MIOLOGIA COMPARADA DO GÊNERO *TRACHYCEPHALUS* TSCHUDI, 1838 (ANURA: HYLIDAE: LOPHYOHYLINI) E SUA CONTRIBUIÇÃO NAS RELAÇÕES FILOGENÉTICAS DA TRIBO LOPHYOHYLINI

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“Um calvinista que busca a Deus, nem por um momento pensa em limitar-se à Teologia e à contemplação, abandonando as outras ciências, como sendo de um caráter inferior [...]; mas pelo contrário, considerando *a ciência como sua tarefa*, a fim de conhecer a Deus em *todas as suas obras*, está consciente de ter sido chamado para sondar, com toda a energia de seu intelecto, as coisas *terrenas* bem como as coisas celestiais.” (Abraham Kuyper)

SUMÁRIO

RESUMO

O gênero *Trachycephalus* atualmente contém 18 espécies de pererecas e notabiliza-se por abrigar representantes com e sem co-ossificação dermal craniana e por ser o grupo natural de Lophyohylini de distribuição mais ampla, ocorrendo desde o México, América Central e Sul a leste dos Andes, até o norte da Argentina e leste do Brasil. O grupo possui diversas questões a serem resolvidas com relação à sua sistemática, e seu monofiletismo é baseado apenas em evidências moleculares. Tendo em vista a escassez de estudos relacionados à sua miologia, bem como a importância desta para a inferência filogenética, o presente trabalho teve como objetivo descrever a anatomia muscular e sua variação para o gênero *Trachycephalus* e, subsequentemente, apresentar uma análise cladística para esse gênero, seguindo uma abordagem de evidência total. O conjunto de dados para a inferência filogenética final incluiu sequências de sete genes mitocondriais e quatro nucleares e 127 caracteres fenotípicos para 16 das 18 espécies de *Trachycephalus*. Os resultados corroboraram a monofilia de *Trachycephalus*, sendo seu saco vocal conspícuo uma sinapomorfia, com uma reversão em *T. hadroceps*. A partir da hipótese de relacionamento obtida, o nome *Trachycephalus spilommus* foi revalidado e o atribuído a populações mexicanas de *T. "vermiculatus"*. Além disso, discutimos a evolução de uma série de caracteres. Verificou-se que os sacos vocais laterais estão associados à reprodução em poças, enquanto os sacos vocais reduzidos e menos conspícuos estão associados à reprodução nos fitotelmos. Também descobrimos que a fragmose é a condição plesiomórfica e associada a espécies com cabeça hiperossificada que usam bromélias como refúgios. Uma reversão teria ocorrido nas espécies restantes, juntamente com uma redução na hiperosificação do crânio. Por fim, observamos que os músculos extensores distais do pé atingem seu desenvolvimento máximo entre em *Phyllodytes*, sugerindo uma possível associação entre redução da SVL e aumento no desenvolvimento desses músculos. Contribui-se, assim, para uma compreensão mais detalhada da história evolutiva do gênero *Trachycephalus*, bem como da tribo Lophyohylini como um todo.

Palavras-chave: Anatomia comparada, Análise cladísitca, Miologia, *Trachycephalus*, Lophyohylini.

ABSTRACT

The genus *Trachycephalus* currently contains 18 species of treefrogs and is notable for harbouring representatives with and without cranial dermal co-ossification and for being the most widely distributed natural group of Lophyohylini, occurring from Mexico, Central and South America to the east of the Andes, to the north of Argentina and eastern Brazil. The group has several issues to be resolved regarding its systematics, and its monophyly is based solely on molecular evidence. In view of the scarcity of studies related to its myology, as well as its importance for phylogenetic inference, the present study aimed to describe the muscular anatomy and its variation for the genus *Trachycephalus* and, subsequently, to present a cladistic analysis for that genus following a total evidence approach. The final dataset for phylogenetic inference included sequences of seven mitochondrial and four nuclear genes and 127 phenotypic characters for 16 of the 18 species of *Trachycephalus*. The results corroborated the monophyly of *Trachycephalus*, with its conspicuous vocal sacs being a synapomorphy, occurring a reversal in *T. hadroceps*. From the relationship hypothesis obtained, the name *Trachycephalus spilommus* was resurrected and attributed to Mexican populations of *T. "vermiculatus"*. Furthermore, we discussed the evolution of a series of characters. Paired lateral vocal sacs were found to be associated with reproduction in ponds, while reduced and less conspicuous vocal sacs are associated with reproduction in phytotelms. We also found that phragmosis is the plesiomorphic condition and associated with species with a hyperossified head that use bromeliads as refuges. A reversal would have occurred in the remaining species, along with a reduction in skull hyperosification. Finally, we observed that the distal extensor muscles of the foot reach their maximum development among lophyohylines in *Phyllodytes*, suggesting a possible association between reduction in SVL and increase in the development of these muscles. Thus, this study contributes to a more detailed understanding of the evolutionary history of the genus *Trachycephalus*, as well as of the tribe Lophyohylini as a whole.

Keywords: Comparative anatomy, Cladistic analysis, Myology, *Trachycephalus*, Lophyohylini.

INTRODUÇÃO

A família Hylidae, a maior da Ordem Anura, é composta por indivíduos conhecidos popularmente como "pererecas". Os hilídeos apresentam uma grande variedade de tamanhos e geralmente possuem discos adesivos em suas falanges terminais que podem auxiliar na escalada, visto que estes animais geralmente habitam em árvores (Vitt & Caldwell, 2013). Atualmente, essa família é constituída por 724 espécies distribuídas pelo continente Americano, região Australo-Papuana, regiões temperadas da Eurásia e extremo Norte da África e no arquipélago japonês (Frost, 2020). Na classificação atual, Hylidae está subdividida em sete tribos (Faivovich *et al.*, 2018; Frost, 2020): Cophomantini, Dendropsophini, Hylini, Lophyohylini, Pseudini, Scinaxini e Sphaenorhynchini. Adicionalmente, o nome *"Hyla" imitator* (Barbour & Dunn, 1921) é o único táxon classificado como *incertae sedis*.

Dentre as tribos de Hylidae, as espécies de Lophyohylini se destacam pela grande diversidade de estados de caracteres relacionados à variação das formas ósseas e grau de ossificação craniana, mesmo em uma curta distância filogenética (ver Faivovich *et al.*, 2005 para revisão). Os espécimes de Lophyohylini possuem distribuição endêmica na região neotropical e estão alocados em 10 gêneros (Frost, 2020; Blotto *et al*, *in press*), sendo estes *Corythomantis* Boulenger, 1896, *Dryaderces* Jungfer, Faivovich, Padial, Castroviejo-Fisher, Lyra, Berneck, Iglesias, Kok, MacCulloch, Rodrigues, Verdade, Torres-Gastello, Chaparro, Valdujo, Reichle, Moravec, Gvoždík, Gagliardi-Urrutia, Ernst, De la Riva, Means, Lima, Señaris, Wheeler, & Haddad, 2013, *Itapotihyla* Faivovich, Haddad, Garcia, Frost, Campbell, & Wheeler, 2005, *Nyctimantis* Boulenger, 1882, *Osteocephalus* Steindachner, 1862, *Osteopilus* Fitzinger, 1843, *Phyllodytes* Wagler, 1830, *Phytotriades* Jowers, Downieb, & Cohen, 2009, *Tepuihyla* Ayarzagüena, Señaris, & Gorzula, 1993 e *Trachycephalus* Tschudi, 1838.

O gênero *Trachycephalus* notabiliza-se por abrigar espécies com e sem co-ossificação dermal craniana e por ser o grupo natural de Lophyohylini de distribuição mais ampla, ocorrendo desde o México, América Central e Sul a leste dos Andes, até o norte da Argentina e leste do Brasil (Frost, 2020). Seus representantes são principalmente arbóreos ou estão associadas à corpos de água, podendo ser encontrados em florestas tropicais e subtropicais, savanas e restingas (IUCN, 2017). Atualmente, somam-se 18 táxons contidos neste gênero, discriminados a seguir (Frost, 2020; Blotto *et al.*, *in press*): *Trachycephalus* "*vermiculatus*" (Cope, 1877), *T. atlas* Bokermann, 1966, *T. coriaceus* (Peters, 1867), *T. cunauaru* Gordo, Toledo, Suárez, Kawashita-Ribeiro, Ávila, Morais, & Nunes, 2013, *T. dibernardoi* Kwet & Solé, 2008, *T. hadroceps* (Duellman & Hoogmoed, 1992), *T. helioi* Nunes, Suárez, Gordo, &

Pombal, 2013, *T. imitatrix* (Miranda-Ribeiro, 1926), *T. jordani* (Stejneger & Test, 1891), *T. lepidus* (Pombal, Haddad, & Cruz, 2003), *T. macrotis* (Eersson, 1945), *T. mambaiensis* Cintra, Silva, Silva, Garcia, & Zaher, 2009, *T. mesophaeus* (Hensel, 1867), *T. nigromaculatus* Tschudi, 1838, *T. quadrangulum* (Boulenger, 1882), *T. resinifictrix* (Goeldi, 1907), *T. typhonius* (Linnaeus, 1758) e *T. venezolanus* (Mertens, 1950).

A primeira proposta de relacionamento filogenético entre os táxons outrora chamados como "pererecas-de-capacete" foi feita por Trueb (1970). À época, *Trachycephalus* incluía os táxons *T. atlas*, *T. jordani*, *T. nigromaculatus*, *T. marmoratus* Duméril & Dibron, 1841 (= *Osteopilus septentrionalis*) e *T. siemersi* (Mertens, 1937) (= *Argenteohyla siemersi*), enquanto *Phrynohyas* Fitzinger, 1843, incluía *P. venulosa* (Laurenti, 1768), *P. ingens* Duellman, 1956, *P. latifasciata* Duellman, 1956, *P. hebes* (Cope, 1862), *P. modesta* Taylor & Smith, 1945 e *P. zonata* (Spix, 1824). A autora concluiu que, dentre o clado dos hilídeos com sacos vocais laterais pareados, *Trachycephalus* e *Osteocephalus* formavam um grupo monofilético que se distinguia de *Phrynohyas* por apresentarem o desenvolvimento de co-ossificação craniana.

Recentemente, ao desenvolver a mais representativa análise filogenética até aquele momento, Faivovich *et al.* (2005) sinonimizaram *Phrynohyas* a *Trachycephalus* para evitar a parafilia deste último, com base em uma filogenia de caracteres moleculares. Assim, *Trachycephalus* passou a ser diagnosticado por 37 transformações de proteínas nucleares e ribossomais, com uma possível sinapomorfia morfológica sendo a presença de sacos vocais pareados que se projetam em ângulo posterior à mandíbula quando inflado (Faivovich *et al.*, 2005). Neste contexto, Smith *et al.* (2007) mapearam os caracteres cranianos considerados por Trueb (1970) em um cladograma também resultante da análise de dados moleculares e propuseram que estes representariam homoplasias com forte sinal filogenético. As hipóteses de relacionamento resultantes das análises de Wiens *et al.* (2010) e Pyron & Wiens (2011) também concordam com o que foi proposto por Faivovich *et al.* (2005), haja visto o uso de muitas das sequências produzidas originalmente neste trabalho. Da mesma forma, *Trachycephalus* foi recuperado como monofilético nos estudos moleculares de Pyron (2014), Ron *et al.* (2016), Duellman *et al*. (2016) e Jetz & Pyron (2018). Além disso, Blotto *et al.* (*in press*) recuperou *Trachycephalus* parafilético em relação a *Aparasphenodon venezolanus*. Para remediar essa situação, eles transferiram *A. venezolanus* para *Trachycephalus* e observaram que o primeiro também apresentava sacos vocais laterais pareados, destacando esse caractere como uma sinapomorfia putativa desse gênero.

Dentre as fontes de dados fenotípicos disponíveis para análises filogenéticas, destaca-se o sistema muscular esquelético devido à sua utilidade (Diogo *et al.*, 2012). Por exemplo, Diogo (2004), ao comparar a contribuição relativa de caracteres miológicos e osteológicos na reconstrução filogenética, demonstrou que, apesar de as estruturas osteológicas apresentarem mais variação, os caracteres miológicos providenciam uma maior proporção de caracteres informativos para a resolução de filogenias.

Em anfíbios, o sistema musculoesquelético pode ser dividido em três unidades convenientes: o crânio, estrutura composta formada pelo esplancnocrânio, condrocrânio e dermatocrânio, e o aparato hiobranquial; o componente axial; e, por fim, o sistema musculoesquelético apendicular (Duellman & Trueb, 1994). O crânio e o aparato hiobranquial formam uma unidade arquitetural complexa e inclui o sistema mecânico que permite a ventilação assim como a captura, manipulação e ingestão do alimento, além da vocalização em anuros (Duellman & Trueb, 1994). O sistema axial provê suporte longitudinal rígido, mas flexível, para a cabeça, as vísceras e o sistema apendicular que, por sua vez, é responsável pela movimentação, tendo os membros anteriores a função primária de elevar e estabilizar o corpo, além de controlar o movimento e a velocidade, enquanto os membros posteriores fornecem a força para a locomoção (Duellman & Trueb, 1994).

Um caractere distinto nos anuros é a presença dos sacos vocais nos machos da maioria das espécies (Tyler, 1971). O saco vocal é uma associação de três estruturas diferentes. Há o revestimento interno do saco, uma mucosa que se origina como uma evaginação do assoalho bucal, formando um divertículo que se comunica com a cavidade bucal através de uma ou duas pequenas aberturas (fendas vocais) laterais à língua. A musculatura submandibular que envolve a mucosa ventralmente e posteriormente com os músculos *mm. intermandibularis* e *interhyoideus*, e dorsalmente com *mm. geniohyoidei* e *m. sternohyoideus*; e o saco gular, uma modificação da pele que é pregueada ou forma bolsas laterais em algumas espécies (Tyler, 1971; Elias-Costa *et al.*, 2017).

Nos anuros, a musculatura em geral é adaptada para o seu modo saltatório de locomoção (Duellman & Trueb, 1994) e o conhecimento mais aprofundado de sua anatomia iniciou-se com os trabalhos de Dugès (1834), Ecker (1889) e Gaupp (1896) que realizaram descrições anatômicas detalhadas de poucas espécies, principalmente do gênero *Rana* Linnaeus, 1758. Além dos estudos de Grobbelaar (1924), sobre a anatomia de *Xenopus laevis*, e Trewavas (1933), acerca da variação do hióideo e da laringe e sua musculatura associada, os artigos

seguintes a estudarem a miologia de anuros se deram a partir da segunda metade do século XX (Ritland, 1955; Dunlap, 1960; Liem, 1970; Tyler, 1971, 1972; Andersen, 1978).

Destacam-se também as importantes contribuições de Davies & Burton (1982) e Burton (1983) acerca da miologia de *Rheobatrachus silus* Liem, 1973 e *Phrynomantis stictogaster* Zweifel, 1973 (= *Callulops stictogaster*), respectivamente. Mais recentemente, Faivovich (2002) utilizou alguns caracteres da musculatura dos pés em um estudo cladístico do gênero *Scinax* (Hylidae), Burton (2004) realizou um estudo aprofundado da musculatura dos pés de Hylidae (que então incluía Hemiphractidae), também realizando uma análise cladística combinando os caracteres obtidos com os utilizados por Duellman (2001). Em sua monografia sobre as relações filogenéticas em Hylidae, Faivovich *et al.* (2005) incluíram os caracteres miológicos de Burton (2004) como o único conjunto de caracteres morfológicos em sua análise. De semelhante modo, o seminal trabalho acerca da "Árvore da Vida dos Anfíbios" (Frost *et al.*, 2006), os únicos dados fenotípicos implementados foram os de Haas (2003), que incluíam grande número de caracteres miológicos de larvas e adultos de anuros. Além disso, diversos estudos também utilizaram caracteres miológicos como parte da base de dados fenotípicos para análises filogenéticas (e.g. Grant *et al.*, 2006; González-Duran *et al.*, 2017). Outros exploraram a variação de um conjunto restrito de caracteres miológicos contexto filogenético amplo, como Blotto *et al.* (2017), com caracteres miológicos dos pés de Odontophrynidae, Targino *et al.* (2019), com caracteres dos sacos vocais de Microhylidae, e Elias-Costa & Faivovich (2019, com caracteres dos sacos vocais de Ranidae.

Com relação a *Trachycephalus*, uma possível sinapomorfia morfológica para este gênero pode ser a presença de sacos vocais pareados (Faivovich *et al.*, 2005). Entretanto, estudos recentes têm notado que este termo nada diz acerca da estrutura interna dos sacos em si, resultado na possibilidade de que uma grande quantidade de variação neste caractere tenha sido negligenciada (Elias-Costa *et al.*, 2017; Elias-Costa & Faivovich, 2019). A monografia de Duellman (1956), acerca de *Phrynohyas*, e o trabalho de Tyler (1971) apresentam as únicas descrições miológicas disponíveis dos sacos vocais de *Trachycephalus*, baseada no exame de espécimes machos de *Phrynohyas spilomma* (= *T. "vermiculatus"*) (Duellman, 1956) e *Trachycephalus jordani* e *T. nigromaculatus* (Tyler, 1971).

Assim sendo, para avaliar a contribuição da miologia comparada de *Trachycephalus* nas relações filogenéticas da tribo Lophyohylini, a presente dissertação foi dividida em dois capítulos. No capítulo 1 nós apresentamos a descrição da miologia de *T. typhonius* e, então, estudamos a variação deste sistema para o gênero *Trachycephalus*¸ oferecendo comparações

com outros grupos de Lophyohylini. No capítulo 2, nós partimos destes dados relativos à anatomia muscular e definimos séries de transformação. Por conseguinte, esses caracteres foram combinados aos de osteologia, biologia reprodutiva, morfologia larval e moleculares (DNA mitocondrial e nuclear), a fim de se realizar a análise cladistíca de *Trachycephalus* sob o paradigma de evidência total. Com isso, tivemos por objetivos testar o monofiletismo de *Trachycephalus* e as relações entre as espécies do gênero. A partir da hipótese filogenética resultante, nós discutimos a evolução de caracteres de interesse como: os sacos vocais e sua relação com o sítio de reprodução; hiperossificação do crânio e comportamento de fragmose; e os músculos extensores distais do pé. Dessa forma, os resultados obtidos nesta dissertação contribuem para uma compreensão mais detalhada da anatomia e da história evolutiva da tribo Lophyohylini.

CAPÍTULO 1 – The musculature of the treefrog genus *Trachycephalus* (Linnaeus, 1758) (Anura, Hylidae, Lophyohylini)[1]

Short running title: The musculature of *Trachycephalus typhonius*

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ABSTRACT

Current knowledge on anuran myology is still mostly restricted to a few seminal works, with most focus being given to hand and foot muscles in the context of phylogenetic analysis. Furthermore, the only putative morphological synapomorphy of the hylid genus *Trachycephalus* is the presence of paired lateral vocal sacs, but variation in its internal structure has been neglected. Given the scarcity of myological investigations in this group, herein we provide a description of the muscular anatomy of *Trachycephalus typhonius* and asses variation within the genus *Trachycephalus*. Muscles of three specimens (two males and one female) of *T. typhonius* were examined, as wells individuals of most nominal species of the genus. Representatives of nine of the 10 genera of Lophyohylini were also examined to provide a broader context of comparisons. We described novel variation in the musculature of the mandibles, with the absence of some adductor muscles in casque-headed species; hands, regarding the close association between flexor muscles of digits III and IV; and feet, with a potential relationship between size reduction and development of distal extensor muscles in some frog species.

Keywords: *Trachycephalus*, myology, Lophyohylini.

1. INTRODUCTION

In anurans, the musculature is generally adapted to its saltatory mode of locomotion (Duellman & Trueb, 1994) and a deeper knowledge of its anatomy began with the works of Dugès (1834), Ecker (1889) and Gaupp (1896), who performed detailed anatomical descriptions of a few species, mainly of the genus *Rana* Linnaeus, 1758. In addition to the studies by Grobbelaar (1924), on the anatomy of *Xenopus laevis*, and Trewavas (1933), on the variation of the hyoid and the larynx and its associated musculature, the articles following the study of anuran myology took place from the second half of the 20th century (Ritland, 1955; Dunlap, 1960; Liem, 1970; Tyler, 1971a, 1972; Andersen, 1978).

The important contributions of Davies & Burton (1982) and Burton (1983) about the myology of *Rheobatrachus silus* Liem, 1973 and *Phrynomantis stictogaster* Zweifel, 1973 (= *Callulops stictogaster*), respectively, also stand out. More recently, Faivovich (2002) used some characters of the foot muscles in a cladistic study of the genus *Scinax* (Hylidae), Burton (2004) carried out an in-depth study of the muscles of the feet of Hylidae (which then included Hemiphractidae), also performing an analysis cladistics combining the characters obtained with those used by Duellman (2001). In their monograph on phylogenetic relationships in Hylidae, Faivovich *et al.* (2005) included Burton's (2004) myological characters as the only set of morphological characters in their analysis. Similarly, in the seminal work on the "The Amphibian Tree of Life" (Frost *et al*., 2006), the only phenotypic data implemented were those of Haas (2003), which included a large number of myological characters from larvae and adult anurans. In addition, several studies have also used myological characters as part of the phenotypic database for phylogenetic analyzes (e.g. Grant *et al.*, 2006; González-Duran *et al.*, 2017). Others explored the variation of a restricted set of myological characters in a broad phylogenetic context, such as Blotto *et al.* (2017), with myological characters of the feet of Odontophrynidae, Targino *et al.* (2019), with characters from the vocal sacs of Microhylidae, and Elias-Costa & Faivovich (2019, with characters from the vocal sacs of Ranidae.

The presence of vocal sacs in males of most species is a distinctive character of anurans (Tyler, 1971a). The vocal sac is an association of three different structures: the inner lining of the sac, a mucosa that originates as an evagination of the buccal floor, forming a diverticulum that communicates with the oral cavity through one or two small openings (vocal apertures) lateral to the tongue; the submandibular musculature that surrounds the mucosa ventrally and posteriorly with the *mm. intermandibularis* and *interhyoideus*, and dorsally with *mm.*

geniohyoidei and *m. sternohyoideus*; and the gular sac, a skin modification that is pleated or forms lateral pockets in some species (Tyler, 1971a; Elias-Costa *et al.*, 2017).

The treefrog genus *Trachycephalus* Tschudi, 1838 is notable for harbouring with and without laterally developed vocal sacs (Faivovich *et al.*, 2005). This group also presents the most widespread distribution among lophyohylines, occurring from the Lowlands of Mexico, Central and South America east of the Andes, south to northern Argentina and eastern Brazil (Frost, 2020). Their representatives are mainly arboreal or associated with water bodies, being found in tropical and subtropical forests, savannas and restingas (IUCN, 2017). Currently, 18 species are allocated to this genus (Frost, 2020; Blotto *et al. in press*) and the only putative morphological synapomorphy is the presence of conspicuous, lateralized vocal sacs. However, recent studies have noted that this term says nothing about the internal structure of the sacs themselves, resulting in the possibility that a large amount of variation in this character has been neglected (Elias-Costa *et al*., 2017; Elias-Costa & Faivovich , 2019). Duellman's (1956) monograph on *Phrynohyas* and Tyler's (1971a) work present the only available myological descriptions of the vocal sacs of *Trachycephalus*, based on examination of male specimens of *Phrynohyas spilomma* (= *T. "vermiculatus"*) (Duellman, 1956) and *Trachycephalus jordani* and *T. nigromaculatus* (Tyler, 1971a).

Herein, we present the description of the muscular anatomy of *Trachycephalus typhonius* and assess variation in this system among the species of the genus. We further provide comparisons with representatives from Lophyohylini, with the goal of contributing to the knowledge on frog anatomy and providing subsidies for further taxonomic and phylogenetic studies.

2. MATERIAL AND METHODS

We chose *Trachycephalus typhonius* as a representative specimen of *Trachycephalus*, providing a thorough description of its overall musculature, because it is the type species of the former *Phrynohyas* (Faivovich *et al.*, 2005). This served as our basis of comparison with the remaining species of the genus. So, we further sampled all nominal species of *Trachycephalus*, except for *T. helioi* and *T. venezolanus*. We note that *T. resinifictrix* and *T. lepidus* were available only for partial dissections. We also included *T. adenodermus* for dissection. Although Lavilla *et al.* (2010) reinforced Duellman (1971) in synonymizing it with *T. typhonius*, Vanzolini (1986) revalidated this taxon based on its reproductive mode, vocalization

and tadpoles, all of which are distinct from *T. typhonius* (see Schiesari *et al*., 2003). The taxon *T. "vermiculatus*" is a placeholder for all names available for former *Trachycephalus typhonius* from Chocoan South America to southern and eastern Mexico (Frost, 2020). Herein, we included under this a specimen collected from eastern Mexico (= *Phrynohyas spilomma* from Duellman, 1956). We also examined representative specimens from 9 of the 10 genera of Lophyohylini, the exception being the monotypic *Phytotriades*, in order to further assess variation with phylogenetic importance. Supplemental information Appendices S1 include a complete list of studied specimens, all of which are preserved in 70% EtOH. Institutional collection abbreviations follow Sabaj (2016).

Terminology followed Duellman & Trueb (1994) for cranial muscles; Gaupp (1896) for axial muscles and fore and hindlimb osteology; Tyler (1971a) for submandibular muscles; Diogo & Ziermann (2014) for fore and hindlimb muscles (except for the *mm. lumbricales breves* and *lumbricales longi* of the hand which follows Burton, 1996, and the *m. adductor pollicis* of the foot which follows Burton, 2004). Anatomical dissections were performed under a ZEISS Discovery V8 stereomicroscope to observe the musculature and topical applications of Bock & Shear's (1972) solution where used when necessary to aid visualization. When necessary, 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.

3. RESULTS

3.1. Description of the musculature of *Trachycephalus typhonius*

Head Musculature

Vocal sac and submandibular musculature: *M. submentalis* (SM; Fig. 1E): this muscle is composed by a mass of continuous fibres, of moderate size and is not separated by a medial raphe. It originates from the lingual surface of the mandible on each side of the mandibular symphysis and inserts with its fibres that pass along the mandible, from one side to the other.

M. intermandibularis (IM; Fig. 1E): the IM has its origin from the lateral lingual surface of the mandible, extending along the whole mandible, from the SM anteriorly, where it is interrupted by the anterior portion of the *m. geniohyoideus lateralis* (GL), until next to the

mandible's articulation, posteriorly. Its fibres also pass along the mandible and meet along a narrow medial raphe. The SM and IM are adjacent at the medial posterior border of the SM.

M. interhyoideus (IH; Fig. 1D-E): this muscle originates from the anterior *cornu* of the hyoid, next to its termination at the skull's *crista parotica*. It accompanies the anterior *cornu* along its ventral path to the region of the post-articular portion of the mandible. The fibres then diverge medially, forming a transverse sheet that unites with the posterior border of the IM. The IH is divided by a medial raphe and presents large supramandibular development with the development of a broad, convoluted, tubular extension. This tubular extension extends posteriorly to the angle of the jaws, covering all the musculature at the dorsal scapular region, and connecting to the *fascia dorsalis* by a wide and thick sheet of connective tissue from the post-mandibular lymphatic septum. In females, there is a strip of connective tissue that extends posteriorly from the IH to the suprascapular region.

The vocal sac apertures are in the form of a gaping hole that is one fifth the length of the mandible in size. The vocal sac extrusions of the collagenous lining of the mouth remain separate, each associated with one aperture and one vocal sac and restricted to the lateral portion of the submandibular region (Fig. 2). These extrusions are also extensively laterally developed. The skin associated with the vocal sacs is differentiated into sacs, where the tegument is thinner and of darker coloration. These sacs cover the expansion of the IH and connects to the surrounding musculature by means of thin connective tissue strips. It is also possible to observe the existence of myointegumental attachment between the IH and the skin, along the posterior margin of this muscle, by means of a post-mandibular septum. This myointegumental attachment is correspondent to the presence of a visible pre-axillary fold. It forms the posterior wall of the submandibular lymph sac and can be categorized as type A *sensu* Tyler (1971).

M. geniohyoideus lateralis (GL; Fig. 1E): The GL originates from the lingual surface of the mandible, just lateral to the SM at the anterior portion of the dentary. They arise on both sides of the *m. geniohyoideus medialis* (GM). These, then, muscles pass posteriorly, contacting the GM from the second third of its length. This muscle is divided into two portions/slips, a lateral and a medial one, that are always in contact until the *m. sternohyoideus* (SH) intrudes between them. This occurs at the level of a line equal to the posterior end of the vocal sac apertures.

M. geniohyoideus medialis (GH; Fig. 1E): This is a paired medial muscle that has its origin dorsal to the SM. Its fibres pass posteriorly along the midline of the throat and are posteriorly covered by the episternum.

Muscles of the mandible: *M. depressor mandibulae* (DM; Fig. 3A): This muscle is divided into three slips. The anterior slip is short and approximately rectangular and originates at the ventral margin of the *annulus tympanicus*. The medial slip is wide and partially covered by the anterior slip and originates at the epimysium of the lateral margin of the *m. levator mandibulae posterior longus* (LPL). The posterior slip is long and approximately triangular, originating from the *fascia dorsalis* at the level of the suprascapula and is in contact with the *m. levator scapulae*. All three slips insert at the articular process of the mandible by a wide, thick and short tendon.

M. levator mandibulae posterior longus (LPL; Fig. 3A): The LPL originates next to the dorsal surface of the frontoparietals, over the dorsal surface of the otoccipitals. Its fibres pass along the otoccipital and converge in a short thick tendon that passes medially to the pterygoid to insert at the angulosplenial. This muscle consists in a mass of fibres and is partially covered by the *m. rhomboideus occipitalis* at its most posterior and internal portion, over the dorsal surface of the frontoparietals.

M. levator mandibulae internus (LMI; Fig. 3B): This muscle consists in fibres that originate from the lateral portion of the frontoparietals, where it is posteriorly in contact with the most anterior and internal portion of the APL. It appears to be greatly reduced, the frontoparietals are exposed, with no muscles covering their dorsal surface.

Axial Musculature

Epaxial musculature: *M. obliquus externus* (OE): This muscle has two points of origin. The first is from the *aponeurosis* covering the long dorsal muscles, and by this from the neural arches of the vertebrae. The second, narrow portion, *portio omo-abominalis* (*sensu* Gaupp, 1896), originates from the posterior border of the scapula by a thin tendon. The *portio omo-abdominalis* becomes broader while running posteroventral to join the anterior border of the larger portion of the OE. The whole muscle is attached by its most anterior fibres to the cartilage of the xiphisternum, the rest passing into an *aponeurosis* which traverses the lower surface of the *m. rectus abdominis* (RA), being connected with the *inscriptiones tendineae*. The anterior margin of the OE covers the posterior margin of the *m. latissimus dorsi* (LAD), which originates from the ventral surface of the *aponeurosis*. This *aponeurosis* also divides laterally in two portions, one of which, the posterior, passes into the OE, while the anterior forms the tendon of origin of the DM.

M. longissimus dorsi (LD): LD originates from the most anterior portion of the urostyle. This muscle passes anterior along the whole dorsum next to the medial line, separated from the muscle of the opposite side by the neural arches of the vertebrae. It is divided by a series of undulated, transversal *inscriptions tendineae* that originate from the vertebral neural arches.

Hypaxial musculature: *M. rectus abdominis* (RA): The RA has its origin by a narrow and robust tendon from the inferior border of the pubis. This muscle is divided by five *inscriptiones tendineae* and, at the first *inscriptio,* divides into two portions. The first, external, portion goes to the *m. pectoralis portio abdominalis* (PA), forming the major lateral division of this muscle that is overlapped by the OE. The second, internal, portion continues as the RA tapering gradually anteriorly.

Forelimbs and Pectoral Girdle Musculature

Muscles of the pectoral girdle and scapula: *M. latissimus dorsi* (LAD): This is a wide, triangular muscle. From the ventral surface of the *fascia dorsalis*, the LAD originates overlapping the *m. dorsalis scapulae* (DS). Its fibres converge alongside with the fibres of the DS, inserting at the *crista deltoideia* of the humerus by a flat tendon.

M. dorsalis scapulae (DS): This muscle appears to have two slips. The anterior slip originates from the most anterior portion of the surface of the suprascapula and overlaps the posterior slip anteriorly. The posterior slip originates from the rest of the surface of the suprascapular and is much wider. The fibres of both slips and the LD converge into the shoulder articulation, to insert at the *crista deltoidei* of the humerus.

M. pectoralis portio abdominalis (PA): This is the largest part of the *m. pectoralis* that can be observed. The PA has broad origin, from the middle of the RA at the region of the second *inscription tendinea* to the xiphisternum. It becomes narrower anteriorly, extending laterally and inserting at the *crista deltoideia* of the humerus with the *m. pectoralis portio sternalis anterior* (PS).

M. pectoralis portio sternalis anterior (PS): This muscle composes the anterior of the *m. pectoralis*, originating along the mesosternum. It becomes narrower as its fibres pass laterally and inserts tendinous at the *crista deltoidei* of the humerus. Medially overlapping the PS are the fibres of the *m. coracoradialis + supracoracoideus* (CO).

M. sternoradialis (SR): Laying ventrally to the PS, this muscle has a broad origin from the omosternum to the base of the epicoracoid cartilage, next to the CO. Its fibres converge

dorsally and outwards to a tendon that inserts at the anterior extremity of the radial portion of the radioulna. This muscle is asymmetric: on the left side of the animal, it overlaps the epicoracoid cartilage, on the right side, is overlapped by said cartilage.

Muscles of the arm: *M. triceps brachii* (TB): This muscle is divided in three branches. The ventral branch originates from the ventrolateral face of the proximal condyle of the humerus; the dorsal branch originates from the dorsolateral base of the proximal condyle of the humerus; and the lateral branch originates by a short, broad tendon from the proximal and posterior border of the suprascapula. The ventral branch gives rise to the elbow *aponeuroris*. The dorsal branch merges with the elbow *aponeurosis*. The lateral branch extends on the lateral surface of the humerus, partially covering the other two branches and inserting on the elbow *aponeurosis*. The TB is broad and covers the entire ventrolateral and dorsolateral surfaces of the humerus.

M. procoracohumeralis (PCH): The PCH muscle is divided in three branches or slips. The *pars episternalis* originates from the base of the omosternum; the *pars clavicularis* originates from the proximal extreme of the epicoracoid cartilage and the *pars scapularis*, the larger slip, originates from the proximal scapulo-clavicular joint. All three branches converge and insert at the distal extreme of the *crista deltoideia* of the humerus. This is a broad and well-developed muscle that covers the entire ventrolateral surface of the humerus.

M. anconeus (AC): The AC lies on the extensor side of the forearm, originating from the lateral epicondyle of the humerus. Its fibres converge and insert at the ulnar surface of the distal condyle of the radioulna.

M. epitrochleoanconeus (EA): This muscle also lies on the extensor side of the forearm, but originates from the distal head of the humerus, at the medial epicondyle. Its fibres converge and insert at the ulnar side of the distal condyle of the radioulna.

M. flexor carpi radialis (FCR): This muscle has a broad origin from the distal half of the humerus, above the medial epicondyle. It becomes narrower and inserts by a wide and flat tendon at the medial side of the radiale. The FCR is robust and well developed, with subtriangular shape. It is located at the radial portion of the forearm.

M. flexor carpi ulnaris (FCU): It arises from the medial epicondyle of the humerus and the common tendon with the *m. flexor digitorum communis longus* (FDCL). This muscle passes along the medial portion of the forearm adjacent to the FDCL internally. Then, it converges with the FCR to the distal radial extremity of the radioulna, presenting a fleshy insertion.

Muscles of the manus (ventral) - Palmar complex: *M. flexor accessorius* (FA; Fig. 4A): This muscle originates from the distal ulnar portion of the radioulna. It then passes medially, transversally on the ventral surface of the *manus*, and connects with the *Tendo superficialis digiti IV* (TSIV) by a thin tendon and by a fleshy portion, under the flexor plate.

M. flexor digitorum communis (FDC; Fig. 4A): The FDC originates from the distal portion of the humerus, below the elbow aponeurosis. It passes along the ventral surface of the forearm, located nearer to the post-axial axis and divides into three slips: lateral, medial and deep. The lateral slip is the most pre-axial and connects with *Tendo superficialis digiti V* (TSV). The medial slip distally gives rise to a ventral portion that relates to the flexor plate and gives rise to two tendinous branches, one that connects to the origin of TSV and one that connects to the origin of TSIII. The larger, dorsal portion of the medial slip connects with the origin of TSIV. The deep slip connects dorsally to the origin of TSV.

Muscles of the manus (ventral) - First layer: *M. flexor indicis superficialis proprius* (FISP; Fig. 4A): This muscle originates from the distal carpal 5-4-3, its fibres pass across the ventral surface of metacarpal II and gives rise to a tendon that inserts on the ultimate phalanx. There appears to be a fine sheet of muscle or connective tissue that arises from the base of the palm and inserts perpendicularly near the base of this muscle.

Tendo superficialis digiti III (TSIII; Fig. 4A): This tendon originates from the flexor plate. See FDC for details. It continues along digit III and inserts at the ultimate phalanx III.

M. caput profundum digiti III (CPIII; Fig. 4A): The CPIII originates from the distal carpal 5-4-3 and its fibres pass across the ventral surface of metacarpal III, inserting at the TSIII which continues to the ultimate phalanx. TSIII passes along and is connected to the pre-axial ventral portion of this muscle.

Tendo superficialis digiti IV (TSIV; Fig. 4A): This tendon originates from the flexor plate. See FDC for details. It passes along the palmar surface of digit IV and inserts at the ultimate phalanx IV.

M. lumbricalis longus digiti IV (LLIV; Fig. 4A): This muscle appears to be divided into two slips. The medial slip originates from the TSIV just distal to the level of the base of metacarpal IV. The lateral slip originates from TSIV at the level of the base of metacarpal IV. Both slips insert each via a thin tendon that accompanies TSIV and inserts at the ultimate phalanx IV. There appears to be a third (Fig. 5), large slip, that is preaxial and more dorsal to

the medial slip, that originates from TSIV at the level of the base of metacarpal IV and inserts along the pre-axial lateral surface of metacarpal IV.

