Programa de Pós-Graduação em Biodiversidade e Conservação
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Análise comparativa da morfologia externa, morfologia oral interna, condrocrânio e aparelho hiobranquial de larvas do gênero *Elachistocleis* Parker 1927 (Anura, Microhylidae)

São Luís
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Orientador: Luiz Norberto Weber

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Aprovada por: Em:

Dedicatória

Dedico este trabalho à minha amada
vó, Maria de Lourdes, pelo apoio
incondicional, amor e carinho
sempre.

Epígrafe

“Tudo posso naquele que me fortalece.”
Filipenses 4:13

Agradecimentos

Primeiramente eu gostaria de agradecer a minha família, que tem me dado todo o suporte e amor que jamais poderia pedir. Em especial à minha mãe, que é difícil ter palavras pra explicar o quanto a amo e o quanto agradeço todo o apoio que ela tem me dado. Hoje eu sou o que sou por causa da senhora e que tem me mostrado a cada dia a superação que eu preciso pra conseguir seguir em frente. Agradeço também aos meus avós, que tanto se preocupam comigo quando me viam com a cara abatida por causa dos tantos artigos e coisas que tinha que dar conta. Amo todos vocês.

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A minha eternamente amada vó, Maria de Lourdes, que tanto se preocupou comigo durante essa etapada da minha vida. Chorou, sorriu, incentivou e me apoiou incrivelmente, sempre dizendo que eu precisava seguir em frente e conquistar as minhas coisas com garra...a senhora não está mais aqui, mas no meu coração será eterna...te amo muito...e só o Senhor sabe o quanto sentiremos sua falta todos os dias...essa etapada da minha vida está acabada finalmente e esse trabalho é seu, somente seu...

Índice

Resumo

O gênero *Elachistocleis* Parker 1927 é composto por 17 espécies e, destas, sete possuem a morfologia externa descritas e duas possuem a morfologia oral interna e o condrocrânio de suas larvas descritas. A falta de trabalhos descritivos que contribuam para o conhecimento dos girinos do gênero *Elachistocleis*, constitui a justificativa deste trabalho. Assim, o objetivo principal deste trabalho foi caracterizar a morfologia externa, morfologia oral interna, condrocrânio e aparelho hiobranquial das larvas do gênero *Elachistocleis*, comparando-as entre si e com espécies descritas na literatura. Foram utilizadas as espécies *E. bumbameuboi*, *E.* cf. *piauiensis*, *E. cesarii* e *E. bicolor* entre os estágios 31 a 39 de Gosner. A morfologia externa dos girinos do gênero *Elachistocleis* é conservativa em muitos aspectos. As diferenças encontradas foram: o formato do corpo em vista dorsal; borda das abas dermais; borda do focinho em vista dorsal; ângulo de emergência da nadadeira dorsal no corpo; linha lateral visível ou não; padrão de pústulas na região posterior à crista mediana; projeção mais acentuada da margem anterior central do corpo da suprarostral; borda posterior das asas da suprarostral em forma de W ou não; borda ventral da fenestra da região occipital levemente ondulada ou claramente ondulada; presença de uma fenestra hipofisária; borda do processo afilado do processo articular do palatoquadrado; presença de expansões digitiformes no processo ventrolateral; formato e inclinação da fenestra subocular; processo subótico único ou levemente bífido; inclinação do processo anterolateral. O estudo comparativo corrobora em muitas das características observadas nos trabalhos descritivos publicados tanto para girinos do gênero *Elachistocleis* quanto para outras espécies de Microhylidae do Velho e Novo Mundo.

