limited. Among the most well-known glands are the pre-cloacal glands, which play crucial roles in intraspecific communication and recognition of home ranges and are commonly used as taxonomic character (López et al., 2000; López & Martín, 2009). These studies of glandular anatomy and physiology underscore the importance of chemoreception in the fossorial lifestyle of amphisbaenians. Among the cephalic glands of amphisbaenians, the supralabial and infralabial gland arrangement resembles that of snakes (Typhlopids) but with distinct structural details (Oliveira & Zaher, 2022). *A. darwinii* is described as having a pair of sublingual glands, similar to those of Scincidae (Woerdemann, 1921), which were not found here, and a premaxillary gland was described in *Trogonophis*, resembling that of chameleons, that was also not observed in species of this study. The position of harderian gland appears consistent across all amphisbaenians, filling the eye orbit, with distinct extension limits.

A “rictal gland” was observed only in *A. microcephala*, the shovel-headed species, and as far as we know, this is the first time this gland is described for this species. As in literature, cephalic glands on Amphisbaenia are poorly studied, it is difficult to establish patterns; for instance, it is not certain whether it is a rictal gland or supralabial accessory,

as for fossorial snakes it is described as morphologically distinct from the supralabial gland. In *A. microcephala*, they appear identical; however, there is a clear separation between one set of glands and the other, demarcated by muscular fibers from both the *m. retractor anguli oris* and the *m. levator anguli oris*.

The various patterns and positions of muscles and glands found in Squamata underscore the importance of studying this system of characters (Martins et al., 2018; Oliveira & Zaher, 2022), highlighting the necessity of investigating additional characters beyond the conservative external morphological characteristics, given the vast diversity of species (Martins et al., 2018). Moreover, employing various methodological techniques, including histological and structural information, is advisable where possible. This array of factors is essential for refining taxonomic hypotheses for the group.

5. CONCLUSIONS

Our study offers a broad description of the cephalic myology and glandular information for four species of *Amphisbaena*, showing the variation between then and among different head shapes representatives here available. The species with rounded heads (*A. darwinii*, *A. dubia* and *A. munoai*) showed more similarity among themselves than the species with shovel-shaped heads (*A. microcephala*). Especially the species *A. darwinii* and *A. munoai*, which are phylogenetically close (Graboski et al., 2022) and exhibited a nearly identical set of muscles.

We identified a novel rictal or supralabial accessory gland in the shovel-headed *A. microcephala*, that is absent in the other three species. This highlights the importance of investigating the glandular characteristics within other cranial types of Amphisbaenia (e.g.,

spaded-headed and keel-headed) present in other species, in order to search for patterns associated with different ecological lifestyles and their phylogenetic relationships.

While our study lays the groundwork for understanding these morphological traits, it is necessary to test the formulated characters within a broader phylogenetic framework, since new phylogenies investigating various sources of characters, such as osteology and myology, which are rare in in Amphisbaenia to date (Navas et al., 2004; Rieppel, 1979; Westphal et al., 2019), should be conducted. This integrative approach will be crucial for advancing our understanding of the evolution of this group.

ACKNOWLEDGMENTS

We are grateful to Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPQ) for support and to the curators Carlos Lucena (MCT-PUCRS) and Thiago Silva Soares (INMA), who provided the loan of specimens under their care.

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FIGURE LEGENDS

Table 1. Presence or absence of specific cephalic glands in the species analyzed.

1. **Figure 1.** Illustrated skull from *Amphisbaena dubia*. Adapted from Gans & Montero (2008).
2. **Figure 2.** Illustrated skull from *Amphisbaena microcephala*. Adapted from Gans & Montero (2008).
3. **Figure 3.** Illustrated schematic diagram of the cephalic muscles of *Amphisbaena dubia* (HCLP-R 065) in dorsal view.
4. **Figure 4.** Illustrated schematic diagram of lateral view of muscles in *Amphisbaena dubia* (HCLP-R 065).
5. **Figure 5.** Illustrated schematic diagram of the cephalic muscles of *Amphisbaena dubia* (HCLP-R 059) in lateral view.
6. **Figure 6.** Illustrated schematic lateral view of cephalic glands in *Amphisbaena dubia* (HCLP-R 65).
7. **Figure 7.** Illustrated schematic lateral view of *m. adductor mandibulae externus medialis* in *Amphisbaena dubia* (HCLP-R 65).
8. **Figure 8.** Illustrated schematic lateral view of *m. adductor mandibulae externus profundus* in *Amphisbaena dubia* (HCLP-R 65).
9. **Figure 9.** Illustrated schematic diagram of ventri-lateral view of *m. intermandibularis posterior* of *Amphisbaena dubia* (HCLP-R 061).
10. **Figure 10.** Illustrated schematic diagram of ventral view of jaw muscles in *Amphisbaena dubia* (HCLP-R 061).
11. **Figure 11.** Illustrated schematic diagram of the cephalic muscles of *Amphisbaena munoai* [MCT 4641] in dorsal view.

TABLES AND FIGURES

Table 1. Presence or absence of specific cephalic glands in the species analyzed.

Gland	*A. dubia*	*A. microcephala*	*A. munoai*	*A. darwini*
Harderian	Present	Present	Present	Present
Supralabial	Present	Present	Present	Present
Infralabial	Present	Present	Present	Present
Rictal	Absent	Present	Absent	Absent

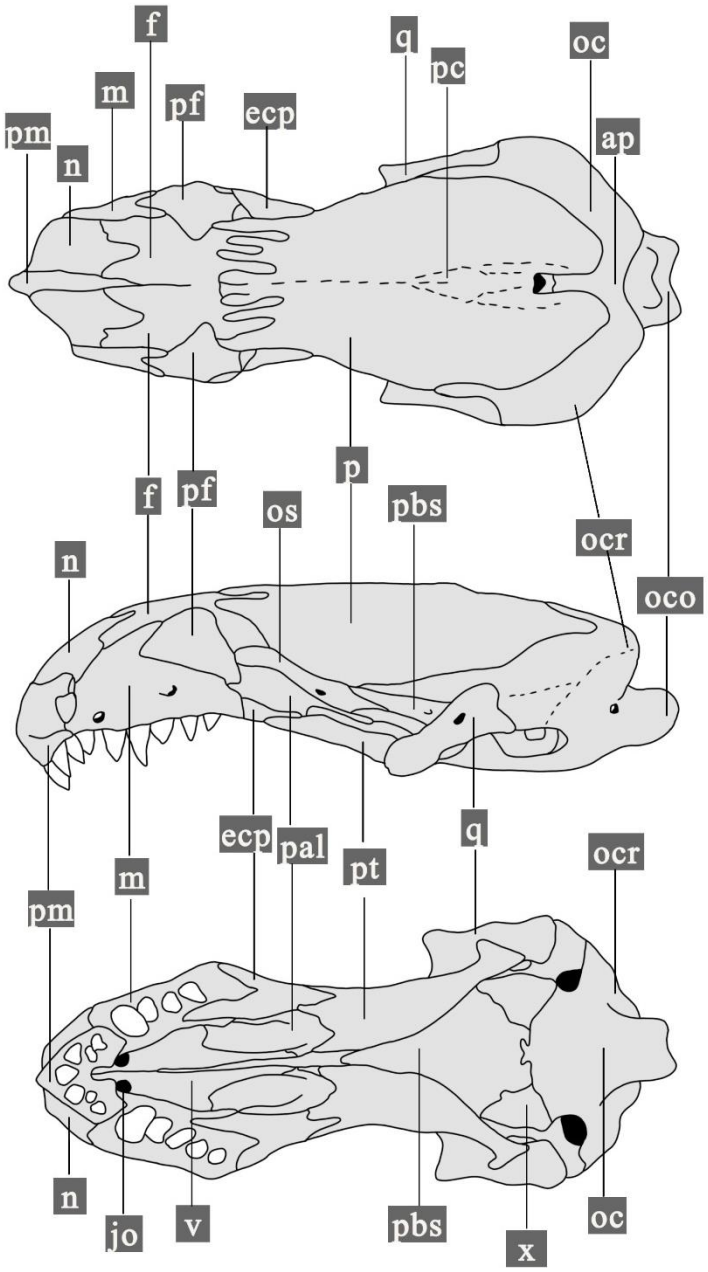

Figure 1. Illustrated skull from *Amphisbaena dubia*. Adapted from Gans & Montero (2008). Abbreviations: ap, ascendent process; ecp, ectopterygoid; f, frontal; jo, foramen for Jacobson's organ; m, maxilla; n, nasal; oc, occipital complex; oco, occipital condyle; ocr, occipital crest; os, orbitosphenoid (or tabulosphenoid); p, parietal; pal, palatine; pbs, parabasisphenoid; pc, parietal crest; pf, prefrontal; pm, premaxilla; pt, pterygoid; q, quadrate; v, vomer; x, element-x.