Tendo superficialis digiti V (TSV; Fig. 4A): TSV originates from the flexor plate, receiving contributions from the lateral slip, ventral portion of the middle slip and deep slip of the FDC. It passes along the palmar surface of digit V and inserts on ultimate phalanx V.

M. lumbricalis longus digiti V (LLV; Fig. 4A): this muscle is divided into two slips. Both slips arise from the TSV at the level of the distal third of metacarpal V and insert each via two thin tendons at the ventral surface of the penultimate phalanx.

Muscles of the manus (ventral) - Second layer: *M. lumbricalis brevis indicis* (LBII; Fig. 4B): LBII originates tendinous from distal carpal 5-4-3 and passes along metacarpal II medially to FISP, inserting broadly at the metacarpophalangeal joint.

M. lumbricalis brevis digiti III (LBIII; Fig. 4B): This muscle originates via a flat tendon from distal carpal 5-4-3 and passes along metacarpal III to insert at the base of the penultimate phalanx III. This muscle runs dorsally to the CPIII and is located more medially.

M. lumbricalis brevis digiti V (LBV; Fig. 4B): This muscle has two slips. The medial slip originates by a flat wide, aponeurotic and discontinuous tendon from the distal carpal 5-4-3. The insertion of this slip is by a tendon on the medial side of antepenultimate phalanx V. The lateral slip has its origin from the tegument by a single tendon. The insertion is also by single insertion on the base of basal phalanx V.

Muscles of the manus (ventral) - Third layer: *M. contrahentes indicis* (CII; Fig. 4C): The CI originates by a tendon from the distal carpal 5-4-3. It passes laterally to the FISP and inserts by a strong tendon at the base of penultimate phalanx II.

M. contrahentes digiti V (CV; Fig. 4C): This muscle has its origin by a short and strong tendon from the distal carpal 5-4-3 and inserts along the palmar surface of metacarpal V.

Muscles of the manus (ventral) - Fourth layer: *M. adductor pollicis* (ADP; Fig. 4D): This muscle is short, wide, robust and originates from a short tendon from distal carpal 5-4-3. It inserts fleshy along the medial border of the prepollical elements.

Medial *m. flexor indicis brevis profundus* (mFIBP; Fig. 4D): It originates by a short and wide tendon from distal carpal 5-4-3 and lies adjacent to the ADP, just medial to it. Its insertion is fleshy along the distal lateroventral border of metacarpal II.

M. intermetacarpalis I (IMI; Fig. 4D): This muscle is composed by a flat sheet of fibres that passes from the basal half of the medial surface metacarpal III and inserts along the length of the lateral surface of metacarpal II. Arising from its distal border is a portion that inserts on the metacarpophalangeal joint by a thin tendon.

M. flexor minimi digiti III (FMIII; Fig. 4D): The FMIII originates from the medial portion of the ventral surface of metacarpal III, dorsally to the IMI. It inserts by a short tendon on the base of penultimate phalanx III.

M. flexor brevis profundus digiti III (FBPIII; Fig. 4D): This muscle has its origin from the distal carpal 5-4-3. It inserts as a fan of fibres along metacarpal III and, distally, by a short tendon on distal portion of metacarpal III and by a longer tendon on basal phalanx III.

M. intermetacarpalis II (IMII; Fig. 4D): This muscle originates from the basal half of the lateral surface of metacarpal III and passes as a flat sheet of fibres to insert at the basal half of the medial surface of metacarpal IV.

M. flexor minimi digiti IV (FMIV; Fig. 4D): FMIV has its origin from the medial portion of the ventral surface of metacarpal IV, dorsally to the IMII and the FBPIV. Its insertion is by a thin tendon on the on the base of penultimate phalanx III.

M. flexor brevis profundus digiti IV (FBPIV; Fig. 4D): This muscle originates from the distal carpal 5-4-3 by a tendon. It inserts along metacarpal IV and, distally, by a long thin tendon at the base of basal phalanx IV.

M. interphalangeus digiti IV (IPIV; Fig. 4D): The IPIV muscle has two slips, a lateral and a medial one. Both slips present fleshy origins from the base of antepenultimate phalanx IV and insert by thin tendons on the base of penultimate phalanx IV.

M. intermetacarpalis III (IMIII; Fig. 4D): This muscle is a flat sheet of fibres that originates from the basal half of the lateral surface of metacarpal III and inserts at the basal half of the medial surface of metacarpal IV.

M. flexor minimi digiti V (FMV; Fig. 4D): The FMV muscle has two heads, both with fleshy origins. One originates from metacarpal IV and another originating from metacarpal V. Its fibres then insert by a short tendon at the base of antepenultimate phalanx V.

M. interphalangeus digiti V (IPV; Fig. 4D): This muscle has two slips, a lateral and a medial one. Both slips, originate fleshy from the base of antepenultimate phalanx V and insert by thin tendons at the base of penultimate phalanx V.

Mm. abductor digiti minimi (ABV; Fig. 4D) and *abductor secundus digiti V* (ASV; Fig. 4D): These muscles are fused together. The portion that topologically corresponds to the ABV

originates from distal carpal 5-4-3 by a short and wide tendon. The portion corresponding to the ASV originates fleshy from the ulnare and, more laterally, from the distal portion of the radioulna. The fibres of these fused muscles converge and insert along metacarpal V.

Muscles of the manus (dorsal) - First layer: *M. extensor digitorum communis longus* (EDCL; Fig. 6A): This muscle originates from the distal condyle humerus and extends along the forearm. It divides into three slips branches before inserting on metacarpals III, IV and V, respectively. The first branch (digit III) inserts by a short tendon on the surface of metacarpal III. The middle branch (digit IV) inserts by a short tendon on the surface of metacarpal IV, just above the insertion of EBSIV. The third branch (digit V) inserts by a short tendon on the laterodorsal surface of metacarpal V. These three branches are of the same width.

M. extensor carpi ulnaris (ECU; Fig. 6A): Arises from the lateral epicondyle of the humerus and the common tendon with the EDCL. This muscle passes along the ulnar surfaces of the radioulna and exhibits two points of insertion. A smaller, more lateral, portion inserts by a small and wide tendon on the ulnare while the rest of the muscle inserts at the post-axial distal carpal 5-4-3. It comes to attention that the ECU is much more developed in width than the EDCL, with ellipsoidal shape, abruptly broadening after its origin.

Muscles of the manus (dorsal) - Second layer: *M. abductor pollicis longus* (ABPL; Fig. 6B): This muscle originates fleshy from the distal three-quarters of the ulnar side of the radioulna. Its fibres converge transversally towards digit II, inserting by a short and flat tendon on the fascia of the EBMII and on metacarpal II.

M. extensor indicis brevis superficialis (EIBS; Fig. 6B): The EIBS originates fleshy from the radiale. Its fibres pass across the wrist and along metacarpal II. Its insertion is by a long tendon at the metacarpophalangeal joint of digit II.

M. extensor brevis superficialis digiti III (EBSIII; Fig. 6B): The EBSIII muscle originates by a tendon from the ulnar side of the radioulna, presenting also a smaller slip that originates fleshy from the ulnare. Its fibres converge to insert fleshy upon metacarpal III.

M. extensor brevis superficialis digiti IV (EBSIV; Fig. 6B): In *T. typhonius*, the EBSIV slip with common origin with EBSIII from radioulna is absent, instead the origin is from the ulnare: This muscle presents two slips, one originating from the ulnare and another from distal carpal 5-4-3. The first, medial slip, originates from the ulnare alongside the smaller slip of EBSIII and inserts upon the inner, lateral surface of metacarpal IV. The second, lateral slip,

originates from distal carpal 5-4-3 and presents two insertions: one by a thin tendon that merges with the insertion tendon of the central *m. dorsometacarpalis proximalis digiti IV* (DMPIV); another by a tendon in common with the tendons of insertion of slips from metacarpals IV and V of the lateral DMPIV.

M. extensor brevis superficialis digiti V (EBSV; Fig. 6B): A small slip that has a common origin with the carpal slip of EBSIV and inserting by a thin tendon that inserts with the tendon of central *m. dorsometacarpalis proximalis digiti V* (DMPV).

Muscles of the manus (dorsal) - Third layer: *M. extensor indicis brevis medius* (EIBM; Fig. 6C): This muscle originates from the radiale, below the EBSII, and inserts fleshy at the distal half of metacarpal II.

M. extensor brevis medius digiti III (EBMIII; Fig. 6C): The EBMIII muscle presents two slips. The first, medial slip, originates from element Y, receives fibres from the lateral slip, and inserts at the metacarpophalangeal joint of digit III. The lateral slip originates from the radiale with part of its fibres converging with the first slip and part inserting fleshy on metacarpal III.

M. extensor brevis medius digiti IV (EBMIV; Fig. 6C): this muscle originates from the radiale and inserts by a long tendon on the metacarpophalangeal joint of digit IV.

Muscles of the manus (dorsal) - Fourth layer *M. abductor indicis brevis dorsalis* (AIBD; Fig. 6D): This muscle originates from element Y and inserts along the prepollex.

M. abductor brevis dorsalis digiti V (ABDV; Fig. 6D): The ABDV originates from the distal carpal 5-4-3 and inserts pennately along the lateral side of metacarpal V.

M. dorsometacarpalis indicis proximalis (DMIP; Fig. 6D): This muscle presents two slips. The medial slip receives fibres from a portion originating from the medial surface of metacarpal II and from a portion originating from the dorsal surface of metacarpal II. This medial slip then inserts by a tendon on the medial side of ultimate phalanx II. The lateral slip originates from distal carpal 2 and inserts by a tendon on the lateral side of ultimate phalanx II.

M. dorsometacarpalis proximalis digiti III (DMPIII; Fig. 6D): This muscle has three slips: medial, central and lateral. The medial slip receives fibres originating from distal carpal 2 and metacarpal III that converge to a common tendon of insertion that reaches ultimate phalanx III. The central slip is composed of fibres that across the dorsal surface of metacarpal III and inserts by a short and wide tendon on the metacarpophalangeal joint. The lateral slip

originates from the lateral portion of metacarpal III and inserts by a tendon that reaches to ultimate phalanx III.

M. dorsometacarpalis proximalis digiti IV (DMPIV; Fig. 6D): This muscle has three slips: medial, central and lateral. The medial slip originates from the lateral border of the base of metacarpal III, its fibres pass along the internal (medial) dorsolateral border of metacarpal IV until the level of the metacarpophalangeal joint, where it gives rise to a long tendon of insertion that inserts at ultimate phalanx IV. The central slip originates centrally on the dorsum of the first third of metacarpal IV, passing along this bone and inserting at the metacarpophalangeal joint by a wide and short tendon. The lateral slip is further composed of two portions: the first portion originates from the base of metacarpal IV and inserts with the common tendon of insertion with EBSIV at the distal interphalangeal joint (this tendon bifurcates at the level of the metacarpophalangeal joint); the second portion originates from the base of metacarpal V by a common tendon of origin with part of the medial and central *m. dorsometacarpalis proximalis digiti V* (DMPV), and presents two insertions. One by a tendon that unites with the common tendon of insertion of the lateral DMPIV and another by a long tendon that arises at the level of the metacarpophalangeal joint and inserts at the distal interphalangeal joint.

M. dorsometacarpalis proximalis digiti V (DMPV; Fig. 6D): The DMPV muscle has three slips: medial, central and lateral. The medial slip has two portions. The first originates from the lateral border of metacarpal IV, its fibres pass along the internal (medial) dorsolateral border of metacarpal V until the midlevel of the antepenultimate phalanx, where it gives rise to a long tendon of insertion that inserts at ultimate phalanx V. The second portion originates from the base of metacarpal V by the common tendon with the lateral DMPIV and central DMPV. This portion extend until the metacarpophalangeal joint, where it gives rise to a thin tendon that inserts at the middorsal of the antepenultimate phalanx V. The central slip originates centrally by the common tendon with the lateral DMPIV, passing along the metacarpal V and inserting at the metacarpophalangeal joint by a wide and short tendon. The lateral slip originates from the first third of the lateral border of metacarpal V and extends until the metacarpophalangeal joint, where it gives rise to a thin tendon that inserts at the first third of the antepenultimate phalanx V.

M. dorsometacarpalis distalis digiti III (DMDIII; Fig. 6D): This muscle presents only a lateral slip, originating fleshy from the distal portion of metacarpal III. Its fibres extend until

the level of the distal portion of penultimate phalanx III, where a thin tendon of insertion arises and inserts at the interphalangeal joint.

M. dorsometacarpalis distalis digiti IV (DMDIV; Fig. 6D): This muscle presents only a lateral slip, originating fleshy from the distal portion of metacarpal IV. Its fibres extend until the level of the distal portion of penultimate phalanx IV, where a thin tendon of insertion arises and inserts at the interphalangeal joint.

M. dorsometacarpalis distalis digiti V (DMDV; Fig. 6D): The DMDV muscle presents a lateral slip and a medial slip. The lateral originating fleshy from the distal portion of metacarpal V. Its fibres extend until the level of the distal portion of penultimate phalanx V, where a thin tendon of insertion arises and inserts at the distal interphalangeal joint. The medial originates fleshy from the distal portion of metacarpal V. Its fibres extend until the level of the distal portion of antepenultimate phalanx V, where a thin tendon of insertion arises and inserts at the distal interphalangeal joint.

Hindlimbs and Pelvic Girdle Musculature

Muscles of the pelvic girdle and the thigh: *M. cruralis* (CRU): The CRU has a broad origin by a short tendon from the anterior surface of the hip joint. This muscle is completely divided internally and is situated on the internal face of the thigh. The division in two portions occurs ventrally, along the frontal plane of the muscle. Its fibres converge to a broad *aponeurosis* that covers the knee and inserts on the femoral epicondyles.

M. extensor iliotibialis B (ITB): This muscle has a fleshy origin with, no accessory tendon, from the dorsolateral border of the ilium. It then inserts by a thin and broad tendon that is fused with the *aponeurosis* of the CRU. The ITB is slender and homogenous in width in all its extension, being thinner than the *m. ischioflexorius* (ISF).

M. extensor iliotibialis A (ITA): The ITA originates from the posterior third of the *ala ossei ilei*. This muscle is slender and poorly developed, being folded dorsoventrally near the point of origin. Is fibres insert fleshy on the first third of the CRU.

M. gracilis major (GCMj): The GCMj originates from a thin, narrow, but short tendon from the posteroventral surface of the pelvic rim in the region of the ischium. This muscle inserts by a common tendon with the GCMn into the *tuberositas tibiae* process of the tibiofibular. Its fibres run adjacently to the *m. adductor femoris* (ADDF) The insertion is covered by the insertion of the *m. pubotibialis B* (PTB).

M. gracilis minor (GCMn): This muscle presents an origin by two heads (*sensu* Dunlap, 1960), one by a thin tendon from the ischiatic region of the pelvis and other from the skin, at the level of the proximal half of the thigh. The ischiatic origin is much more developed. Its fibres converge with those from the GCMj forming a common tendon.

M. ischioflexorius (ICF)*:* The ICF presents a fleshy origin from the ventrolateral surface of the ischium. This muscle is not divided and tapers in direction to its insertion point. The insertion is by a short tendon at the medioventral surface of the femur, adjacent to the tibiofibular. Its proximal portion is twice as wide as its distal portion.

M. tenuissimus (TN): This muscle arises from the posteroventral border of the *ala ossei ilei* by a short tendon, ventral and posterior to the origin of the ITB. This muscle is thin, with its first half being covered by the ITB and the ICF. Its fibres insert at the deep *aponeurosis* of the knee joint via a short tendon distally expanded.

M. pubotibialis A (PTA): The origin of the PTA is by a wide, flat tendon from the inguinal region of the antero-lateral border of the ventral pelvic rim in the iliac and slightly in the pubic region. The portion of the insertion that is superficially visible is by a narrow, flat tendon that attaches to the insertion of the GCMj at the knee. Another ventral portion of insertion converges with the *aponeurosis* of the CRU. This muscle is wide and thin, extending distally and medially, being of equal width along all its length until it tapers into a narrow tendon at the insertion.

M. adductor longus (ADDL): The ADDL originates by a tendon from the anterior lower portion of the pubic symphysis. This muscle inserts below the middle of the femur, converging with the *adductor femoris* (ADDF). Also, this muscle is under the (PTA), being covered by it with only the point of origin visible.

M. adductor femoris (ADDF): This muscle arises by two heads divided by the tendon of the PTB. The ventral head from the ventral border of the pubic portion of the pelvic rim, by a short tendon. The dorsal head has a fleshy origin from the ischiatic border of the pelvic rim. The larger ventral branch of this muscle converges with the PTB at about two thirds of the length of the femur, receiving the insertion of the ADDL and inserting along the medioventral border of the femur. The smaller dorsal branch converges with the latter branches and inserts into the distal half of the inner surface of the femur. This muscle passes over the ventral surface of the thigh medially and is covered by the PTB in all the second half of its length. Superficially, it is broad and flat.

Muscles of the leg: *M. flexor digitorum communis longus* (FCLp): Arises from two heads. The dorsal, long, slender head originates from the dorso-distal border of the *aponeurosis* of the knee and covers the insertion of the TN. It then inserts by a thick, flat tendon that spreads through the plantar surface of the *pes* to form the *aponeurosis plantaris*, from which several muscles of the tarsus and *pes* originate. This muscle is reasonably thin, presenting, dorsally, a width comparable to that of the *m. peroneus* (PRS).

M. peroneus (PRS): The *m. peroneus* originates from a short, thick tendon from the extensor surface of the knee joint. It inserts with a double tendinous insertion. One involving a well-defined tendon at the base of the malleolus of the tibiofibula. The second tendon inserts broadly at the lateral surface of the malleolus and in the lateral border of the fibulare.

M. extensor cruris tibialis (ECT): The ECT originates by a long and slender tendon from the ventral surface of the medial condyle of the femur. This is a thin, slender muscle that lies at the latero-ventral margin of the leg, ventral to the *m. tibialis anticus longus* (TAL). The insertion is fleshy along the proximal three-quarters of the latero-ventral surface of the tibiofibular.

M. tibialis anticus longus (TAL): This muscle has its origin from the latero-ventral surface of the medial condyle of the femur by a long and slender tendon. This is a long muscle that tapers along the lateral surface of the leg, bordered above by the PRN and below by the ECT. It divides into two bellies near the middle of the leg. The insertion occurs by two tendinous heads. One lateral head insert at the dorsolateral surface of the proximal portion of the fibulare. The other, medial head inserts at the medial border of the proximal portion of the tibiale.

M. tibialis anticus brevis (TAB): The TAB has a fleshy origin from the dorsolateral surface of the middle of the tibiofibula. This is a long and slender muscle that is positioned along the anterior surface of the distal half of the leg. The insertion occurs by a short tendon at the proximo-medial surface of the tibiale, just dorsal to the insertion of the TAL.

M. cruroastragalus (CRA): This muscle originates fleshy along all the medial surface of the shank of the tibiofibula. It is mostly covered by the FCLp along its extension. The insertion is by a long and slender tendon formed near the articulatory head of the tibiofibula. This tendon attaches to the tibiale, laterally and proximally to the insertion of the TAL, after passing around the malleolus and the dorso-medial surface of the tibiale.

Muscles of the pes (ventral) - First layer: *Tendines superficiales* (TS; Fig. 7A): From a narrow, slightly raised strip of tendon in the *aponeurosis plantaris*, near the base of digit III, arises a set of three slender tendons. These pass distally along digits III, IV and V and insert on

the dorsal surface of the skin of the subarticular tubercles at the metatarsophalangeal joints. The TS are strong tendons that pass along the plantar side of each digit and insert on the ultimate phalanges. The TSI and TSII arise from the distal margin of the *aponeurosis plantaris*, whereas those TSIII, TSIV and TSV arise from the trifurcation of the distal tendon of the *m. flexor brevis superficialis*. Distally, as each tendon passes over the ventral surface of the ultimate phalanx it divides into a 'Y' shape and inserts on the lateral and medial sides of the plantar surface of the ultimate phalanx.

M. lumbricalis longus digiti III (LLIII; Fig. 7A): The LLIII has its origin from the distal margin of the *aponeurosis plantaris* by a narrow tendon and from a supplementary tendon from TSIII. This muscle passes along metatarsal III and inserts by a strong tendon at the first interphalangeal joint.

Mm. lumbricalis longus (LLIV; Fig. 7A) and *lumbricalis longissimus digiti IV* (LGIV; Fig. 7A): The LGIV muscle originates from TSIV. It is slender and fusiform, being surrounded by the LLIV at its origin. It inserts by a single long tendon that adheres closely to TSIV and inserts on the base of the plantar surface of penultimate phalanx IV. LLIV is also a fusiform muscle, originating from the base of TSIV and inserting, by a long tendon, on the plantar surface of the antepenultimate phalanx IV.

M. lumbricalis longus digiti V (LLV; Fig. 7A): This muscle originates from TSV, passes along the ventral surface of metatarsal V and inserts by a long tendon that adheres directly to TSV. It is medial in relation to the *m. lumbricalis brevis digiti V* (LBV).

Muscles of the pes (ventral) - Second layer: *M. lumbricalis brevis hallucis* (LBI; Fig. 7A): LBI originates broadly and penniform from the distal margin of *aponeurosis plantaris.* This muscle's lateral margin is adjacent to TSI and its fibres converge to a small tendon that inserts at the basal phalanx I.

M. lumbricalis brevis digiti II (LBII; Fig. 7B): This muscle originates broadly and penniform from the *aponeurosis plantaris* medial to the base of TSII. Its fibres insert by a short tendon proximally on basal phalanx II.

M. lumbricalis brevis digiti III (LBIII; Fig. 7B): The LBIII has its origin from a narrow tendon on the distal margin of *aponeurosis plantaris*. Its fibres pass along metatarsal III, medially to TSIII, and insert by a long tendon on the plantar surface of penultimate phalanx III.

Mm. lumbricales breves digiti IV (LBIV; Fig. 7B): The LBIV has two portions: a lateral and a medial muscle. The medial muscle originates from the distal border of *aponeurosis*

plantaris near the origin of LLIII. It inserts by a short tendon on the base of basal phalanx IV and, indirectly by a mass of connective tissue, is connected at the metatarsophalangeal joint to a long tendon that inserts on antepenultimate phalanx IV. The lateral muscle originates from the cartilage over the surface of the distal condyle of the tibiale by a tendon. It inserts on the lateroplantar surface of basal phalanx IV, also possessing an indirect connection to a tendon that inserts on the base of antepenultimate phalanx IV.

Mm. lumbricales breves digiti V (LBV; Fig. 7B): LBV also has two portions: a lateral muscle and a medial muscle that divides into two slips. The medial muscle originates from a common tendon with the lateral LBIV. This muscle divides into 1) a fusiform medial slip that has a long tendon of origin and a short tendon of insertion proximally onto the medioventral surface of the metatarsophalangeal joint. Also, a slender tendon from the base of TSIV attaches to the surface of this slip; and 2) a lateral slip with a broad fibrous base and a long, flat, narrow tendon of insertion onto the lateroventral side of the metatarsophalangeal joint, adjacent to that of the lateral muscle. The lateral muscle arises from the distal condyle of the fibulare via a tendon and inserts by a short tendon proximally on the lateroplantar surface of basal phalanx V.

Muscles of the pes (ventral) - Third layer: *M. contrahentes pedis hallucis* (CHP; Fig. 7C): This muscle originates from distal tarsal 2-3 by a short tendon. It is a fusiform muscle that passes along metatarsal I, laterally to TSI, and inserts on the proximal portion of basal phalanx I.

Muscles of the pes (ventral) - Fourth layer: *M. abductor brevis plantaris hallucis* (ABH; Fig. 7D): ABH originates from the medial margin of the *aponeurosis plantaris*. Its fibres converge to a very thin tendon that passes just laterally to the prehallux and inserts one-third of the way along the medial side of metatarsal I.

M. flexor hallucis accessorius (OH; Fig. 7D): This muscle originates by a short and flat tendon from distal tarsal 2–3. Its fibres fan out as they pass medially, to form a flat muscle with a broad, penniform insertion along the whole length of the medioventral surface of metatarsus I and the prehallux.

M. flexor minimi digiti II (FMII; Fig. 7D): The FMII has a penniform origin on the distal three-quarters of the medioventral surface of metatarsal I. Its most proximal fibres lie dorsal to

the *m. intermetatarsalis* I. This muscle inserts via a short tendon onto the proximal end of basal phalanx II.

M. flexor brevis profundus digiti II (FBPII; Fig. 7D): This muscle has a common origin with the *m. flexor brevis profundus digiti III* by a short and flat tendon from the distal condyle of the fibulare, near the base of metatarsal V. This tendon divides into two flat tendons, which give rise to the FBPII and FBPIII. Its fibres fan out to form a broad muscle that inserts along the whole length of metatarsal II on its lateroplantar margin.

M. flexor minimi digiti III (FMIII; Fig. 7D): The FMIII has a penniform origin along the distal three-quarters of the medioventral surface of metatarsal III and inserts via a short tendon on the base of basal phalanx III.

M. flexor brevis profundus digiti III (FBPIII; Fig. 7D): This muscle originates by a flat tendon from the condyle of the fibulare close to the base of metatarsal V, in common with the FBPII. Its fibres spread to a penniform insertion along the whole length of the lateroplantar margin of metatarsal III.

M. flexor minimi digiti IV (FMIV; Fig. 7D): The FMIV muscle originates from the distal three-quarters of the ventral surface of metatarsal IV. The proximal part of it is dorsal to the most distal fibres of *m. intemetatarsalis III*. The insertion is by a short tendon onto the proximal end of basal phalanx IV.

M. flexor brevis profundus digiti IV (FBPIV; Fig. 7D): This muscle arises from a long, flat tendon from the distal end of the fibulare, at the base of digit V. The tendon of origin arises more proximally than the common tendon of origin of FBPII and FBPIII and crosses the plantar side of that common tendon. Its fibres fan out and insert along the length of the lateroventral surface of metatarsal IV. This muscle is ventral to *m. intermetatarsalis IV*.

M. flexor digiti minimi V (FMV; Fig. 7D): This muscle originates penniform on the distal two-fifths of the plantar surface of metatarsal V, inserting by a short tendon at the proximal end of basal phalanx V.

M. flexor brevis profundus digiti V (FBPV; Fig. 7D): The FBPV originates by a broad tendon from the distal condyle of the fibulare and inserts pennately along the entire length of the lateral surface of metatarsal V.

M. intermetatarsalis I (IMTI; Fig. 7D): This muscle is broad and flat, composed by a sheet of fibres that originate from the proximal two-fifths of the medial surface of metatarsal II and insert similarly on the entire length of the lateral surface of metatarsal I.

M. intermetatarsalis II (IMTII; Fig. 7D): The IMTII originates from the proximal two-fifths of the medioventral surface of metatarsal III and inserts on the proximal half of the lateroplantar surface of metatarsal II.

M. intermetatarsalis III (IMTIII; Fig. 7D): This muscle has its origin from the proximal third of the medioplantar surface of metatarsal IV and inserts on the proximal half of the lateroplantar surface of metatarsal III.

M. intermetatarsalis IV (IMTIV; Fig. 7D): This muscle originates from the distal half of metatarsal V and inserts on the distal half of the lateroplantar surface of metatarsal IV.

Muscles of the pes (dorsal) - First layer: *M. extensor digitorum communis longus* (EDCLp; Fig. 8A): This muscle originates by a long, narrow tendon from the distal surface of the tibiofibular. It inserts by three tendons. Two short, robust tendons on the dorsal surfaces of metatarsals II and III. The insertion on metatarsal III is much thicker than that on metatarsal II. A third, long thin tendon arises from the post-axial side of this muscle and passes over the dorsal surface of the foot to insert at the distal portion of the dorsum metatarsal IV, alongside the medial *m. extensor brevis superficialis digiti IV*.

M. abductor digiti minimi (ABDM; Fig. 8A): This muscle has a penniform origin on the entire dorsolateral surface of the fibulare. Its insertion is by a short and robust tendon on the dorsum of metatarsal V, proximal to the origins of the *mm. dorsometatarsales proximales*. This muscle runs along the lateral margin of the EDCLp and is slightly broader than it.

Muscles of the pes (dorsal) - Second layer: *M. extensor brevis superficialis hallucis* (EBSH; Fig. 8B): This muscle originates from a broad origin on the fibulare. Its fibres pass medially and distally as a broad, thick parallel sheet across the dorsal surface of the foot. The EBSH inserts by a tendon onto the middorsum of the metatarsophalangeal joint.

M. extensor brevis superficialis digiti II (EBSII; Fig. 8B): EBSII also originates from broad and penniform origin on the fibulare, distal to EBSH. Its fibres are parallel and pass medially and distally as a broad, thick sheet across the dorsal surface of the foot. This creates, with EBSH, a large sheet of muscle. This muscle presents two insertions: one by a tendon that bifurcates proximally and inserts at the metatarsophalangeal joint; and another by a tendon that passes between digits I and II and inserts ventrally, on *m. lumbricalis brevis digiti II*.

M. extensor brevis superficialis digiti III (EBSIII; Fig. 8B): This muscle originates by a tendon common with the medial *m. extensor brevis superificialis digiti IV* from the fibulare. Its

fibres pass along metatarsal III and, at the midlevel of this bone, it gives rise to two insertions. The medial insertion is by a broad tendon that inserts at the distal end of the metatarsal IV. The lateral insertion is by a long tendon on penultimate phalanx III.

M. extensor brevis superficialis digiti IV (EBSIV; Fig. 8B): This muscle is composed of two portions, a lateral and a medial one. The medial EBSIV originates by the common tendon with EBSIII, its fibres pass medially on the dorsum of metatarsal IV and insert by a wide, flat tendon on the metatarsophalangeal joint. The lateral EBSIV originates by a tendon from the fibulare, just adjacent to the common tendon previously mentioned. Its fibres extend along the dorsolateral side of metatarsal IV and insert by a long tendon at the antepenultimate phalanx IV.

Muscles of the pes (dorsal) - Third layer: The *mm. extensores breves medii* are difficult to separate from the *mm. extensores breves superficiales* of digits I and II (Burton, 2004). An objective criterion that can be followed was suggested by Blotto (pers. comm.): there is an evident blood vessel that passes along the fibulare, ventrally to the EBS muscles and dorsally to the EBM muscles.

M. extensor brevis medius hallucis (EBMH; Fig. 8C): This muscle originates from the distal condyle of the fibulare, just distal to the origin of the EBSH. Its fibres pass medially and distally, extending until the first third of the metatarsal I. The insertion is by a common tendon with EBSH, onto the middorsum of the metatarsophalangeal joint.

M. extensor brevis medius II (EBMII; Fig. 8C): The EBMII originates adjacently to EBMH, from the distal condyle of the fibulare. This muscle divides in two portions: a small, medial portion that inserts on the base of metatarsal II and a larger, lateral portion that inserts by a bifurcated tendon, common with EBSII, on the metatarsophalangeal joint.

Muscles of the pes (dorsal) - Fourth layer: *M. dorsometatarsalis hallucis proximalis* (DMHP; Fig. 8D): The DMHP is composed of a medial and a lateral muscle. The medial muscle has three slips: one originating from the prehallux, a second originating from element Y, and a third slip arising from metatarsal I. Fibres from all three slips converge to a common tendon of insertion at the laterodorsal side of ultimate phalanx I. The lateral muscle originates from the base of metatarsal I and inserts by a long tendon at the middorsum of the ultimate phalanx I.

M. dorsometatarsalis proximalis digiti II (DMPII; Fig. 8D): DMPII is also composed of two muscles, a lateral and a medial. The medial DMPII originates by a tendon from metatarsal II, its fibres pass along metatarsal II and insert by a tendon at the base of the basal phalanx II.

The lateral DMPII has a fleshy origin from the lateral side of the base of metatarsal II and inserts by a long tendon that bifurcates distally, inserting at the ultimate phalanx II.

M. dorsometatarsalis proximalis digiti III (DMPIII; Fig. 8D): The DMPIII, as those mentioned above, is also composed of a medial and a lateral muscle. The medial DMPIII originates by a tendon from the base of metatarsal II, just medial to the origin of DMPII. Its fibres pass along the laterodorsal portion of metatarsal III and presents two insertions: one by a tendon at the metatarsophalangeal joint and another, also by a tendon, on the proximal interphalangeal joint. The lateral DMPIII originates from the laterodorsal side of metatarsal III and inserts by a long tendon on the dorsum of ultimate phalanx III.

M. dorsometatarsalis proximalis digiti IV (DMPIV; Fig. 8D): The DMPIV is composed of a medial and a lateral muscle. The medial DMPIV originates by a long tendon from the distal fused end of the tibiale and fibulare. It presents two insertions: one is by a short tendon on the metatarsophalangeal joint, while the remaining fibres insert by a long tendon on the distal interphalangeal joint. The lateral DMPIV originates from fleshy from the proximal lateral side of metatarsal IV and inserts by a long tendon that runs along the laterodorsal side of digit IV and inserts on the dorsum of ultimate phalanx IV.

M. dorsometatarsalis proximalis digiti V (DMPV; Fig. 8D): The DMPV consists of three muscles: medial, central and lateral. The medial DMPV originates from the mediodorsal surface of metatarsal V and inserts by a long tendon on ultimate phalanx V. The central DMPV originates from the middorsum of metatarsal V and inserts by a short tendon at the base of the metatarsophalangeal joint. The lateral DMPV has its origin from the proximal laterodorsal surface of metatarsal V and inserts by a long tendon that on the dorsum of penultimate phalanx V.

3.2. Muscular anatomical variation in *Trachycephalus* and Lophyohylini

Variation in the muscular anatomy among species of *Trachycephalus* and other genera of Lophyohylini is summarized below

Head Musculature

Variation in the head musculature among the studied specimens was restricted to the subgular region, which represented a large amount of the overall variation we observed, and a few aspects regarding the muscles of the mandible.

***M. intermandibularis*, relation with *m. submentalis*.** In almost all species of *Trachycephalus*, the *m. intermandibularis* is adjacent to or partially overlaping the *m. submentalis* posteriorly. The same is observed for *Dryaderces*, *Osteocephalus*, most *Osteopilus, Itapotihyla, Nyctimantis pomba* and *Tepuihyla.* A *m. intermandibularis* clearly separated from the *m. submentalis* is occurs in *T. cunauaru*, the remaining *Nyctimantis* and *Op. ocellatus*. All remaining species exhibit a *m. intermandibularis* connected to *m. submentalis* posterolaterally.

***M. intermandibularis*, medial portion, morphology.** The medial portion of the *m. intermandibularis* in *Trachycephalus* most commonly presents a medial raphe, as well as *N. galeata, N. rugiceps* and *Op. ocellatus.* The remaining species present a medial *aponeurosis* with variable morphology: it can be homogeneous in its width, as in *O. leprieurii*; widened in its medial portion, as in *T. nigromaculatus*, *T. "vermiculatus"*, *T. adenodermus*, *N. pomba, N. siemersi, Phyllodytes* and most *Osteocephalus*; widened in its distal portion, as in *T. coriaceus, T. cunauaru, N. brunoi* and *Osteopilus*; and widened in its proximal portion, as in *Corythomantis* and *Tepuihyla*.

***M. intermandibularis*, differentiated supplementary apical portion.** Among lophyohylines, the supplementary apical portion is only differentiated in *Phyllodytes*, being absent on the rest of the species.

***M. interhyoideus*, medial portion, morphology.** The great majority of lophyohylines present a *m. interhyoideus* with a medial raphe, albeit a medial *aponeurosis* is present in *T. cunauaru, N. siemersi, Op. dominicensis* and *Op. septentrionalis.*

***M. interhyoideus* and vocal sac mucosae, lateral extension.** Lateral extension was not present when the mucosa or mucosae do not exceed the mandibular joint from a ventral view. This was observed in studied specimens of *Nyctimantis brunoi* and *Phyllodytes*, for example. The *m. interhyoideus* and the mucosa or mucosae can project ventrolaterally in some species. This lateral elongation of a subgular structure represents the first degree of lateralization observed in Lophyohylini and is present in *Corythomantis*. In many species, the *m. interhyoideus* and, with it, the vocal sac mucosae develop further dorsally to occupy a position just posterior to the mandibular joint. Fibres of the this muscle, which in most frogs are transversely oriented (from their origins in the distal portion of the anteromedial processes of the hyoid, near the otic capsules, to their insertion in the midline), radiate posteriorly in these species. Moderate lateral extensions of vocal sac mucosae occur in *Nyctimantis siemersi*, *Itapotihyla*, most *Osteocephalus* and *Osteopilus*, and *Tepuihyla edelcae*. Supramandibular

lobes of the *m. interhyoideus*, or lateral extensions of paired vocal sacs behind the angles of the jaw were also reported for *Dryaderces pearsoni* (Trueb & Duellman, 1971) and are observed in *Oc. verruciger*. In *Trachycephalus* (except for *T. hadroceps* and *T. helioi*), the lateral extensions of the *m. interhyoideus* and the mucosae extend further dorsally exceeding the post-tympanic fold, forming a broad and convoluted dorsal sac. The reference of the post-tympanic fold must not be confused with the extension of the skin modification. In most *Trachycephalus*, the inflatable portions of the skin, evident externally due to their dark pigmentation and high degree of folding, are delimited dorsally by the post-tympanic fold. It must be noted that this does not imply a limit for the internal anatomy.

***M. interhyoideus*, insertion on the *fascia dorsalis*.** In anurans, myo-integumental attachments, formed by sheets of connective tissue, connect the skin to the adjacent muscles generating a series of compartments, which constitutes the lymph sacs (Tyler, 1971b). The submandibular lymph sac is delimited posteriorly by the postmandibular septum, which possibly retracts the skin associatted with the vocal sac in the course of its deflation. This septum normally originates from the posterolateral margin of the skull and is attached to the *m. interhyoideus* in two points: near its origin and, ventrally, at the posterior submandibular margin of this muscle (Tyler, 1971b). When there is no lateral development of *m. interhyoideus*, this lymphatic septum is transverse and extends approximately between the two mandibular joints. In frogs that present lateral extensions of the *m. interhyoideus*, the postmandibular septum is projected posterodorsally. In this case, the septum can either accompany the muscular lobes, connecting them with the surrounding skin; or form a sheath around the muscular lobes that associates with the *fascia dorsalis*, providing secondary anchorage. This association can be even further developed as some fibers of the *m. interhyoideus* insert directly in the *fascia*. We observed this condition in all examined species of *Trachycephalus* with the exception of *T. resinifictrix*. Other species that presented association of the *m. interhyoideus* with the *fascia dorsalis* were *Osteocephalus leprieurii*, *Oc. planiceps*, *Oc. taurinus*, *Oc. oophagus*, *Oc. verruciger* and *Tepuihyla edelcae.* In all remaining species this association was absent or could not be observed.