Abstract

The gender *Elachistocleis* Parker in 1927 consists of 17 species , and of these, seven have the external morphology described and two have the internal oral morphology and the chondrocranium of its larvae described. Lack of descriptive works that contribute to the knowledge of the tadpoles of *Elachistocleis* is the justification of this work. Therefore, the goal of this work was to characterize the external morphology, internal oral morphology, chondrocranium and hiobranquial apparatus of tadpoles of Elachistocleis, comparing them with each other and with other species described in the literature. We used *E. bumbameuboi*, *E.* cf. *piauiensis*, *E. cesarii* and *E. bicolor* between 31-39 of Gosner stage . The external morphology of *Elachistocleis* is conservative in many aspects. Differences found were: Body format in dorsal view; edge of dermal flaps; snout edge in dorsal view; emergency angle of the dorsal fin in the body; lateral line visible or not; pustules pattern on the posterior region of the median ridge; pronunciation of the projection in the anterior medial margin of the suprarostral corpus; posterior margin of the suprarostral alae in W-shaped or not; ventral margin of the fenestra in the occipital region slightly or clearly wavy; presence of *fenestra hypophyseos*; margin of the thin process in the articular process; Presence of finger-like expansions in the ventrolateral process; shape and inclinatione of the subocular fenestra; subótico process single or slightly bifid; inclination of the anterolateral process. The comparative study corroborates with most of the characteristics observed in descriptive works published for the gender *Elachistocleis* and for other species of Microhylidae from the Old and New World.

Apresentação

A família Microhylidae Günther, 1858 é a quarta maior família de anuros (depois de Hylidae, Strabomantidae e Bufonidae) (de Sá *et al.* 2012). Atualmente constitui 564 espécies amplamente distribuídas nas Américas do Norte e Sul, África Subsaariana, e a faixa que se estende da Índia e Coréia até o norte da Austrália (Frost 2015; Duellman 1993).

O gênero *Elachistocleis* Parker, 1927 constitui-se de 17 espécies válidas, das quais 12 são encontradas no Brasil (Frost 2015). Recentemente, Caramaschi (2010) apresentou um estudo taxonômico sobre o gênero. Neste artigo, Camaraschi discute a problemática envolvendo a taxonomia de *Elachistocleis ovalis, Elachistocleis bicolor* e *Elachistocleis surinamensis*, considerando *E. ovalis* um *nomem dubium*. Além disso, o artigo descreve cinco novas espécies (*Elachistocleis helianneae, Elachistocleis surumu, Elachistocleis carvalhoi, Elachistocleis matogrosso*), dentre elas *Elachistocleis bumbameuboi*, cuja distribuição conhecida se restringe a duas localidades no Estado do Maranhão. Não são abordadas características larvares no artigo.

No Estado do Maranhão, além de *E. bumbameuboi*, é encontrada *E. piauiensis* em região mais ao Sul do Estado. Outra espécie do gênero, *E. carvalhoi*, possui distribuição próximo à fronteira do Estado, na região Sudeste do Pará.

O uso de caracteres larvários em estudos taxonômicos de anfíbios anuros pode auxiliar na resolução de problemas taxonômicos. O primeiro trabalho em sistemática anura envolvendo a morfologia larval foi feito por Lataste (1879). Desde então, muitos pesquisadores têm utilizado caracteres larvares em seus estudos sistemáticos.

Orton (1953; 1957) propôs uma classificação das larvas de anuros baseada em quatro tipos larvários: Tipo 1, que inclui a família Pipidae e Rhinophrynidae, com girinos que possuem espiráculo emparelhado e ausência de partes da boca queratinizadas; Tipo 2, que consiste da família Microhylidae, com ausência de partes da boca queratinizadas e um único espiráculo mediano; Tipo 3, que inclui as famílias Ascaphidae e Discoglossidae, com partes da boca queratinizadas e um espiráculo mediano; Tipo 4, que inclui todas as demais famílias, com girinos com partes da boca queratinizadas e espiráculo sinistral.

Starret (1973), após um estudo mais aprofundando sobre a sistemática dos anuros, o qual em seu trabalho ainda incluiu novos dados sobre o condrocrânio, a musculatura, as câmeras branquiais e a localização do primórdio do membro anterior, propôs nomes para os 4 grupos definidos por Orton: Xenoanura para o Tipo 1, Scoptanura para o Tipo 2, Lemmanura para o Tipo 3 e Acosmanura para o Tipo 4.

A revisão da filogenia das larvas de anuros de Sokol (1975), junto com o trabalho de Starret (1973), trouxeram a aceitação da classificação nos 4 tipos larvares de Orton, mas trabalhos subsequentes mostraram a descoberta de girinos com características que não se encaixavam nos tipos larvares de Orton, principalmente quanto ao Tipo 2 (Blommers-Schlösser 1975; Wassersug 1984; Pyburn 1980), levando-se a conclusão da necessidade de redefinição desse tipo larvar.