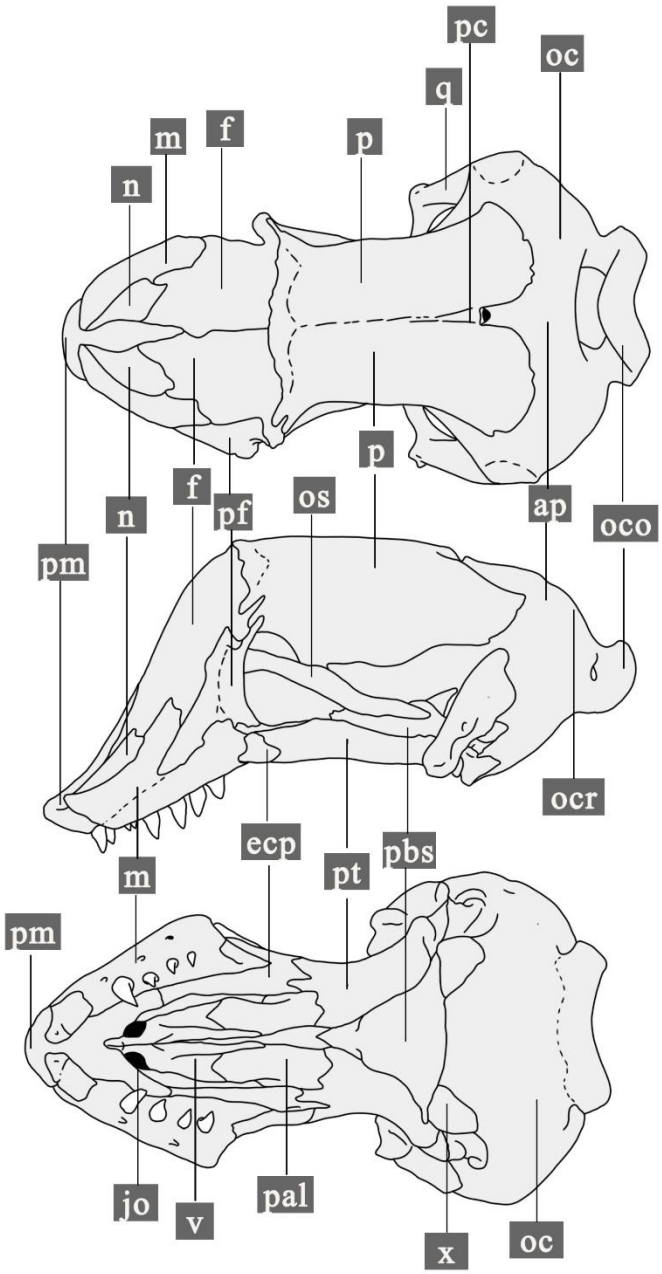

Figure 2. Illustrated skull from *Amphisbaena microcephala.* Adapted from Gans & Montero (2008). Abbreviations: ap, ascendent process; ecp, ectopterygoid; f, frontal; jo, foramen for Jacobson's organ; m, maxilla; n, nasal; oc, occipital complex; oco, occipital condyle; ocr, occipital crest; os, orbitosphenoid (or tabulosphenoid); p, parietal; pal, palatine; pbs, parabasisphenoid; pc, parietal crest; pf, prefrontal; pm, premaxilla; pt, pterygoid; q, quadrate; v, vomer; x, element-x.

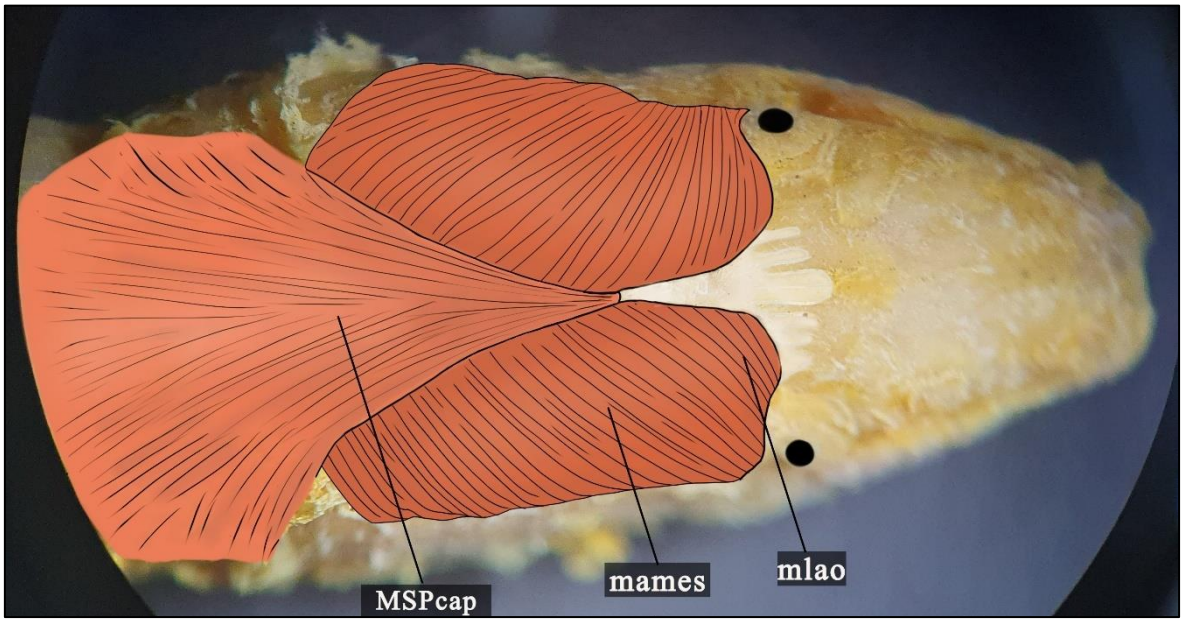

Figure 3. Illustrated schematic diagram of the cephalic muscles of *Amphisbaena dubia* (HCLP-R 065) in dorsal view. The white area corresponds to the "*canthus-rostralis*" region to illustrate the position of the muscle in relation to the parietal bone. Abbreviations: mlao, *m. levator anguli oris*; mames, *m. adductor mandibulae externus superficialis*; MScap, *m. spinalis capitis.*