Vocal sac mucosae, internal disconnection. Adult males with single and paired and vocal sac cavities were observed. Single vocal sac cavities (i.e. fused mucosae) were observed in *Phyllodytes*, and *Oc. Taurinus* (Fig. 9A). In all remaining species examined, mucosae remained disconnected in adult males.

Vocal sac mucosae, degree of medial extension. The occurrence of paired vocal sac cavities by itself does not correlate with externally paired vocal sacs. The morphology of each projection of the buccal floor can greatly affect the overall shape of the inflated vocal sac, as it defines the area available for air to occupy. In species in which mucosae develop greatly towards the midline, the inflatable portion of the vocal sac is extended to the centre of the gular region. In extreme cases, paired mucosae contact mutually in the midline, functionally resembling a single-cavity vocal sac. In this way, disconnection of vocal sac mucosae was observed in species with single subgular, bilobated, paired subgular or paired lateral vocal sacs, all which show a variable degree of medial development of each contralateral element. In *Nyctimantis brunoi*, *Corythomantis*, *Osteocephalus planiceps*, *Oc. verruciger*, *Osteopilus dominicensis*, *Tepuihyla edelcae*, *Trachycephalus jordani*, *T. hadroceps*, *T. helioi* and *T. lepidus* mucosae are disconnected, but greatly developed medially (Fig. 9B-C). As a general reference, paired mucosae occupy the area defined laterally by the insertions of the *m. submentalis*, ventral to the sternum. They can even establish mutual contact, functionally resembling a single-cavity vocal sac. Inflation of the subgular portion of the vocal sac, regardless of the presence of lateral lobes, is important. In *Nyctimantis siemersi*, *Itapotihyla*, *Trachycephalus nigromaculatus*, *T. resinifictrix*, *T. cunauaru*, *T. macrotis* and *T. adenodermus* moderate medial development was observed (Fig. 9D). Contralateral elements occupy the area defined medially by insertion of the *m. submentalis* and, laterally, by the lateral limit of the *m. geniohyoideus lateralis*. In some species, paired mucosa are extremely lateral and do not exceed the *m. geniohyoideus lateralis* from a ventral view (Fig. 93E-F). This was observed in the remaining *Trachycephalus*.

***M. geniohyoideus lateralis*, extension of the division of this muscle in medial and lateral portions.** The *m. geniohyoideus lateralis* can be divided along its whole length in two portions, a medial and lateral one, as in *Itapotihyla langsdorffii*, *Phyllodytes* and *Trachycephalus typhonius, T. mesophaeus, T. atlas, T. nigromaculatus, T. imitatrix, T. cunauaru, T. macrotis, T. quadrangulum T. vermiculatus* and *T. adenodermus.* On the remaining species examined, the division of the *m. geniohyoideus lateralis* in two portions only occurs distally, near the *m. intehyoideus*.

***M. depressor mandibulae*, slip originating from the *fascia dorsalis* at the level of the *m. dorsalis scapulae*.** In *Phyllodytes*, the *m. depressor madibulae* muscle has only two slips, with the absence of a slip originating from the *fascia dorsalis* and *m. dorsalis scapulae.* In the remaining species, this slip has variable extension, since it can: overlap only the first half of the

m. deltoideus scapularis, as in *Dryaderces pearsoni*, *Trachycephalus*, *Tepuihyla* and *Oc. oophagus*; overlap the whole *m. dorsalis scapulae*, but does not extend pass it, as in *Itapotihyla*, *N. pomba*, *N. siemersi*, *Op. dominicensis* and the remaining *Osteocephalus*; or overlap the whole *m. dorsalis scapulae* and extend until the *m. latissimus dorsi*.

M. levator mandibulae posterior longus. In species with extensive hyperossification of the frontoparietals, such as *Trachycephalus jordani*, *T. atlas*, *T. nigromaculatus* and *Nyctimantis*, the region where the *m. levator mandibulae posterior longus* is usually found is completely occupied by bones. Thus, in these species, the *m. levator mandibulae posterior longus* was referred to as encovered by the bones.

M. levator mandibulae internus. A similar condition as stated above, observed in *Trachycephalus jordani*, *T. atlas*, *T. nigromaculatus*, *Corythomantis* and *Nyctimantis*.

Axial Musculature

Axial musculature was found to be considerably conservative in morphology among examined specimens.

***M. rectus abdominis*, point of division in two slips.** The only variation was observed in *C. greeningi* and *Itapotihyla*, where the *m. rectus abdominis* divides in two slips at the level of the second *inscription tendinea*, instead of the first.

Pectoral Girdle Musculature

***M. pectoralis portio abdominalis*, relation with the *m. obliquus externus*.** In most species examined, the *m. pectoralis portio abdominalis* is overlapped by *m. obliquus externus*. The expections are *C. greeningi*, *D. pearsoni*, *N. pomba*, *N. siemersi*, *Op. dominicensis*, *Oc. verruciger and Oc. planiceps*, where *m. pectoralis portio abdominalis* is overlapping *m. obliquus externus*.

M. pectoralis portio axillaris. This muscle is composed by only a few fibres, originating from the posterior portion of the *m. pectoralis p. abdominalis* and inserting medially on the humerus, distally to the insertion of the *m. pectoralis p. abdominalis.* It is present in all species, except for *T. typhonius*, *T. mesophaeus*, *T. atlas*, *T. imitatrix*, *T. coriaceus*, *N. brunoi*, *N. pomba*, *N. siemersi* and *Itapotihyla.*

***M. sternoradialis*, symmetry in relation with the epicoracoid cartilage.** In all species examined, the *m. sternoradialis* is asymmetric, overlapping the epicoracoid cartilage on one

side and, conversely, being overlapped by it on the other. In most species, this muscle is overlapped by the cartilage on the right side of the individual, but it is overlapped by the cartilage on the left side in *T. mesophaeus, T. cunauaru, T. quadrangulum, Phyllodytes, Tepuihyla, Oc. oophagus* and *N. siemersi.*

Forelimb Musculature

Lateral *m. lumbricalis brevis digiti V*. In most *Trachycephalus*, except for *T. jordani, T. nigromaculatus* and *T. macrotis*, the lateral *m. lumbricalis brevis digiti V* originates from the tegument, as well as in *Phyllodytes, Oc. oophagus* and *O. planiceps.* In the remaining species examined, this muscle presents only a slip originating from distal carpal 5-4-3.

***M. extensor digitorum communis longus*, internal branch (FIII), number of insertion points.** The internal branch of the *m. extensor digitorum communis longus*, that inserts onto Digit III, can be single or divided at its tip. Thus, presenting one insertion point, as in most species, or two insertion points, as in *T. nigromaculatus* and *Itapotihyla*.

***M. extensor digitorum communis longus*, medial branch (FIV), point of insertion.** In *Phyllodytes tuberculosus* and *P. melanomystax*, the medial branch of the *m. extensor digitorum communis longus* inserts on the insertion tendon of the *m. extensor brevis medius digiti IV*. In the remaining species, the main insertion of this branch is on the dorsum of metacarpal IV. A secondary insertion might be present, with this branch being divided at its tip, in *T. lepidus, Itapotihyla* and *Op. dominicensis.*

***M. extensor digitorum communis longus*, lateral branch (FV), number of insertion points.** The internal branch of the *m. extensor digitorum communis longus*, that inserts onto Digit V, can also be single or divided at its tip, as described above. The only case where two insertions were observed (divided at its tip) was in *T. lepidus*.

***M. caput profundum III*, number of slips, and *m. lumbricalis brevis digiti III*.** In some species of Lophyohylini, such as *Nyctimantis siemersi, Osteocephalus planiceps, O. oophagus* and *Osteopilus dominicensis*, the *m. caput profundum III* can be divided into two slips. Interestingly, when *m. caput profundus* is divided its medial slip inserts along metacarpal III where the *m. lumbricalis brevis digiti III* would usually be located.

***M. lumbricalis longus digiti IV*, extra slip that is preaxial to the medial slip.** In some species of *Trachycephalus*, a third, extra slip of *m. lumbricalis longus digiti IV* can be present. It is large slip, that is preaxial and more dorsal to the medial slip, that originates from *Tendo superficialis digiti IV* at the level of the base of metacarpal IV and inserts along the pre-axial

lateral surface of metacarpal IV, where *m. lumbricalis brevis digiti IV* would usually be. This occurs in *T. jordani, T. dibernadoi, T. adenodermus* and *T. hadroceps.*

M. lumbricalis brevis digiti IV. This muscle is present only in *Trachycephalus mesophaeus, T. atlas, T. nigromaculatus* and *T. lepidus.* In most lophyohylines, the *m. lumbricalis brevis digiti IV* is absent. Instead, its position is occupied either by the medial slip of *m. lumbricalis longus digiti IV,* or by a third, extra slip of *m. lumbricalis longus digiti IV*.

Mm. dorsometacarpales distales. These muscles originate from a penniform origin from the anterior portion of metacarpals and insert by a tendon upon the ultimate phalanx. All species present theses muscles on the lateral sides of metacarpals III-V and on the medial side of metacarpal V. The lateral *m. dorsometacarpals distales digiti II* is absent only in *Trachycephalus*, except for *T. mesophaeus*. When they are present, the length of their fibres may vary, eventhough they maintain fixed origin and insertion points.

Hindlimb Musculature

***M. extensor iliotibialis B,* relation with *m. ischioflexorius,* relative width.** The *m. extensor iliotibialis B* is wider than the *m. ischioflexorius* in *T. jordani, T, nigromaculatus, T. dibernadoi, T. cunauaru, T. quadrangulum, T. adenodermus, N. brunoi, Itapotihyla, Op. dominicensis, Tepuihyla, Oc. taurinus* and *Oc. verruciger.* The remaining species present a *m. ischioflexorius* that is wider than *m. iliotibialis B.*

***M. tenuissimus,* relation with *mm. extensor iliotibialis B* and *ischioflexorius*.** In most species examined the *m. tenuissimus* is covered by *mm. iliotibialis B* e *ischioflexorius* at the first half of its length. But it can either be completely evident, as in *T. quadrangulum, T. "vermiculatus", N. pomba, Op. dominicensis, Oc. oophagus* and *D. pearsoni*; or covered by *mm. iliotibialis B* e *ischioflexorius* only at the first third of its length, as in *T. coriaceus, T. cunauaru, T. macrotis, T. hadroceps, N. brunoi, N. siemersi, Corythomantis, Tepuihyla* and the remaining *Osteocephalus*.

***M. extensor cruris tibialis*, length of point of insertion.** The most common state observed is the *m. extensor cruris tibialis* inserting along the proximal 3/4 of the tibiofibular. Nevertheless, the *m. extensor cruris tibialis* can insert along the proximal 1/3 of the tibiofibular, as in *Phyllodytes melanomystax*; along the proximal 1/2 to 2/3 of the tibiofibular, as in *Itapotihyla, Phyllodytes tuberculosus, P. edelmoi,* and *Osteocephalus*; along the whole length of the tibiofibular, as in *Corythomantis, Nyctimantis brunoi* and *N. pomba*.

***M. tibialis anticus longus*, point of division in two bellies.** The *m. tibialis anticus longus* divides in two bellies at the half of the tibiofibular in most species examined. In *T. dibernadoi, T. macrotis, T. hadroceps, Op. dominicensis, Tepuihyla* and *Osteocephalus* this division occurs at the proximal 1/3 of the tibiofibular.

***M. tibialis anticus brevis*.** In *Phyllodytes* this muscle is clearly larger and more developed than in the remaining species examined. We also observed variation regarding its point of origin, which can be at the half of the tibiofibular, as in the majority of species examined; or at the distal 1/3 of the tibiofibular, as in *T. typhonius, T. mesophaeus, T. atlas, T. quadrangulum, Osteopilus dominicensis, Osteocephalus taurinus* and *N. siemersi*.

***Tendo superficialis hallucis* and *tendo superficialis digiti II*, accessory muscular head.** In *T. mesophaeus* and *Phyllodytes*, a muscular head from the *m. transversus plantae distalis*, that originates from distal tarsal 2–3, is present and inserts on the lateral side of the *Tendo superificalis hallucis*. A similar situation is observed regarding the *tendo superficialis digiti II* in *T. cunauaru, T. "vermiculatus", Tepuihyla* and *Oc. taurinus.*

***M. extensor digitorum communis longus*, insertion.** Most lophyohylines exhibit a *m. extensor digitorum communis longus* with triple insertion, on metatarsi II, III and IV. In *T. "vermiculatus"* and *D. pearsoni*, only the insertion on metatarsal III is present. A double insertion, on metatarsi II and III, is present in *Itapotihyla, Oc. planiceps* and *N. siemersi*. On the other hand, double insertion, on metatarsi III and IV, is present in *Oc. verruciger* and *Oc. oophagus.*

***M. flexor teres hallucis*.** The *m. flexor teres hallucis* is a deep flexor muscle that originates from metatarsal I and insertes on the metatarsophalangeal joint of the hallux. Among the studied specimens, it was present only in the non-casque-headed species of *Trachycephalus*.

***M. intermetatarsalis digiti IV*.** The *m. intermetatarsalis digiti IV* is present in all species, except for *Phyllodytes, Tepuihyla* and *Op. dominicensis*.

Mm. dorsometatarsales distales. These muscles present a clear topological similarity with the *mm. dorsometacarpales distales* of the hands, having a penniform origin from the anterolateral or anteromedial margin of the metatarsal bones and inserting on the ultimate phalanges. Only *Phyllodytes* presented these muscles, on the lateral sides of metatarsi I-IV and on the medial side of metatarsal V.

4. DISCUSSION

Knowledge on vocal sac structure in the tribe was mainly represented by studies that considered these characters in a taxonomic context (e.g. Duellman, 1956 and Trueb & Tyler, 1974), or specifically focused on their anatomy (Tyler, 1971a). Still, a great amount of variation has been overlooked, especially when it comes to the vocal sacs' internal anatomy (as noted by Elias-Costa *et al.*, 2017 and Elias-Costa & Faivovich, 2019). Our observations of subgular musculature expand what was known from the previous works of Duellman (1956) and Tyler (1971a) for *Trachycephalus*, highlighting the unique morphology of the *m. interhyoideus* in this genus, which is greatly laterally developed. Furthermore, although the vocal sac of *T. hadroceps* presents an external morphology similar to "single and subgular" (as described by Duellman & Hoogmoed, 1992), its *m. interhyoideus* and the associated mucosae still presents a moderate lateral extension.

The first mentions of the disconnection of paired internal mucosae in adult males date back to Inger & Greenberg (1956) for *Ptychadena porossissima* (Ptychadenidae), and McCallister (1959) for *Spea* (Scaphiopodidae). Nevertheless, it was first used in a phylogenetic context for Hylodidae and related groups (Elias-Costa *et al*., 2017), and for Victoranura (Elias-Costa & Faivovich, 2019). The vocal sac mucosae are internally disconnected in all *Trachycephalus* and in most lophyohylines, which sheds light on the observation by Tyler (1971a) that in the vocal sac of *Nyctimantis brunoi* and *Corythomantis greeningi* "medial adhesions prevent communication between the two portions of the paired submandibular structure", most likely referring to the disconnection of paired mucosae in these species. The varying degree of medial development of the internal paired mucosae should also provide useful for further phylogenetic and functional studies.

Regarding the mandible musculature, Manzano *et al.* (2003) described variation in the *m. depressor mandibulae* for Anura and noted that in hylids this muscle may present up to three slips: one originating from otic process of the squamosal, a second slip originating from the *fascia dorsalis* at the level of the *m. dosalis scapulae*, and a third slip originating from the *annulus tympanicus*. Among Lophyohylini, *Phyllodytes* apparently presents an exception for being the only group with absence of the second slip, while all other specimens present all three slips. A similar morphology was observed in *Boana riojana* of Cophomantini (Manzano *et al.*, 2003). The occlusion of the *mm. levator mandibulae anterior longus* and *levator mandibulae internus* in species with hyperossified skulls was previously unreported, and further, deeper dissections, could elucidate if they are in fact absent and functionally replaced by other muscles, or are simply repositioned.

The fore and hindlimb musculature of anurans has long been explored and utilized in systematic contexts (Hoyos & Salgar, 2016). For hylids, some representative works are those of Andersen (1978) and Burton (1996, 1998) regarding hand muscles, and Dunlap (1960) and Burton (2004) regarding foot musculature. Furthermore, Da Silva (1998) also described hand muscles in hylids and Faivovich (2002) employed myological characters from the hand and foot to assess the phylogeny of *Scinax*. Most of the variation we observed was already described by these authors, although a few aspects stand out. The close association between *m. caput profundum III* and the *m. lumbricalis brevis digiti III* was already mentioned by Burton (1996), but the apparent functioning of the *m. caput profundum III* as a substitute of the *m. lumbricalis brevis digiti III* was previously unreported. Similar is the case regarding the *m. lumbricalis longus digiti IV* and the *m. lumbricalis brevis digiti IV* in *Trachycephalus*, and these observations highlight the plasticity that can be exhibited by hand muscles while the overall function is retained (Burton, 1996, 1998).

The distal extensor muscles of the foot, *mm. dorsometatarsales distales*, were described by Faivovich (2002) for some species of *Smilisca*, *Scinax* and *Ololygon*, although ambiguous references to them were already present in the literature (Dunlap, 1960; Andersen, 1978). Attention was drawn to their topological similarity with the *mm. dorsometacarpales distales* of the hand, described by Burton (1996) who considered them as probable adaptations to climbing and arboreality (Burton, 1998). In his assessment of hylid foot musculature, Burton (2004) stated that, although the distal extensors of the foot could also be associated with climbing, he encountered a high degree of variation. In fact, his observation that variability in *mm. dorsometatarsales distales* (= *mm. extensores breves distales*) occurred amongst arboreal species and even between different feet of the same frog, was one of the factors that led him to consider that natural selection allows a "good deal of slack" in the anatomy of foot muscles (Burton, 2004). Herein, we observed that only *Phyllodytes* presented these muscles.

The fact that reduction in snout-vent length is a putative synapomorphy of this genus in comparison to most clades of Lophyohylini (Blotto *et al., in press*) could imply a possible association between body size reduction and presence of the distal extensor muscles of the foot. These muscles insert dorsally in the ultimate phalanges together with the *mm. dorsometatarsales proximales*, and their contraction aids probably aids in peeling the toe discs from the substrate, as occurs in their correlates in the hands (Burton, 1998). Whether smaller frogs would require additional muscles for a finer control of the toe discs or if the presence of these muscles are related to other environmental factors requires further studies.

5. CONCLUSION

Our survey of the muscular anatomy among *Trachycephalus* and representative lophyohylines highlights the already proposed diversity and value of character states related to the vocal sacs internal structure. Furthermore, we described novel variation in the musculature of the mandibles, with the absence of some adductor muscles in casque-headed species; hands, regarding the close association between flexor muscles of digits III and IV; and feet, with a potential relationship between size reduction and development of distal extensor muscles in some frog species. From a systematic perspective, the skeletal muscular system stands out due to its usefulness (Diogo *et al.*, 2012). For example, Diogo (2004), when comparing the relative contribution of myological and osteological characters in the phylogenetic reconstruction, demonstrated that, although the osteological structures present more variation, the myological characters provide a greater proportion of informative characters for the resolution of phylogenies. Thus, comparative myology studies can provide important subsidies for future phylogenetic studies, as we note is the case for Anura. Another valuable aspect still to be explored is the functional value of these variations observed among anurans.

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FIGURES

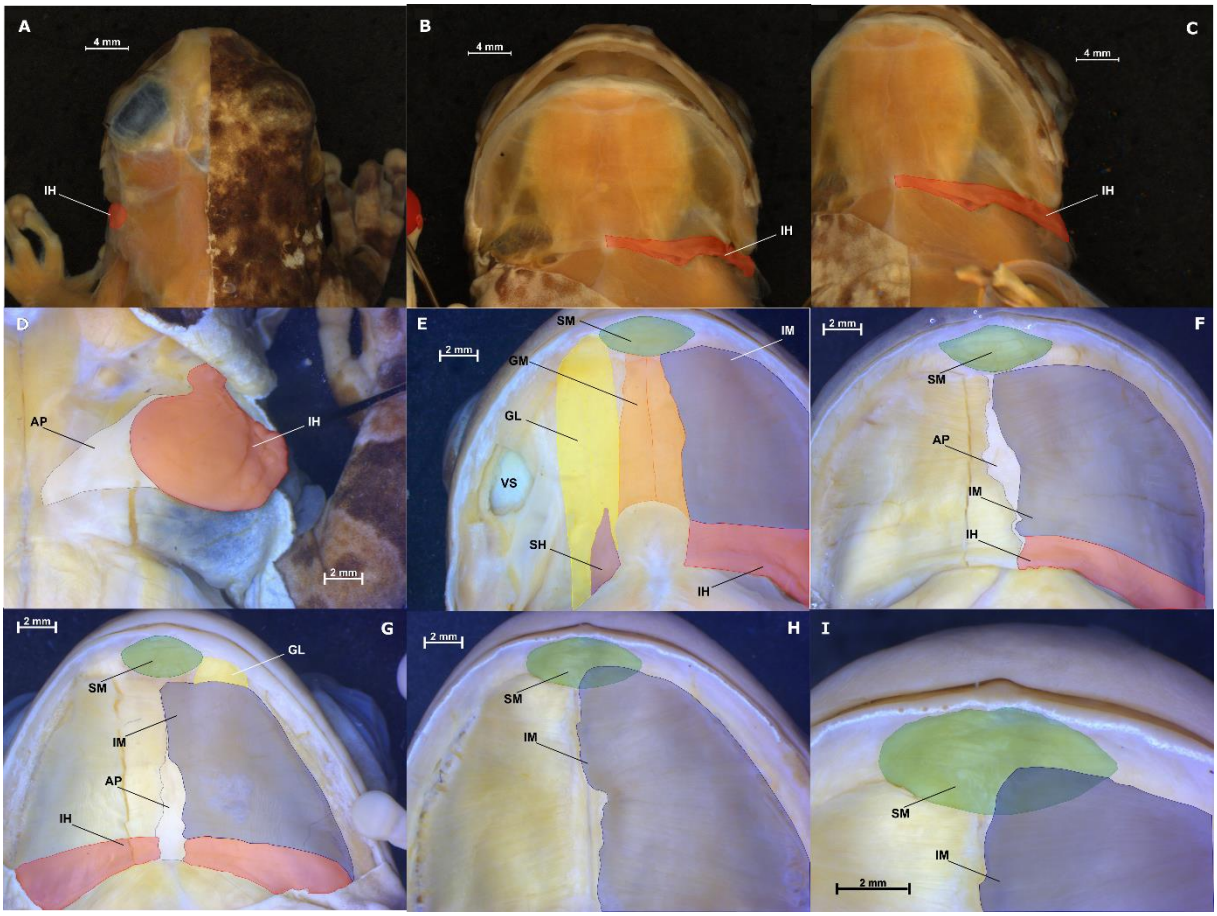

Figure 1. Submandibular musculature in *Trachycephalus*. A-C) *T. hadroceps* (MNHNP 1999.8601); D-E) *T. typhonius* (MNRJ 20362); F) T. jordani (MNRJ 73366); G) *T. cunauaru* (MNRJ 74127); H-I) *T. macrotis* (MNRJ 78675). Abbreviations are as in the text.

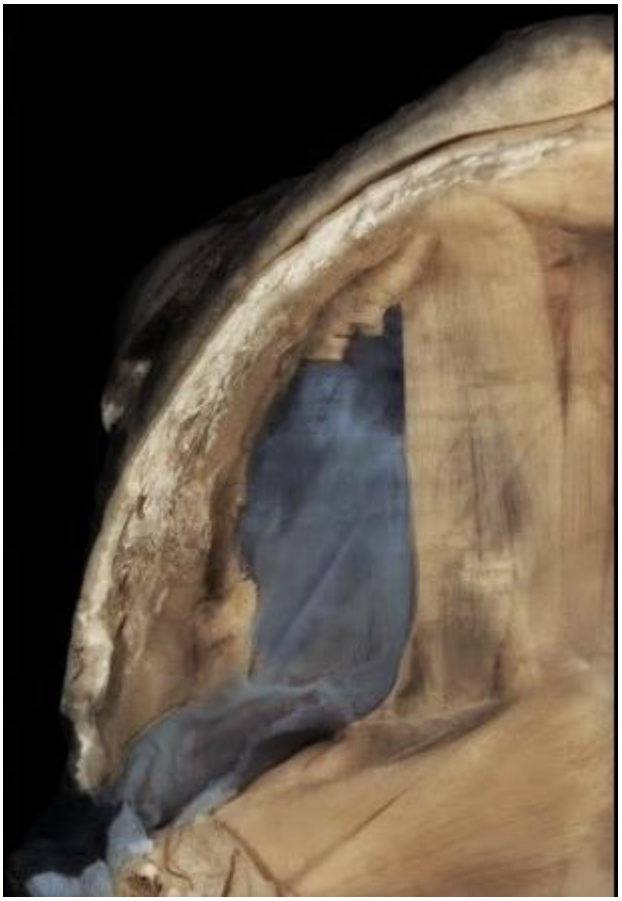

Figure 2. Vocal sac mucosa of *T. typhonius* (MACN 40540). The vocal sac mucosa is highlighted in blue. Image by: Agustín J. Elias-Costa.

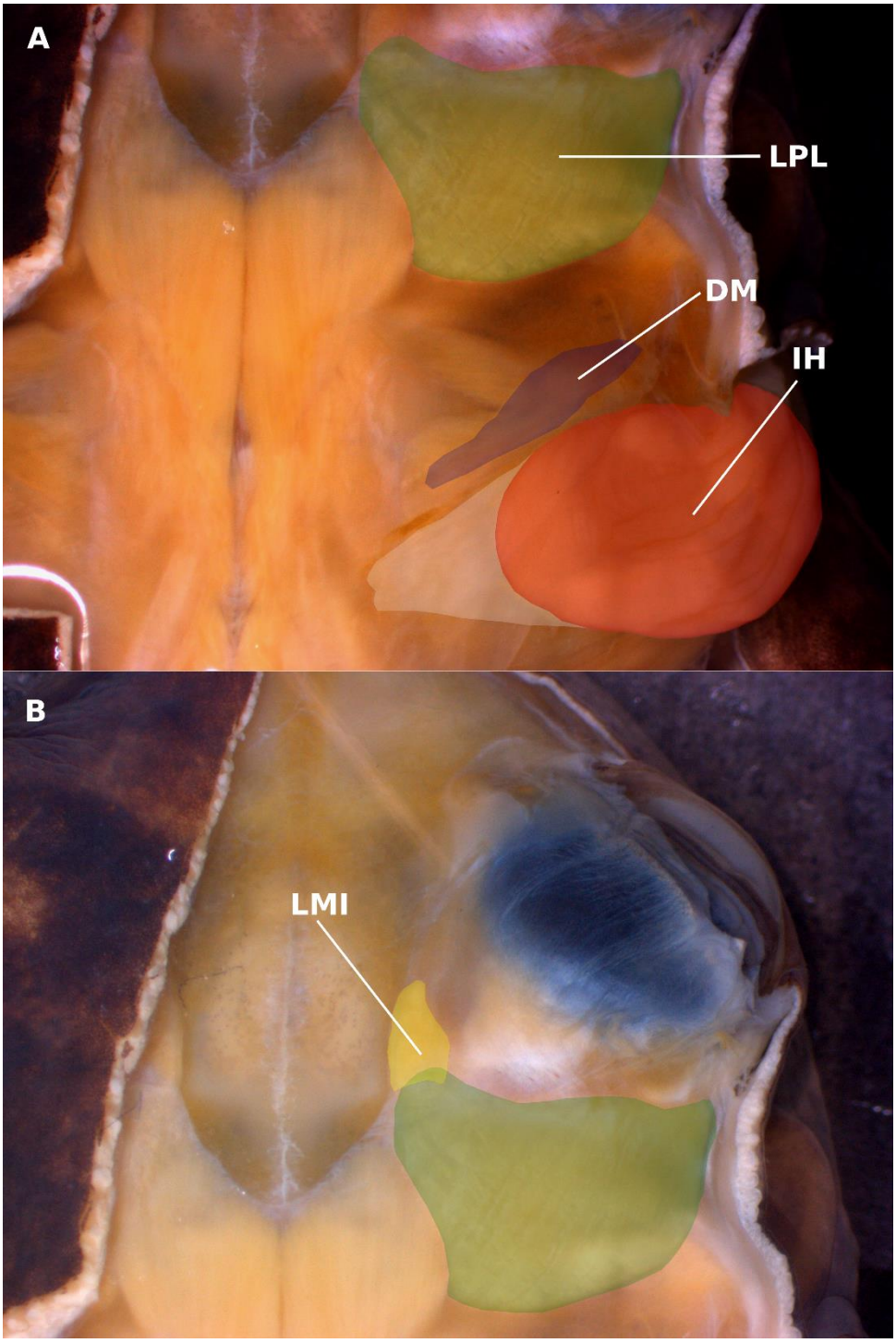

Figure 3. Dorsal head musculature of *Trachycephalus typhonius* (MNRJ 78678). Abbreviations are as in the text.

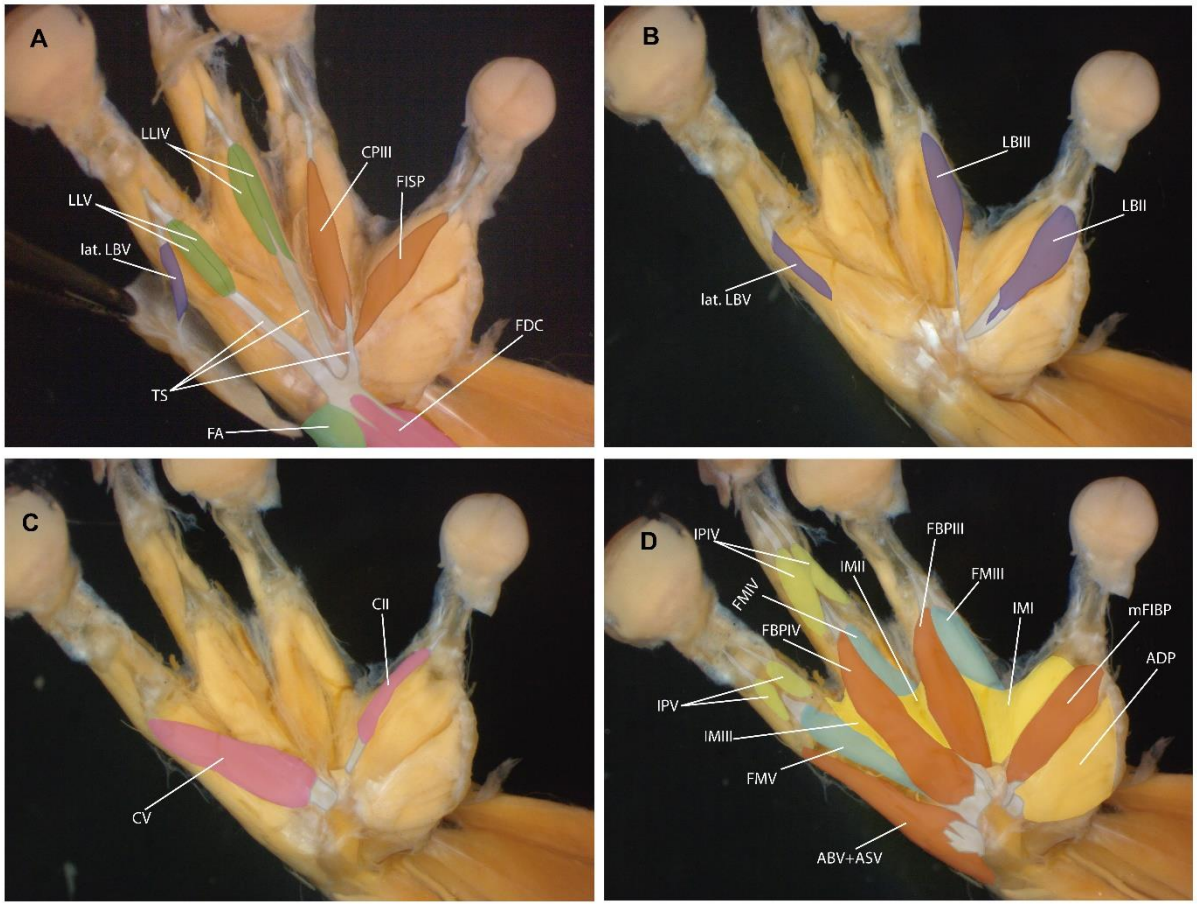

Figure 4. Ventral view of the hand of *Trachycephalus typhonius* (MNRJ 43172). A) First layer. B) Second layer. C) Third layer. D) Fourth layer. Abbreviations are as in the text.

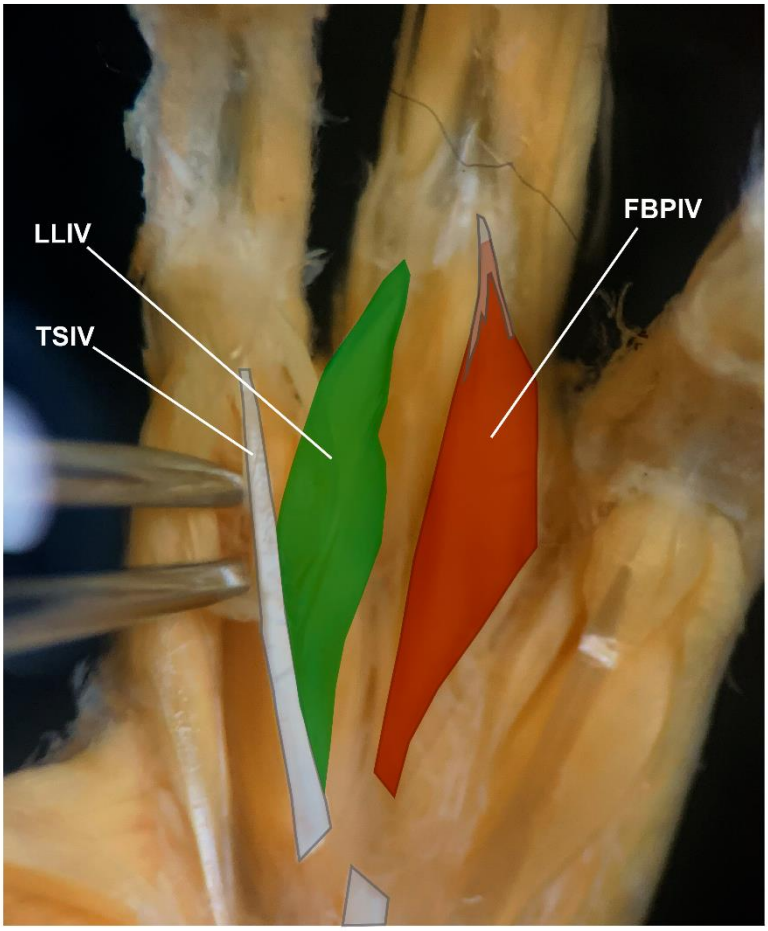

Figure 5. Ventral view of the hand of *Trachycephalus typhonius* (MNRJ 78678). Abbreviations are as in the text.

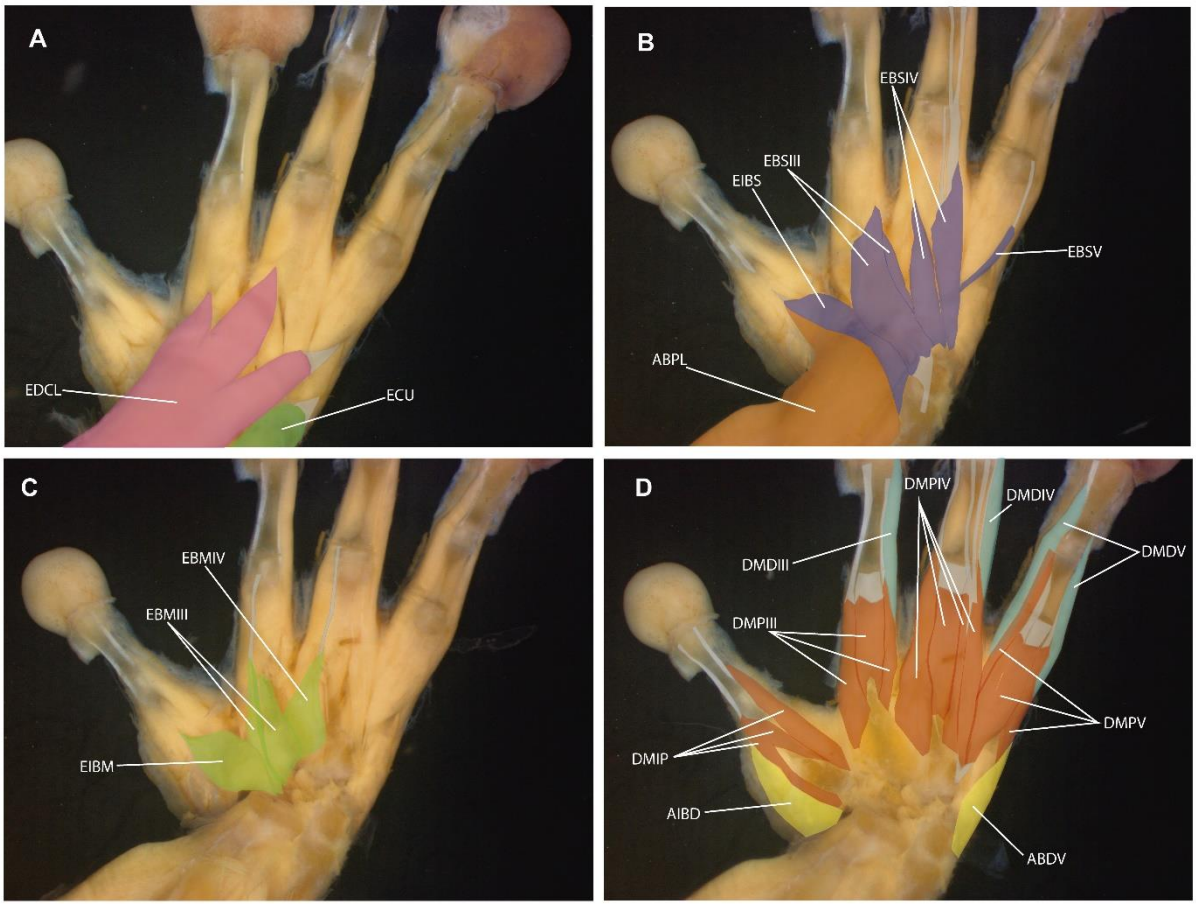

Figure 6. Dorsal view of the hand of *Trachycephalus typhonius* (MNRJ 43172). Abbreviations are as in the text.