Wassersug (1976) argumenta que a chance de divergência entre as famílias é alta devido aos poucos caracteres utilizados na definição dos tipos larvares de Orton, necessitando na busca por caracteres diagnósticos adicionais que possam validar ainda mais esses tipos larvares. Desse modo, em seu trabalho sobre a descrição geral e a terminologia a ser usada em morfologia oral interna de larvas de anuros, buscou padronizar as características apresentadas na cavidade oral interna das larvas, além da busca por novas características diagnósticas.

Desse modo, o trabalho de Wassersug ampliou o conhecimento que se tem sobre as estruturas que os girinos apresentam e que são de extrema importância no delineamento das espécies conhecidas. Os argumentos apresentados por ele do porquê utilizar a morfologia oral interna das larvas de anuros reflete a repercussão que seu trabalho teve nos futuros projetos com girinos: a facilidade em trabalhar com as estruturas orais, visto que grande parte dos tecidos das larvas estão envolvidas na digestão e o contato direto que estas estruturas têm com o ambiente na aquisição de nutrientes.

Características larvares da morfologia oral interna, do condrocrânio e do aparelho hiobranquial têm recebido relativa atenção nos últimos anos por serem consideradas conservativas em muitos grupos de anuros (Lavilla 1992). Wassersug (1976) sugere que a morfologia interna das larvas seja mais conservativa do que a morfologia externa. Em contrapartida, Andrade *et al.* (2007) afirmam que esta última sofra mais intensamente as imposições ambientais.

O esqueleto larval dos anuros é composto do crânio e notocórdio. O crânio é comumente divido em três partes (Hyman 1942): condrocrânio ou neurocrânio, dermatocrânio e o esplancnocrânio. Além disso, o esqueleto hiobranquial é composto pelo conjunto dos elementos derivados dos arcos hióide e branquiais. O primeiro trabalho sobre o esqueleto larval em Microhylidae foi feito por Ridewood (1898), onde foi feita uma breve descrição do aparelho hiobranquial de *Microhyla ornata*. Lavilla & Langone (1991) iniciaram um estudo sobre as mudanças ontogenéticas no espiráculo e a orientação do tubo anal e também do esqueleto cefálico de *Elachistocleis bicolor*, além da descrição do condrocrânio e aparelho hiobranquial (Lavilla & Langone 1995).

Com referência ao gênero *Elachistocleis*, somente *E. surinamensis* (Kenny, 1969), *E. erythrogaster* (Kwet & Di-Bernardo 1998), *E. pearsei* (Lynch 2006), *E. cesarii* (Magalhães *et al.* 2012) e *E. haroi* (Pereyra *et al.* 2013) possui descritas sua morfologia externa, junto com *E. bicolor* (Williams & Gudynas, 1987; Rossa-Feres & Nomura, 2006) e *E. panamensis* (Candioti 2006, 2007) únicas espécies com a morfologia oral interna (D'Heursel & Baldissera Junior 1999; Echeverría & Lavilla 2000; Candioti 2007), além do condrocrânio (Lavilla & Langone 1995; Candioti 2006; 2007) descritos.

Dessa forma, pretendemos descrever a morfologia externa; a morfologia oral interna; o condrocrânio e o aparelho hiobranquial de larvas do gênero *Elachistocleis*, visando descobrir possíveis caracteres diagnósticos que auxiliem na taxonomia das espécies do gênero da família Microhylidae.

Objetivos

Geral

Descrever aspectos da morfologia e taxonomia do gênero *Elachistocleis* com ênfase em estudos da morfologia externa, morfologia oral interna, do condrocrânio e do aparelho hiobranquial de larvas.

Específicos

(1) Descrever e comparar o condrocrânio, o aparelho hiobranquial, morfologia externa e a morfologia oral interna de larvas do gênero *Elachistocleis*;

(2) Distinguir possíveis caracteres que possam ser utilizados na taxonomia e sistemática da família Microhylidae.

Referencial teórico

Características larvais e a sistemática anura

Van Bambeke (1863) apud Altig & McDiarmid (1999) foi o primeiro a notar que havia diferenças interespecíficas entre anuros europeus baseando-se em traços larvais que incluíam caracteres taxonômicos tal como descrição do aparato bucal de girinos. A partir daí, Lataste (1879, 1888) apud Altig & McDiarmid (1999) buscou utilizar a morfologia larvária de anuros na sistemática do grupo com base na posição do espiráculo e propôs nomes às famílias de anuros com base nesse traço morfológico (e.g. Mediogyrinidae).