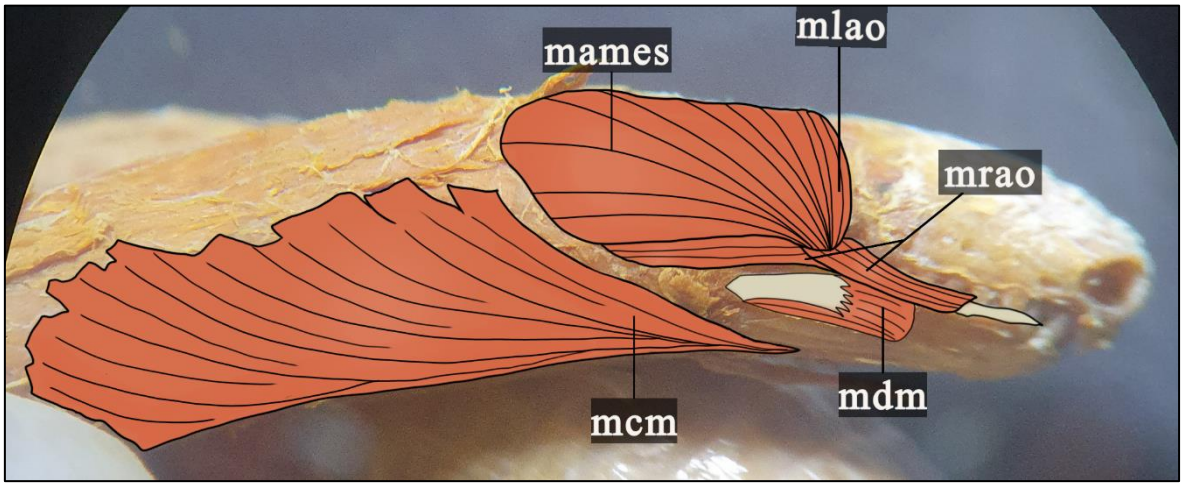

Figure 4. Illustrated schematic diagram of lateral view of muscles in *Amphisbaena dubia* (HCLP-R 065). Abbreviations: mames, *m. adductor mandibulae externus superficialis*; mlao, *m. levator anguli oris*; mrao, *m. retractor anguli oris*; mcm, *m. cervico-mandibularis*; mdm, *m. depressor mandibulae*.

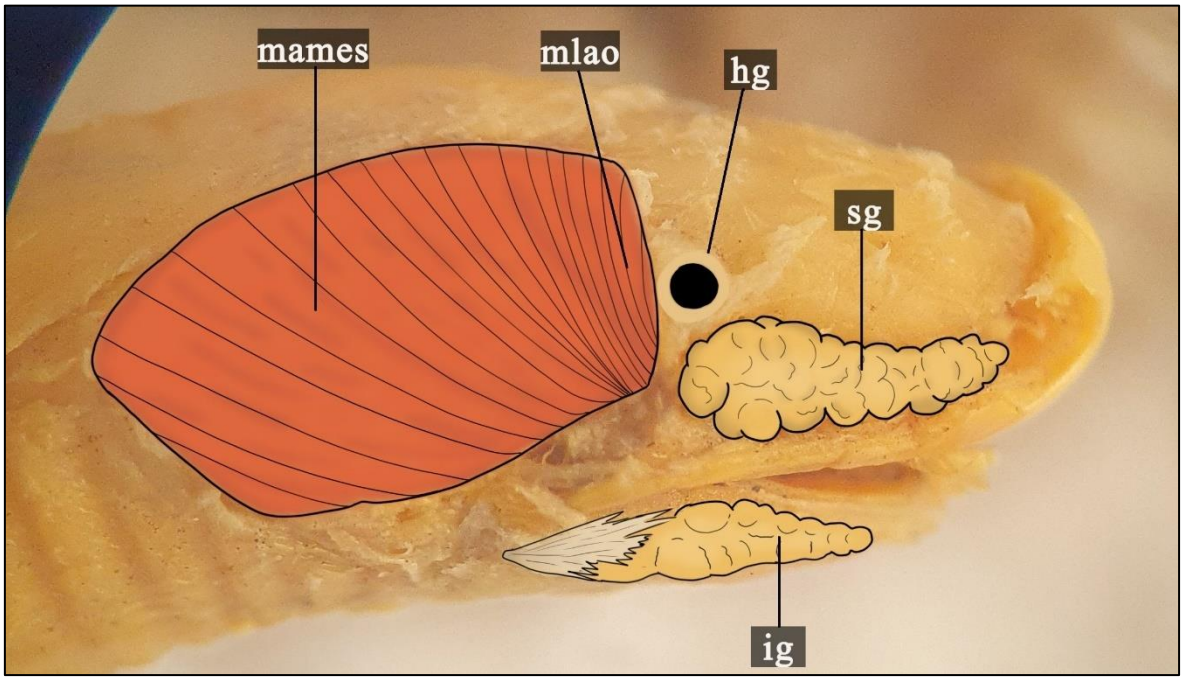

Figure 5. Illustrated schematic diagram of the cephalic muscles of *Amphisbaena dubia* (HCLP-R 059) in lateral view. Complex of the *m. adductor mandibulae externus superficialis*; sg, supralabial glands, visible after removal of the *m. retractor anguli oris* and associated fascia; ig, infralabial glands, visible after removal of some fibers of the *m. depressor mandibulae associated with a layer of fascia.*

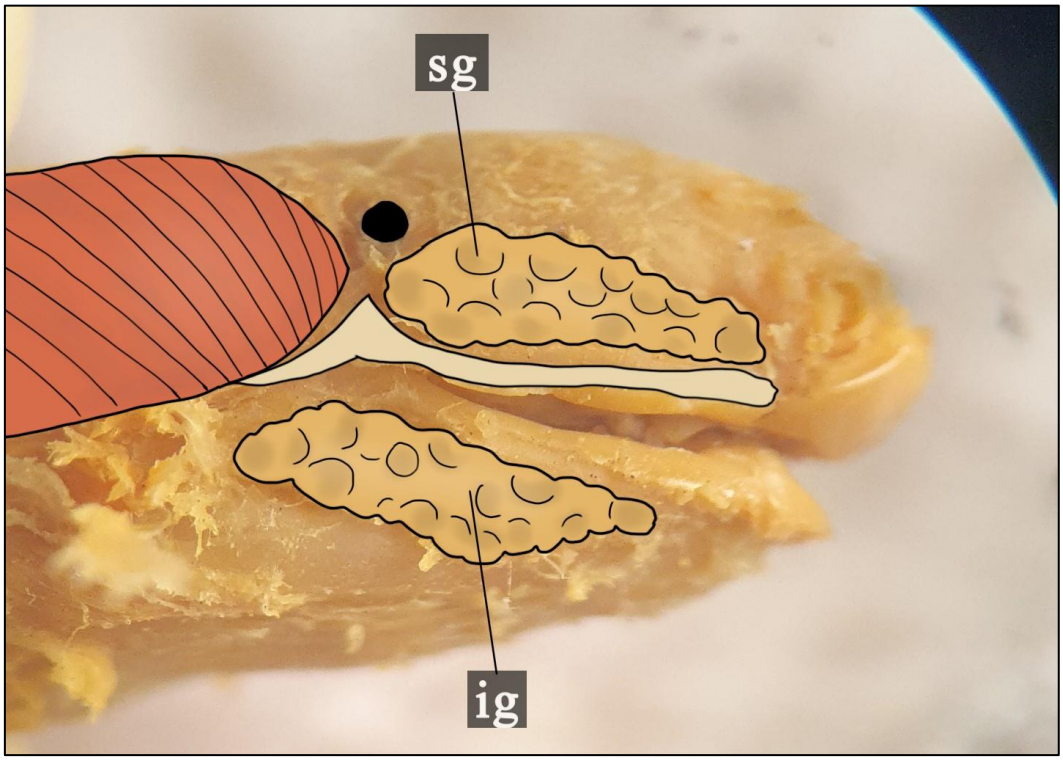

Figure 6. Illustrated schematic lateral view of cephalic glands in *Amphisbaena dubia* (HCLP-R 65). Abbreviations: sg, supralabial gland; ig, infralabial gland.