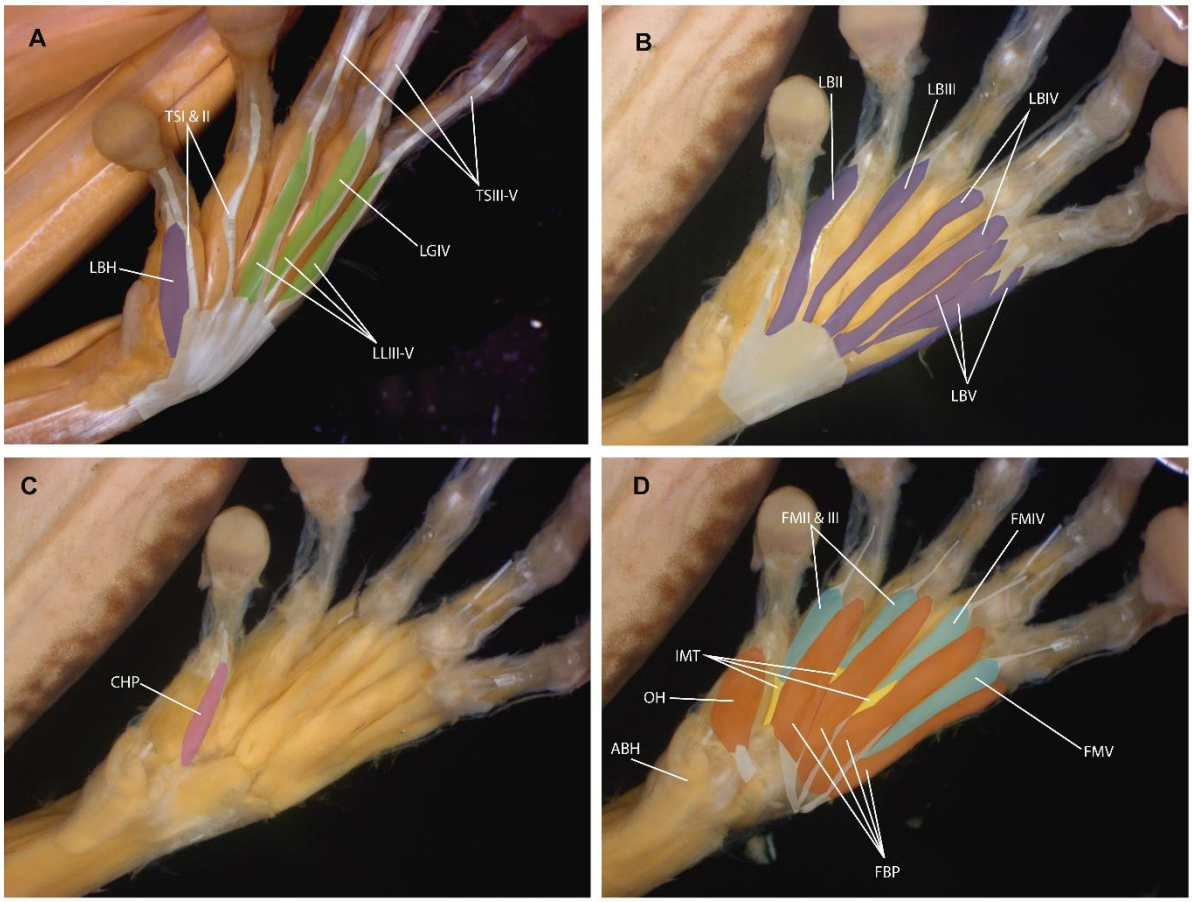

Figure 7. Ventral view of the foot of *Trachycephalus typhonius* (MNRJ 43172). Abbreviations are as in the text.

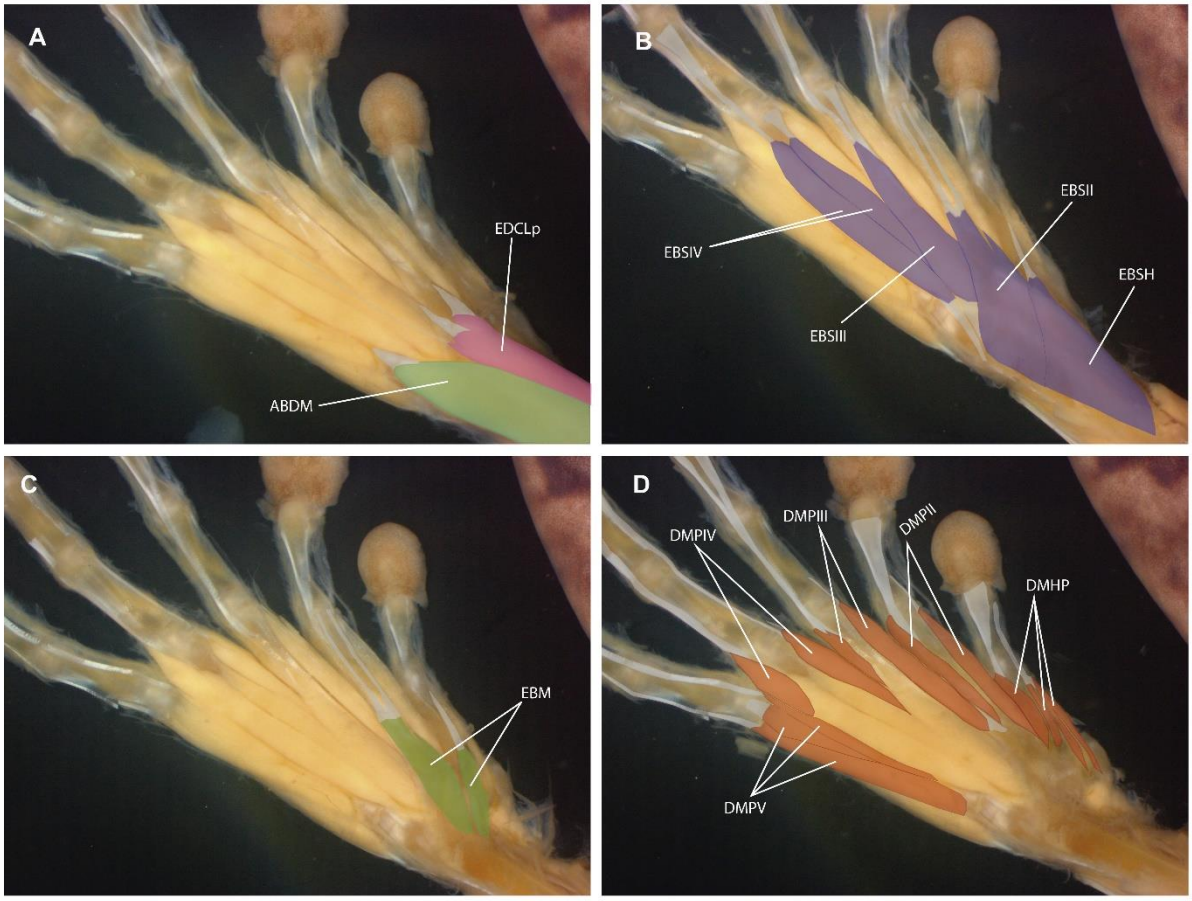

Figure 8. Dorsal view of the foot of *Trachycephalus typhonius* (MNRJ 43172). Abbreviations are as in the text.

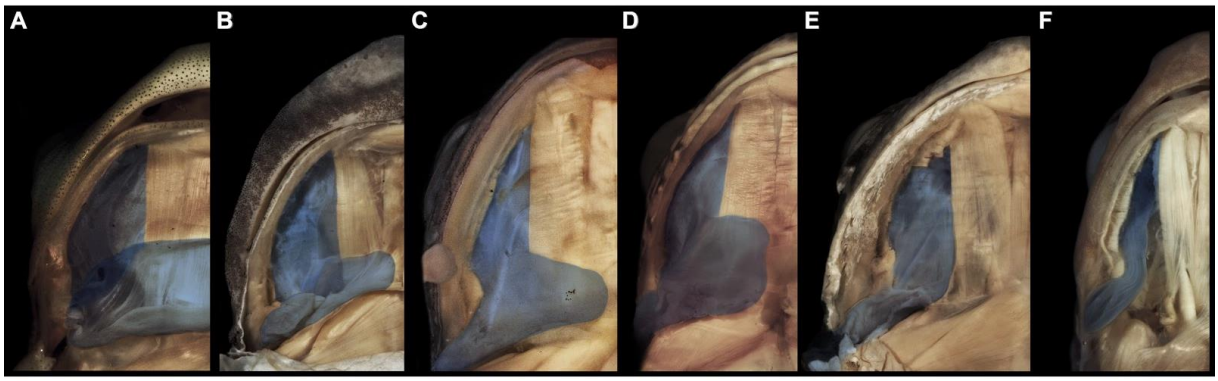

Figure 9. Diversity in the shape of vocal sac mucosae and its influence on the inflated vocal sac morphology. A-F: Ventral view of adult males with gular skin and *mm. intermandibularis* and *interhyoideus* removed to show the development of the vocal sac internal mucosae. A: *Phyllodytes melanomystax* (CFBH 35707) showing the plesiomorphic condition for Lophyohylini, where fused mucosae without lateral development produce a single subgular vocal sac. B: *Corythomantis greeningi* (CFBH 16129), showing bilobated vocal sacs. Mucosae are disconnected but their medial development enables exhaled air to access the centre of the gular region, functionally resembling a single-cavity vocal sac. Slight lateral, subgular extensions of the mucosae and the *m. interhyoideus* produce bilobulation of the inflated vocal sac. C: *Osteocephalus verruciger* also presents inflation in the centre of the gular region, but the mucosae and the *m*. *interhyoideus* extend dorsolaterally reaching the post-tympanic fold. The resulting inflated vocal sac does not fit any of the traditional categories used to classify the vocal sacs of frogs, since it combines both lateralization and subgular inflation. D: In *Itapotihyla langsdorffi* (CFBH 12724), medial development is comparatively smaller, resulting in paired lateral vocal sacs with only slight subgular inflation. E: In *Trachycephalus typhonius* (MACN 40540), mucosae are restricted to an extremely dorsolateral position, with negligible medial development and dorsolateral lobes exceeding the post-tympanic fold. F: In *Hylodes phyllodes* (MACN 17252), mucosae do not extend medially but show only slight lateral development, resulting in paired subgular vocal sacs. Image by Agustín J. Elias-Costa.

APPENDIX S1. List of studied specimens.

Abbreviations are as follows: M – specimen examined for complete musculature; PM – specimen only partially examined.

Lophyohylini: ***Corythomantis greeningi*****:** MNRJ 15866 (M). ***Dryaderces*** **aff.** ***pearsoni:*** MNRJ 86410 (M). ***Itapotihyla langsdorffii*****:** MNRJ 63214 (M). ***Nyctimantis brunoi*****:** MNRJ 45781 (M). ***Nyctimantis pomba*****:** MZUFV 11519 (M). ***Nyctimantis siemersi*****:** MACN 6635 (M). ***Nyctimantis galeata*****:** MNRJ 27174 (PM). ***Nyctimantis rugiceps:*** KU 150489 (PM). ***Osteocephalus leprieurii:*** KU 126630 (PM). ***Osteocephalus oophagus:*** MNRJ 56625 (M). ***Osteocephalus planiceps:*** MNRJ 40385 (M). ***Osteocephalus taurinus:*** MNRJ 52966 (M). ***Osteocephalus verruciger:*** MNRJ 78672 (M). ***Osteopilus dominicensis:*** MNRJ 72169 (M). ***Osteopilus ocellatus:*** KU 287866 (PM). ***Osteopilus septentrionalis:*** KU 265364 (PM). ***Phyllodytes edelmoi:*** MNRJ 54396 (M). ***Phyllodytes melanomystax:*** MNRJ 32955 (M). ***Phyllodytes tuberculosus:*** MNRJ 46472 (M). ***Tepuihyla edelcae:*** MNRJ 78673 (M).

Trachycephalus**:** ***Trachycephalus "vermiculatus":*** MNRJ 78677 (M). ***Trachycephalus adenodermus:*** MNRJ 89969 (M). ***Trachycephalus atlas:*** MNRJ 19291 (M). ***Trachycephalus coriaceus:*** MNRJ 73316 (M). ***Trachycephalus cunauaru:*** MNRJ 74127 (M). ***Trachycephalus dibernadoi:*** MNRJ 55151 (M). ***Trachycephalus hadroceps:*** MNHNP 1999.8601 (M). ***Trachycephalus imitatrix:*** MNRJ 5130 (M). ***Trachycephalus jordani:*** MNRJ 73366 (M). ***Trachycephalus lepidus:*** MZUSP 136548 (PM). ***Trachycephalus macrotis:*** MNRJ 78675 (M). ***Trachycephalus mesophaeus:*** MNRJ 15863 (M); MNRJ 8839 (M). ***Trachycephalus nigromaculatus:*** MNRJ 50992 (M). ***Trachycephalus quadrangulum:*** MNRJ 78676 (M). ***Trachycephalus resinifictrix:*** MNRJ 74126 (PM). ***Trachycephalus typhonius:*** MNRJ 20362 (M); MNRJ 43172 (M); MNRJ 78678 (M).

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CAPÍTULO 2 - Total evidence cladistic analysis of *Trachycephalus* treefrogs (Hylidae, Lophyohylini)[2]

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Short running title: Total evidence analysis of *Trachycephalus*

Authors: Moura and Nunes

[2] This manuscript is formatted according to the requirements of the journal "Zoologica Scripta". For further details see: https://onlinelibrary.wiley.com/page/journal/14636409/homepage/forauthors.html.

ABSTRACT

The treefrog genus *Trachycephalus* (Anura, Hylidae, Lophyohylini) currently contains 18 species, and is notable for harbouring representatives with and without laterally developed vocal sacs as well as dermal co-ossification of the cranium. Until now, the only putative morphological synapomorphy for this genus was the presence of paired lateral vocal sacs that protrude posteriorly to the angles of the jaws when inflated. Herein we present a cladistic analysis of *Trachycephalus* following a total evidence approach. Our dataset included sequences of seven mitochondrial and four nuclear genes, and 127 phenotypic characters for 16 of the 18 species of *Trachycephalus*, representatives of almost all other lophyohyline genera (except for *Phytotriades*), and representatives of Hylini. Our results support the monophyly of *Trachycephalus* and corroborates its conspicuous lateralized vocal sac as a synapomorphy, with a reversal in *T. hadroceps*, and probably *T. helioi* (not included here). We also resurrected *Trachycephalus spilommus* and assigned it to Mexican populations of *T. "vermiculatus"*. Based on our phylogenetic hypothesis, we discuss the evolution of vocal sacs in association with breeding site, cranial co-ossification and phragmosis and aspects of the distal extensor musculature of the foot.

Keywords: Myology, phylogeny, *Trachycephalus*, Lophyohylini.

INTRODUCTION

Lophyohylini Miranda-Ribeiro, 1926, is an anuran tribe remarkable for exhibiting great diversity of character states associated with cranial ossification (see Faivovich *et al.*, 2005 for review). Among them, the treefrog genus *Trachycephalus* Tschudi, 1838 is notable for harbouring with and without laterally developed vocal sacs as well as casquing (dermal co-ossification of the cranium, Trueb 1970). This group also presents the most widespread distribution among lophyohylines, occurring from the Lowlands of Mexico, Central and South America east of the Andes, south to northern Argentina and eastern Brazil (Frost, 2020). Their representatives are mainly arboreal or associated with water bodies, being found in tropical and subtropical forests, savannas and restingas (IUCN, 2017). Currently, 18 species are allocated to this genus (Frost, 2020; Blotto *et al. in press*): *Trachycephalus* "*vermiculatus*" (Cope, 1877), *T. atlas* Bokermann, 1966, *T. coriaceus* (Peters, 1867), *T. cunauaru* Gordo, Toledo, Suárez, Kawashita-Ribeiro, Ávila, Morais, & Nunes, 2013, *T. dibernardoi* Kwet & Solé, 2008, *T. hadroceps* (Duellman & Hoogmoed, 1992), *T. helioi* Nunes, Suárez, Gordo, & Pombal, 2013, *T. imitatrix* (Miranda-Ribeiro, 1926), *T. jordani* (Stejneger & Test, 1891), *T. lepidus* (Pombal, Haddad, & Cruz, 2003), *T. macrotis* (Eersson, 1945), *T. mambaiensis* Cintra, Silva, Silva, Garcia, & Zaher, 2009, *T. mesophaeus* (Hensel, 1867), *T. nigromaculatus* Tschudi, 1838, *T. quadrangulum* (Boulenger, 1882), *T. resinifictrix* (Goeldi, 1907), *T. typhonius* (Linnaeus, 1758) and *T. venezolanus* (Mertens, 1950).

The first evolutionary relationship hypothesis for the taxa formerly known as “casque-headed treefrogs” was proposed by Trueb (1970a). At that time, *Trachycephalus* was composed by *T. atlas, T. jordani, T. nigromaculatus, T. marmoratus* Duméril & Bibron, 1841 (= *Osteopilus septentrionalis*) and *T. siemersi* (= *Nyctimantis siemersi*). The remaining species of *Trachycephalus* were harboured in the genus *Phrynohyas* Fitzinger, 1834, included *P. venulosa* (Laurenti, 1768), *P. ingens* Duellman, 1956, *P. latifasciata* Duellman, 1956, *P. hebes* (Cope, 1862), *P. modesta* Taylor & Smith, 1945, and *P. zonata* (Spix, 1824). The author concluded that, among the clade of hylids with paired lateral vocal sacs, *Trachycephalus* and *Osteocephalus* formed a monophyletic group that was distinguished from *Phrynohyas* by the development of cranial co-ossification.

More recently, with the most representative phylogenetic analysis up to that date, Faivovich *et al*. (2005) synonymized *Phrynohyas* to *Trachycephalus* to avoid the paraphilia of the latter, based on a phylogeny of molecular characters. Thus, *Trachycephalus* was diagnosed

by 37 nuclear and mitochondrial and ribosomal protein gene transformations, with a possible morphological synapomorphy being the presence of paired vocal sacs that protrude posteriorly to the mandible when inflated (Faivovich *et al*., 2005). In this context, Smith *et al*. (2007) mapped the cranial characters considered by Trueb (1970a) in a cladogram also resulting from the analysis of molecular data and proposed that they represent homoplasies with strong phylogenetic signal. The relationship hypotheses resulting from the analyses by Wiens *et al.* (2010) and Pyron & Wiens (2011) also agreed with what was proposed by Faivovich *et al.* (2005), since they used many of the sequences originally produced in that work. Similarly, *Trachycephalus* has been recovered as monophyletic in the molecular studies of Pyron (2014), Ron *et al.* (2016), Duellman *et al.* (2016) and Jetz & Pyron (2018). Moreover, Blotto *et al.* (*in press*) recovered a paraphyletic *Trachycephalus* with respect to *Aparasphenodon venezolanus*, while presenting the most taxonomically densely sampled phylogeny of Lophyohylini available. To remedy this situation of non-monophyly, they transferred *A. venezolanus* to *Trachycephalus* and observed that the former also presented paired lateral vocal sacs, further highlighting this character as a putative synapomorphy of this genus. Regarding the internal relations, Blotto *et al.* (*in press*) recovered clades that are overall congruent with the results provided by Ron *et al.* (2016).

The systematics of *Trachycephalus* still presents several questions to be resolved. Gordo *et al.* (2013) highlights the insufficient delimitation of species and that there are species complexes with wide geographical distribution, such as *T. typhonius*. Duellman (1956) initially defined *Phrynohyas venulosa* (= *Trachycephalus typhonius*) as a species complex, recognizing two new taxa (*P. ingens* and *P. latifasciata*) and revalidating five others: *P. hebes*, *P. inflata* (Taylor, 1944), *P. modesta* (Taylor & Smith, 1945), *P. spilomma* (Cope, 1877) and *P. zonata*. Subsequently, McDiarmid (1968) considered *P. latifasciata*, *P. inflata* and *P. spilomma* as synonyms of *P. venulosa* in Central America, being only variations of the same species. In 1971 Duellman, in a new revision of *Phrynohyas*, corroborated what was established by McDiarmid (1968) and further concluded that the genus would contain only four species: *P. coriacea* (Peters, 1867), *P. imitatrix* (Miranda-Ribeiro, 1926), *P. mesophaea* (Hensel, 1867), and *P. venulosa*. Thus, in addition to presenting 26 synonyms (Lavilla *et al*., 2010), the distribution of *T. typhonius* overlaps that of the entire genus *Trachycephalus* (Frost, 2020). Ron *et al*. (2016) initiated the breakdown of *T. typhonius*, revalidating two other species (*T. quadrangulum* and *T. macrotis*). Blotto *et al.* (*in press*) corroborated these results and stressed the need of further studies regarding populations assigned to *T. typhonius*, as well as taxonomic revisions for the

complexes of (i) *T. atlas*, *T. mambaiensis* and *T. nigromaculatus*, and (ii) *T. dibernadoi* and *T. imitatrix*.

The only putative morphological synapomorphy for *Trachycephalus* is the presence of paired vocal sacs (Faivovich *et al.*, 2005). However, this term says nothing about the internal structure of the sacs themselves, resulting in the possibility that a great deal of variation in this character has been neglected (Elias-Costa *et al.*, 2017; Elias-Costa & Faivovich, 2019). Duellman's (1956) monograph on *Phrynohyas* and Tyler's (1971) work present the only available anatomical descriptions of vocal sacs of *Trachycephalus*, based solely on the examination of male specimens of *Phrynohyas spilomma* (= *T.* "*vermiculatus*") (Duellman, 1956) and *Trachycephalus jordani* and *T. nigromaculatus* (Tyler, 1971). Until now, this character remains untested in a phylogenetic context and lacks a detailed anatomical study across a large number of species. Therefore, considering that *Trachycephalus* is defined only by molecular synapomorphies and that the only proposed morphological synapomorphy has not been tested in a phylogenetic context, this group is an interesting object for systematic studies based on phenotypic data.

In the present study, we present a cladistic analysis for *Trachycephalus* following a total evidence approach with the goals of: (i) testing the monophyly of *Trachycephalus*; (ii) testing the relationships between the species of genus; and (iii) studying the evolution of morphological characters in light of the resulting phylogeny.

MATERIAL AND METHODS

Taxon sampling

We sampled all nominal species of *Trachycephalus*, except for *T. helioi* and *T. venezolanus*. We also included *T. adenodermus* as a terminal in our analysis. Although Lavilla et al. (2010) reinforced Duellman (1971) in synonymizing it with *T. typhonius*, Vanzolini (1986) revalidates this taxon based on its reproductive mode, vocalization and tadpoles, all of which are distinct from *T. typhonius* (see Schiesari *et al*., 2003). The taxon *T. "vermiculatus*" is a placeholder for all names available for former *Trachycephalus typhonius* from Chocoan South America to southern and eastern Mexico (Frost, 2020). Herein, we included morphological and molecular data for specimens collected from Potrero Viejo, Veracruz State, eastern Mexico, which would fall under this name. Duellman (1956) considered the type

specimen of *Hyla vermiculata* Duméril & Bibron, 1841, to be probably from eastern Venezuela or the Guianas, based on external morphology. Instead, he attributed to eastern Mexican populations the name *Phrynohyas spilomma* (Cope, 1877), based on morphological similarity with the type of *Hyla spilomma*, described by Cope (1877) from an immature specimen collected at Cosamaloapan, Veracruz, Mexico. Thus, based on the morphological similarity with our specimens (IN pers. comm.), we considered our individuals to be representatives of *P. spilomma sensu* Duellman (1956), therefore restricting the name *T. "vermiculatus"* to northern South America morphotypes.

In order to test the monophyly of *Trachycephalus*, we selected outgroups based on previous phylogenetic hypotheses published by Faivovich *et al*. (2005), Ron *et al*. (2016), Duellman *et al*. (2016) and Blotto *et al*. (*in press*). Thus, our outgroup included representative species from 9 of the 10 genera of Lophyohylini, the exception being the monotypic *Phyototriades*, and *Acris crepitans*, *Pseudacris regilla* and *Duellmanohyla rufioculis* of Hylini. The trees were rooted with *D. rufioculis*. Supplemental information Appendices S1 include a complete list of studied specimens. Institutional collection abbreviations follow Sabaj (2016).

Phenotypic character sampling

Terminology followed McDiarmid & Altig (1999) for larval morphology; Duellman & Trueb (1994) for cranial muscles; Gaupp (1896) for axial muscles and fore and hindlimb osteology; Tyler (1971) for submandibular muscles; Diogo & Ziermann (2014) for fore and hindlimb muscles (except for the *mm. lumbricales breves* and *lumbricales longi* of the hand which follows Burton, 1996, and the *m. adductor pollicis* of the foot which follows Burton, 2004); Trueb (1970a, 1973, 1993) for cranial and post-cranial osteology; and Fabrezi (1992, 2001) for carpal and tarsal osteology. Anatomical dissections were performed to observe the musculature and topical applications of Bock & Shear's (1972) solution where used when necessary to aid visualization. Osteological characters were coded either from dry skeletons, x-rays or cleared and double stained specimens (Taylor & Van Dyke, 1985). Some osteological and tadpole characters were coded from available literature (Appendix S2). Not all specimens were available for myological dissections. A list of studied specimens indicating the types of preparation and which were examined for myological characters is presented in Appendix S1. The final phenotypic dataset included 127 characters, 17 of which were considered additive (Appendices S3 and S4).

Molecular character sampling

We included eight mitochondrial DNA fragments (*12S*, *16S*, *COI*, *tRNA^{Leu}*, *tRNA^{Gln}*, *tRNA^{Ile}*, *Cytb*, *ND1*) and four nuclear DNA fragments (*POMC*, *RAG1*, *RHO*, *TYR*) available from GenBank for Lophyohylini and the Hylini specimens. New *COI* fragments were generated for our eastern Mexican representatives of *Trachycephalus* (see Laboratory protocols below). Gene fragments were individually aligned using the web version of MAFFT v. 6.903 (Katoh *et al.*, 2005) under the Q-INS-i strategy (for *12S* and *16S*; secondary structure of RNA is considered) and the G-INS-i strategy (all other fragments; global homology is considered) with default parameters. The final molecular dataset included 6968 characters for 34 terminals. GenBank accession numbers are included in Appendix S5. Molecular samples were not available for *Trachycephalus atlas*, *T. mambaiensis*, *T. lepidus*, *T. dibernadoi*, *T. adenodermus*, *Nyctimantis pomba*, *N. galeata*, *Phyllodytes tuberculosus*, *P. edelmoi* and *Tepuihyla luteolabris*. The sequences for these species will be available at the moment of the publication of Blotto *et al.* (*in press*) (IN pers. Comm.).

Laboratory protocols

We extracted whole genomic DNA from ethanol-preserved muscle tissue using the Wizard DNA extraction kit (Promega, Madison, WI). The final sample was stored at -20ºC. We used PCR to amplify a fragment of 649 bp at the 5' of the mtDNA cytochrome oxidase subunit I gene (COI) using universal amphibian primers by Che *et al*. (2012) as modified by Jennings *et al.* (2015):

Chmf4M13-21 5'–TGTAAAACGACGGCCAGTTYTCWAC
WAAYCAYAAAGAYATCGG–3'; and
Chmr4M13-29 5'–CAGGAAACAGCTATGACCACYTCRGG
RTGRCCRAARAATCA–3'.

DNA amplification (PCR) procedures followed Jennings *et al.* (2015): [(94 ºC for 1:30) x 1 cycle], [(94 ºC for 0:30, 50ºC for 45 s, 70 ºC for 1:00) x 35 cycles], and (70 ºC for 10:00). The presence of single target PCR bands in each reaction was confirmed using agarose gel electrophoresis. PCR products were treated with Exo-SAP before sequencing. Sequencing was performed at Macrogen, Inc. (Seoul, South Korea). A fragment of the COI gene was sequenced

for two specimens of *Trachycephalus* from Potrero Viejo, Veracruz State, Mexico (as *Trachycephalus spilommus*, see results and Appendix S6; GenBank accession numbers: CC520 and CC527) resulting in 649 bp. A consensus sequence for each DNA fragment was generated through the comparison of complementary strands using BioEdit (Hall, 1999). The resulting sequences were submitted to Blast searching (Altschul *et al.*, 1997) in GenBank to ensure the required sequence had been sequenced.

Cladistic analyses

For the purpose of comparison, we initially performed separate analysis of the molecular (molecular dataset: MD) characters, and, subsequently, a total evidence (TE) analysis. All matrices were edited in Mesquite v. 3.61 (Maddison & Maddison, 2019) and inapplicable and unknown character states were coded as "-" and "?", respectively. We treated every character state transformation event under their own implicit weights (i.e. under equal weights), since differential weighting of characters leads to a less efficient distribution of characters states and to greater incongruence (Grant & Kluge, 2003). Multistate characters were considered as additive or non-additive and all phenotypic characters were coded following Sereno (2007). Moreover, every sequenced specimen was treated as an individual terminal for TE. In this sense, we followed Grant *et al.* (2006) and duplicated the morphological entries for the species of each molecular terminal.

The optimality criterion employed was maximum parsimony, given that it maximizes explanatory power by minimizing the number of transformation events necessary to explain character states of terminal taxa as homology hypotheses (Kluge & Grant, 2006; Grant & Kluge, 2009). Given the non-deductive character of phylogenetic inference, the requirement of total evidence is applied, which stipulates that all relevant available evidence must be considered for a given inference (Fitzhugh, 2006). Hence, we considered the phylogenetic hypothesis obtained with TE to be the hypothesis that best explains the evidence. Therefore, discussions of character evolution and synapomorphies are based on the results of TE.

All cladistic analyses were performed with the software TNT v. 1.1 (Goloboff *et al.*, 2008). The most parsimonious trees were found using the heuristic search method, with 1000 rounds of random addition sequences retaining 10 trees per replicate, and then submitting them to a round of tree bisection and reconnection (TBR) branch swapping. The resulting trees saved in RAM memory were then submitted to a second round of TBR. All nodes whose minimum

length was zero were collapsed. Clade support was estimated by calculating parsimony Jackknife absolute frequencies (Farris *et al.*, 1996) and Goodman-Bremer values (Goodman *et al.*, 1982; Bremer, 1988; Grant & Kluge, 2008) in TNT v. 1.1 (Goloboff *et al.*, 2008). Jackknife values were calculated using "Traditional Search" with the same parameters described above and 1000 replicates. Goodman-Bremer values were estimated with the option "absolute supports" and calculated with TBR from all suboptimal trees with relative fit difference ≤ 10. Character states were considered synapomorphies if they optimized unambiguously, since preferential selection for ACCTRAN or DELTRAN optimization is arbitrary (see Agnarsson & Miller, 2008).

RESULTS

Cladistic analyses

The MD parsimony analysis resulted in one most parsimonious tree with 7866 steps, Retention Index = 0.45 and Consistency Index = 0.46 (Fig. 1). The genus *Trachycephalus* was recovered as a well-supported monophyletic group (Jackknife = 86%; GB = 9), sister taxon to *Corythomantis* with which it forms a monophyletic group with *Itapotihyla* + *Nyctimantis*. In this topology, *Trachycephalus jordani* + *Trachycephalus coriaceus* is the earliest diverging clade and sister to all remaining *Trachycephalus*. Most interspecific relations present low support values (average Jackknife < 50%; GB < 4), as well as intergeneric relations.

The TE analysis resulted in three most parsimonious trees with 15505 steps, Retention Index = 0.44 and Consistency Index = 0.37. Strict consensus tree is shown in Figure 2. *Trachycephalus* was recovered as a well-supported monophyletic group (Jackknife = 93%; GB = 7), sister taxon to *Corythomantis* + *Nyctimantis galeata* + *N. pomba* with which it forms a monophyletic clade with *Itapotihyla* + (*N. brunoi* + (*N. rugiceps* + *N. siemersi*)). Interspecific relations are low supported in general (average Jackknife < 50%; GB < 3), as well as intergeneric relations (Fig. 2). Topological differences are restricted to the internal relations in *Phyllodytes* and the clade formed by *Itapotihyla* + (*Nyctimantis brunoi* + (*Nyctimantis rugiceps* + *Nyctimantis siemersi*)). In all topologies, *T. jordani* is the earliest diverging taxon, followed by a clade formed by the remaining casque-headed species from the Caatinga, Cerrado and Atlantic Forest (*T. atlas* + *T. mambaiensis* + *T. nigromaculatus*), which is sister to the remaining species of the genus. This subsequent clade has *T. dibernadoi* as its earliest diverging taxon,

followed by a clade composed of *T. imitatrix* + *T. lepidus* and, furthermore, a clade composed of *T. mesphoaeus* + *T. coriaceus*. The trans-Andean, Amazonian and Guyanese species form the most derived clade (*T. typhonius* + *T. adenodermus, T. resinifictrix* + *T. hadroceps, T. cunauaru* + *T. quadrangulum, T. macrotis*) with the Mexican specimens nested well within it (Fig. 2).

Differences between the MD and TE topologies are related to, besides the inclusion of species for which DNA sequences weren't available, the position of some *Trachycephalus* species. In MD, *T. coriaceus* is sister to *T. jordani,* and *T. mesophaeus* is sister to the remaining species of the genus; and *T. resinifictrix* + *T. hadroceps* is sister to a clade which includes *T. typhonius* (compare Figs. 1 and 2).

DISCUSSION

The monophyly of Trachycephalus *and comments on outgroup relations*

We found *Trachycephalus* as a monophyletic group supported by two synapomorphies: *m. interhyoideus* and vocal sac mucosae presenting great lateral extension, exceeding the post-tympanic fold (chs. 5.3 and 7.3). These character states are responsible for what is usually described as "paired lateral vocal sacs that protrude behind the angle of the jaw" and our results represent the first robust test of these conditions as synapomorphies for this genus. Duellman (1956) already noted that the paired lateral vocal sacs of what was then considered two separate genera, *Phrynohyas* and *Trachycephalus*, were essentially the same (see Tyler, 1971 also) and provided morphological evidence that they evolved from a common ancestor. Similarly, Trueb (1970a) included these two genera in the same clade on the same basis, although not in a phylogenetic context. Faivovich *et al.* (2005) redefined *Trachycephalus* as to include all species of *Phrynohyas*, given the polyphyly of the former, and were the first to propose said putative morphological synapomorphy. Subsequent studies also employed a molecular perspective and were congruent, given that the majority of DNA sequences they used were the same (Wiens *et al.*, 2006, 2010; Pyron & Wiens, 2011; Pyron, 2014; Duellman *et al.*, 2016; Ron *et al.*, 2016; Jetz & Pyron, 2018). Blotto *et al.* (*in press*) performed the most comprehensive phylogenetic analysis of Lophiohylini to date based on molecular data and discussed the evolution of some non-molecular features (level of co-ossification and chin-tucking for phragmosis behaviour, for

instance). Hence, our study represents the first corroboration of these molecular results that approached the issue from a total evidence framework.

Although testing the relationship of *Trachycephalus* with other lophyohylines was not our main goal, the recovery of *Trachycephalus* as sister taxon to *Corythomantis* recapitulates previous results of Ron *et al.* (2016), Duellman *et al.* (2016) and Jetz & Pyron (2018), although no unambiguous synapomorphy was recovered. In addition, there is the paraphyly of *Nyctimantis* due to the grouping of *N. pomba* and *N. galeata* with *Corythomantis* in a polytomy. The exception is *Blotto et al.* (*in press*), which recovered *Trachycephalus* as sister clade of *Corythomantis + Nyctimantis*. Another difference of our study to *Blotto et al.* (*in press*) is the nesting of *N. pomba* and *N. galeata* within *Corythomantis* supported by the presence of nasal bones that occlude the allary process of the premaxillae (ch. 64.1). This character state was reported by Trueb (1970a), which Faivovich *et al.* (2005) identified as an autapomorphy for *Corythomantis*, promping the initial inclusion of *N. galeata* in this genus (Pombal *et al.*, 2012). Nevertheless, we refrain from taking any nomenclatural acts here since we didn't have DNA samples available for *N. pomba* and *N. galeata*, which limits our discussion at this point.

Furthermore, the grouping of *Trachycephalus*, *Corythomantis*, *Nyctimantis* and *Itapotihyla* as a clade was also recovered by Duellman *et al.* (2016) and, in the present study, was supported by the occluded *m. levator mandibulae posterior longus* (ch. 17.0; see Appendix S4 for comment), a character state indirectly associated with cranial hyperossification. In our optimization, secondary exposure of this muscle occurs in *C. greening* and the non-casque-headed *Trachycephalus*. The absence of *Phytotriades* in this study, a genus with which *Itapotihyla* was grouped in previous analyses (Pyron, 2014; Blotto *et al.*, *in press*), and the low support obtained for this clade (Jackknife = 21%; GB = 7) stresses the need for further studies in this regard. Interestingly, although given the scope and limitations of our sampling, the presence of four or more posterior tooth rows in the tadpole oral disc (ch. 123.1) was recovered as a synapomorphy of Lophyohylini (Jackknife = 100%; GB = 10), corroborating what was suggested by Faivovich *et al.* (2005).