Subsequentemente a esses trabalhos, a relevância de se considerar caracteres larvais em sistemática anura foi feita principalmente por Noble (1925, 1927). Noble (1925) já havia considerado as características larvais como tão importantes quanto às dos adultos em diagnosticar relações de parentesco e, no ano seguinte, utilizou características larvares e sua importância na elucidação de contradições entre anuros, principalmente da família Brevicipitidae e outras espécies da África do Sul.

Na década de 50, temos trabalhos de grande relevância que até hoje têm parte na terminologia utilizada na taxonomia larvária anura. Orton (1953, 1957) propôs uma classificação com base em dois caracteres morfológicos e agrupou os girinos em 4 grandes grupos designados à uma família ou grupos de famílias: Tipo 1, que inclui a família Pipidae e Rhinophrynidae, com girinos que possuem espiráculo emparelhado e ausência de partes da boca queratinizadas; Tipo 2, que consiste da família Microhylidae, com ausência de partes da boca queratinizadas e um único espiráculo mediano; Tipo 3, que inclui as famílias Ascaphidae e Discoglossidae, com partes da boca queratinizadas e um espiráculo mediano; Tipo 4, que inclui todas as demais famílias, com girinos com partes da boca queratinizadas e espiráculo sinistral.

Em seguida, Starret (1973), após um estudo mais aprofundando sobre a sistemática dos anuros, o qual em seu trabalho ainda incluiu novos dados sobre o condrocrânio, a musculatura, as câmeras branquiais e a localização do primórdio do membro anterior, propôs nomes para os 4 grupos definidos por Orton: Xenoanura para o Tipo 1, Scoptanura para o Tipo 2, Lemmanura para o Tipo 3 e Acosmanura para o Tipo 4.

A revisão da filogenia das larvas de anuros de Sokol (1975), junto com o trabalho de Starret (1973), trouxeram a aceitação da classificação nos 4 tipos larvares de Orton, mas trabalhos subsequentes mostraram a descoberta de girinos com características que não se encaixavam nos tipos larvares de Orton, principalmente quanto ao Tipo 2 (Blommers-Schlösser 1975; Wassersug 1984; Pyburn 1980), levando-se a conclusão da necessidade de redefinição desse tipo larvar.

Wassersug em 1976 argumenta que a chance de divergência entre as famílias é alta devido aos poucos caracteres utilizados na definição dos tipos larvares de Orton, necessitando na busca por caracteres diagnósticos adicionais que possam validar ainda mais esses tipos larvares. Desse modo, verificou que muitas das descrições de estruturas orais de girinos eram inconsistentes, com estruturas recebendo denominações diferentes ou especulações quanto à função de diferentes partes, e resolveu publicar uma descrição geral das estruturas orais de girinos. Neste trabalho, termos antigos são redefinidos e novas estruturas são descritas em uma terminologia que buscou a descrição geral das estruturas orais em girinos, baseado mais precisamente no Tipo 4 de Orton, com relação a larvas nos estágios 26-39 (Gosner, 1960).

Assim, o trabalho de Wassersug ampliou o conhecimento que há sobre as estruturas que os girinos apresentam e que são de extrema importância no delineamento das espécies conhecidas. Os argumentos apresentados por ele do porquê utilizar a morfologia oral interna das larvas de anuros reflete a repercussão que seu trabalho teve nos futuros projetos com girinos: a facilidade em trabalhar com as estruturas orais, visto que grande parte dos tecidos das larvas estão envolvidas na digestão e; o contato direto que estas estruturas têm com o ambiente na aquisição de nutrientes. Assim, trabalhos subsequentes que primeiramente descreviam as estruturas orais de girinos foram sendo publicados (Parker 1881; Savage 1952; Gradwell 1970, 1972).