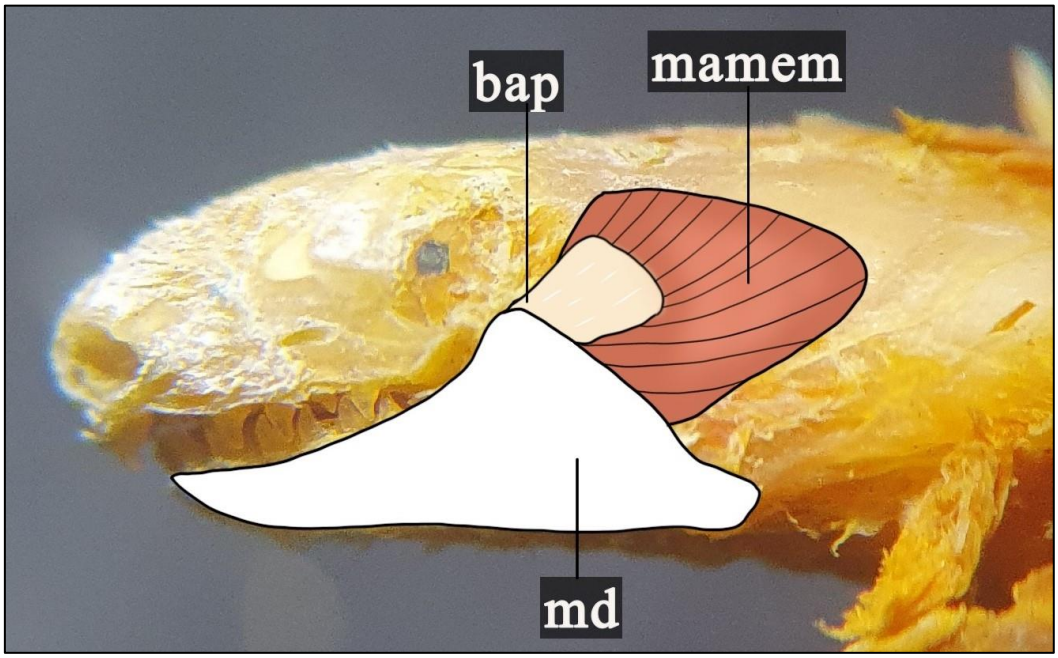

Figure 7. Illustrated schematic lateral view of *m. adductor mandibulae externus medialis* in *Amphisbaena dubia* (HCLP-R 65). Abbreviations: bap, basal aponeurosis; mamem, m. adductor mandibulae externus medialis; md, mandible.

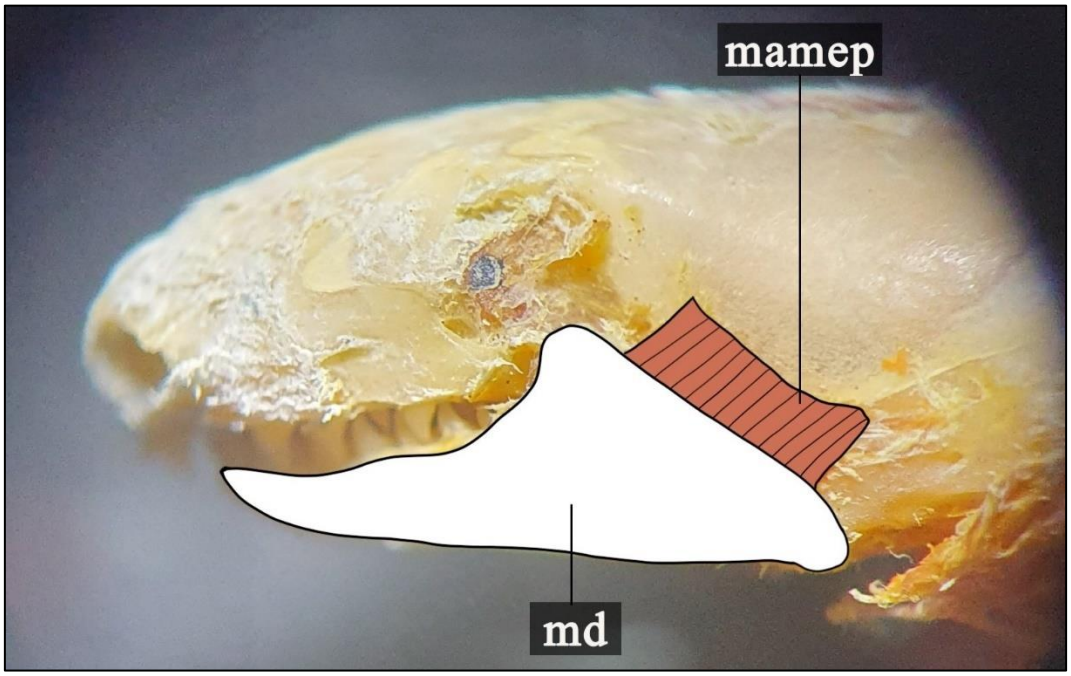

Figure 8. Illustrated schematic lateral view of *m. adductor mandibulae externus profundus* in *Amphisbaena dubia* (HCLP-R 65). Abbreviations: md, mandible; mamep: *m. adductor mandibulae externus profundus*.

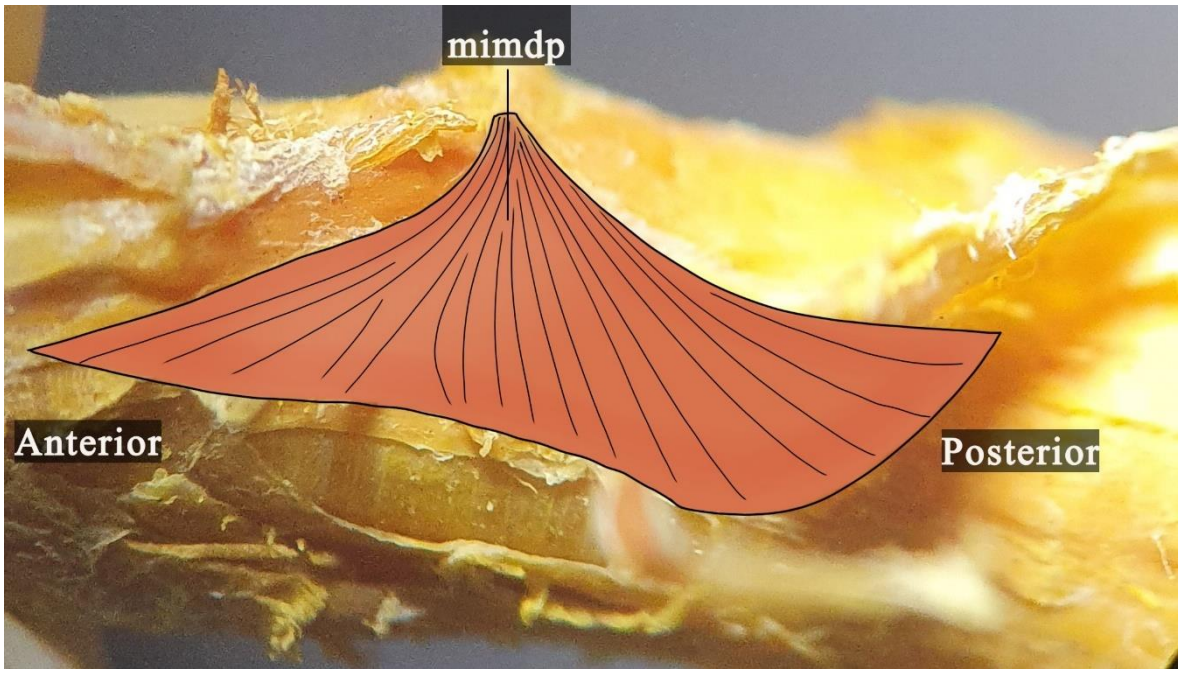

Figure 9. Illustrated schematic diagram of ventri-lateral view of *m. intermandibularis* posterior of *Amphisbaena dubia* (HCLP-R 061).