Internal relations in Trachycephalus

As already mentioned, internal relations in *Trachycephalus* showed pervasive low support possibly due to absence of molecular data for a few species (*Trachycephalus atlas, T. mambaiensis, T. lepidus, T. dibernadoi* and *T. adenodermus*), but wildcard behaviour was not

observed, in which its positioning becomes unstable due to the large number of missing data (Nixon & Wheeler, 1992). Thus, the positioning of all taxa was consistent among different TE topologies. The recovery of *T. jordani* as the earlier diverging species recapitulates previous studies (e.g. Faivovich *et al.*, 2005; Ron *et al.*, 2016; Blotto *et al.*, *in press*), presenting as an autapomorphy the presence of an interruption on the posterior region of the medial ventral crest of the cultriform process of the parasphenoid, which has been previously compared to an odontoid (ch. 94.1; see Trueb, 1970). The next diverging clade is formed by the remaining casque-headed *Trachycephalus* (except for *T. venezolanus*, not included in this study) and is supported by a sphenethmoid with completely ossified orbital plane (ch. 100.3). The grouping of these three species together is congruent with Blotto *et al.* (*in press*) and the topology here presented implies that in *T. mambaiensis* a secondary loss of the orbital flange of the frontoparietals and of the posterior ridge of the otoccipital occurred, explaining why this species presents what appears to be an intermediate condition regarding hyperossification (Bokermann, 1966; Cintra *et al.*, 2009). However, taxonomic revision of this group is still needed given small genetic distances among them and the widespread distribution of *T. nigromaculatus* (Blotto *et al.*, *in press*).

Then, all non-casque-headed *Trachycephalus* species are nested together in a clade, but with low support (Jackknife = 38%; GB = 7). These species are generally known as "milk-frogs" (Frost, 2020) and were previously included in the genus *Phrynohyas* (Duellman, 1956; McDiarmid, 1968; Duellman, 1971; Duellman & Hoogmoed, 1992; Pombal *et al.*, 2003) by the absence of dermal co-ossification of the cranium and the presence of paired lateral vocal sacs. In our analysis, the presence of the *m. flexor teres hallucis* is a synapomorphy for this group among lophyohylines, although this character is present at least in *Osteopilus septentrionalis* (Burton, 2004; myology not examined in our study). This is already suggested by the distribution of this character in Burton (2004), whose sampling included *T. jordani*, *T. nigromaculatus* and *T. typhonius* (= *P. venulosa*), corroborated herein.

Positioned as sister to the remaining *Trachycephalus* milk-frogs, *T. dibernadoi* is distinguished from its proposed sister-species, *T. imitatrix* (Kwet & Solé, 2008; Blotto *et al.*, *in press*), by some homoplastic character-states: fibres of the medial *m. dorsometacarpalis distalis digiti V* reaching the distal margin of penultimate phalanx V (ch. 40.2; fibres reach the distal margin of the antepenultimate phalanx V in *T. imitatrix*); *m. tibialis anticus longus* divides into two bellis at the proximal third of the tibiofibular (ch. 44.0; it separates into two bellies at ½ of the tibiofibular in *T. imitatrix*); absence of exostosis on the nasals (ch. 72.0; present in *T.*

imitatrix); and absence of exostosis on the frontoparietals (ch. 73.1; present in *T. imitatrix*). This would provide further evidence for the full species status of *T. dibernadoi*, but since Blotto *et al.* (*in press*) notes that their sequences from both species are identical and given that our sampling didn't encompass the whole distribution and variability of *T. imitatrix*, further taxonomic studies are warranted. On the other hand, *T. lepidus*, which is recovered as sister to all *Trachycephalus* by Blotto *et al.* (*in press*), is sister to *T. imitatrix* with low support (Jackknife = 15%). Character-states that support this relationship are the homoplasies: translucent frontoparietal fenestra (ch. 67.0; present also in *T. hadroceps*); and allary process of the premaxillae with expanded base (ch. 81.0) and presence of a crest on the spinous process of the atlas vertebra (ch. 114.0), both also present in *T. mesophaeus*.

The next monophyletic group is congruent with the findings of both Ron *et al.* (2016) and Blotto *et al.* (*in press*), and includes *Trachycephalus typhonius*, *T. adenodermus*, *T. resinifictrix*, *T. hadroceps*, *T. cunauaru*, *T. quadrangulum*, *T. macrotis* and the eastern Mexican specimens. However, internal topology is divergent and overall poorly supported, with *T. typhonius* and *T. adenodermus* forming a clade sister to the remaining species, followed by *T. hadroceps* + *T. resinifictrix* (Jackknife = 74%; GB = 1), *T. cunauaru* + *T. quadrangulum* (Jackknife = 74%; GB = 2) and *T. macrotis* + eastern Mexican *Trachycephalus* (Jackknife = 58%; GB = 2). Blotto *et al.* (*in press*) already stressed out that there is no reason to expect that populations attributed to *T. typhonius* throughout its widespread distribution are going to be recovered as more closely related to *T. typhonius sensu stricto* (see Ron *et al.*, 2016). Herein, our samples of *Trachycephalus* from Veracruz, Mexico, formed a clade with high support (Jackknife = 99%; GB = 10) and were nested with *T. macrotis*, a species from Amazonian Ecuador and Northeastern Peru.

These are distinguishable from *T. macrotis* by a series of myological characters: *m. intermandibularis* with medial aponeurosis that widens distally (ch. 2.1; medial raphe present in *T. macrotis*); a completely evident *m. tenuissimus* (42.0; covered by the *mm. iliotibialis B* and *ischioflexorius* in *T. macrotis*); presence of a muscular head in *tendo superficialis digiti II* of the *pes* (ch. 48.1; absent in *T. macrotis*); and absence of insertions of the *m. extensor digitorum communis longus* of the *pes* on metatarsals II and IV (chs. 49.0 and 51.0; both present in *T. macrotis*). This, alongside further evidence presented in Appendix S6, prompts us to elevate this population as a full species. To solve the status of these populations we resurrect *Hyla spilomma* Cope, 1877 from its synonymy with *T. typhonius*, resulting in *Trachycephalus spilommus* **comb. n.** (Cope, 1877) (see Appendix S6 for full account).

Character Evolution

Vocal sac evolution and reproductive biology: Lophyohyline frogs call attention for their vocal sac diversity. For instance, in these treefrogs the vocal sacs range from single and subgular (e.g. *Phyllodytes* and some *Osteocephalus*) to paired, dorsal vocal sacs (e.g. in most *Trachycephalus*), with a wide array of intermediate forms. Furthermore, vocal sac-related characters played, and still play, an important role in determining relationships at the genus or species levels in Lophyohylini (Duellman, 1956; Duellman, 1970; Trueb, 1970a; Trueb, 1970b; Trueb & Duellman, 1971; Tyler, 1971a; Trueb & Tyler, 1974; Faivovich *et al.*, 2005; Jungfer *et al.*, 2013). The knowledge on vocal sac structure in the tribe was mainly represented by studies that considered these characters in a taxonomic context (e.g. Duellman, 1956 and Trueb & Tyler, 1974), or specifically focused on their anatomy (Tyler, 1971a). Still, a great amount of variation has been overlooked, especially when it comes to the vocal sacs' internal anatomy (as noted by Elias-Costa *et al.*, 2017 and Elias-Costa & Faivovich, 2019).

The external shape that the anuran vocal sacs acquire when inflated is determined by a combination of the anatomy of the *m. interhyoideus* and the vocal sac mucosae (Elias-Costa & Faivovich, 2019). In this sense, the conspicuous vocal sacs of *Trachycephalus* correspond to the great lateral extension of both the *m. interhyoideus* and the associated mucosae, recovered as synapomorphies for this genus as stated above (chs. 5.3 and 7.3). Optimization of these characters (Fig. 3) shows that species with apparently no externally developed vocal sac, as *T. hadroceps*, indeed represents a case of reversal by the reestablishment of their ancestral state, as suggested by Nunes *et al.* (2013). Curiously, however, although the vocal sac of *T. hadroceps* presents an external morphology similar to "single and subgular" (as described by Duellman & Hoogmoed, 1992), its *m. interhyoideus* and the associated mucosae still presents a moderate lateral extension.

The anatomical variation also calls attention to the importance of the degree of medial development of the vocal sac mucosae (ch. 9), a determinant factor on the degree of subgular expansion exhibited when the sacs are inflated (Elias-Costa & Faivovich, 2019). Although optimization of this character is ambiguous (Fig. 4), its distribution shows that *Trachycephalus jordani* and *T. lepidus* presents mucosae with pronounced medial development (ch. 9.0), while different degrees of reduction of medial development is observed in the remaining species. It is noteworthy that most species of casque-headed *Trachycephalus* exhibit some degree of medial

development of vocal sac mucosae, while all species that present mucosae that are restricted to the lateral portion of the submandibular region (ch. 9.2) are not casque-headed. This explains Duellman's (1956) observation that *Trachycephalus* (*sensu* Duellman, 1956) was distinguished from *Phrynohyas* by the presence of subgular expansion of the vocal. Still, the presence of medial development of this structure in some non-casque-headed *Trachycephalus* contradicts the notion that this could be used to distinguish them.

Furthermore, paired vocal sacs originated several times in Anura, including a wide array of forms with variable degree of independence (Liu, 1935). In most of these species, paired vocal sacs remain subgular and form two small spheres below the angles of the jaw, as seen in *Euphlyctis* + *Hoplobatrachus* + *Nannophrys* (Dicroglossidae); *Pseudis minuta* + *P. cardosoi* (Hylidae); Hylodidae (except for *M. goeldii*); many *Gephyromantis* (Mantellidae); Odontobatrachidae; Ptychadenidae; and many species of *Amolops*, *Huia*, *Humerana*, *Hydrophylax*, *Meristogenys*, *Odorrana*, and *Staurois* (Ranidae). Furthermore, in other lineages vocal sacs developed dorsally occupying the post-tympanic region. This occurred in some *Limnonectes* (Dicroglossidae); some Lophyohylini (Hylidae); the *Boophis albilabris* species group (Mantellidae); *Glyphoglossus molossus* (Microhylidae); *Nyctibatrachus* (Nyctibatrachidae); *Pelophylax*, and many *Lithobates* and *Rana* (Ranidae, Boulenger, 1882; Liu, 1935; Inger, 1954; Glaw & Vences, 2007; Biju *et al*., 2011; Barej *et al*., 2015; Elias-Costa *et al*., 2017; Elias-Costa & Faivovich, 2019; Targino *et al*., 2019). Vocals sacs in the diverse genus *Nyctibatrachus* and the *Boophis albilabris* species group resemble those of *Nyctimantis siemersi*, *Itapotihyla*, *Osteocephalus*, *Osteopilus* and *Tepuihyla* inasmuch as paired lobes of the *m. interhyoideus* develop dorsally behind the mandibular joint. The variable degree of gular inflation among these species (lesser in *Boophis* but extensive in *Nyctibatrachus jog*, Gururaja, 2012; Köhler *et al*., 2017; Vences *et al*., 2010) could be likely related to varied medial development of internal mucosae.

Among all frog lineages, extreme dorsal projection of lateral vocal sacs originated only three times in Anura: in *Trachycephalus*, *Pelophylax* and *Lithobates areolatus*, each with a distinctive pattern. In *Pelophylax* and *L. areolatus*, vocal sacs do not exceed greatly the margins of the head but a highly localized skin modifications enables that vocal sacs are inflated vertically. In some *Trachycephalus*, on the other hand, vocal sacs can reach great volumes above the head. This dorsal projection is enabled by extreme lateral development of the paired lobes of the *m. interhyoideus*, which insert in the *fascia dorsalis*, forming a unique anatomical

pattern. Hence, skin modification in *Trachycephalus* is seemingly related to the maximum volume of the inflated vocal sacs, rather than their overall shape.

The complex function of anuran vocal sacs has been thoroughly revisited in recent years. Traditionally, they were thought to act as resonating chambers, but this hypothesis was rejected and they are currently thought to play a much bigger role in the reproductive biology of frogs, including facilitating lung re-inflation, reducing the impedance mismatch with the external medium and the performance of diverse multimodal displays (Ryan, 1985; Rand & Dudley, 1993; Rosenthal *et al.*, 2004; Pauly *et al.*, 2006; Taylor *et al.*, 2008; Sternberger *et al.*, 2014; Elias-Costa *et al.*, 2017). It has been suggested that paired vocal sacs act to maximize the exposure of the internal medium to the atmosphere, reducing the impedance mismatch between these media and facilitating sound transmission during vocalization in the water-air surface (Prestwich *et al.*, 1989; Wells, 2007).

In fact, as is shown by optimization of breeding site (ch. 119), males of most species of *Trachycephalus* breed in ponds and are known to vocalize while floating on water (Fig. 5). Furthermore, our results indicate that breeding in phytotelmata evolved twice in *Trachycephalus*: once in the clade formed by *T. resinifictrix* and *T. hadroceps*, and another in *T. cunauru*. This is convergent with Blotto *et al.* (*in press*), which also obtained two instances of evolution of phytotelm breeding in this group. Interestingly, *T. hadroceps* and *T. helioi* (not included in this study, but also breeds in tree holes; Nunes *et al.*, 2013) are the only two species of this genus that present reductions in the degree of lateralization of the vocal sacs (Nunes *et al.*, 2013).

Previous studies have implied that vocal sacs are secondarily reduced in species that breed in phytotelmata, due to the limited volume which hinders the inflation of large structures (Jungfer *et al.*, 2013; Duellman, 2019). An example is *Osteocephalus oophagus*, which contrasts with the pond-breeding species of its group inasmuch as they possess small body sizes, less developed vocal sacs, small egg clutches in small intervals, and lack dorsal tubercles, all characters putatively associated with their reproduction in bromeliads (Jungfer & Weygoldt, 1999; Jungfer *et al.*, 2013). A similar scenario is observed in the *Osteocephalus planiceps* species group (Jungfer *et al.*, 2013). Our results provide further evidence for this association between vocal sac morphology and breeding/calling site in Lophyohylini, which would act as an important evolutionary pressure (Fig. 5). Thus, an evolutionary scenario emerges where, from an ancestral state of vocal sacs without any degree of lateralization, there is the development of posterolaterally bilobate sacs and, subsequently, of extremely lateralized sacs.

However, in the cramped environments of phytotelms, there would be a selective pressure for smaller, more discrete vocal sacs, with less lateral expansion.

Skull hyperossification and chin-tucking for phragmosis: The evolution of skull hyperossification was extensively discussed by Blotto *et al.* (*in press*) for Lophyohylini. Nevertheless, differences in our topology render a few comments regarding *Trachycephalus*. Specifically, the nesting of the clade formed by *T. atlas, T. mambaiensis* and *T. nigromaculatus* as the subsequent diverging group after *T. jordani* renders hyperossification as plesiomorphic for this genus. Thus, a reversal to non-hyperossified skulls would have occurred in the remaining *Trachycephalus*, which then retained varying degrees of exostosis. Albeit the case for *T. atlas, T. mambaiensis* and *T. nigromaculatus* remaining the same (as expected by Blotto *et al.*, *in press*), this scenario is more congruent with what was put forward by Faivovich *et al.* (2005). The absence of *T. venezolanus* in our analysis adds uncertainty, but should future studies recover a similar topology with this species grouped alongside the remaining casque-headed *Trachycephalus*, the resulting evolutionary scenario would be more parsimonious than previous hypotheses.

Similarly, the behaviour of chin-tucking for phragmosis is recovered as plesiomorphic in *Trachycephalus*, occurring in all casque-headed species. Phragmosis, or phragmotic behaviour, is the behaviour of using its own body as a barrier to defend itself in a burrow-like shelter (see Toledo *et al.*, 2011 for an overview of defensive behaviours in frogs). In frogs, this is related to modifications of the ends of the body to use the head to close tubular burrows (Wheeler, 1927). This behaviour has previously been associated with co-ossification (see Blotto *et al., in press*) and also considered to be an antipredator behaviour or a mechanism to avoid dehydration (Lutz & Lutz, 1939; Duellman & Klass, 1964; Jared *et al.*, 2005; Toledo *et al.*, 2011). Although with the same reservations described above, our results provide a more parsimonious scenario when compared with previous hypotheses, where phragmosis is the plesiomorphic condition and associated with casque-headed species that use bromeliads as refuges. A reversal would have occurred in the remaining species, together with reduction in skull hyperossification. Since the head is the most exposed part of the frog in bromeliads, strengthening of the skull would be a consistent evolutionary response (Trueb, 1973; Blotto *et al., in press*).

Distal extensor muscles of the foot: The distal extensor muscles of the foot, *mm. dorsometatarsales distales*, were described by Faivovich (2002) for some species of *Smilisca*, *Scinax* and *Ololygon*, although ambiguous references to them were already present in the literature (Dunlap, 1960; Andersen, 1978). Attention was drawn to their topological similarity with the *mm. dorsometacarpales distales* of the hand, described by Burton (1996) who considered them as probable adaptations to climbing and arboreality (Burton, 1998). In his assessment of hylid foot musculature, Burton (2004) stated that, although the distal extensors of the foot could also be associated with climbing, he encountered a high degree of variation. In fact, his observation that variability in *mm. dorsometatarsales distales* (= *mm. extensores breves distales*) occurred amongst arboreal species and even between different feet of the same frog, was one of the factors that led him to consider that natural selection allows a "good deal of slack" in the anatomy of foot muscles (Burton, 2004).

Notwithstanding, our optimizations of these characters (chs. 56-60) among the examined lophyohylines (all of which present arboreal habits) shows that only *Phyllodytes* presented these muscles. The fact that reduction in snout-vent length is a putative synapomorphy of this genus in comparison to most clades of Lophyohylini (Blotto *et al.*, *in press*) could imply a possible association between body size reduction and presence of the distal extensor muscles of the foot. These muscles insert dorsally in the ultimate phalanges together with the *mm. dorsometatarsales proximales*, and their contraction aids probably aids in peeling the toe discs from the substrate, as occurs in their correlates in the hands (Burton, 1998). Whether smaller frogs would require additional muscles for a finer control of the toe discs or if the presence of these muscles are related to other environmental factors requires further studies. A larger sampling among hylids and other arboreal anurans would also help further establish this putative association between SVL and distal extensor musculature of the feet.

CONCLUDING REMARKS

Herein we presented the description of the muscular anatomy of *Trachycephalus typhonius* and a cladistic analysis of *Trachycephalus* using a total evidence approach. Our final dataset used for the phylogenetic reconstruction encompassed 127 phenotypic characters from myology, osteology, larval morphology and reproductive biology, as well as DNA sequences for seven mitochondrial and four nuclear loci, and 44 terminals (17 ingroup species, 26 outgroup species). We recovered a monophyletic *Trachycephalus* with high support and

synapomorphies being *m. interhyoideus* and vocal sac mucosae presenting great lateral extension, exceeding the post-tympanic fold (chs. 5.3 and 7.3). These character states are responsible for what is usually described as "paired lateral vocal sacs that protrude behind the angle of the jaw" and our results represent the first robust test of their condition as synapomorphy for this genus.

Our results further subsidised the resurrection of *Hyla spilomma* (Cope, 1877) for eastern Mexican populations of *Trachycephalus*, resulting in *Trachycephalus spilommus* (Cope, 1877) **comb. n.** Furthermore, based on our phylogenetic hypothesis we discussed the evolution of a series of characters. Paired lateral vocal sacs were found to be associated with reproduction in ponds, while reduced and less conspicuous vocal sacs are associated with reproduction in phytotelms. Regarding skull hyperossification and phragmosis, we found that phragmosis is the plesiomorphic condition and associated with species with a hyperossified head that use bromeliads as refuges. A reversal would have occurred in the remaining species, along with a reduction in skull hyperosification. Finally, we observed that the distal extensor muscles of the foot reach their maximum development among lophyohylines in *Phyllodytes*, suggesting a possible association between reduction in SVL and increase in the development of these muscles. Despite our sampling, support values among interspecific relations in *Trachycephalus*, as well as among intergeneric relations was low. Further studies would benefit by the inclusion of all *Trachycephalus* species currently recognised (*sensu* Blotto *et al.*, *in press*) and a larger character sampling.

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FIGURES

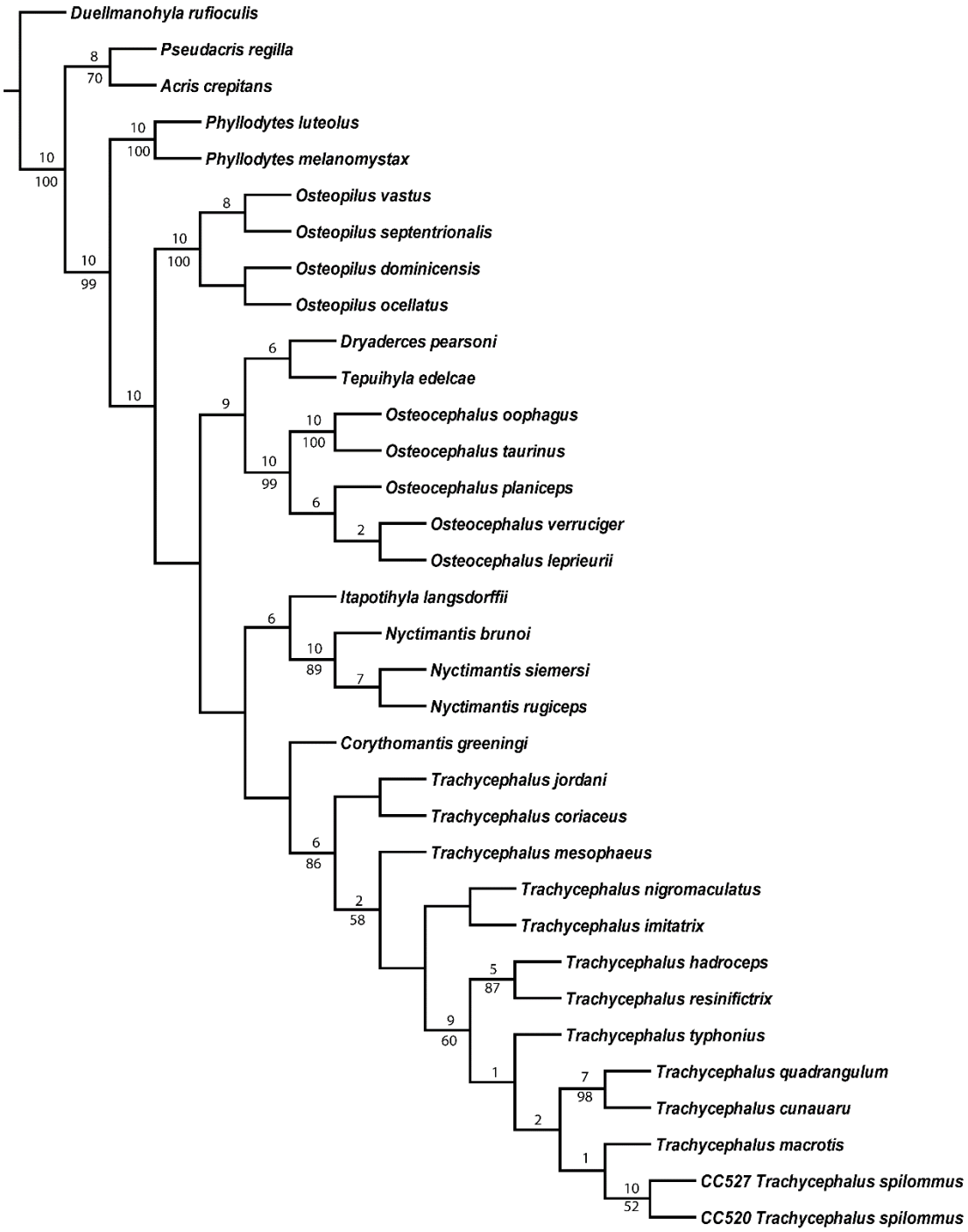

Figure 10. Most parsimonious tree obtained with MD analysis. Goodman-Bremer (above) and Jackknife (below) support values are shown at the nodes.

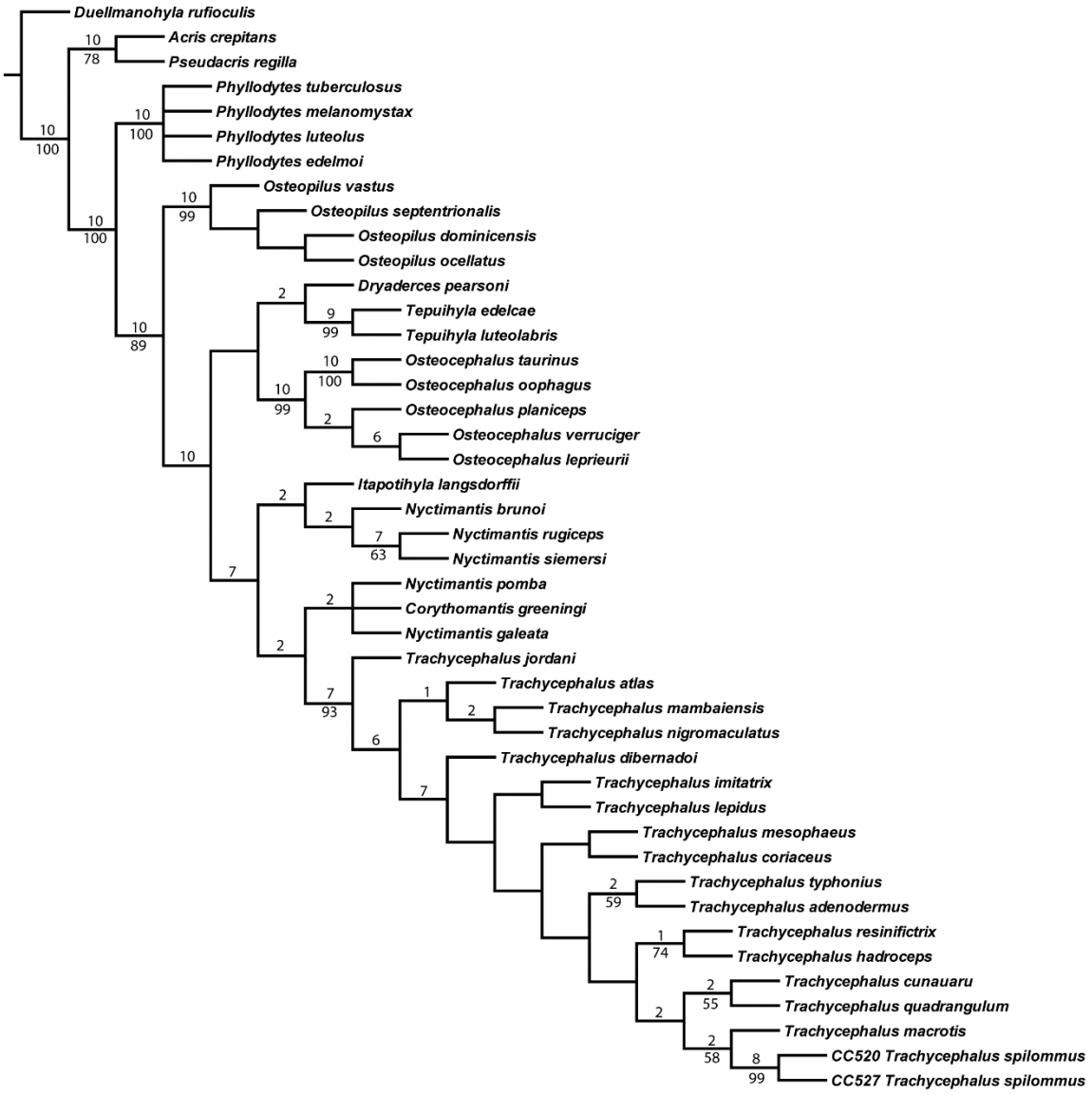

Figure 2. Strict consensus tree obtained with TE analysis. Goodman-Bremer (above) and Jackknife (below) support values are shown at the nodes.

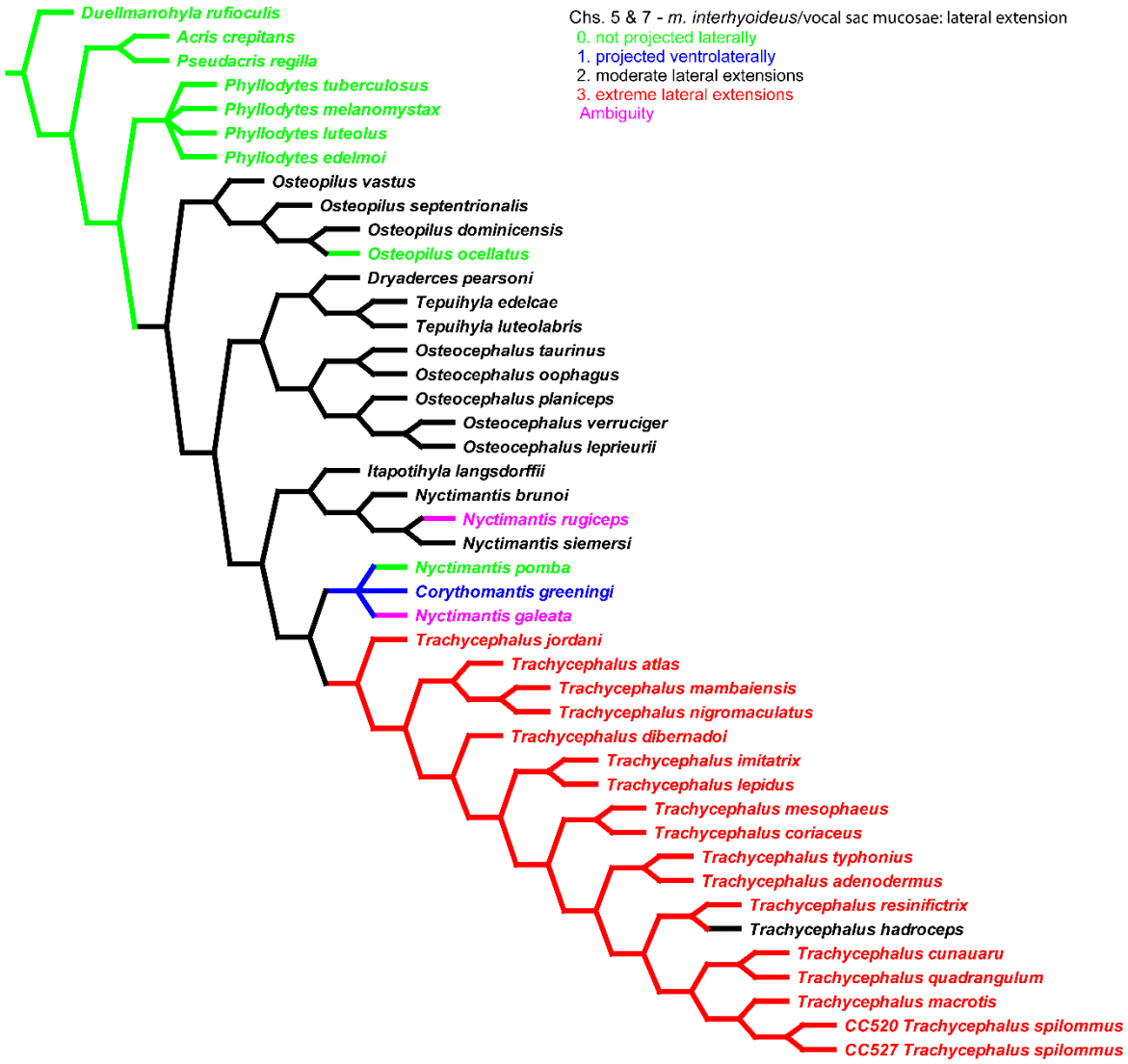

Figure 3. Optimization of characters 5 and 7 on the consensus topology obtained from the TE analysis.

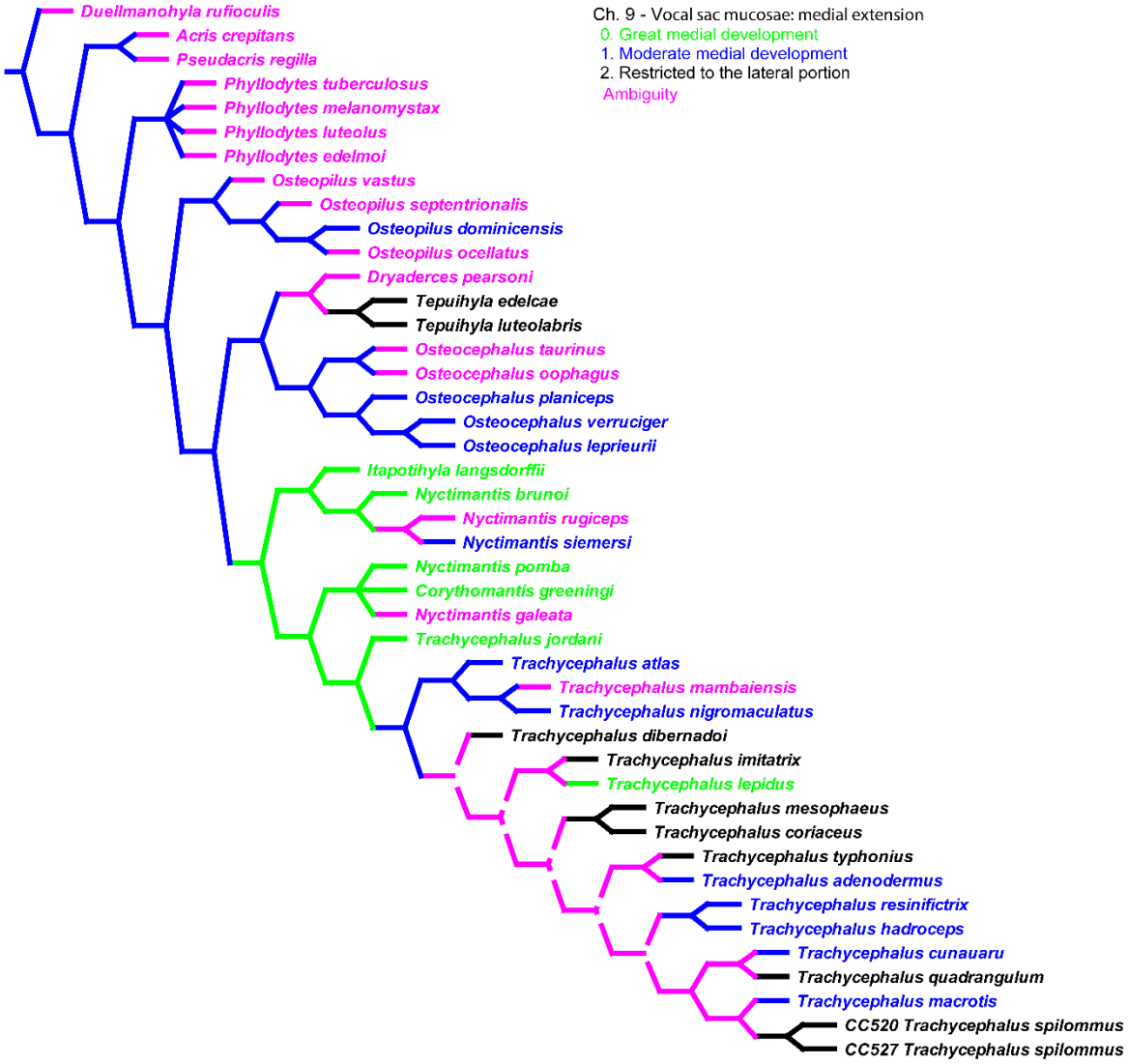

Figure 4. Optimization of character 9 on the consensus topology obtained from the TE analysis.

Figure 511. Optimization of characters 5 and 7, on the left side, and character 119, on the right side, on the consensus topology obtained from the TE analysis.

APPENDIX S1. List of studied specimens.

Abbreviations are as follows: M – specimen studied for myology. C – cleared and double stained. DS – dry skeleton. X – x-ray.

Outgroup: ***Acris crepitans:*** MNRJ 12558 (M). ***Pseudacris regilla:*** MNRJ 38022 (M). ***Duellmanohyla rufioculis:*** MNRJ 14644 (M). ***Corythomantis greening*****:** MNRJ 76754 (DS); MNRJ 15867 (C); MNRJ 15866 (M). ***Dryaderces pearsoni:*** KU 164086 (C); KU 164087 (C). ***Dryaderces* aff. *pearsoni:*** MNRJ 86410 (M). ***Itapotihyla langsdorffii*****:** MNRJ 43910 (C); MNRJ 58062 (C); MNRJ 63214 (M). ***Nyctimantis brunoi*****:** KU 92213 (C); MNRJ 247 (holotype); MNRJ 15705 (DS); MNRJ 45781 (M). ***Nyctimantis pomba*****:** MZUFV 11519 (M). ***Nyctimantis siemersi*****:** KU 116937 (C); MACN 6635 (M). ***Nyctimantis galeata*****:** MNRJ 27173-27174; MNRJ 27175 (C). ***Nyctimantis rugiceps:*** KU 150489 (C); KU 164087 (C). ***Osteocephalus leprieurii:*** KU 126628 (C); KU 126630 (C). ***Osteocephalus oophagus:*** MNRJ 56625 (M). ***Osteocephalus planiceps:*** MNRJ 40385 (M). ***Osteocephalus taurinus:*** KU 99421 (C); KU 175501 (C); MNRJ 52966 (M). ***Osteocephalus verruciger:*** KU 143218 (C); KU 164413 (C); MNRJ 78672 (M). ***Osteopilus dominicensis:*** KU 84699 (C); MNRJ 72169 (M). ***Osteopilus ocellatus:*** KU 287866 (C). ***Osteopilus septentrionalis:*** KU 265364 (C). ***Osteopilus vastus:*** KU 84710 (C). ***Phyllodytes edelmoi:*** MNRJ 54396 (M). ***Phyllodytes luteolus:*** KU 92212 (C); KU 187476 (C); MNRJ 23298 (C); MNRJ 33711 (C); MNRJ 36531 (C). ***Phyllodytes melanomystax:*** MNRJ 51719 (C); MNRJ 32955 (M). ***Phyllodytes tuberculosus:*** MNRJ 46472 (M). ***Tepuihyla edelcae:*** MNRJ 78673 (M; X).