Após isso, as características larvares não somente da morfologia oral interna, mas do condrocrânio e do aparelho hiobranquial receberam relativa atenção nos últimos anos por serem consideradas conservativas em muitos grupos de anuros (Lavilla 1992; Candioti 2007; Haas 1995). Wassersug (1976) sugere que a morfologia interna das larvas seja mais conservativa do que a morfologia externa. Em contrapartida, Andrade *et al.* (2007) afirmam que esta última sofra mais intensamente as imposições ambientais.

Aparentemente, o primeiro trabalho envolvendo o condrocrânio de anfíbios foi feito por Dugès (1834) apud Canatella (1999), que descreveu o girino de *Pelobates* *fuscus*. Dentre o crescente número de trabalhos subsequentes que descrevem a estrutura do condrocrânio e aparelho hiobranquial de diversos girinos (Noble 1929; Ramaswami 1940; Starret 1975; Sokol 1975; Wassersug & Hoff 1979 1982; Lavilla 1992; Candioti 2006, 2007), podemos destacar o trabalho de Haas (2003), que atualmente constitui o maior trabalho envolvendo a filogenia de anuros com base em uma alta gama de dados larvais que, utilizando 156 caracteres larvais, envolvendo a morfologia externa, condrocrânio, musculatura, biologia reprodutiva, além de caracteres adultos, buscou agrupar os diversos táxons de anuros que ainda são sustentados principalmente por características oriundas de espécimes adultos.

O trabalho deu suporte adicional às características apomórficas apresentadas pela família Microhylidae, mas utilizou somente três espécies que representassem os microhilídeos americanos, dentre elas o *E. bicolor,* que foram agrupadas em um clado com base em três sinapomorfias: divisão do *m. levator arcuum branchialum III* em dois feixes de cruzamento; ausência do *processos muscularis*; presença de um distinto processo ventral do palatoquadrado rostralmente ao *m. orbitohyoideus*.

A família Microhylidae e o gênero *Elachistocleis*

A família Microhylidae Günther, 1858 é a quarta maior família de anuros (depois de Hylidae, Strabomantidae e Bufonidae) (de Sá *et al.* 2012). Atualmente constitui 564 espécies amplamente distribuídas nas Américas do Norte e Sul, África Subsaariana, e a faixa que se estende da Índia e Coréia até o norte da Austrália (Frost 2015; Duellman 1993).

De acordo com Ford & Canatella (1993), as sinapomorfias de Microhylidae que sustentam o monofiletismo da família incluem 2 a 3 dobras do palato em adultos e as seguintes características larvais: ausência de dentículos cornificados, velum ventral dividido medianamente, a glote totalmente exposta no assoalho bucal, narinas não perfuradas, cristas secretoras das malhas branquiais de alimentos com uma linha única de células secretoras nas ápices (Wassersug 1984).

Parker (1934) apresenta uma revisão monográfica da família ao apresentar o agrupamento de 191 espécies em 7 subfamílias definidas em 12 caracteres morfológicos não-sinapomórficos. Após este trabalho, estudos subsequentes ficaram restritos a revisão de poucas subfamílias (Burton 1986; Carvalho 1954) e o reconhecimento de novas subfamílias, caso de Phrynomerinae (Lynch 1973), Scaphiophryninae (Bloomers-Schlösser, 1975) e Otophryninae (Wasssersug & Pyburn, 1987).

A sistemática de Microhylidae é aberta a muitas discussões quanto à sua monofilia e trabalhos subsequentes tiveram discussões fechadas quanto ao parentesco com Ranidae (Griffiths 1963; Lynch 1973; Trueb 1973; Starrett 1973; Savage 1973; Sokol 1975), enquanto alguns trabalhos descreveram chaves taxonômicas e notas sobre Microhylidae restritos a determinadas regiões (Barker *et al.* 1995; Manthey & Grossman 1997; Fei *et al.* 2009).

Com a utilização de técnicas moleculares no auxílio da resolução de problemas taxonômicos, alguns trabalhos buscaram resolver parte dos problemas na divisão das subfamílias de Microhylidae (Matsui *et al.* 2011). Pyron & Wiens (2011) em seu estudo em larga escala sobre a filogenia anfíbia, corrobora os resultados encontrados por van der Meijden (2007) sobre a delimitação atual das subfamílias de Microhylidae, além de diversos gêneros inclusos com o status de *incertae sedis*. De Sá *et al.* (2012) atualmente constitui o trabalho com maior abrangência de espécies num estudo detalhado que buscou a resolução filogenética com base em amostras de 200 espécies, representando 91% das subfamílias e 95% dos gêneros do Novo Mundo reconhecidos no momento.