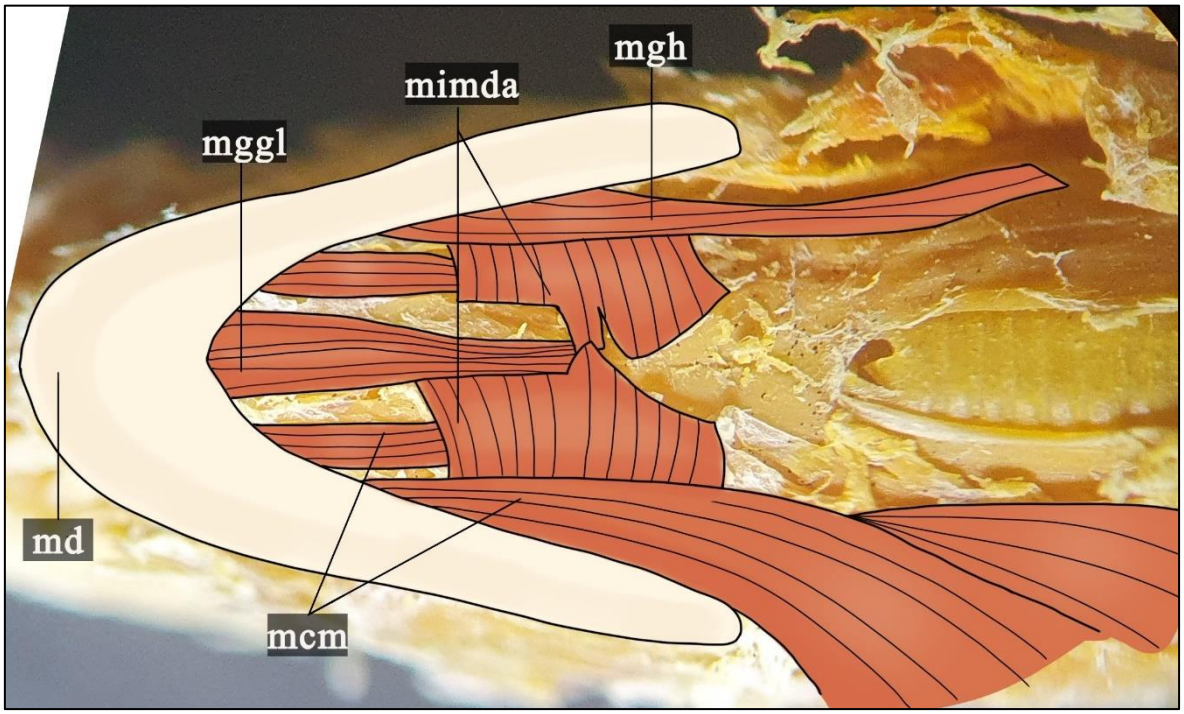

Figure 10. Illustrated schematic diagram of ventral view of jaw muscles in *Amphisbaena dubia* (HCLP-R 061). Abbreviations: md, mandible; mggl, *m. genioglossus*; mimda, *m. intermandibularis anterior*; mgh, *m. geniohyoideus*; mcm, *m. cervico-mandibularis*

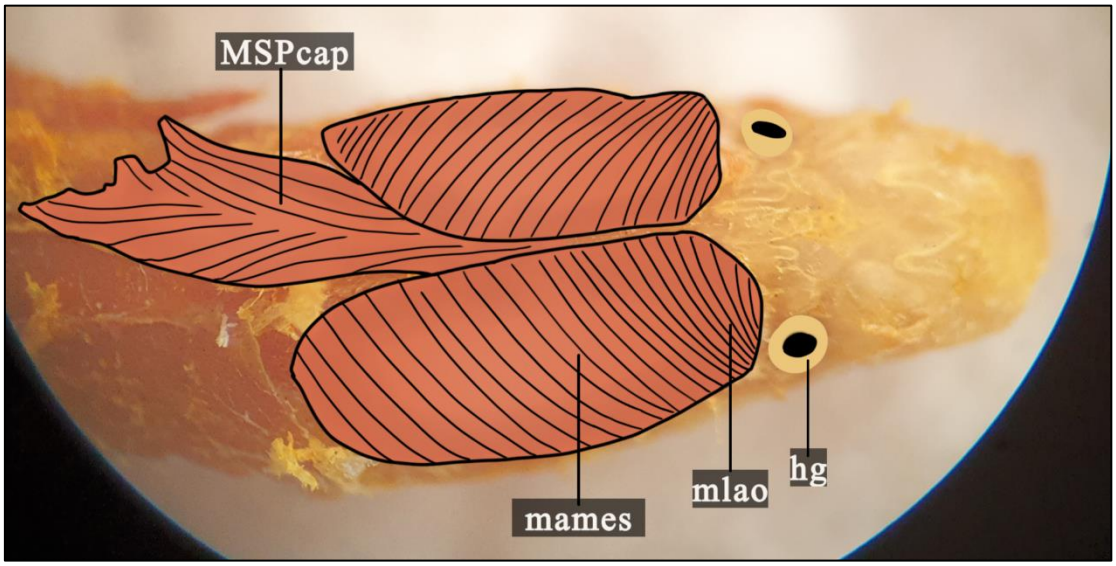

Figure 11. Illustrated schematic diagram of the cephalic muscles of *Amphisbaena munoai* [MCT 4641] in dorsal view. Abbreviations: MSPcap, *m. semispinalis capitis*; mames, *m. adductor mandibulae externus superficialis;* mlao, *m. levator anguli oris;* hg, harderian gland.

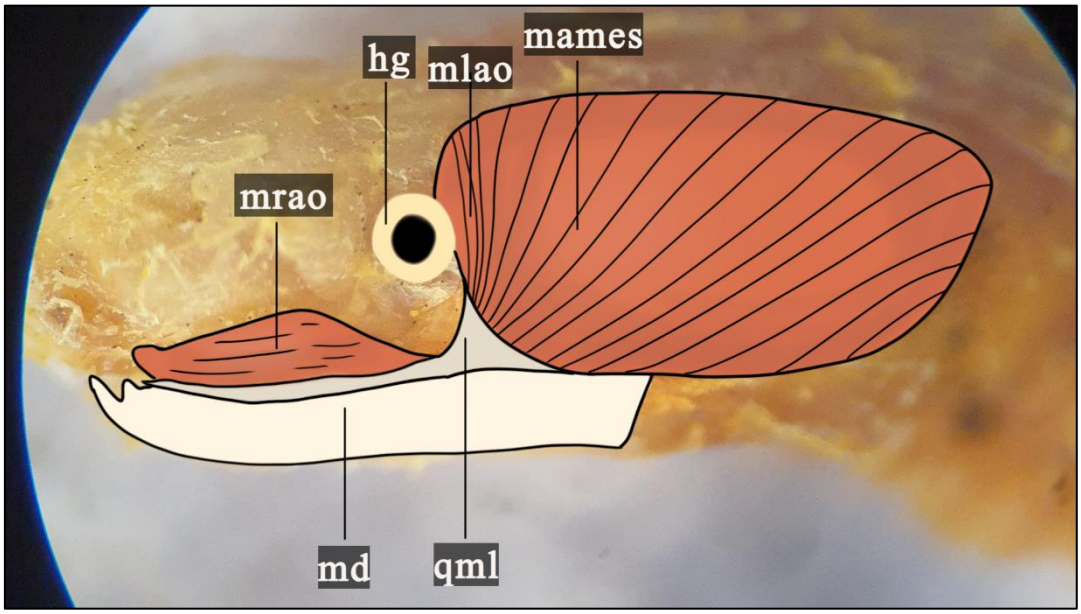

Figure 12. Illustrated schematic diagram of the cephalic muscles of *Amphisbaena munoai* (MCT 4641) in lateral view. Abbreviations: md, mandible; qml, quadrato-maxillary ligament; mrao, *m. retractor anguli oris*; hg, harderian gland; mlao, *m. levator anguli oris*; mames, *m. adductor mandibulae externus superficialis*.