Ingroup: ***Trachycephalus "vermiculatus" (spilommus):*** MNRJ 78677 (M); USNM 167743-167749; USNM 194904; USNM 194886-194901; AMNH 55660; AMNH 109281; UMMZ 71211; AMNH 74377-74390; AMNH 101053-101110; UMMZ 75301-75309.6; USNM 25141-25142; CAS 69726-69731; TCWC 16782-16791; USNM 117601-117602; USNM 225147-225175; USNM 241876-241969; AMNH 54910; USNM 514292; USNM 563423; 563951; AMNH 54915-54916; USNM 217565-217581; USNM 225109-225146; 242826-243061; USNM 514293-514295; USNM 523469; AMNH 54799; USNM 559232-559237; USNM 563422; USNM 565386; USNM 570500; USNM 573824; USNM 573825-573826; USNM 523186-523188; MCZ 26426; MCZ 26450; TCWC 19180-19184; AMNH 176420; AMNH 176429; MZUSP 76227; USNM 115013 (holotype of *Acrodytes modesta*); USNM 115010–115012; USNM 115015–115025 (paratypes of *Acrodytes modesta*); USNM

266253-266255; UMMZ 80018; UMMZ 108019; FMNH 100046 (holotype of *Acrodytes inflata*); AMNH 59198-59199; UMMZ 104814; UIMNH 67060 (holotype of *Phrynohyas corasterias*); AMNH 73367-73370; AMNH 78836; AMNH 109287-109296; AMNH 176422, USNM 46917; AMNH 176424-176428; AMNH 52954; AMNH 57740-57742; AMNH 71419; AMNH 176371-176373; AMNH 182549; USNM 114979; USNM 244726-244727; BMNH 83.2.7.1 holotype of *Phrynohyas latifasciata*); AMNH 60314-60316; AMNH 176417; AMNH 176419; USNM 115009; AMNH 176374; AMNH 6300; AMNH 52147; AMNH 71418; AMNH 73418-73419; AMNH 109282-109286; AMNH 176375; AMNH 176403-176416; AMNH 176418; AMNH 176421; AMNH 176423; MZUSP 77289-77290; UMMZ 88796.1-88797.5; USNM 38264-38265; USNM 38303; USNM 114980-115008; USNM 315393; ANSP 15430. ***Trachycephalus adenodermus:*** MNRJ 89969 (M); MNRJ 4054. ***Trachycephalus atlas:*** MNRJ 19291 (M); MNRJ 4028 (X); MZUSP 119815 (DS). ***Trachycephalus coriaceus:*** MNRJ 73316 (M); MNRJ 77314 (X); MZUSP 60165 (DS). ***Trachycephalus cunauaru:*** MNRJ 74127 (M). ***Trachycephalus dibernadoi:*** MNRJ 55150; MNRJ 55151 (M). ***Trachycephalus hadroceps:*** MNHNP 1999.8601 (M; X). ***Trachycephalus imitatrix:*** MNRJ 108928(DS); MNRJ 5129 (X); MNRJ 5130 (M). ***Trachycephalus jordani:*** MNRJ 73366 (M; X). ***Trachycephalus lepidus:*** MZUSP 136548 (M); MZUSP 136549-136551; CFBH 2447 (C). ***Trachycephalus macrotis:*** MNRJ 78675 (M); AMNH 72372-72375; AMNH 79325-79331; AMNH 34071; AMNH 68090; MZUSP 105906; AMNH 82133-82137; USNM 152012-152016; USNM 152198; MZUSP 12629-12630; USNM 165977-165978; USNM 165981-165986; USNM 320883; MZUSP 77568-77569; AMNH 42393; USNM 317377-317394; USNM 560337-560345; USNM 568774-568784; AMNH 42349-42351; AMNH 42607; AMNH 127169; MZUSP 10340; USNM 325139; USNM 342605; USNM 342966; AMNH 42164; USNM 80612; UMMZ 57397; MZUSP 105303; UMMZ 55570 (holotype of *Phrynohyas ingens*); UMMZ 55567–55569 (paratypes of *Phrynohyas ingens*). ***Trachycephalus mambaiensis:*** MZUSP 135713 (DS); ***Trachycephalus mesophaeus:*** MNRJ 15863 (M); MNRJ 8839 (M); MNRJ 51514 (DS). ***Trachycephalus nigromaculatus:*** MNRJ 50992 (M). ***Trachycephalus quadrangulum:*** MNRJ 78676 (M). ***Trachycephalus resinifictrix:*** MNRJ 74126, MZUSP 57345. ***Trachycephalus typhonius:*** MNRJ 20362 (M); MNRJ 43172 (M); MNRJ 78678 (M); (MNRJ 55221); AMNH 94199; USNM 566123; USNM 162939-162941); MCZ 2618; USNM 164179; AMNH 141142-141143; AMNH 167105-167106; AMNH 23128; AMNH 70977; UMMZ 55834; UMMZ 80495.

APPENDIX S2. Literature references for character scoring.

Species	Character	Reference
Acris crepitans	Chs. 120-127	Altig & McDiarmid (2015)
Pseudacris regilla	Chs. 120-127	Altig & McDiarmid (2015)
Duellmanohyla rufioculis	Chs. 120-127	Campbell & Smith (1992)
Corythomantis greeningi	Chs. 120-127	Juncá et al. (2008)
Itapotihyla langsdorffii	Chs. 120-127	Pimenta & Canedo (2007)
Nyctimantis brunoi	Chs. 120-127	Wogel et al. (2006)
Nyctimantis siemersi	Chs. 120-127	Sá (1983)
Osteocephalus taurinus	Chs. 120-127	Schiesari et al. (1996)
Osteocephalus verruciger	Chs. 120-127	Ron et al. (2010)
Osteocephalus oophagus	Chs. 120-127	Jungfer & Schiesari (1995)
Osteopilus dominicensis	Chs. 120-127	Galvis et al. (2014)
Osteopilus septentrionalis	Chs. 120-127	Lanoo et al. (1987)
Osteopilus vastus	Chs. 120-127	Galvis et al. (2014)
Osteopilus ocellatus	Chs. 120-127	Lanoo et al. (1987)
Phyllodytes	Chs. 120-127	Bokerman (1966); Caramaschi et al. (1992); Magalhães et al. (2015)
Tepuihyla edelcae	Chs. 120-127	Myers & Donnelly (2008)
Trachycephalus typhonius	Chs. 120-127	Duellman (1970); Schiesari et al. (1996)
Trachycephalus mesophaeus	Chs. 120-127	Lutz (1973); Carvalho-e-Silva et al. (2002); Prado et al. (2003)
Trachycephalus jordani	Chs. 120-127	McDiarmid & Altig (1989-1990)
Trachycephalus atlas	Chs. 120-127	Barreto et al. (2015)
Trachycephalus nigromaculatus	Chs. 120-127	Wogel et al. (2000)
Trachycephalus coriaceus	Chs. 120-127	Schiesari & Moreira (1996); Lescure et al. (1996)
Trachycephalus cunauaru	Chs. 120-127	Grillitsch (1992)
Trachycephalus resinifictrix	Chs. 120-127	Hero (1990); Schiesari et al. (1996)
Trachycephalus adenodermus	Chs. 120-127	Nunes (2012)
Trachycephalus spilommus	Chs. 120-127	Pyburn (1967)

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APPENDIX S3. Phenotypic characters. Novel characters are marked with an asterisk (*).

Head Musculature

1. ***M. intermandibularis*, relation with *m. submentalis*.** Variation in this musculature was described by Tyler (1971a) and employed in a phylogenetic study by Da Silva (1998) for Hylids. Tyler (1985) discussed these characters for Sooglossidae. Herein, We followed Da Silva's definition of character states unless otherwise noted (see his characters 96 to 100): 0. *M. intermandibularis* is separated from *m. submentalis*; 1. *M. intermandibularis* is adjacent to or partially overlaping the *m. submentalis* posteriorly; 2. *M. intermandibularis* is connected to *m. submentalis* posteromedially; 3. *M. intermandibularis* is connected to *m. submentalis* posterolaterally.
2. ***M. intermandibularis*, medial portion, morphology.** We modified Da Silva's definition and included additional character states describing variation in the medial *aponeurosis*: 0. *M. intermandibularis* with medial *aponeurosis* that is homogeneous in its width; 1. *M. intermandibularis* with medial *aponeurosis* that is heterogeneus in width, widened in its medial portion; 2. *M. intermandibularis* with medial *aponeurosis* that is heterogeneous in width, widened in its distal portion; 3. *M. intermandibularis* with medial *aponeurosis* that is heterogeneous in width, widened in its proximal portion; 4. *M. intermandibularis* with medial raphe; 5. Araphic *M. intermandibularis*.
3. ***M. intermandibularis*, differentiated supplementary apical portion.** We followed Da Silva's definition of character states in treating the IM supplementary portions as independent muscles: 0. Absent; 1. Present.
4. ***M. interhyoideus*, medial portion, morphology.** We modified Da Silva's definition and included an additional character states for the presence of a medial *aponeurosis* on the *m. interhyoideus*: 0. Araphic *m. interhyoideus*; 1. *M. interhyoideus* with medial raphe; 2. *M. interhyoideus* with medial *aponeurosis*.
5. ***M. interhyoideus*, lateral extension.** Tyler (1971a) described the different morphologies for this muscle and Da Silva (1998) used the same variation as characters. Herein we only dealt with lateral extension, treating it as an independt character from subgular development, as Favivovich (2002). The lateral extension of the vocal sac mucosae was also considered a separate character. Lateral extension was not present when the mucosa or mucosae do not exceed the mandibular joint from a ventral view. This was observed in studied specimens of *Nyctimantis brunoi* and *Phyllodytes*, for example. The *m. interhyoideus* and the mucosa or mucosae can project ventrolaterally in some species. This lateral elongation of a subgular structure represents the first degree of lateralization observed in Lophyohylini and is present in *Corythomantis*. In many species, the *m. interhyoideus* and, with it, the vocal sac mucosae develop further dorsally to occupy a position just posterior to the mandibular joint. Fibres of the this muscle, which in most frogs are transversely oriented (from their origins in the distal portion of the anteromedial processes of the hyoid, near the otic capsules, to their insertion in the midline), radiate posteriorly in these species. Moderate lateral extensions of vocal sac mucosae occur in *Nyctimantis siemersi*, *Itapotihyla*, most *Osteocephalus* and *Osteopilus*, and *Tepuihyla edelcae*. Supramandibular lobes of the *m. interhyoideus*, or lateral extensions of paired

vocal sacs behind the angles of the jaw were also reported for *Dryaderces pearsoni* (Trueb & Duellman, 1971) and are observed in *Oc. verruciger* (Jungfer *et al.*, 2013). In *Trachycephalus* (except for *T. hadroceps* and *T. helioi*), the lateral extensions of the *m. interhyoideus* and the mucosae extend further dorsally exceeding the post-tympanic fold, forming a broad and convoluted dorsal sac. The reference of the post-tympanic fold must not be confused with the extension of the skin modification. In most *Trachycephalus*, the inflatable portions of the skin, evident externally due to their dark pigmentation and high degree of folding, are delimited dorsally by the post-tympanic fold. It must be noted that this does not imply a limit for the internal anatomy. 0. *M. interhyoideus* not projected laterally; 1. *M. interhyoideus* projected ventrolaterally; 2. *M. interhyoideus* with moderate lateral extensions; 3. *M. interhyoideus* with lateral extensions exceeding the post-tympanic fold.

6. ***Vocal sac mucosae, lateral extension.** 0. Mucosae not projected laterally; 1. Mucosae projected ventrolaterally; 2. Mucosae with moderate lateral extensions; 3. Mucosae with lateral extensions exceeding the post-tympanic fold.
7. ******M. interhyoideus*, **insertion on the** ***fascia dorsalis*****.** In anurans, myo-integumental attachments, formed by sheets of connective tissue, connect the skin to the adjacent muscles generating a series of compartments, which constitutes the lymph sacs (Tyler, 1971b). The submandibular lymph sac is delimited posteriorly by the postmandibular septum, which possibly retracts the skin associatted with the vocal sac in the course of its deflation. This septum normally originates from the posterolateral margin of the skull and is attached to the *m. interhyoideus* in two points: near its origin and, ventrally, at the posterior submandibular margin of this muscle (Tyler, 1971b). When there is no lateral development of *m. interhyoideus*, this lymphatic septum is transverse and extends approximately between the two mandibular joints. In frogs that present lateral extensions of the *m. interhyoideus*, the postmandibular septum is projected posterodorsally. In this case, the septum can either accompany the muscular lobes, connecting them with the surrounding skin; or form a sheath around the muscular lobes that associates with the *fascia dorsalis*, providing secondary anchorage. This association can be even further developed as some fibers of the *m. interhyoideus* insert directly in the *fascia*. Duellman (1956) already observed this condition in *Trachycephalus "vermiculatus"* (= *Phrynohyas spilomma* in his study), reporting that the vocal sac was attached to the musculature of the suprascapular region, over the posterior part of the *m. depressor mandibulae* and most of the *m. latissimus dorsi* (state 1, Fig. S3.1). We observed this condition in all examined species of *Trachycephalus* with the exception of *T. resinifictrix* and *T. venezolanus*. Other species that presented association of the *m. interhyoideus* with the *fascia dorsalis* were *Osteocephalus leprieurii*, *Oc. planiceps*, *Oc. taurinus*, *Oc. oophagus*, *Oc. verruciger* and *Tepuihyla edelcae.* In all remaining species this association was absent or could not be observed (state 0). 0. Absent; 1. Present.
8. ***Vocal sac mucosae, internal disconnection.** Adult males with single and paired and vocal sac cavities were observed. First mentions of the disconnection of paired internal mucosae in adult males date back to Inger & Greenberg (1956) for *Ptychadena porossissima* (Ptychadenidae), and McCallister (1959) for *Spea* (Scaphiopodidae). Nevertheless, it was first used in a phylogenetic context for Hylodidae and related groups (Elias-Costa *et al.*,

2017), and for Victoranura (Elias-Costa & Faivovich, 2019). Tyler (1971a) mentioned that in the vocal sac of *Nyctimantis brunoi* and *Corythomantis greeningi* "medial adhesions prevent communication between the two portions of the paired submandibular structure", most likely referring to the disconnection of paired mucosae in this species. Elias-Costa *et al.* (2017) reported disconnected mucosae in *Trachycephalus typhonius*, used as outgroup for their study. Single vocal sac cavities (i.e. fused mucosae) were observed in *Phyllodytes*, and *Oc. Taurinus* (Fig. S3.2A). In all remaining species examined, mucosae remained disconnected in adult males. 0. Absent, mucosae internally connected forming a single cavity; 1. Present, mucosae internally disconnected.

9. ***Vocal sac mucosae, degree of medial extension.** The occurrence of paired vocal sac cavities by itself does not correlate with externally paired vocal sacs. The morphology of each projection of the buccal floor can greatly affect the overall shape of the inflated vocal sac, as it defines the area available for air to occupy. In species in which mucosae develop greatly towards the midline, the inflatable portion of the vocal sac is extended to the centre of the gular region. In extreme cases, paired mucosae contact mutually in the midline, functionally resembling a single-cavity vocal sac. In this way, disconnection of vocal sac mucosae was observed in species with single subgular, bilobated, paired subgular or paired lateral vocal sacs, all which show a variable degree of medial development of each contralateral element. In *Nyctimantis brunoi*, *Corythomantis*, *Osteocephalus planiceps*, *Oc. verruciger*, *Osteopilus dominicensis*, *Tepuihyla edelcae*, *Trachycephalus jordani*, *T. hadroceps* and *T. helioi*, mucosae are disconnected, but greatly developed medially (Fig. S3.2B-C). As a general reference, paired mucosae occupy the area defined laterally by the insertions of the *m. submentalis*, ventral to the sternum. They can even establish mutual contact, functionally resembling a single-cavity vocal sac. Inflation of the subgular portion of the vocal sac, regardless of the presence of lateral lobes, is important. In *Nyctimantis siemersi*, *Itapotihyla*, *Trachycephalus nigromaculatus* and *T. resinifictrix*, moderate medial development was observed (state 1, Fig. S3.2D). Contralateral elements occupy the area defined medially by insertion of the *m. submentalis* and, laterally, by the lateral limit of the *m. geniohyoideus lateralis*. In some species, paired mucosa are extremely lateral and do not exceed the *m. geniohyoideus lateralis* from a ventral view (Fig. S3.2E-F). This state was observed in *Trachycephalus venezolanus*, *T. coriaceus*, *T. typhonius*. 0. Great medial development, exceeding the lateral limit of the *m. geniogyoideus medialis;* 1. Moderate medial development, not exceeding the lateral limit of the *m. geniohyoideus medialis*; 2. Mucosae restricted to the lateral portion, not exceeding the lateral limit of the *m. geniohyoideus lateralis*. This character was considered additive.
10. **Vocal apertures, shape.** Variation in this structure was described by Tyler (1971a) and employed in a phylogenetic study by Da Silva (1998) for Hylids. We followed Da Silva's definition of character states (character 102). 0. Vocal apertures in the shape of a small orifice; 1. Vocal apertures with slit shape; 2. Vocal apertures in the shape of a gaping hole. This character was considered additive.
11. ***M. geniohyoideus lateralis*, extension of the division of this muscle in medial and lateral portions.** The *m. geniohyoideus lateralis* can be divided along its whole length in two portions, a medial and lateral one, as in *Itapotihyla langsdorffii*, *Phyllodytes* and some

Trachycephalus. On the remaining species examined, the division of the *m. geniohyoideus lateralis* in two portions only occurs distally, near the *m. intehyoideus*. 0. *m. geniohyoideus lateralis* divided only on in its distal portion; 1. *m. geniohyoideus lateralis* divided along its whole length.

12. ***M. geniohyoideus lateralis*, point of intrusion of the *m. sternohyoideus*, relation with the episternum**. 0. Point of intrusion of the *m. sternohyoideus* completely visible, not covered by the episternum; 1. Point of intrusion of the *m. sternohyoideus* covered by the episternum.
13. ***M. geniohyoideus lateralis*, relation with the *m. geniohyoideus medialis*:** 0. *m. geniohyoideus lateralis* is in contact with the *m. geniohyoideus medialis* from the first third of its length; 1. *m. geniohyoideus lateralis* is in contact with the *m. geniohyoideus medialis* only at the final third of its length; 2. *m. geniohyoideus lateralis* and *m. geniohyoideus medialis* not in contact. This character was considered additive.
14. ***M. depressor mandibulae*, slip originating from the *annulus tympanicus*.** Manzano *et al.* (2003) described variation in the *m. depressor mandibular* for Anura and noted that in hylids this muscle may present up to three slips: one originating from otic process of the squamosal, a second slip originating from the *fascia dorsalis* at the level of the *m. dorsalis scapulae*, and a third slip originating from the *annulus tympanicus*. In the outgroup, the slip originating from the *annulus tympanicus* is Absent. This character was employed by Faivovich (2002; character 41). 0. Absent; 1. Present.
15. ***M. depressor mandibulae*, slip originating from the *fascia dorsalis* at the level of the *m. dorsalis scapulae*.** This character is similar to character 40 of Faivovich (2002). In *Phyllodytes*, the *m. depressor madibulae* muscle has only two slips, with the absence of a slip originating from the *fascia dorsalis* and *m. deltoideus scapularis.* 0. Absent; 1. Present.
16. ***M. depressor mandibulae*, slip originating from the *fascia dorsalis* at the level of the *m. dorsalis scapulae*, extension.** 0. Fibres of the scapular slip of *m. depressor madibulae* overlap only the first half of the *m. deltoideus scapularis*; 1. Fibres of the scapular slip of *m. depressor madibulae* overlap the whole *m. deltoideus scapularis*, but does not extend pass it; 2. Fibres of the scapular slip of *m. depressor madibulae* overlap the whole *m. deltoideus scapularis* and extend until the *m. latissimus dorsi*. This character was considered additive.
17. ***M. levator mandibulae posterior longus.*** In species with extensive hyperossification of the frontoparietals, such as *Trachycephalus jordani, T. atlas, T. nigromaculatus* and some *Nuctimantis*, the region where the *m. levator mandibulae posterior longus* is usually found is completely occupied by bones, due to dermal coossification. Thus, in these species, the *m. levator mandibulae posterior longus* was considered to be occluded. 0. Occluded; 1. Exposed.
18. ***M. levator mandibulae internus.*** In species with extensive hyperossification of the frontoparietals, such as *Trachycephalus jordani, T. atlas, T. nigromaculatus, Corythomantis* and some *Nyctimantis*, the region where the *m. levator mandibulae internus* is usually found is completely occupied by bones, due to dermal coossification. Thus, in these species, the *m. levator mandibulae internus* was considered to be occluded. 0. Occluded; 1. Exposed.

Axial Musculature

19. *_M. rectus abdominis_, point of division in two slips. 0. *M. rectus abdominis* divides into two slips at the level of the first *inscriptio tendinea*; 1. *M. rectus abdominis* divides into two slips at the level of the second *inscriptio tendinea*.

Pectoral Girdle Musculature

20. *M. pectoralis portio abdominalis*, relation with the *m. obliquus externus*. This character was employed by Da Silva (1998; character 103) and Faivovich (2002; character 39). 0. *m. pectoralis portio abdominalis* is overlapped by *m. obliquus externus*; 1. *m. pectoralis portio abdominalis* is overlapping *m. obliquus externus*.

21. *M. pectoralis portio axillaris.* Da Silva (1998) notes that this muscle is composed by only a few fibres, originating from the posterior portion of the *m. pectoralis p. abdominalis* and inserting medially on the humerus, distally to the insertion of the *m. pectoralis p. abdominalis*. 0. Absent; 1. Present.

Forelimb Musculature

22. *Lateral *m. lumbricalis brevis digiti V*, slip with origin from tegument. 0. Absent; 1. Present.

23. *Lateral *m. lumbricalis brevis digiti V*, slip with origin from distal carpal 5-4-3. 0. Absent; 1. Present.

24. *M. extensor digitorum communis longus*, medial branch (FIV), point of insertion. The medial branch of the *m. extensor digitorum communis longus* can insert directly on the dorsum of metacarpal IV or can insert with the insertion tendon of the *m. extensor brevis medius digiti IV*. This character was used by Faivovich (2002, character 45). 0. Midbranch of the *m. extensor digitorum communis longus* inserts on the insertion tendon of *m. extensor brevis medius digiti IV*; 1. Midbranch of *m. extensor digitorum communis longus* inserts directly on the dorsum of metacarpal IV.

25. *_M. extensor digitorum communis longus_, internal branch (FIII), number of insertion points. The internal branch of the *m. extensor digitorum communis longus*, that inserts onto Digit III, can be single or divided at its tip. Thus, Presenting one or two insertion points. 0. Internal branch of *m. extensor digitorum communis longus* with single insertion point; 1. Internal branch of *m. extensor digitorum communis longus* with two insertion points.

26. *_M. extensor digitorum communis longus_, medial branch (FIV), number of insertion points. The internal branch of the *m. extensor digitorum communis longus*, that inserts onto digit IV, can be single or divided at its tip. Thus, presenting one or two insertion points. This character was used by Faivovich (2002; character 44). 0. Medial branch of *m. extensor digitorum communis longus* with single insertion point; 1. Medial branch of *m. extensor digitorum communis longus* with two insertion points.

27. *_M. extensor digitorum communis longus_, lateral branch (FV), number of insertion points. The internal branch of the *m. extensor digitorum communis longus*, that inserts onto Digit V, can be single or divided at its tip. Thus, Presenting one or two insertion points. 0. Lateral branch of *m. extensor digitorum communis longus* with single insertion point; 1. Lateral branch of *m. extensor digitorum communis longus* with two insertion points.

28. ***M. caput profundum III*, number of slips.** In some species of Lophyohylini, such as *Nyctimantis siemersi, Osteocephalus planiceps, O. oophagus* and *Osteopilus dominicensis,* the *m. caput profundum III* can be divided into two slips. Interestingly, when *m. caput profundus* is divided its medial slip inserts along metacarpal III where the *m. lumbricalis brevis digiti III* would usually be located. 0. *M. caput profundum III* with single slip; 1. *M. caput profundum III* with two slips.
29. ***M. lumbricalis brevis digiti III*.** As mentioned above, when *M. caput profundum III* is divided the *m. lumbricalis brevis digiti III* is absent. Instead, the medial slip of *M. caput profundum III* occupies its place. 0. Absent; 1. Present.
30. ***M. lumbricalis longus digiti IV*, extra slip that is preaxial to the medial slip.** In some species of *Trachycephalus,* a third, extra slip of *m. lumbricalis longus digiti IV* can be present. It is large slip, that is preaxial and more dorsal to the medial slip, that originates from *Tendo superficialis digiti IV* at the level of the base of metacarpal IV and inserts along the pre-axial lateral surface of metacarpal IV, where *m. lumbricalis brevis digiti IV* would usually be (Fig. S3.3). 0. Absent; 1. Present.
31. ***M. lumbricalis brevis digiti IV*.** Interestingly, in most lophyohylines, the *m. lumbricalis brevis digiti IV* is absent. Instead, its position is occupied either by the medial slip of *m. lumbricalis longus digiti IV* or by a third, extra slip of *m. lumbricalis longus digiti IV*. 0. Absent; 1. Present.
32. **Lateral *m. dorsometacarpalis distalis digiti II*.** The group of *mm. dorsometacarpalis distalis* were described by Burton (1996) and used in a phylogenetic analysis by Faivovich (2002) for *Scinax.* These muscles originate from a penniform origin from the anterior portion of metacarpals and insert by a tendon upon the ultimate phalanx. In this study We coded for variation in the following aspects: They can be either absent or present in the medial or lateral sides of each metacarpal; when they are present, the length of their fibres may vary, eventhough they maintain fixed origin and insertion points. Following Faivovich (2002), characters related to fibre length were treated as additive. 0. Absent; 1. Present.
33. **Lateral *m. dorsometacarpalis distalis digiti III*.** 0. Absent; 1. Present.
34. ***Lateral *m. dorsometacarpalis distalis digiti III*, length of the fibres.** 0. Fibres reach the half length of the penultimate phalanx III; 1. Fibres reach the distal margin of penultimate phalanx III.
35. **Lateral *m. dorsometacarpalis distalis digiti IV*.** 0. Absent; 1. Present.
36. ***Lateral *m. dorsometacarpalis distalis digiti IV*, length of the fibres.** 0. Fibres reach the distal margin of antepenultimate phalanx IV; 1. Fibres reach half the length of penultimate phalanx IV; 2. Fibres reach the distal margin of penultimate phalanx IV. This character was considered as additive.
37. **Lateral *m. dorsometacarpalis distalis digiti V*.** 0. Absent; 1. Present.
38. ***Lateral *m. dorsometacarpalis distalis digiti V*, length of the fibres.** 0. Fibres reach the half length of antepenultimate phalanx V; 1. Fibres reach the distal margin of antepenultimate phalanx V; 2. Fibres reach the distal margin of penultimate phalanx V. This character was considered as additive.
39. **Medial *m. dorsometacarpalis distalis digiti V*.** 0. Absent; 1. Present.

40. ***Medial *m. dorsometacarpalis distalis digiti V*, length of the fibres.** 0. Fibres reach the distal margin of antepenultimate phalanx V; 1. Fibres reach the half length of penultimate phalanx V; 2. Fibres reach the distal margin of penultimate phalanx V. This character was considered as additive.

Hindlimb Musculature

41. ***M. extensor iliotibialis B*, relation with *m. ischioflexorius*, relative width.** 0. *m. extensor iliotibialis B* is wider than *m. ischioflexorius*; 1. *M. ischioflexorius* is wider than *m. iliotibialis B.*
42. ***M. tenuissimus*, relation with *mm. extensor iliotibialis B* and *ischioflexorius*.** 0. *m. tenuissimus* completely evident; 1. *m. tenuissimus* covered by *mm. iliotibialis B* e *ischioflexorius* at the first third of its length; 2. *m. tenuissimus* covered by *mm. iliotibialis B* e *ischioflexorius* at the first half of its length.
43. ***M. extensor cruris tibialis*, point of insertion, length.** Variation on the length of the insertion point of the *m. extensor cruris tibialis* was described by Dunlap (1960) for Anura. We observed the same variation amongst lophyohylines, so we coded the character states following his original scheme. 0. Along the proximal 1/3 of the tibiofibular; 1. Along the proximal 1/2 to 2/3 of the tibiofibular; 2. Along the proximal 3/4 of the tibiofibular; 3. Along the whole length of the tibiofibular. This character was considered as additive.
44. ***M. tibialis anticus longus*, point of division in two bellies.** Variation on the point of division of the *m. tibialis anticus longus* in two bellies was described by Dunlap (1960) for Anura. We observed the same variation amongst lophyohylines, so we coded the character states following his original scheme. 0. At the proximal 1/3 of the tibiofibular; 1. At the 1/2 of the tibiofibular; 2. At the distal 1/3 of the tibiofibular. This character was considered as additive.
45. ***M. tibialis anticus brevis*, point of origin.** Variation on the point of origin of the *m. tibialis anticus brevis* was described by Dunlap (1960) for Anura. We observed the same variation amongst lophyohylines, so we coded the character states following his original scheme. 0. At the 1/2 of the tibiofibular; 1. At the distal 1/3 of the tibiofibular.
46. ***M. tibialis anticus brevis*, degree of development.** Variation on the degree of development of the *m. tibialis anticus brevis* was described by Dunlap (1960) for Anura. In *Phyllodytes, Acris crepitans* and *Pseudacris regilla* this muscle is clearly larger and more developed than in the remaining species examined. 0. Well developed; 1. Poorly developed.
47. ***Tendo superficialis hallucis*, muscular head from *m. transversus plantae distalis* originating from distal tarsal 2–3 that inserts on the lateral side of this tendon.** In some species a muscular head from the *m. transversus plantae distalis,* that originates from distal tarsal 2–3, can be present and insert on the lateral side of the *Tendo superificalis hallucis* (see Burton, 2004; character 28). 0. Absent, 1. Present.
48. ***Tendo superficialis digiti II*, muscular head from *m. transversus plantae distalis* originating from distal tarsal 2–3 that inserts on the lateral side of this tendon.** Similary as for the *Tendo superificialis hallucis,* a muscular head inserting on *Tendo superficialis digiti II* may also be present. 0. Absent, 1. Present.
49. ***M. extensor digitorum communis longus*, insertion on metatarsal II.** Burton (2004) described variation in the number and positions of insertions of the *m. extensor digitorum*

communis longus. Within hyloids, the *m. extensor digitorum communis longus* inserts by strong tendons on the dorsa of one or more metatarsi (Burton 2004), with this muscle exhibiting either a single insertion on metatarsal II or metatarsal III, double insertion on both metatarsi II and III or III and IV, or even triple insertion, on metatarsi II, III and IV (see Burton, 2004; character 48). Herein we considered each of these insertions as being independent. 0. Absent; 1. Present.

50. ***M. extensor digitorum communis longus*, insertion on metatarsal III.** 0. Absent; 1. Present.
51. ***M. extensor digitorum communis longus*, insertion on metatarsal IV.** 0. Absent; 1. Present.
52. ***M. flexor teres hallucis*.** The *m. flexor teres hallucis* is a deep flexor muscle that originates from metatarsal I and insertes on the metatarsophalangeal joint of the hallux (Burton, 2004). It may be present or absent amongst hylids. I employed this character similarly as Burton (2004; character 29). 0. Absent; 1. Present.
53. ***M. intermetatarsalis digiti IV*.** We modified character 44 of Burton (2004), coding just the absence or presence of this muscle, since we found no significant variation among species where it is present. 0. Absent; 1. Present.
54. ***M. flexor brevis superficialis*, structure of tendon of insertion.** Variation in the tendon of the *m. flexor brevis superficialis* was described by Burton (2004). This author also employed this in his phylogenetic analysis (see Burton, 2004; character 26). We employed the same coding as that study regarding variation observed among the studied specimens. 0. Single, undivided; 1. Divided along its length into a medial tendon, from which arises *Tendo superficialis digiti IV*, and a lateral tendon from which arise TSIII and TSV, with cross-tendons between the divisions.
55. **Lateral *M. dorsometatarsalis hallucis distalis.*** Faivovich (2002) described a muscle with penniform origin from the anterolateral margin of the metatarsal bone of digit I that inserts on the ultimate phalanx. Due to ambiguous references to this muscle in earlier literature (see Dunlap, 1960; Andersen, 1978), Faivovich (2002) employed the name *m. extensores brevis distalis digiti I* to refer to this muscle and serial homologues of remaining toes, because of its topological similarity with the *mm. extensores breves distales* of the hand. Burton (2004) followed this strategy and so do we (although we used the revised nomenclature of Diogo & Ziermann, 2014). We found variation regarding absence or presence of these muscles. 0. Absent; 1. Present.
56. **Lateral *M. dorsometatarsalis distalis digiti II*.**0. Absent; 1. Present.
57. **Lateral *M. dorsometatarsalis distalis digiti III.*** 0. Absent; 1. Present.
58. **Lateral *M. dorsometatarsalis distalis digiti IV*.** 0. Absent; 1. Present.
59. **Medial *M. dorsometatarsalis distalis digiti V.*** 0. Absent; 1. Present.

Cranium osteology

60. **Pre-nasal bone**. The pre-nasal bone is an additional element of the skull table that was described by Trueb (1970) for *Triprion* and *Aparasphenodon*. This bone lies anterior to the premaxillaries and articulates with the maxillaries, nasals and the *pars dentalis* of the pre-maxilla. Alcalde (2017), examining the cranium of *Rhinella fernandezae*, found a single triangular bone, that appeared postmetamorphically as a diffuse centre of dermal

ossification, which he considered homologous with the prenasals. This character was adapted from Da Silva (1998; character 1). 0. Absent; Present.

61. ***Nasal bones, medial articulation**. 0. Nasal bones not in contact; 1. Nasal bones in contact, articulating medially.
62. **Nasal bones, degree of posterior development**. The antorbital plane is a cartilaginous or calcified wall that extends laterally from the anterolateral border of the sphenethmoid to the maxilla (Trueb, 1973; Da Silva, 1998). This character was adapted from Da Silva (1998; character 4) and was considered additive. 0. Nasals do not reach the antorbital plane; 1. Nasal reach antorbital plane; 2. Nasals exceed the antorbital plane.
63. **Nasal bones, shape**. This character follows character 5 of Da Silva (1998). 0. Nasals in "L" shape; 1. Nasals rounded; 2. Nasals arched.
64. **Pre-maxilla, allary process.** This character follows Trueb (1970). 0. Allary process of the pre-maxilla exposed; 1. Allary process of the pre-maxilla ocluded.
65. **Dermal Sphenethmoid**. The dermal sphenethmoid was described by Trueb (1970) for casque-headed hylids. It is a single, diamond-shaped dermal element articulating with the posteromedial margins of the nasals anteriorly and the antero-medial borders of the frontoparietals posteriorly (Fig. S3.4). This bone develops from a separate and membranous origin dorsal to the cartilaginous sphenethmoid (Trueb, 1973). 0. Absent; 1. Present.
66. **Frontoparietal bones, lateral margins, shape**. Follows character 9 of Da Silva (1998). 0. Lateral margins of the frontoparietals oblique; 1. Lateral margins of the frontoparietals parallel.
67. ***Frontoparietal fenestra, degree of development of ossification.** See Figure S3.5 for photos illustrating the character states. 0. Transluscent fenestra with only periferal ossification; 1. fenestra with narrow transluscent portion and developed periferal ossification; 2. Ossified fenestra with anterior cartilagenous portion; 3. Ossified fontanelle with posterior cartilagenous portion; 4. fenestra completely ossified. This character was considered additive.
68. **Frontoparietal bones, porterolateral development.** This character follows Da Silva (1998; character 11). We followed him and it considered additive also. 0. Posterior margins of frontoparietals do not reach the epiotic eminences; 1. Posterior margins of frontoparietals reach the orbital half of the epiotic eminences; 2. Posterior margins of frontoparietals covering the orbital half of the epiotic eminences; 3. Posterior margin of the frontoparietals covering the otic capsule dorsally.
69. **Frontoparietal bones, relation between frontoparietal and nasal bones.** This character was also used by Da Silva (1998; character 12). We followed him and considered it additive also. 0. Frontoparietal bones not articulating with nasals; 1. Frontoparietal bones articulating with nasals.
70. **Frontoparietal bones, development of perpendicular lamina.** Character 15 of Da Silva (1998). 0. Absent or poorly developed; 1. Perpendicular lamina of frontoparietals with extensive development, marking the superior portion of the ocular fenestra.
71. **Pre-maxilla, exostosis.** The development of dermal ornamentation in can be characterized by three types, evident in dermal investing bones (Trueb, 1973): a) exostosis, b) casquing and c) co-ossification. Exostosis is reflected by the presence of sculptured patterns on the

surface of the bone, due to appositional growth of dermal bone. Casquing involves the proliferation and expansion of dermal bones, forming a helmeted appearance (Trueb, 1970; 1973). The extreme condition of ornamentation is co-ossification, which involves the integration of skin overlaying dermal bones into the bone structure (Trueb, 1970; 1973). There are different options to code this variation (see the discussion of Blotto *et al.*, *in press*). Herein we coded the presence or absence of exostosis as independent for each boné (e.g. see chs. 17-21 in Da Silva, 1998). 0. Absent; 1. Present.