O trabalho de De Sá (2012) oferece percepções acerca da evolução da família Microhylidae com ênfase nas espécies pertencentes ao Novo Mundo. Todas as espécies *incertae sedis* pertencentes ao Novo Mundo foram incluídas na subfamília Gastrophryninae, com exceção de *Synapturanus*, gênero considerado um parente distante dos outros microhilídeos do Novo Mundo e excluído de Gastrophryninae (van der Meijden *et al.* 2007) e recuperado como táxon irmão de *Otophryne* de Otophryninae, a exemplo de Pyburn & Wiens (2011).

Em relação ao gênero *Elachistocleis*, De Sá *et al.* (2012) demonstraram que *Chiasmocleis panamensis* é mais próximo do gênero *Elachistocleis* do que outras espécies do gênero *Chiasmocleis*, além de incluir em *Elachistocleis* o gênero *Relictivomer*.

O gênero *Elachistocleis* Parker, 1927 constitui-se de 17 espécies válidas, das quais 11 são encontradas no Brasil (Frost 2014). O gênero é palco de diversas discussões e controvérsias quanto à validação taxonômica de suas espécies, principalmente por causa de problemas de identificação envolvendo *E. ovalis* e *E. bicolor* desde 1841 (Lavilla *et al.* 2003). Na descrição original de Schneider (1799) e nem nas subsequentes (Shaw 1802; Merren 1820) de *E. ovalis*, nenhuma localidade tipo foi dada à espécie.

Para *E. bicolor* (Guerín de Méneville 1838; Valenciennes 1838), a descrição original aponta sua localidade-tipo como América Meridional. Posteriormente, Lavilla (2003) argumenta que *E. bicolor* possui evidências indiretas sobre a suposição de sua localidade-tipo específica, devido à indivíduos analisados por Duméril & Bibron (1841) com base na descrição figurativa de Guerín de Méneville (1838), suportando a hipótese levantada por Dunn (1949) sobre a localidade tipo ser Buenos Aires, Argentina.

Lavilla (2003) argumenta que o conjunto de dados que caracterizam os anfíbios como *Elachistocleis*, como por exemplo a coloração ventral imaculada, constitui um grande complexo de espécies que necessita de uma revisão mais aprofundada.

Recentemente, Caramaschi (2010) apresentou um estudo taxonômico sobre o gênero. Neste artigo, Camaraschi discute a problemática envolvendo a taxonomia de *E. ovalis, E. bicolor* e *Elachistocleis surinamensis*, considerando *E. ovalis* um *nomem dubium*. Além disso, o artigo descreve cinco novas espécies (*Elachistocleis helianneae, Elachistocleis surumu, Elachistocleis carvalhoi, Elachistocleis matogrosso*), dentre elas *Elachistocleis bumbameuboi*, cuja distribuição conhecida se restringe a duas localidades no Estado do Maranhão.

De acordo com Frost (2014), esta afirmação sobre *E. ovalis* tem o efeito de deixar as populações do Panamá, Colômbia, Venezuela, Peru, Equador, Trinidade e Tobago, Guiana, Guiana Francesa e o Suriname sem um nome. Desse modo, estas populações necessitariam de uma revisão adequada com base nas características e suas variações que compõem as espécies de *Elachistocleis*.

Nos últimos anos, o número de espécies descritas para o gênero tem crescido consideravelmente. Lavilla (2003) que descreve *Elachistocleis skotogaster* para Argentina e, no Brasil, temos Caramaschi (2010) com a cinco novas espécies, Toledo (2010) com a descrição de *Elachistocleis magnus* para o município de Rondônia e a descrição recente de *Elachistocleis muiraquitan* para o município do Acre (Nunes-de-Almeida & Toledo 2012).

Por último, Pereyra *et al*. (2013) descreveram a espécie *E. haroi* para a Argentina, com base em caracteres morfológicos, osteológicos, vocalização, além da descrição da morfologia externa do girino.