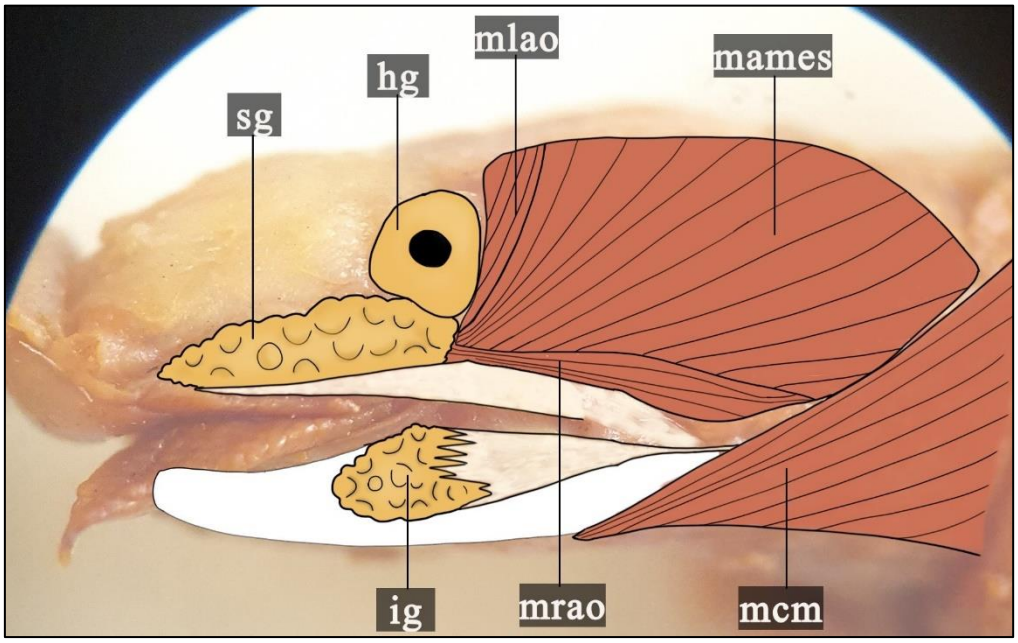

Figure 13. Illustrated schematic diagram of the cephalic muscles of *Amphisbaena darwinii* (MCT 1197) in lateral view. Abbreviations: mrao, *m. retractor anguli oris*; mlao, *m. levator anguli oris*; mames, *m. adductor mandibulae externus superficialis*; mcm, *m. cervico-mandibularis*; hg, harderian gland; sg, supralabial glands; ig, infralabial glands;

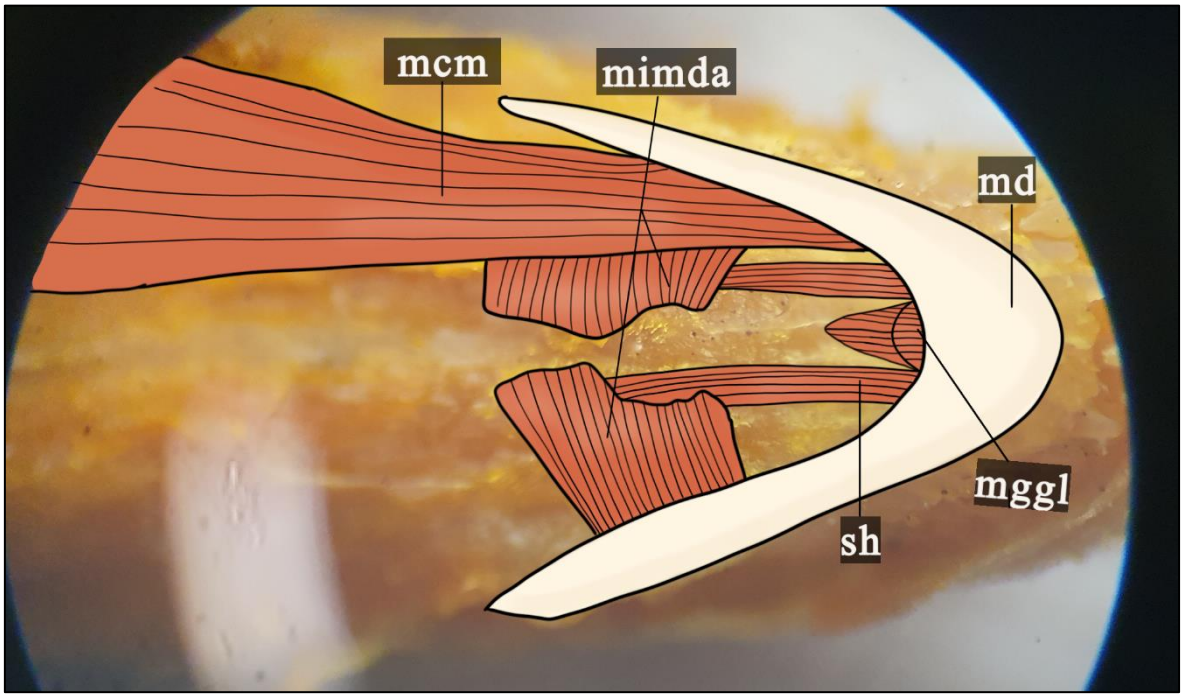

Figure 14. Illustrated schematic diagram of ventral view of muscles in *Amphisbaena munoai* (MCT 1197). Abbreviations: mcm, *m. cervico-mandibularis*; mimda, *m. intermandibularis anterior*; md, mandible; mggl, *m. genioglosss*; msh, *m. sternohyoideus.*

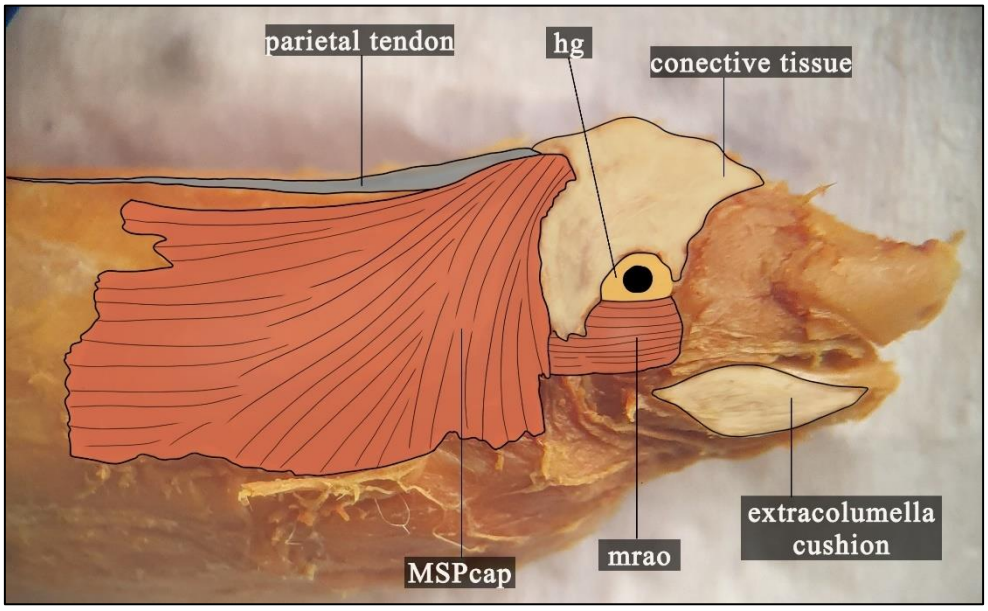

Figure 15. Illustrated superficial musculature of *Amphisbaena microcephala* (MBML 2955), in lateral view after skin removal. Muscles: *m. semispinalis capitis; m. retractor anguli oris.*