72. **Nasal, exostosis.** 0. Absent; 1. Present.
73. **Frontoparietal bone, exostosis.** 0. Absent; 1. Present.
74. **Squamosal bone, exostosis.** 0. Absent; 1. Present.
75. **Maxilla, exostosis.** 0. Absent; 1. Present.
76. **Level of elaboration of exostosis.** This character was considered additive. 0. Absent; 1. Poorly elaborated, comprising irregular, slightly thickened and pitted bone; 2. Elaborated.
77. **Dermal co-ossification.** 0. Absent; 1. Present.
78. **Maxilla, shape of the *pars facialis*.** This is the same variation described by character 22 of Da Silva (1998). 0. *Pars facialis* of the maxilla single; 1. *Pars facialis* of the maxilla composed of two pieces, one dorsal and one ventrolateral, with a bony ridge separating them.
79. **Maxillary teeth, relative range.** This is the same variation described by character 23 of Da Silva (1998). 0. Maxillary teeth absent at the anterior portion of the maxilla and extending, at least, to the middle of the pterygoid fossa; 1. Maxillary teeth extending to the anterior extremity of the maxilla.
80. **Maxilla, degree of overlap between maxilla and pre-maxilla.** This is the same variation described by character 26 of Da Silva (1998). 0. Minimal overlap; 1. Extensive overlap, with maxilla covering the pre-maxilla.
81. **Pre-maxilla, alary process, shape.** Character 27 of Da Silva (1998). 0. Narrow with parallel edges; 1. Expanded base, approximately triangular shape.
82. **Squamosal bone, zygomatic ramus, relative length.** The squamosal is a dermal bone that is triradiate in lateral view, presenting three rami: zygomatic (anterior), ventral and otic (posterior) (Trueb, 1973). The zygomatic ramus extends anteriorly to the orbital region, sometimes articulating with the maxilla. Character 28 of Da Silva (1998). 0. Short; 1. Zygomatic ramus long and curved in direction of maxilla, but without articulation; 2. Zygomatic ramus long, articulating with pterygoid; 3. Zygomatic ramus long, articulating with maxilla.
83. **Squamosal bone, relation with prootic bone.** 0. Squamosal does not articulate with the prootic; 1. Squamosal articulates with epiotic crest.
84. **Pterygoid bone, medial ramus, relative length.** The pterygoid is also triradiate and is a paired, dermal palatal bone, located at the posterior corner of the skull (Trueb, 1973). We followed the same definition of character 31 of Da Silva (1998). 0. Medial ramus of pterygoid short, not in contact with otic capsule; 1. Medial ramus of pterygoid reaches the otic capsule; 2. Medial ramus of pterygoid articulates with parasphenoid. This character was considered additive.

85. **Vomer bone, dentigerous process, shape.** We followed the same definition of character 32 of Da Silva (1998). 0. Dentigerous process of the vomer transverse; 1. Dentigerous process of the vomer angulated; 2. Dentigerous process of the vomer elongated.
86. **Vomer bone, anterior process, orientation.** We followed the same definition of character 33 of Da Silva (1998). 0. Anterior process of the vomer oriented towards the articulation between maxilla and pre-maxilla; 1. Anterior process of the vomer oriented only towards the pre-maxilla.
87. **Vomer bone, relation with maxillary arch.** We followed the same definition of character 34 of Da Silva (1998). 0. Anterior process of the vomer separated from the maxillary arch; 1. Anterior process of the vomer articulated with maxillary arch.
88. **Vomer bones, medial articulation.** We followed the same definition of character 35 of Da Silva (1998). 0. Vomer bones not articulated medially; 1. Vomer bones articulated medially.
89. **Neopalatine bones, relative length.** We followed the same definition of character 36 of Da Silva (1998). 0. Neopalatines reach the anterior process of antorbital plane; 1. Neopalatines extend to the medial portion of sphenethmoid.
90. **Neopalatine bones, texture.** 0. Smooth; 1. Serrated.
91. **Parasphenoid bone, transverse ridge of *ala parasphenoidalis.*** We followed the same definition of character 38 of Da Silva (1998). 0. Absent; 1. Present.
92. **Parasphenoid bone, *ala parasphenoidalis*, relative orientation.** We followed the same definition of character 39 of Da Silva (1998). 0. Perpendicular to the axis of the body; 1. *Ala parasphenoidalis* angulated posteriorly.
93. **Parasphenoid bone, cultriform process, medial ventral crest.** Character 40 of Da Silva (1998). 0. Absent; 1. Presence of a poorly developed longitudinal bony crest.
94. ***Parasphenoid bone, medial ventral crest of cultriform process, interruption.** 0. Absent; 1. Present.
95. **Parasphenoid bone, cultriform process, relative length.** Character 41 of Da Silva (1998). 0. Anterior extremity of cultriform process is posterior to the orbitonasal foramen; 1. Anterior extremity of cultriform process is at the level of the orbitonasal foramen; 2. Anterior extremity of the cultrifomr process is anterior to the orbitonasal foramen.
96. **Parasphenoid bone, anterior extremity of cultriform process, shape.** Character 42 of Da Silva (1998). 0. Truncate; 1. Pointed.
97. **Parasphenoid bone, dorsolateral flange.** The parasphenoid may present a dorsolateral expansion extending from the cultriform process in some casque-headed species. Character 43 of Da Silva (1998). 0. Absent; 1. Present.
98. **Parasphenoid bone, posteromedial process.** Character 44 of Da Silva (1998). 0. Absent; 1. Present.
99. **Sphenethmoid bone, degree of anterior mineralization.** The sphenethmoid is an endochondral element anteriorly housing the brain (Trueb, 1993; Da Silva 1998). Variation is related to the degree of mineralization of this element and we followed characters 46-49 of Da Silva (1998). 0. Anterior mineralization posterior to or at the level of the antorbital plane; 1. Anterior mineralization extending to the middle of the choanae; 2. Anterior mineralization extending to the anterior portion of the choanae.

100. Sphenethmoid bone, degree of lateral mineralization. 0. Antorbital plane entirely cartilaginous; 1. Mineralization of the orbital plane reaches the nasal septum; 2. Mineralization of the orbital plane includes the nasal septum; 3. Orbital plane completely ossified.

101. Sphenethmoid bone, degree of posterior mineralization. 0. Ocular fenestra filled with connective tissue; 1. Region immediately anterior to ocular fenestra mineralized; 2. Mineralized portion of sphenethmoid extending to the ocular fenestra.

102. Sphenethmoid bone, *crista supraorbital*. 0. Absent; 1. Present.

103. Otoccipital bone, posterior ridge. 0. Absent; 1. Present.

104. Otoccipital bone, posterior ridge, degree of development. 0. Poorly developed; 1. Greatly developed.

105. *Annulus tympanicus*, relation with the *crista parotica* of the skull. In anurans, the auditory apparatus is composed of the stapes, *annulus tympanicus* (tympanic ring) and the operculum (Duellman & Trueb, 1994). Variation in this system was coded by Da Silva (1998) for hylids. We followed his character's 52 and 54-56. 0. *Annulus tympanicus* not in contact with *crista parotica*; 1. Posterior region of *annulus tympanicus* fused to the postero-lateral process of *crista parotica.*

106. *Pars externa plectri*, shape. The stapes is an endochondral rod that connects the tympanum to the inner ear, its external portion is named *pars externa plectri* (Duellman & Trueb, 1994). 0. Elongated; 1. Arched and dorso-ventrally flattened; 2. Rounded.

107. Operculum, degree of calcification. 0. Cartilaginous; 1. Calcified.

108. Occipital canal. The occipital canal acts as a pathway for the occipital artery and, when the frontoparietals are greatly developed posteriorly, may be intercepted (Da Silva, 1998). 0. Absent; 1. Present.

109. Dentary bones, odontoids. Among lophyohylines, this character is only present in *Phyllodytes* and is traditionally considered a synapomorphy (e.g. Peters, 1873; Faivovich *et al*., 2005). Absent; 1. Present.

Hindlimb (*Pes*) osteology

110. Achilles tendon, sesamoid. Character 83 of Da Silva (1998). 0. Absent; 1. Present.

111. *Ligamentum calcanei*, sesamoid. Character 84 of Da Silva (1998). 0. Absent; 1. Present.

112. Posterior process of pre-hallux. Character 83 of Da Silva (1998). See Figure S3.6 for photos illustrating this character. 0. Absent; 1. Present.

113. Distal pre-hallux, number of elements anterior to distal pre-hallux. Character 89 of Da Silva (1998). 0. One; 1. Two; 2. Three.

Axial (Vertebra) osteology

114. Cervical vertebra atlas, spinous process, crest. Character 91 of Da Silva (1998). 0. Absent; 1. Present.

115. Pre-sacral vertebra VIII, lateral process, orientation. Character 92 of Da Silva (1998). See Figure S3.7 for photos illustrating this character. 0. Anteriorly oriented; 1. Orthogonal to the axis of the body.

116. Urostyle, relation with sacrum. Character 93 of Da Silva (1998). 0. Intercondylar space exposed; 1. Intercondylar space covered by the urostyle.

117. Pre-sacral vertebra II, lateral process, size. Character 94 of Da Silva (1998). See Figure S3.8 for photos illustrating this character. 0. Same dimension as lateral process of pre-sacral vertebra III, 1. Laterally expanded, broader than lateral process of pre-sacral vertebra III.

Ecology and behaviour

118. Phragmotic behaviour. Phragmotic behaviour was defined by Wheeler (1927) as blocking of the entrance of a burrow or a hole using part of the body. Blotto *et al.* (*in press*) discussed the evolution of phragmosis in Lophyohylini. We also considered both "experimentally induced phargmosis" (when the behaviour was only observed in captivity; Jared *et al.*, 1999; Navas *et al.*, 2002) and "semi-phragmotic behaviour" (when the animal assumes a phragmotic posture, but is unable of displaying phragmotic behaviour *per se* because of the available natural refuges; Cajade *et al.*, 2017) under the character state "present". 0. Absent; 1. Present.

119. Reproductive site. Blotto *et al.* (*in press*) provided extensive discussion regarding evolution of phytotelm breeding in this group and summarized available information on reproductive biology. In lophyohylines, species may breed in pools or ponds, streams, or phytotelmata (small cavities filled with water in some plant structures, such as leaf axils of bromeliads, tree holes or fruit capsules). Breeding in pools or ponds is exhibited by most species of Lophyohylini, while breeding in streams is scarce (Ron *et al.*, 2012; Jungfer *et al.*, 2013). Polymorphism in breeding site is observed in *Corythomantis greeningi* and *Osteopilus dominicensis* for example, which reproduce in both pools and streams of slow-moving water (Jared *et al.*, 1999; Juncá *et al*., 2008; Díaz *et al*., 2015; Blotto *et al*., *in press*). In these cases, character states were coded as 0&1. The remaining species of the tribe shows breeding in phytotelmata: breeding in tree-holes is observed in *Trachycephalus resinifictrix* (Goeldi, 1907), *T. cunauaru* (Gordo *et al.*, 2013), *T. hadroceps* (Lecure & Marty, 2000), *T. helioi* (Nunes *et al*., 2013), *Nyctimantis rugiceps* (Duellman & Trueb, 1976), *Tepuihyla shushupe* and *Tp. tuberculosa* (Ron et al., 2016); use of bromeliads for reproduction is widespread in all studied species of *Phyllodytes* (Bokermann, 1966, 1968; Kenny, 1969), as in *Osteocephalus oophagus* (Jungfer & Weygoldt, 1999; Jungfer *et al.*, 2013) and the *Oc. planiceps* species group (Jungfer *et al*., 2013). In the latter, the use of nut capsules has been reported for *Oc. castaneicola* (Moravec et al., 2009). 0. Pools or Ponds; 1. Streams; 2. Phytotelmata.

Tadpole (Larva) morphology

120. Tadpole tail, ratio between tail length and body length. 0. 3/5; 1. 2/3; 2. 3/4.

121. Tadpole oral disc, position. 0. Ventral; 1. Anteroventral; 2. Terminal.
122. Tadpole oral disc, submarginal papillae. 0. Absent; 1. Present.
123. Tadpole oral disc, number of posterior tooth rows. 0. Three or less; 1. Four or more.
124. Tadpole oral disc, anterior jaw sheath, shape. 0. "U" shape; 1. Arch shape; 1. "M" shape, presence of medial projection.
125. Tadpole oral disc, posterior jaw sheath, shape. 0. "V" shape; 1. "U" shape.
126. Tadpole eyes, position. 0. Lateral; 1. Dorsolateral.
127. Tadpole nostrils, position. 0. Nearer the eyes; 1. Half distance between eyes and snout; 2. Nearer the snout.

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FIGURES

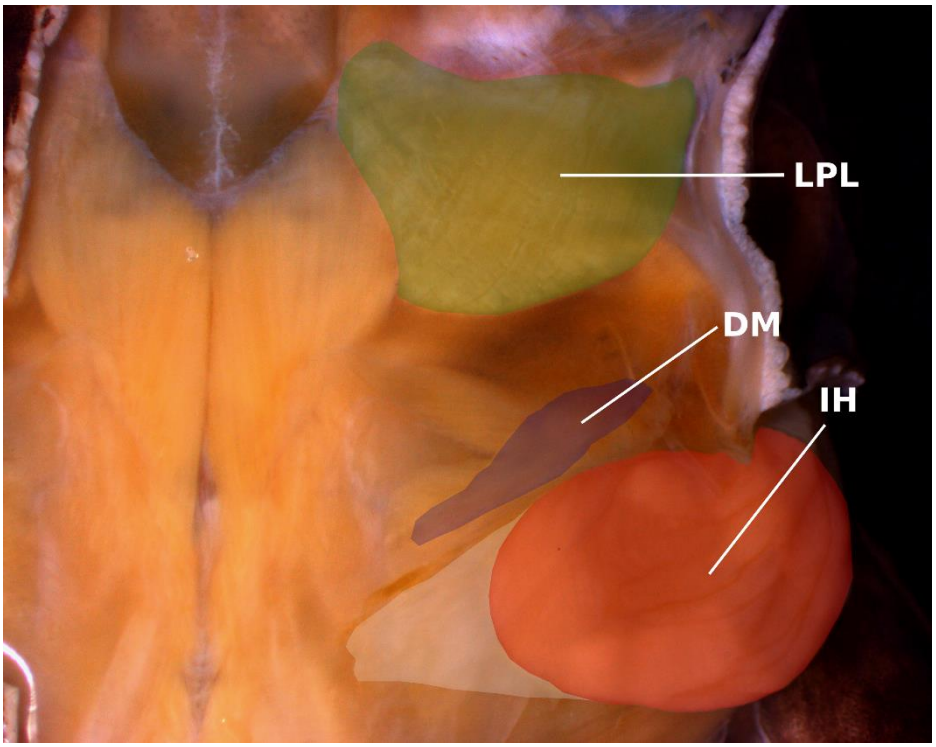

Figure S3.1. Dorsal head musculature showing the insertion of the *m. interhyoideus* (IH) on the *fascisa dorsalis.*

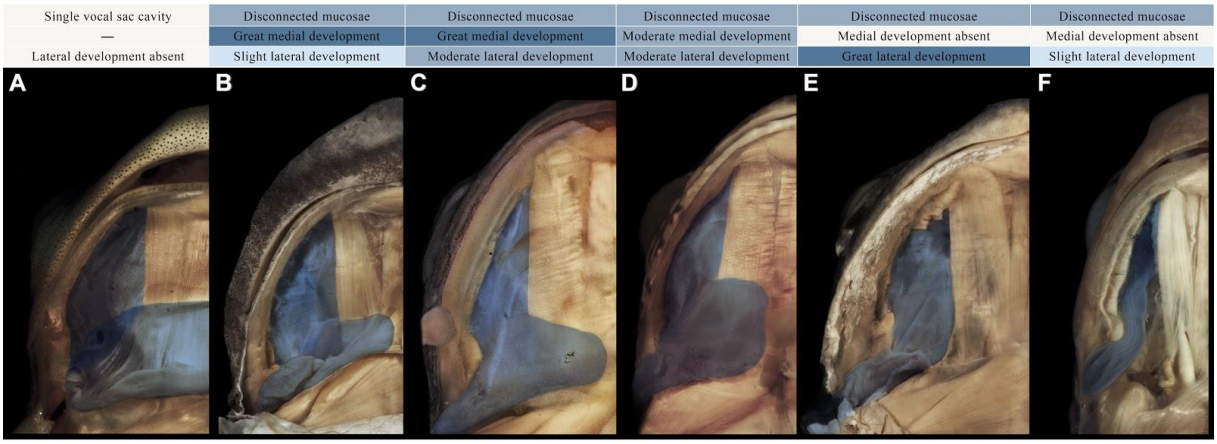

Figure S3.2. Diversity in the shape of vocal sac mucosae and its influence on the inflated vocal sac morphology. A-F: Ventral view of adult males with gular skin and *mm. intermandibularis* and *interhyoideus* removed to show the development of the vocal sac internal mucosae. A: *Phyllodytes melanomystax* (CFBH 35707) showing the plesiomorphic condition for Lophyohylini, where fused mucosae without lateral development produce a single subgular vocal sac. B: *Corythomantis greeningi* (CFBH 16129), showing bilobated vocal sacs. Mucosae are disconnected but their medial development enables exhaled air to access the centre of the gular region, functionally resembling a single-cavity vocal sac. Slight lateral, subgular extensions of the mucosae and the *m. interhyoideus* produce bilobulation of the inflated vocal sac. C: *Osteocephalus verruciger* also presents inflation in the centre of the gular region, but the mucosae and the *m. interhyoideus* extend dorsolaterally reaching the post-tympanic fold. The resulting inflated vocal sac does not fit any of the traditional categories used to classify the vocal sacs of frogs, since it combines both lateralization and subgular inflation. D: In *Itapotihyla langsdorffi* (CFBH 12724), medial development is comparatively smaller, resulting in paired lateral vocal sacs with only slight subgular inflation. E: In *Trachycephalus typhonius* (MACN 40540), mucosae are restricted to an extremely dorsolateral position, with negligible medial development and dorsolateral lobes exceeding the post-tympanic fold. F: In *Hylodes phyllodes* (MACN 17252), mucosae do not extend medially but show only slight lateral development, resulting in paired subgular vocal sacs. Image by Agustín J. Elias-Costa.

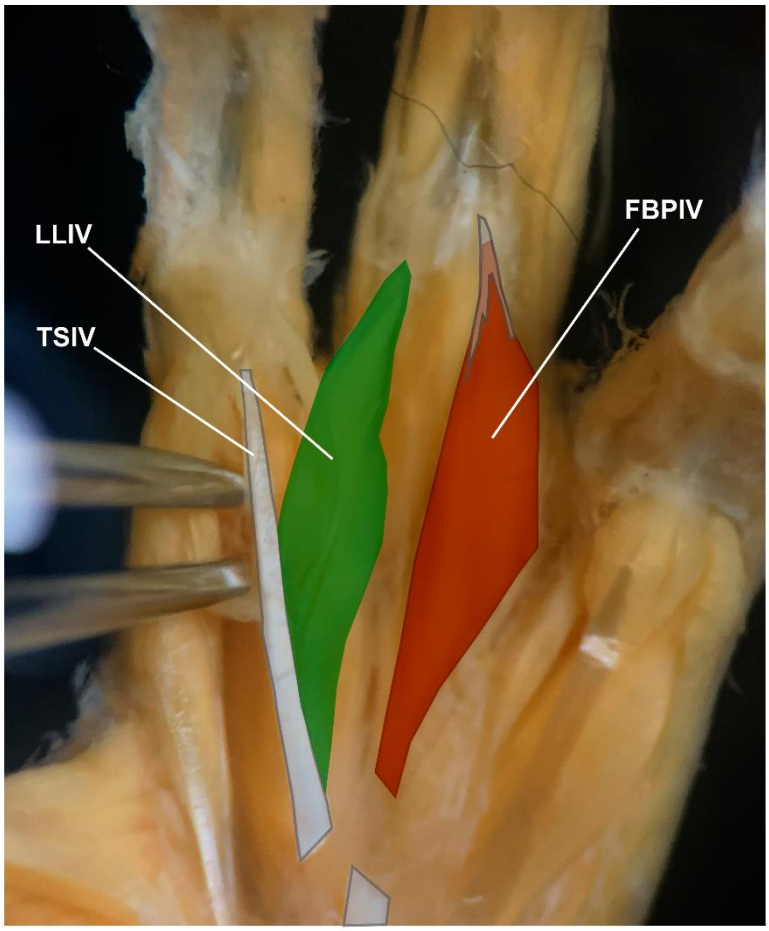

Figure S3.3. Ventral view of the hand of *Trachycephalus typhonius* (MNRJ 78678). Highlighted in green is the extra slip of the LLIV that inserts along metacarpal IV (ch. 31.1). FBPIV: *m. flexor brevis profundus digiti IV*; LLIV: *m. lumbricalis longus digiti IV*; TSIV: *Tendo superficialis digiti IV*.

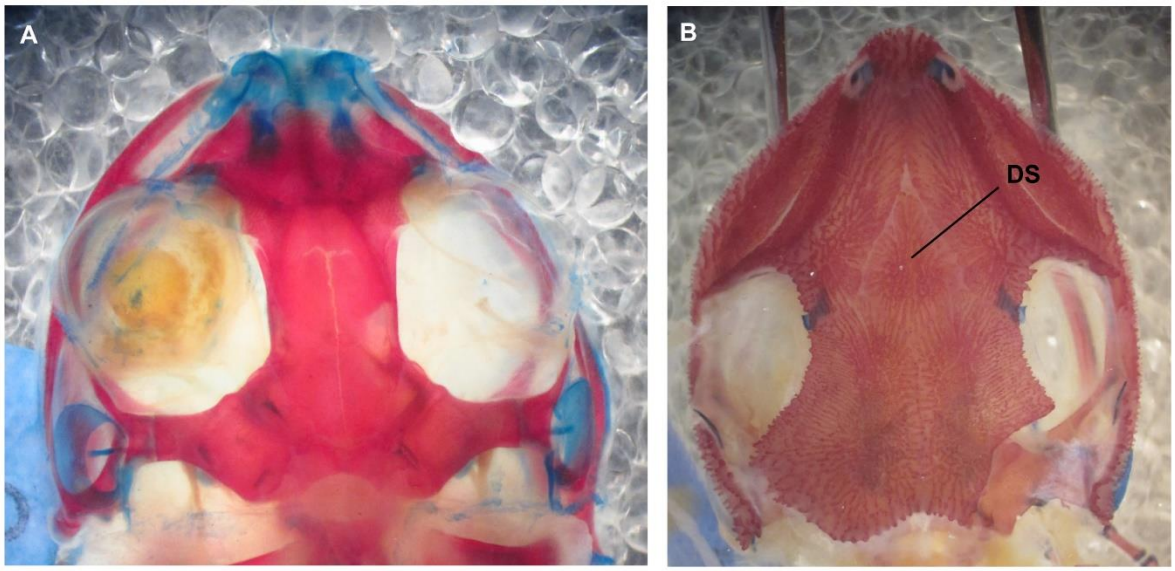

Figure S3.4. Cleared and stained dorsal view of the skull illustrating character 65 (Dermal Sphenethmoid). A) Ch. 65.0, absence of dermal sphenethmoid (*Trachycephalus coriaceus*; KU 207658). B) Ch. 65.1, presence of dermal sphenethmoid (*Nyctimantis brunoi*; KU 92213). DS: dermal sphenethmoid.

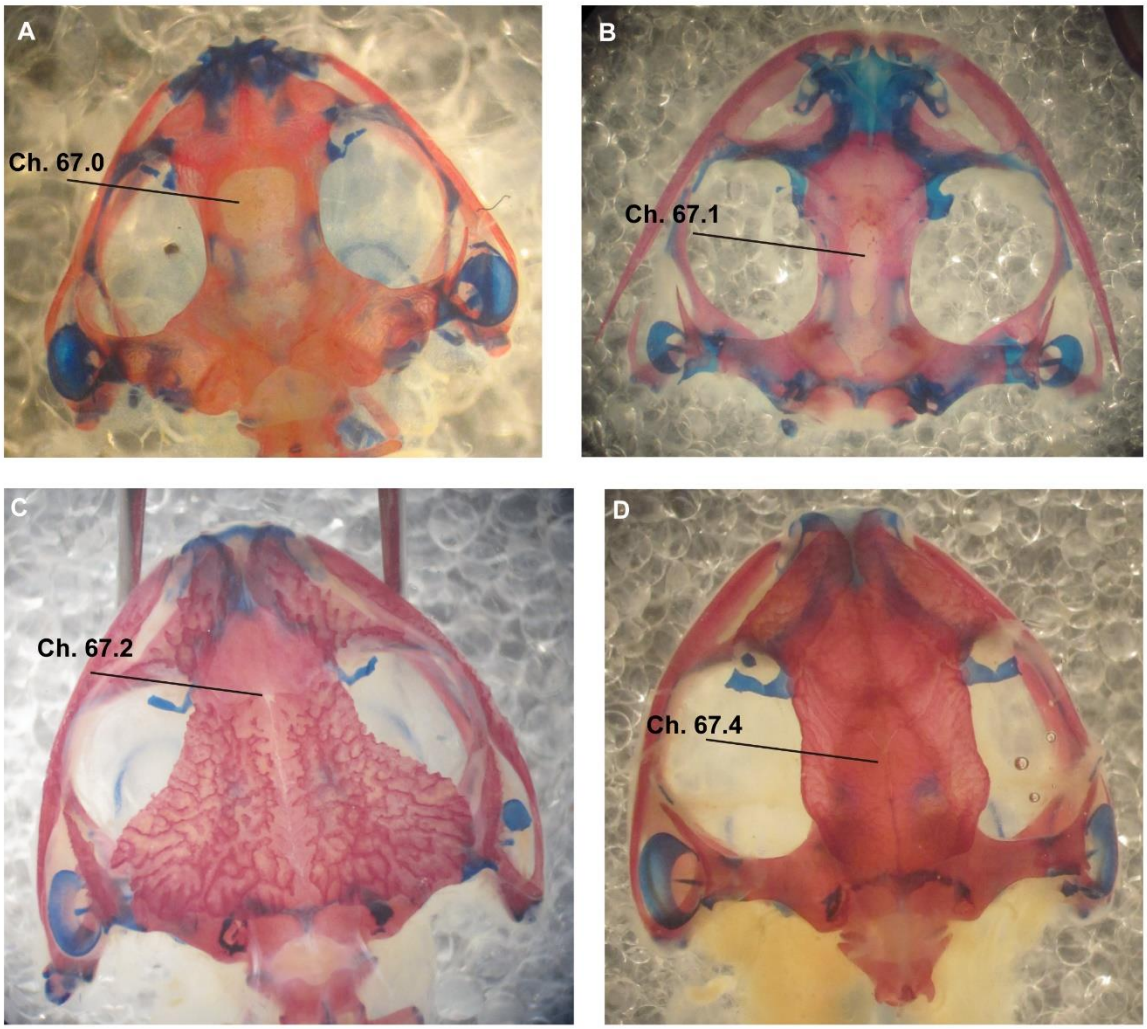

Figure S3.5. Cleared and stained dorsal view of the skull illustrating character 67 (Frontoparietal fenestra). A) Ch. 67.0: Transluscent fenestra with only periferal ossification (*Phyllodytes luteolus*; KU 187476). B) Ch. 67.1: Fenestra with narrow transluscent portion and developed periferal ossification (*Osteopilus vastus*; KU 84710). C) Ch. 67.2: Ossified fenestra with anterior cartilagenous portion (*Nyctimantis siemersi*; KU 116937). D) Ch. 67.4: Fenestra completely ossified (*Osteocephalus taurinus*; KU 175501).

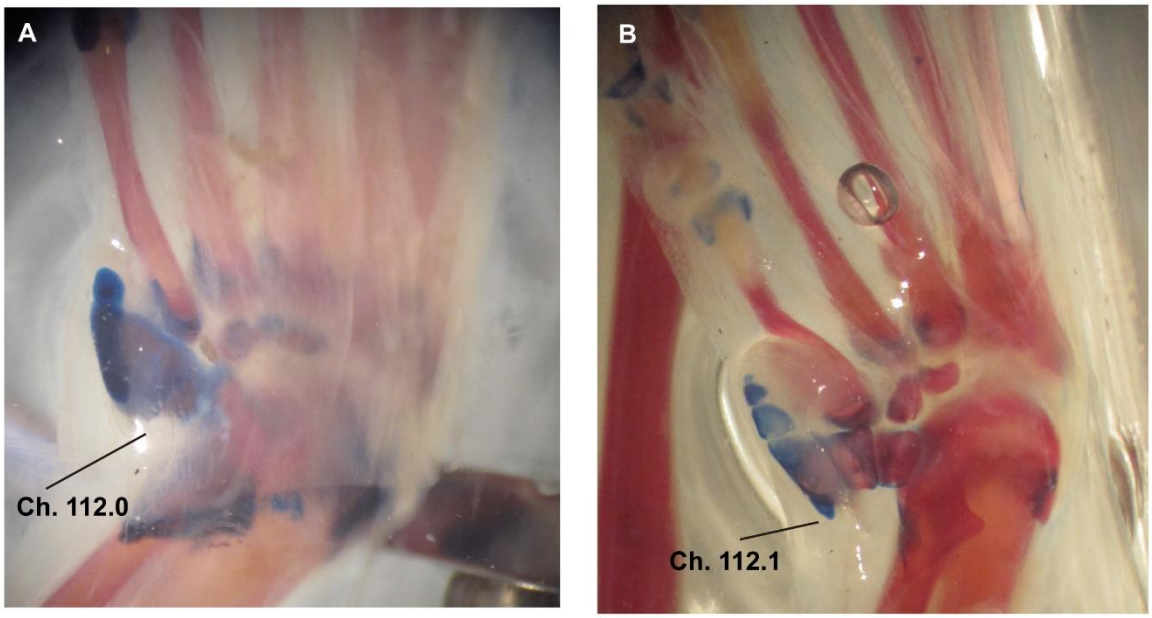

Figure S3.6. Cleared and stained dorsal view of the left foot illustrating character 112 (Posterior process of prehallux). A) Ch. 112.0: Posterior process of prehallux absent (*Nyctimantis siemersi*; KU 116937). B) Ch. 112.1: Posterior process of prehallux present (*Trachycephalus typhonius;* KU 92261).

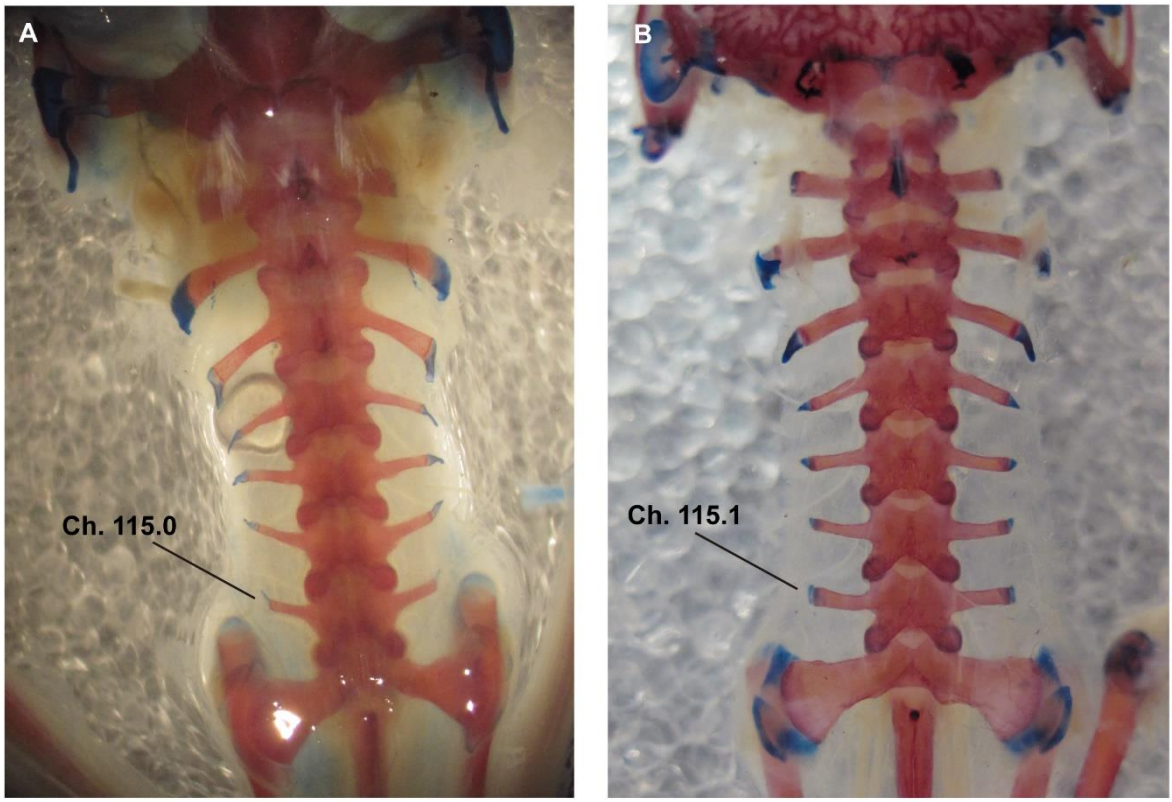

Figure S3.7. Cleared and stained dorsal view of the vertebral column illustrating character 115 (Pre-sacral vertebra VIII, lateral process, orientation.). A) Ch. 115.0: Lateral process of pre-sacral vertebra VIII anteriorly oriented (*Trachycephalus typhonius*; KU 92261). B) Ch. 115.1: Lateral process of pre-sacral vertebra VIII orthogonal to the axis of the body (*Nyctimantis siemersi*; KU 116937).

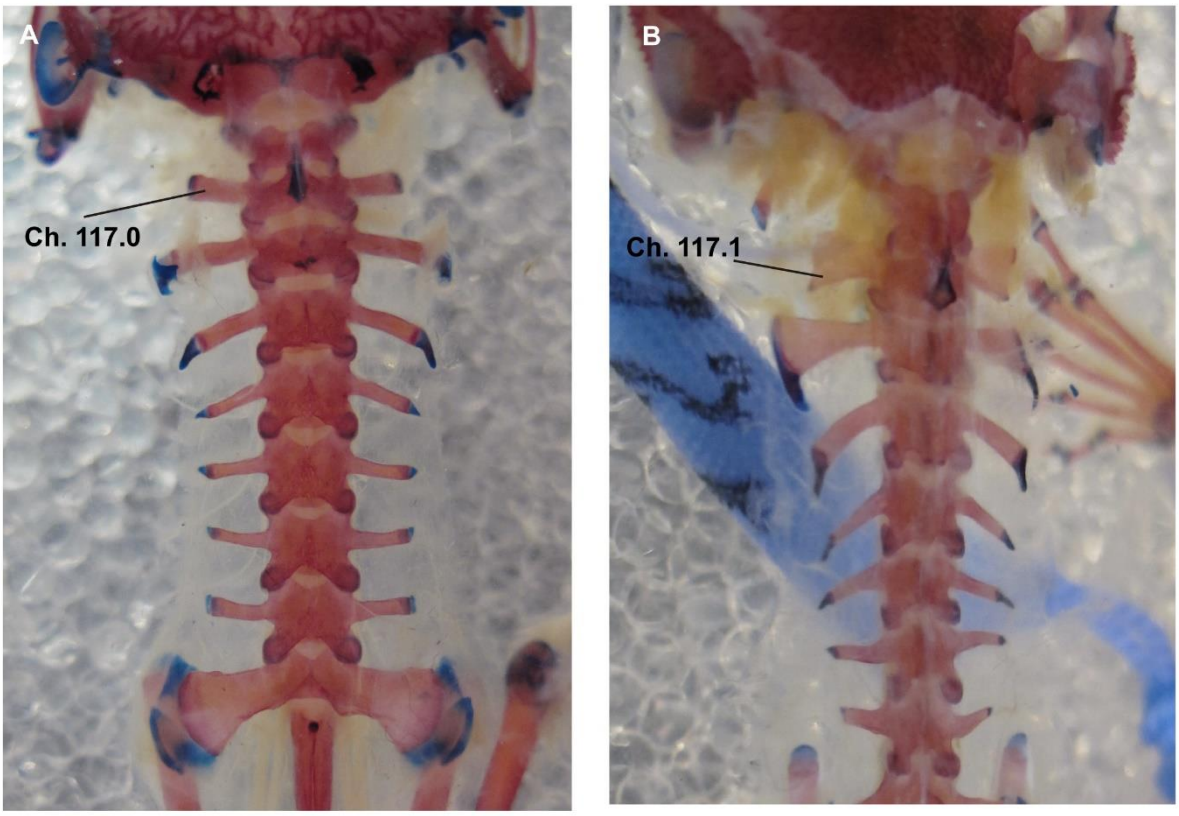

Figure S3.8. Cleared and stained dorsal view of the vertebral column illustrating character 117 (Pre-sacral vertebra II, lateral process, size). A) Ch. 117.0: Lateral process of pre-sacral vertebra II with same dimension as lateral process of pre-sacral vertebra III (*Nyctimantis siemeri*; KU 116937). B) Ch. 117.1: Lateral process of pre-sacral vertebra II laterally expanded, broader than lateral process of pre-sacral vertebra III (*Nyctimantis brunoi*; KU 92213).

APPENDIX S4. Phenotypic character matrix.