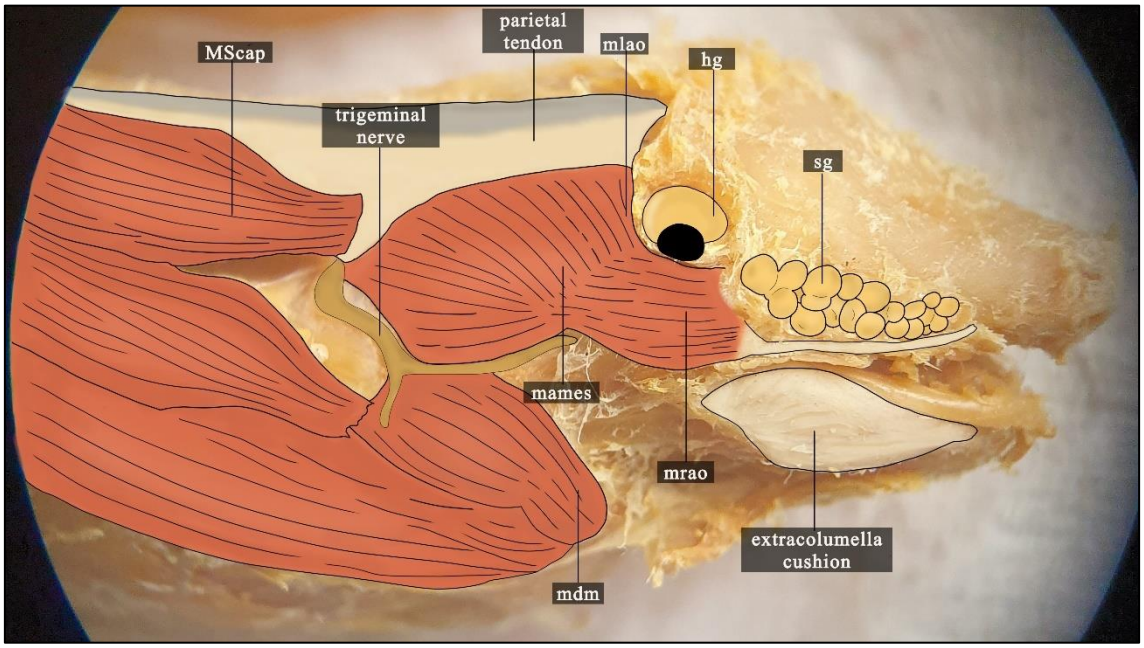

Figure 16. Illustrated musculature of *Amphisbaena microcephala* (MBML 2956) in lateral view after removal of the *mspcap*. Complex of the superficial external mandibular adductor musculature. II. Partially removed *m. retractor anguli oris* muscle for observation of the supralabial glands. III. Right side of the split cephalic tendon. IV. Trigeminal nerve. V. Dermic layer covering the extracolumella. VI. *m. depressor mandibulae*.

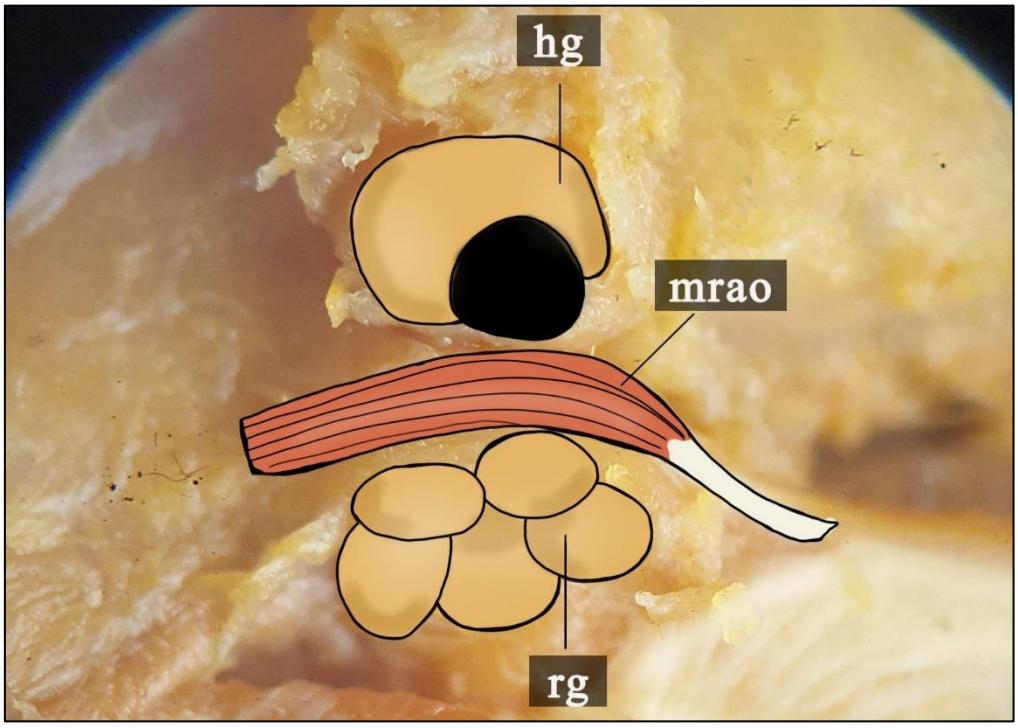

Figure 17. Illustrated schematic diagram of lateral view of cephalic glands in *Amphisbaena microcephala* (MBML 2955). Abbreviations: hg, harderian gland; rg, rictal gland; mrao, *m. retractor anguli oris*.

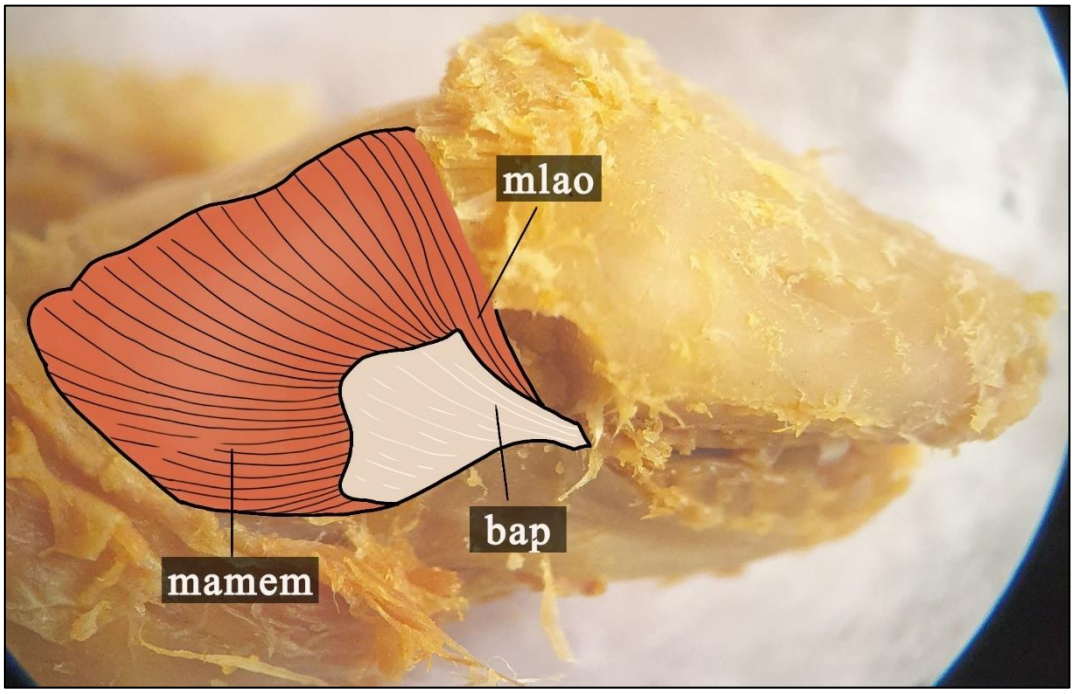

Figure 18. Illustration of lateral view of the *m. adductor mandibulae* in *A. microcephala* (MBML 2956). Abbreviations: mlao, *m. levator anguli oris*; mamem, *m. adductor mandibulae externus medialis*; bap, *basal aponeurosis.*

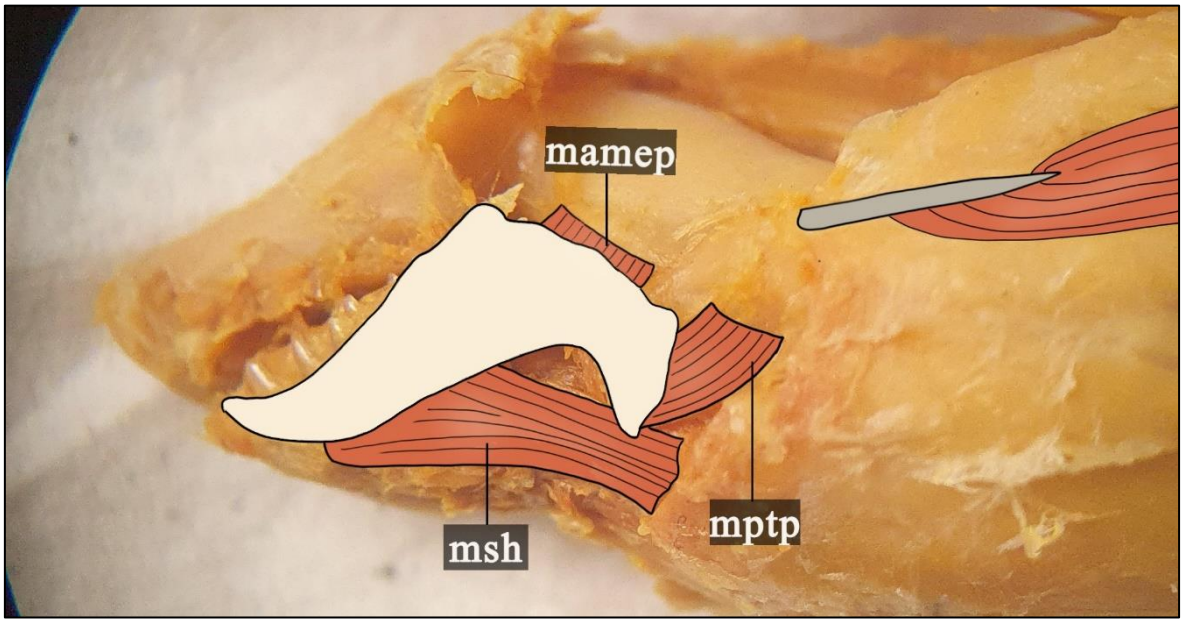

Figure 19. Illustrated schematic diagram of deep layers muscles in *A. microcephala.*

Abbreviations: msh, *m. sternohyoiudeus*; mamep, *m. adductor mandibulae externus profundus*; mptp, *m. pterygoideus profundus*.

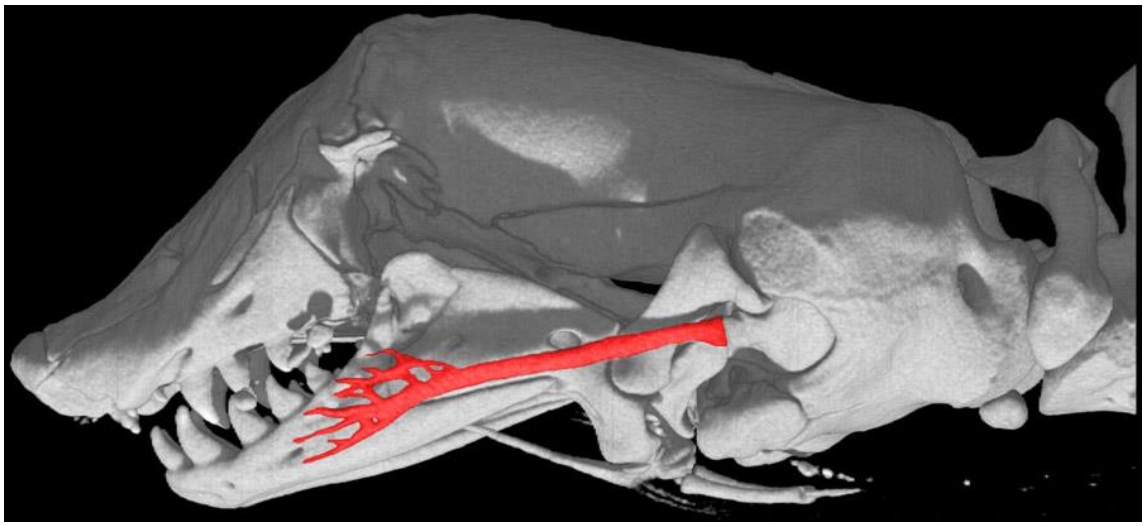

Figure 20. In red, extracolumella structure in *Amphisbaena microcephala.* This structure is present in all amphisbaenians except for the genera Blanus and Bipes. It is a cartilaginous structure that extends from the columella to attach along a dermal layer on the mandible. The extracolumella functions in sound/vibration transmission, enabling the detection of underground vibrations to identify prey. (Image obtained from: www.digimorph.org.)

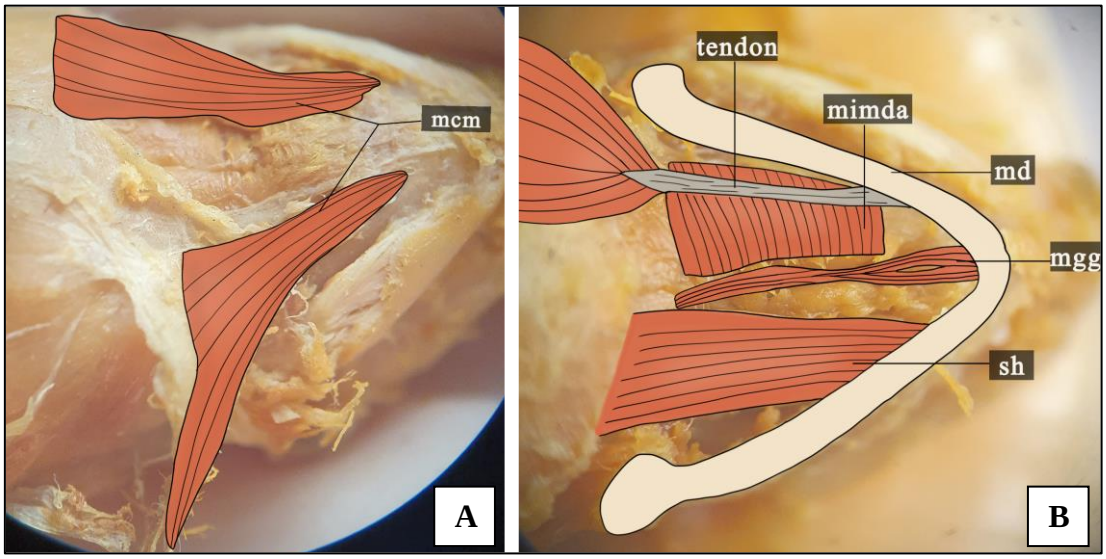

Figure 21. Illustrated schematic diagram of ventral view of muscles in *Amphisbaena microcephala* (MBML 2956). A: position of *m. cervico-mandibularis*. **B:** muscles after removal of *m. cervico-mandibularis* (left side). The muscle sternohyoideus is only visible after removal of the tendon and *m. intermandibularis anterior* (right side). Abbreviations as are in the text.

APPENDIX 1. List of studied specimens

Amphisbaena dubia: HCLP-R 059; HCLP-R 061; HCLP-R 065. ***Amphisbaena darwinii:*** MCT 4641. ***Amphisbaena munoai:*** MCT 1197; MCT 1198. ***Amphisbaena microcephala:*** MBML 2955; MBML 2956

APPENDIX 2. List of abbreviations

Institutional abbreviations

HCLP: Herpetologia do Câmpus do Litoral Paulista

INMA: Instituto Nacional da Mata Atlântica

MCT-PUCRS: Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul

MCTI: Ministério da Ciência, Tecnologia e Inovações

UNESP: Universidade Estadual Paulista "Júlio de Mesquita Filho"

Anatomical abbreviations

mame: *m. adductor mandibulae externus*

mames: *m. adductor mandibulae externus superficialis*

mamep: *m. adductor mandibulae externus profundus*

mamem: *m. adductor mandibulae externus medialis*

mlao: *m. levator anguli oris*

mrao: *m. retractor anguli oris*

mimda: *m. intermandibularis anterior*

mimdp: *m. intermandibularis posterior*

hg: harderian gland

sg: supralabial gland

ig: infralabial gland

rg: rictal gland

rp: rictal plate

mdm: *m. depressor mandibulae*

mlpt: *m. levator pterygoidei*

mppt: *m. protector pterygoidei*

mrpt: *m. retractor pterygoidei*

mpsts: *m. pseudotemporalis superficialis*

mpstp: *m. pseudotemporalis profundus*

mptp: *m. pterygoideus profundus*

mcm: *m. cervico-mandibularis*

msc: *m. sphincter colli*

mggl: *m. genioglossus*

mld: *m. longissimus dorsi*

mscap: *m. spinalis capitis*

mspcap: *m. semispinalis capitis*

mrca: *m. rectus capitis anterior*