Terminals	Characters																																															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**
Trachycephalus typhonius	1	4	0	1	3	1	?	0	2	1	1	0	0	1	1	0	1	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	1	1	1	2	1	2	1	0	1	2	2	1	1	1	0	0	1
Trachycephalus mesophaeus	1	4	0	1	3	1	?	0	2	1	1	0	0	1	1	0	1	1	0	0	0	1	0	1	0	0	0	0	1	0	1	1	1	1	1	0	1	0	1	2	1	2	2	1	1	1	1	0	1
Trachycephalus jordani	1	4	0	1	3	1	3	0	0	1	0	1	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0	0	1	1	1	2	1	2	1	0	0	2	2	1	0	1	0	0	1
Trachycephalus atlas	1	4	0	1	3	1	3	0	1	1	1	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	1	1	1	0	1	1	1	0	1	2	2	1	1	1	0	0	1
Trachycephalus mambaiensis	1	4	0	1	3	1	3	0	-	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Trachycephalus nigromaculatus	1	1	0	1	3	1	3	0	1	1	1	0	0	1	1	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	0	1	1	1	0	1	1	1	1	0	0	2	1	0	1	0	0	1
Trachycephalus imitatrix	1	4	0	1	3	1	3	0	2	1	1	0	0	1	1	0	1	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	1	1	1	0	1	2	2	1	0	1	0	0	1
Trachycephalus lepidus	1	4	0	1	3	1	3	0	0	1	?	?	?	?	?	?	?	?	?	?	?	1	0	1	0	1	1	0	1	0	1	0	1	1	1	0	1	1	1	1	?	?	?	?	?	1	0	0	1
Trachycephalus dibernadoi	1	4	0	1	3	1	3	0	2	1	0	0	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	0	1	1	0	0	1	1	1	0	1	1	1	2	0	2	2	0	0	1	0	0	1
Trachycephalus coriaceus	1	2	0	1	3	1	3	0	2	1	?	?	?	1	1	0	1	1	0	0	0	1	0	1	?	0	0	0	1	0	0	0	1	1	1	1	1	1	1	2	1	1	2	1	0	1	0	0	1
Trachycephalus cunauaru	0	2	0	2	3	1	3	0	1	1	1	0	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	1	0	1	2	0	1	2	1	0	1	0	1	1
Trachycephalus resinifictrix	1	4	0	1	3	0	3	0	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Trachycephalus macrotis	3	4	0	1	3	1	3	0	1	1	1	0	0	1	1	0	1	1	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	1	1	0	1	1	1	2	1	1	2	0	0	1	0	0	1
Trachycephalus quadrangulum	1	4	0	1	3	1	3	0	2	1	0	0	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	1	1	1	1	0	0	2	1	1	1	0	0	1
CC520 Trachycephalus spilommus	3	1	0	1	3	1	3	0	2	1	1	0	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	1	1	1	1	1	0	2	1	0	1	0	1	0
CC527 Trachycephalus spilommus	3	1	0	1	3	1	3	0	2	1	1	0	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	1	1	1	1	1	0	2	1	0	1	0	1	0
Trachycephalus adenodermus	3	4	0	1	3	1	3	0	1	1	1	0	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	0	1	1	0	0	1	1	1	2	1	2	1	0	0	2	2	1	0	1	0	0	1
Trachycephalus hadroceps	1	4	0	1	2	1	2	0	1	1	0	0	0	1	1	0	1	1	0	0	1	?	?	1	?	?	?	0	1	1	0	?	?	?	?	?	?	?	?	?	1	1	2	0	0	1	0	0	1
Nyctimantis brunoi	0	2	0	1	2	0	2	0	0	1	0	0	0	1	1	2	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	1	1	0	1	1	1	0	0	1	3	1	0	1	0	0	1
Nyctimantis pomba	1	1	0	1	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	0	1	1	1	0	1	0	3	1	0	1	0	0	1
Corythomantis greeningi	3	3	0	1	1	0	1	0	0	2	0	1	0	1	1	2	1	0	1	1	1	0	1	1	0	0	0	0	1	1	0	0	1	1	1	0	1	1	1	0	1	1	3	1	0	1	0	0	1
Nyctimantis galeata	0	4	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Itapotihyla langsdorffii	1	1	0	1	2	0	2	0	0	2	1	0	0	1	1	1	0	1	1	0	0	0	1	1	1	1	0	0	1	0	0	0	1	1	1	0	1	1	1	0	0	2	1	1	0	1	0	0	1
Nyctimantis rugiceps	0	4	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Phyllodytes tuberculosus	1	1	1	1	0	0	0	1	-	2	1	0	0	1	0	-	1	1	0	0	1	1	0	0	0	0	0	0	1	1	0	1	1	1	1	2	1	1	1	2	1	2	1	1	0	0	1	0	1
Phyllodytes melanomystax	1	1	1	1	0	0	0	1	-	2	1	0	0	1	0	-	1	1	0	0	1	1	0	0	0	0	0	0	1	1	0	1	1	1	1	2	1	1	1	2	1	2	0	1	0	0	1	0	1
Phyllodytes luteolus	1	1	1	1	0	0	0	1	-	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Phyllodytes edelmoi	1	1	1	1	0	0	0	1	-	2	1	0	0	1	0	-	1	1	0	0	1	1	0	1	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	2	1	2	1	1	0	0	1	0	1
Osteopilus dominicensis	1	2	0	2	2	0	2	0	1	1	0	0	0	1	1	1	1	0	0	1	1	0	1	1	0	1	0	1	0	1	0	1	1	1	1	0	1	1	1	1	0	0	2	0	1	1	0	0	1
Osteopilus septentrionalis	1	2	0	2	2	0	2	1	-	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Osteopilus ocellatus	0	4	1	1	0	0	0	1	-	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Osteopilus vastus	1	2	0	1	2	0	2	1	-	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tepuihyla edelcae	1	3	0	1	2	1	2	0	2	0	0	0	0	1	1	0	1	1	0	0	1	0	1	1	0	0	0	0	1	0	0	1	1	1	1	0	1	1	1	2	0	1	2	0	0	1	0	1	1
Tepuihyla luteolabris	1	3	0	1	2	1	2	0	2	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Osteocephalus taurinus	1	1	0	1	2	1	2	1	-	2	0	0	0	1	1	1	1	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	1	1	1	0	1	1	1	1	0	1	1	0	1	1	0	1	1
Osteocephalus verruciger	1	1	0	1	2	1	2	0	0	1	0	0	0	1	1	1	1	1	0	1	1	0	1	1	0	0	0	1	0	1	0	1	1	1	1	0	1	1	1	1	0	1	1	0	0	1	0	0	0
Osteocephalus oophagus	1	1	0	1	2	1	2	1	-	1	0	0	0	1	1	0	1	1	0	0	1	1	0	1	0	0	0	1	0	1	0	1	1	1	1	0	1	1	1	2	1	0	1	0	0	1	0	0	0
Osteocephalus planiceps	1	1	0	1	2	1	2	0	0	1	0	0	0	1	1	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1
Osteocephalus leprieurii	1	0	0	1	2	1	2	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Dryaderces pearsoni	1	1	0	1	2	0	2	0	?	?	0	0	0	1	1	0	1	0	0	1	1	0	1	1	0	0	0	0	1	1	0	1	1	1	1	0	1	1	1	2	1	0	2	1	0	1	0	0	0
Nyctimantis siemersi	3	1	0	2	2	0	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	?	?	1	?	?	?	0	1	0	0	?	?	?	?	?	?	?	?	?	1	1	2	1	1	1	?	?	1
Acris crepitans	1	5	1	0	0	0	0	1	-	0	0	0	1	0	1	0	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	0	-	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0
Pseudacris regilla	2	4	1	1	0	0	0	1	-	1	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	-	0	-	0	-	0	-	0	1	1	0	0	0	0	0	0
Duellmanohyla rufioculis	2	3	1	1	0	0	0	1	-	1	0	0	0	0	1	0	1	1	0	0	1	0	1	1	0	1	0	0	1	1	0	1	1	1	1	0	0	-	1	0	0	1	1	0	0	1	1	0	1

(Continuation) Phenotypic character matrix.

Terminals	Characters																																																		
	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Trachycephalus typhonius	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	2	1	0	1	1	1
Trachycephalus mesophaeus	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	3	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	2	1	0	1	1	1
Trachycephalus jordani	1	1	0	1	1	0	0	0	0	0	0	1	2	2	0	1	1	4	3	1	0	1	1	1	1	1	2	1	1	1	0	1	2	1	0	0	0	1	0	1	1	1	0	1	1	2	1	0	1	1	1
Trachycephalus atlas	1	1	0	1	1	0	0	0	0	0	0	1	2	2	0	1	1	4	3	1	1	1	1	1	1	1	2	1	1	1	0	1	2	0	0	0	0	1	1	1	0	0	0	1	0	2	1	1	1	1	3
Trachycephalus mambaiensis	?	?	?	?	?	?	?	?	?	?	0	1	2	2	0	1	1	4	3	1	0	1	1	1	1	1	2	1	1	1	0	1	2	0	0	0	0	1	1	1	1	0	0	1	0	2	1	0	1	2	3
Trachycephalus nigromaculatus	1	1	0	1	1	0	0	0	0	0	0	1	2	2	0	1	1	4	3	1	0	1	1	1	1	1	2	1	1	1	0	1	2	1	0	0	0	1	1	1	1	0	0	1	0	2	1	0	1	2	3
Trachycephalus imitatrix	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	2	1	0	1	1	1
Trachycephalus lepidus	1	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	2	1	0	1	1	1
Trachycephalus dibernadoi	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	?	0	0	0	0	1	0	0	?	0	0	2	1	0	1	1	1
Trachycephalus coriaceus	1	1	?	1	1	0	0	0	0	0	0	0	1	0	0	0	1	2	1	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	1	0	0	2	1	0	1	1	1
Trachycephalus cunauaru	1	1	?	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	?	0	0	0	0	1	0	0	?	0	0	2	1	0	1	1	1
Trachycephalus resinifictrix	?	?	?	?	?	?	?	?	?	?	0	1	1	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	2	1	0	1	1	1
Trachycephalus macrotis	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	?	0	0	0	0	1	0	0	?	0	0	2	1	0	1	1	1
Trachycephalus quadrangulum	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	?	0	0	0	0	1	0	0	?	0	0	2	1	0	1	1	1
CC520 Trachycephalus spilommus	1	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	?	0	0	0	0	1	0	0	?	0	0	2	1	0	1	1	1
CC527 Trachycephalus spilommus	1	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	?	0	0	0	0	1	0	0	?	0	0	2	1	0	1	1	1
Trachycephalus adenodermus	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	?	0	0	0	0	1	0	0	?	0	0	2	1	0	1	1	1
Trachycephalus hadroceps	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	2	1	0	1	1	1
Nyctimantis brunoi	1	1	0	1	1	0	0	0	0	0	1	1	2	2	0	1	1	4	3	1	1	0	1	1	1	1	2	1	1	1	1	1	2	0	1	1	1	1	0	1	1	1	0	0	0	2	1	1	1	2	2
Nyctimantis pomba	1	1	0	1	1	0	0	0	0	0	0	1	2	2	1	1	1	4	3	1	1	0	1	1	1	1	2	1	0	1	1	1	?	0	?	1	?	?	0	1	1	?	?	?	?	2	1	1	1	?	?
Corythomantis greeningi	1	1	0	0	1	0	0	0	0	0	0	1	2	2	1	0	1	4	3	1	1	0	1	1	1	1	2	1	0	1	1	1	1	0	0	2	0	0	0	1	0	1	0	0	0	2	1	1	1	1	0
Nyctimantis galeata	?	?	?	?	?	?	?	?	?	?	0	1	2	2	1	1	1	4	3	1	1	0	1	1	1	1	2	1	0	1	1	1	1	0	0	2	0	0	0	1	0	1	0	0	0	2	1	1	1	1	0
Itapotihyla langsdorffii	1	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	1	4	1	1	0	0	1	1	1	1	2	1	0	0	0	1	0	0	2	1	0	0	1	1	0	0	1	0	0	2	1	0	1	2	1
Nyctimantis rugiceps	?	?	?	?	?	?	?	?	?	?	0	0	1	2	0	0	1	4	2	0	0	0	1	1	1	1	2	1	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	2	2
Phyllodytes tuberculosus	1	1	0	0	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	2	0	0	0	2	0
Phyllodytes melanomystax	1	1	0	0	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	2	0	0	0	2	0
Phyllodytes luteolus	?	?	?	?	?	?	?	?	?	?	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	2	0	0	0	2	0
Phyllodytes edelmoi	1	1	0	0	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	2	0	0	0	2	0
Osteopilus dominicensis	1	1	0	0	1	0	0	0	0	0	0	1	2	2	0	0	1	4	3	1	0	0	1	1	1	1	2	1	0	1	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	2	1	1	1	2	0
Osteopilus septentrionalis	?	?	?	?	?	?	?	?	?	?	0	1	2	2	0	0	1	4	3	1	0	0	1	1	1	1	2	1	0	1	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	2	1	0	1	1	0
Osteopilus ocellatus	?	?	?	?	?	?	?	?	?	?	0	1	2	2	0	1	1	4	3	1	0	0	1	1	1	1	2	1	0	1	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	2	1	0	1	1	1
Osteopilus vastus	?	?	?	?	?	?	?	?	?	?	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	2	1	0	1	1	0
Tepuihyla edelcae	1	1	0	0	1	0	0	0	0	0	0	0	1	2	0	0	0	0	1	0	?	0	1	0	0	0	1	0	0	0	0	1	2	0	0	1	0	0	0	0	0	0	1	0	0	2	1	?	1	?	2
Tepuihyla luteolabris	?	?	?	?	?	?	?	?	?	?	0	0	1	2	0	0	0	0	1	0	?	?	?	?	?	?	?	?	0	0	0	1	2	0	0	1	0	0	0	0	0	0	1	0	0	2	1	?	1	?	2
Osteocephalus taurinus	1	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	1	4	1	1	0	0	1	1	0	0	2	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0	1	0	0	2	1	0	1	1	1
Osteocephalus verruciger	1	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	1	0	0	1	0	0	2	1	0	1	2	1
Osteocephalus oophagus	1	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	1	3	1	1	0	0	1	0	0	0	1	0	0	0	0	1	?	0	?	1	0	0	0	1	0	0	1	0	0	2	1	0	1	?	?
Osteocephalus planiceps	1	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	1	4	1	1	0	0	1	1	0	0	1	0	0	0	0	1	?	0	?	1	0	0	0	1	0	0	1	0	0	2	1	0	1	?	?
Osteocephalus leprieurii	?	?	?	?	?	?	?	?	?	?	0	0	1	0	0	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	0	0	1	0	0	1	0	0	2	1	0	1	2	1
Dryaderces pearsoni	1	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1	0	0	1	1	0	0	1	0	0	0	0	1	?	0	?	1	0	0	0	1	0	0	1	0	0	2	1	0	1	?	?
Nyctimantis siemersi	1	0	?	?	1	0	0	0	0	0	0	0	2	2	0	1	1	2	3	1	0	0	1	1	1	1	2	1	0	1	0	1	3	0	1	0	0	1	0	1	0	0	1	0	0	2	1	0	1	1	0
Acris crepitans	1	0	0	1	0	0	0	0	0	0	0	0	1	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0
Pseudacris regilla	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	?	1	0	0	0	0	1	0	0	1	0	0	2	1	0	1	0	0
Duellmanohyla rufioculis	0	0	0	1	0	1	1	1	1	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	2	1	0	0	0	0

(Continuation) Phenotypic character matrix.

Terminals	Characters																										
	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127
Trachycephalus typhonius	1	1	0	-	0	0	1	0	0	0	0	1	3	1	0	1	0	0	0	0	1	1	1	1	0	0	2
Trachycephalus mesophaeus	2	1	0	-	0	0	1	0	0	0	0	1	3	0	0	1	0	0	0	1	1	1	1	1	0	0	2
Trachycephalus jordani	2	1	1	1	0	0	0	0	0	0	0	1	2	0	0	1	1	1	0	2	1	1	1	1	0	0	2
Trachycephalus atlas	2	1	1	1	0	0	0	0	0	0	0	1	2	1	0	0	1	1	0	1	1	1	1	1	1	0	0
Trachycephalus mambaiensis	2	1	0	-	0	0	1	0	0	0	0	1	2	1	0	1	1	1	0	?	?	?	?	?	?	?	?
Trachycephalus nigromaculatus	2	1	1	1	0	0	1	0	0	0	0	1	2	1	0	1	1	1	0	1	1	1	1	0	1	0	2
Trachycephalus imitatrix	2	1	0	-	0	0	1	0	0	0	0	1	3	0	0	1	0	0	0	?	?	?	?	?	?	?	?
Trachycephalus lepidus	2	1	0	-	0	0	1	0	0	0	0	1	3	0	0	1	0	0	0	?	?	?	?	?	?	?	?
Trachycephalus dibernadoi	2	1	0	-	0	0	1	0	0	0	0	1	?	?	0	?	0	0	0	?	?	?	?	?	?	?	?
Trachycephalus coriaceus	2	1	0	-	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	2	1	1	1	1	0	0	2
Trachycephalus cunauaru	2	1	0	-	0	0	1	0	0	0	0	1	?	?	0	?	0	0	2	0	1	1	1	1	0	0	2
Trachycephalus resinifictrix	1	1	0	-	0	0	1	0	0	0	0	1	3	1	0	1	0	0	2	0	1	1	0	1	0	0	2
Trachycephalus macrotis	1	1	0	-	0	0	1	0	0	0	0	1	?	?	0	?	0	0	0	?	?	?	?	?	?	?	?
Trachycephalus quadrangulum	1	1	0	-	0	0	1	0	0	0	0	1	?	?	0	?	0	0	0	?	?	?	?	?	?	?	?
CC520 Trachycephalus spilommus	1	1	0	-	0	0	1	0	0	0	0	1	?	?	0	?	0	0	0	0	1	1	1	1	1	0	0
CC527 Trachycephalus spilommus	1	1	0	-	0	0	1	0	0	0	0	1	?	?	0	?	0	0	0	0	1	1	1	1	1	0	0
Trachycephalus adenodermus	1	1	0	-	0	0	1	0	0	0	0	1	?	?	0	?	0	0	0	0	1	1	1	1	0	0	2
Trachycephalus hadroceps	1	1	0	-	0	0	1	0	0	0	0	1	3	0	0	0	0	0	2	?	?	?	?	?	?	?	?
Nyctimantis brunoi	1	1	1	0	0	0	0	1	0	0	0	1	2	1	1	1	1	1	0	1	1	1	1	1	0	0	0
Nyctimantis pomba	?	?	1	?	?	?	0	1	0	?	?	?	?	?	?	?	?	1	?	?	?	?	?	?	?	?	?
Corythomantis greeningi	1	1	1	1	0	0	0	1	0	0	0	1	1	1	0	1	1	1	0&1	1	0	1	1	2	0	1	0
Nyctimantis galeata	1	1	1	1	0	0	0	1	0	0	0	1	1	1	0	1	1	1	0	?	?	?	?	?	?	?	?
Itapotihyla langsdorffii	1	1	0	-	0	0	0	0	0	0	0	1	3	1	1	1	1	0	0	1	1	1	1	0	0	0	0
Nyctimantis rugiceps	1	1	0	-	0	0	0	0	0	0	0	1	3	0	1	1	0	0	2	?	?	?	?	?	?	?	?
Phyllodytes tuberculosus	1	1	0	-	1	2	0	0	1	1	0	0	0	0	0	1	0	0	2	1	0	0	1	1	0	0	2
Phyllodytes melanomystax	1	1	0	-	1	2	0	0	1	1	0	0	0	0	0	1	0	0	2	1	0	0	0	1	0	0	2
Phyllodytes luteolus	1	1	0	-	1	2	0	0	1	1	0	0	0	0	0	1	0	0	2	1	0	0	1	1	0	0	2
Phyllodytes edelmoi	1	1	0	-	1	2	0	0	1	1	0	0	0	0	0	1	0	0	2	1	0	0	1	1	0	0	2
Osteopilus dominicensis	2	1	0	-	0	0	0	1	0	0	0	1	2	0	0	0	0	0	0&1	0	1	0	1	1	0	1	1
Osteopilus septentrionalis	1	1	0	-	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0&1	0	1	0	1	1	0	1	1
Osteopilus ocellatus	2	1	0	-	0	0	0	0	0	0	0	1	2	0	1	1	0	0	2	2	0	0	0	1	0	1	1
Osteopilus vastus	1	1	0	-	0	0	0	0	0	0	0	1	3	0	1	0	0	0	0&1	1	1	1	1	0	0	1	1
Tepuihyla edelcae	1	1	0	-	0	?	?	0	0	0	0	1	0	?	1	1	0	0	0	1	0	1	0	1	1	1	1
Tepuihyla luteolabris	1	1	0	-	0	?	?	0	0	0	0	1	0	?	1	?	0	0	0	?	?	?	?	?	?	?	?
Osteocephalus taurinus	1	1	0	-	0	0	0	0	0	0	0	1	2	1	0	0	1	0	0	2	1	1	1	1	0	1	1
Osteocephalus verruciger	1	1	0	-	0	0	0	0	0	0	0	1	2	1	1	1	0	0	1	1	1	1	1	1	0	1	1
Osteocephalus oophagus	?	1	0	-	0	?	0	0	0	0	0	1	2	?	?	?	?	0	2	0	1	1	0	1	0	1	0
Osteocephalus planiceps	?	1	0	-	0	?	0	0	0	0	0	1	2	?	?	?	?	0	2	?	?	?	?	?	?	?	?
Osteocephalus leprieurii	1	1	0	-	0	0	0	0	0	0	0	1	2	1	1	0	0	0	0	?	?	?	?	?	?	?	?
Dryaderces pearsoni	?	1	0	-	0	?	0	0	0	0	0	1	2	?	?	?	?	0	0	?	?	?	?	?	?	?	?
Nyctimantis siemersi	1	1	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0
Acris crepitans	0	0	0	-	0	2	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	0	2
Pseudacris regilla	0	1	0	-	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	1	1	0	0
Duellmanohyla rufioculis	0	1	0	-	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1

APPENDIX S5. GenBank accession numbers.

Taxon	12S	16S	COI	Cytb	ND1	tRNA-Leu	tRNA-Ile	tRNA-Gln	POMC	RAG-1	RHO	TYR
Ac. crepitans	KM273797	MN135368	MN135582	AY843782	GQ366290			-	MH004171	AY844358	AY844533	EF988302
N. brunoi	AY843567	KU495133	KU494337	AY843789	KF002246				-	AY844364	AY844541	AY44023
N. rugiceps	AY843780	AY843781	-	AY843945	-	-	-	-	-	-	-	-
N. siemersi	AY843570	AY843570	-	AY843792	-	-	-	-	-	AY844367	AY844544	AY844026
C. greeningi	AY843578	AY843578	-	AY843800	KF002247				-	AY844374	AY844551	AY844030
Dh. rufioculis	MH004066	AY843583	-	AY549368	DQ388749		-	-	DQ388705	AY844377	AY844556	-
Dr. pearsoni	KF002017	KF002006	KF001884	KF001947	KF002189				-	-	-	-
I. langsdorffii	KY202804	KU495310	KU494514	AY843951	AY819511		-	-	JQ868470	AY844482	AY844697	-
Oc. oophagus	EF376030	MG805968	KF001902	KF001975	JQ742405	KF002249			MH378002	AY844484	AY844699	MH377912
Oc. planiceps	KY211976	FJ965294	MG030720	-	KF002223				EU034118	EU034134	-	-
Oc. taurinus	KF002165	MG805971	KU494616	AY843954	KF002240				MH378078	AY844485	-	MH377981
Oc. verruciger	JX847100	KF002170	JX875856	-	KF002241				EU034119	-	-	-
Oc. leprieurii	KF002061	JQ742237	KU494613	KF001966	JQ742404	KF002214			JQ868498	AY844483	KP011015	AY844138
Op. dominicensis	AY819443	AY843711	EU034053	AY843956	EU034085		-	-	EU034122	AY844486	AY844701	AY844141
Op. vastus	DQ380384	-	EU034057	EU034075	EU034091	-	-	-	EU034128	EU034144	-	AY844143
Op. septentrionalis	EU034035	-	EU034056	AY843957	AY819513		-	-	EU034127	EU034141	-	AY844142
Op. ocellatus	DQ380382	EU034083	EU034051	EU034067	EU034083		-	-	EU034120	EU034136	-	-
Ph. luteolus	AY843721	MF002006	KU494629	AY843966	GQ366314	-	-	-	GQ366043	AY844494	AY844708	-
Ph. melanomystax	-	MH004306	-	-	-	-	-	-	-	MH004374	-	-
Ps. regilla	AY819376	MN135396	MN135610	AY843983	AY819508		-	-	AY819126	AY844504	AY844725	AY844165
Tp. edelcae	KP011130	KT390939	-	-	KT390948	KP010959	-	-	KP011090	AY844530	KP010999	-
Tr. coriaceus	KY013376	EF376068	-	-	KY013420			-	EU034130	-	-	-
Tr. cunauaru	KY013388	KY013440	KY013407	-	KY013440				KY013462	-	-	-
Tr. hadroceps	AT843717	AY843717	-	AY843962	-	-	-	-	-	AY844490	AY844704	AY844146
Tr. imitatrix	EU034036	-	-	-	-	-	-	-	-	-	-	-
Tr. jordani	JX847091	AY843771	EU034060	AY844015	KF002248				AY819145	AY844531	AY844758	AY844190
Tr. macrotis	KY013377	KY013423	KY013407	-	KY013429				KY013446	-	-	-
Tr. mesophaeus	KY202832	KU495602	KU494809	AY843963	-	-	-	-	-	AY844491	AY844705	AY844147
Tr. nigromaculatus	AY843772	AY843772	-	AY844016	-	-	-	-	-	-	AY844759	AY844191
Tr. quadrangulum	KY013383	KY013432	KY013403	-	KY013438				KY013454	-	-	-
Tr. resinifictrix	JQ868529	KU495604	KU494811	AY843964	-	-	-	-	JQ868482	AY844492	AY844706	AY844148
Tr. spilommus	-	-	X	-	-	-	-	-	-	-	-	-
Tr. typhonius	KY013391	KP149406	KP149201	AY549415	JX875626			-	JX875780	AY844493	-	-

APPENDIX S6. Taxonomic account of *Trachycephalus spilommus* comb. n.

Genus *Trachycephalus* Tschudi, 1838
Trachycephalus spilommus (Cope, 1877) **comb. n.**

Hyla spilomma Cope, 1877: 86.
Hyla paenulata Brocchi, 1879: 21. New synonymy.
Hyla nigropunctata Boulenger, 1882: 366. New synonymy.
Acrodytes inflata Taylor, 1944: 63. New synonymy.
Acrodytes spilomma — Taylor (1944): 64. New synonymy.
Acrodytes modesta Taylor & Smith, 1945: 594. New synonymy.
Hyla modesta — Mertens (1952): 30. New synonymy.
Phrynohyas inflata — Duellman (1956): 22. New synonymy.
Phrynohyas latifasciata Duellman (1956): 24. New synonymy.
Phrynohyas modesta — Duellman (1956): 25. New synonymy.
Phrynohyas spilomma — Duellman 1956: 28. New synonymy.
Phrynohyas corasterias Shannon & Humphrey, 1957: 15. New synonymy.
Trachycephalus venulosus (part) — Faivovich *et al.* (2005): 111.
Trachycephalus typhonius (part) — Lavilla *et al*. 2010: 22.
Trachycephalus typhonius (part) — Duellman *et al*. 2016: 22.
Trachycephalus typhonius (part) — Blotto *et al*. (*in press*).

Remarks. The taxon *Hyla spilomma* was described by Cope (1877) from an immature individual collected by Sumichrast from Cosamoaloapam, Veracruz State, Mexico. Kellog (1932) synonymized *Hyla spilomma* to *Hyla venulosa* Laurenti (= *T. typhonius*) and was included in the Official List of Specific Names in Zoology by Anonymous (1958). Posterior combinations include *Acrodytes spilomma* Taylor (1944) and *Phrynohyas spilomma* Duellman (1956). Frost (2020) considered *Trachycephalus* "*vermiculatus*" as a place-holder taxon for all names available for former *Trachycephalus typhonius* from Chocoan South America to southern and eastern Mexico, including *Hyla spilomma*. Duellman (1956) considered the type specimen of *Hyla vermiculata* Duméril & Bibron, 1841, to be probably from eastern Venezuela or the Guianas, based on external morphology. Instead, he attributed to eastern Mexican populations the name *Phrynohyas spilomma* (Cope, 1877), based on morphological similarity

with the type of *Hyla spilomma*, described by Cope (1877) from an immature specimen collected at Cosamoloapan, Veracruz, Mexico. We follow Duellman (1956) and restrict the name *T. "vermiculatus"* to northern South America morphotypes. Furthermore, McDiarmid (1968) synonymized *P. spilomma* to *P. venulosa* based primarily on variation on colouration pattern and the size of the populations, a decision with which Duellman (1971) agreed. Our results provide subsidy to consider these eastern Mexican *Trachycephalus* as a full species, with *Hyla spilomma* as the proper name to be applied to them. This results in *Trachycephalus spilommus* (Cope, 1877) **comb. n.** However, the holotype that should be in the USNM according to Frost (2011), is currently lost (Kellogg, 1932; K. Tighe pers. comm.). It was collected by Francis Sumichrast in "Cosamaloapam, Veracruz", date of collection unknown. The collection site is precisely in Cosamaloapan, City of Boca del Río, Veracruz, Mexico, at coordinates ~ 19º 06'57" S, 96º 06'59" W, ~ 30 m above sea level (WGS84 datum). This led us to designate a neotype, in accordance with article 75.3 of ICZN (1999). This neotype is designated with the express purpose of clarifying the taxonomic status of a nominal taxon (Art. 75.3.1), is described and compared with relevant species (Arts. 75.3.2 -3,5). In addition, as stated above, the original holotype is lost (Art. 75.3.4) and the neotype was collected near the original locality of the type (Art. 75.3.6) and is housed at the USNM (Art. 75.3.7).

Neotype. USNM 114981, m, MEXICO, Potrero Viejo (~18º 52' 44" S, 96º 50' 23" W), Veracruz State, 600 m, 1938, H. Smith.

Other material. See APPENDIX S1.

Diagnosis. Morphological terminology Follows Duellman *et al.* (2001) and Myers & Duellman (1982) for adults and McDiarmid & Altig (1999) for larvae: A member of *Trachycephalus* as revealed by the presence of *m. interhyoideus* and vocal sac mucosae presenting great lateral extension, exceeding the post-tympanic fold. It is similar to *T. macrotis, T. mesophaeus, T. quadrangulum* and *T. typhonius,* and can be diagnosed by the following combination of characters: (1) large size for the genus, male SVL 55.0–96.0 mm and female SVL 54.5–93.3 mm; (2) semi-circular snout in dorsal view; (3) rounded snout with high profile in lateral view; (4) absence of co-ossification; (5) absence of casquing; (6) *canthus rostralis* not marked; (7) posterior crest of frontoparietals absent; (8) supratympanic fold not ossified and well developed; (9) absence of odontoids on parasphenoid; (10) well developed paratoid gland; (11) milky secretion present; (12) males present paired lateral vocal sacs (*m. interhyoideus* and vocal sac mucosae presenting great lateral extension, exceeding the post-tympanic fold); (13) spines on dorsum absent; (14) axillary membrane absent; (15) webbing formula of the hand II

vestigial III 1–2 IV 2–2 V; (16) absence of tubercles on forearm; (17) absence of ulnar fold; (18) webbing formula of the foot I 1–2 II 1–2 III 1–2 IV 2–1 V; (19) absence of tarsal fold; (20) absence of fringes on the lateral region of the foot and tarsus; (21) dorsum brown to uniform dark brown or with a beige frame that goes from the head, in front of the eyelids, continuing irregularly on the flanks to the inguinal region, having irregular shapes in the sacral region, in addition to black punctuations throughout the back and flanks; (22) absence of red punctuations on the dorsum; (23) absence of black spot on the flank; (24) beige interdigital membrane; (25) iris yellow to orange, with black cross and/or reticulations; (26) *m. intermandibularis* with medial aponeurosis that widens distally; (27) a completely evident *m. tenuissimus*; (28) presence of a muscular head in *tendo superficialis digiti II* of the *pes*; (29) absence of insertions of the *m. extensor digitorum communis longus* of the *pes* on metatarsals II and IV; (30) reproduction in ponds; (31) tadpole body ovoid to pyriform in profile; (32) double rows of marginal papillae on the oral disc, except for the anterior gap; (33) submarginal papillae present and in large numbers at the lateral portion of the posterior labium; (34) LTRF 4(1,2,3)/6-7(1); (35) jaw sheaths in "U" shape; (36) dorsal fin initiating at the posterior third of the body; (37) dorsal and ventral fins concave, higher than the body and tapering at the posterior third; (38) nostrils closer to the eyes than to snout; (39) tadpole colouration with brown dorsum with dark punctuations, presence of a lateral dark spot at the junction of the myomeres of the tail; (40) advertisement call with 150 to 175 pulses/s; (41) advertisement call with multipulsed structure.

Comparisons. Trachycephalus spilommus shares exclusively with *T. macrotis, T. mesophaeus, T. quadrangulum* and *T. typhonius* the combined characters of absence of dermal cranial co-ossification, reproduction in ponds and iris yellow to bronze, with black reticulations (except for *T. macrotis*, which presents uniform dark brown iris). *T. spilommus* can be distinguished from *T. typhonius* by the high profile of the snout (low profile in *T. typhonius*); different dorsum colour (dorsum with dense black reticulations in *T. typhonius*); loreal region uniform with dorsal region of the head (loreal region darker in *T. typhonius*); absence of granules on the forearm (present in *T. typhonius*); LTRF 4(1,2,3)/6-7(1) (LTRF 3(1,3)/4-5(1,2,3) in *T. typhonius*); tadpole coloration with brown dorsum, with darkened spots, presence of a dark lateral spot at the junction of the tail myomeres (tadpole with yellowish-brown dorsum with mottled brown spots throughout the body and tail in *T. typhonius*); advertisement call with 150 to 175 pulses/s (123 to 138 pulses/s in *T. typhonius*). It can be further distinguished from *T. macrotis* by *m. intermandibularis* with medial aponeurosis that widens distally (medial raphe present in *T. macrotis*); a completely evident *m. tenuissimus* (covered by the *mm. iliotibialis B*

and *ischioflexorius* in *T. macrotis*); presence of a muscular head in *tendo superficialis digiti II* of the *pes* (absent in *T. macrotis*); and absence of insertions of the *m. extensor digitorum communis longus* of the *pes* on metatarsals II and IV (both present in *T. macrotis*).

Description. A redescription of *T. spilommus* can be found in Duellman (1956).

Colour. In life, brown to dark brown dorsum, uniform or densely reticulated, with a smooth mustard brown frame running from the head to the front of the eyelids, continuing irregularly on the flanks, up to the inguinal region; iris yellow to orange, with black cross and/or reticulations. In preservative, all colors acquire shades of brown at the base and beige in the patterns.

Measurements of the neotype (in mm; precision 0.05). Follows Heyer *et al.* (1990) and Napoli (2005): SVL 70.7; HL 22.6; HW 23.0; ED 6.1; END 5.3; TD 4.2; ETD 3.2; UEW 7.0; IOD 6.0; IND 5.4; NSD 3.8; FAL 13.9; HL 21.3; DF3 4.1; THL 32.8; TBL 33.0; FL 45.5; DT4 3.31.

Variation. Specimens from southern Mexico to lower latitudes do not present the tarsal fold. The two basic colour patterns determined for *T. spilommus* present frequencies closely related to their distribution and, consequently, to altitude. The coloured pattern with frame occurs, in its great majority (<90%), in the lower regions of northern and central Mexico, Belize, and Atlantic coastal regions of Guatemala and north-western Honduras. The colour pattern with uniformly coloured dorsum with punctuations throughout the dorsum and flanks occurs, in the great majority (> 90%), in the mountainous regions of "Sierra Madre Sul" and "Sierra Central", which covers southern Mexico to Nicaragua in Central America. Females are slightly larger and more robust than males.

Vocalization. Porter (1962) first mentioned the vocalization of *T. spilommus.* Duellman (2001), described the vocalization of the species for Mexican populations has a multipulsed physical structure, duration from 0.23 to 0.36 s, emission of 150.0 to 175.0 pulses/s and dominant frequency from 1.39 to 1.95 kHz.

Tadpole. Pyburn (1967) described the tadpole of *T. spilommus* (as *Phrynohyas spilomma*) from Tuxtla, Veracruz Department, Mexico. The tadpole (stage 36 for oral disc and stage 38 for body) has an oval to piriform body in lateral view; double rows of marginal papillae throughout the oral disc, except the anterior gap; submarginal papillae present in large numbers on the lateral portion of the posterior labium; LTRF 4(1,2,3)/6-7 (1); "U" shaped jaw sheaths; dorsal fin starting at the posterior third of the body; concave dorsal and ventral fins, higher than the body, and tapering in the posterior third; nostrils closer to the eyes than the snout; tadpole

coloration with brown dorsum, with darkened punctuations, presence of a dark side spot at the junction of the tail myomeres. Duellman (2001) described the tadpole for Tepic, Nayarit Department, Mexico, and the characteristics agree with Pyburn (1967). Altig (1987) used Duellman's data (1971) to produce a key to Mexican tadpoles, with *T. spilommus* among them.

Habitat and Natural History. This frog inhabits forested or open regions and reproduces explosively right at the beginning of rainy seasons (Stuart, 1935; Duellman, 1956, 1960; Pyburn, 1967). Couples in an axillary amplexus can be found floating in water ponds, where they lay their eggs. Females place their heads under the water while they extend their hind limbs also into the water, exposing their cloaca above the water line. Upon spawning, the female makes lateral movements that apparently stimulate the male. The eggs look like an elongated mass and spread on the surface of the water like a film (Pyburn, 1967).

Distribution. Lowlands of Mexico, in coastal regions with little altitude that border the flanks of the "Sierra Madre", passing through lowland regions and altitude in Belize, Guatemala, Honduras and El Salvador, up to the altitude regions of the "Sierra Central" in Nicaragua.

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CONCLUSÕES

- A descrição da anatomia muscular de *Trachycephalus typhonius* é apresentada pela primeira vez. A partir da variação deste sistema em *Trachycephalus* e no grupo externo, foi possível definir 59 séries de transformação (caracteres), incluídos em uma análise cladística de evidência total;
- A hipótese filogenética resultante corroborou o monofiletismo de *Trachycephalus*, com alto valor de suporte;
- A análise filogenética mostrou que *Trachycephalus* apresenta duas sinapomorfias morfológicas relacionadas aos sacos vocais laterais e pareados: o *m. interhyoideus* e a mucosa dos sacos vocais que exibem extenso desenvolvimento lateral, ultrapassando a dobra pós-timpânica (caracteres 5.3 e 7.3);
- A análise filogenética mostrou que há uma reversão nestes caracteres em *Trachycephalus hadroceps*;
- A análise filogenética subsidiou a revalidação de um táxon: *Trachycephalus spilommus*;
- Com base na hipótese filogenética, foi possível estudar a evolução dos sacos vocais e sua relação com o sítio de reprodução, de modo que sacos vocais laterais e pareados estão associados à reprodução em poças, enquanto sacos vocais reduzidos e menos conspícuos estão associados à reprodução em fitotelmos;
- Com base na hipótese filogenética, foi possível estudar a evolução da hiperossificação craniana e sua relação com o comportamento de fragmose em *Trachycephalus*, propondo-se um cenário mais parcimonioso com relação a hipóteses anteriores, no qual a fragmose é a condição plesiomórfica e associada a espécies com cabeça hiperossifica e que utilizam bromélias como refúgios. Uma reversão teria ocorrido nas espécies restantes, juntamente com a redução da hiperossificação do crânio;
- Com base na hipótese filogenética, foi possível estudar a evolução dos músculos extensores distais dos pés, de forma que estes atingem seu desenvolvimento máximo observado em *Phyllodytes*, sugerindo-se uma possível associação entre redução no comprimento rostro-cloacal e aumento no desenvolvimento destes músculos.

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