Com referência a descrição de girinos para o gênero, somente *E. surinamensis* (Kenny, 1969), *E. bicolor* (Williams & Gudynas, 1987; Rossa-Feres & Nomura, 2006), *E. erythrogaster* (Kwet & Di-Bernardo 1998), *E. panamensis* (Candioti 2006), *E. pearsei* (Lynch 2006), *E. cesarii* (Magalhães *et al.* 2012) e *E. haroi* (Pereyra *et al.* 2013) possuem sua morfologia externa descrita.

Elachistocleis bicolor possui sua morfologia oral interna descrita por D'Heursel & Baldissera Júnior (1999), Echeverría e Lavilla (2000) e Candioti (2007) e o condrocrânio e aparelho hiobranquial descritos por Lavilla & Langone (1995) e Candioti (2007), enquanto *E. panamensis* tem sua morfologia oral interna e o condrocrânio e aparelho hiobranquial descritos por Candioti (2006; 2007).

Quanto ao condrocrânio, Candioti (2007), verifica que a principal diferença entre as espécies residiu num longo e bem desenvolvido processo ventrolateral do palatoquadrado em *E. bicolor* em comparação com *D. muelleri* e *E. panamensis*, descritas no estudo, apesar de não haver diferenças marcantes quanto ao aparelho hiobranquial.

Caracterização da Morfologia Oral Interna para Microhylidae

A cavidade oral pode ser dividida em duas grandes regiões: a região anterior, chamada de cavidade bucal, e a posterior, cavidade faringeana. Essas cavidades são separadas por estruturas não musculares que são essencialmente contínuas ao longo do assoalho e teto bucal, o velum ventral e o velum dorsal (Wassersug 1976).

Assoalho Bucal (Figura 1):

A região anterior consiste na presença da região interna do lábio inferior, correspondente à infrarostral; A presença do rudimento lingual, desprovido de papilas. Na região lateral, há a presença das bolsas bucais bem desenvolvidas, formações epiteliais presentes em espaços laterais entre a margem posterolateral dos ceratohiais e a margem anterior dos primeiros ceratobranquiais. As bolsas bucais apresentam papilas e pustulações, chamadas de papilas das bolsas bucais.

A arena do assoalho bucal, que se inicia após o rudimento lingual é praticamente desprovida de qualquer estrutura. É limitada posteriormente por uma série, transversa e curva de papilas longas de cada lado da abertura da glote. A glote é estreita e alongada, totalmente visível dorsalmente; lábios glotais pouco proeminentes. Lateralmente aos lábios glotais, desde a região anterior da glote, dois condutos alongados que se estendem até o final do velum, envolvendo a glote numa forma levemente circular e estreitando-se à medida que se estende ao velum, dividindo-o em porção direita e esquerda.

A região posterior do assoalho bucal é marcada pela presença do velum ventral, que constitue uma estrutura longa, lisa e delicada, sem espículas de sustentação; sua face ventral é parcialmente presa às placas filtrantes. A margem inferior do velum é livre e encurvada dorsalmente. A superfície velar é irregular com projeções suaves e largas.

Teto bucal (Figura 2):

Dividido em três regiões. A primeira, região anterior, chamada de área pré-coanal, consiste na presença de uma papila em sua região mediana, chamada de papila da arena pré-coanal. Na região mediana há a apresenta das coanas, estruturas correspondentes as narinas, não perfuradas e que se projetam abaixo as superfície rostral. Estas possuem estruturas projetando-se da sua margem posterior, que são estruturas provavelmente homólogas às projeções coanais valvulares (narial valve projections) (Candioti 2007; Wassersug 1980). Na região posterolateral das coanas, temos a presença de uma papila relativamente longa, seguida de pequenas pústulas que seguem o contorno anterior da borda das coanas.

A segunda região, chamada pós-coanal, é praticamente ausente e está inserida anteriormente pelas coanas e posteriormente pela crista mediana. A crista mediana é caracterizada como uma aba transversal e corresponde a um importante ponto de referência.

Por fim, temos a terceira região, chamada de arena do teto bucal, uma área fracamente definida que corresponde a arena do assoalho bucal ventralmente. Esta começa atrás da crista mediana e possui pequenas pústulas distribuídas irregularmente em toda sua extensão. Posteriormente há a presença do velum dorsal, um fino e delicado epitélio que carece de espículas cartilaginosas e seu extenso tecido conectivo suporta o velum ventral.

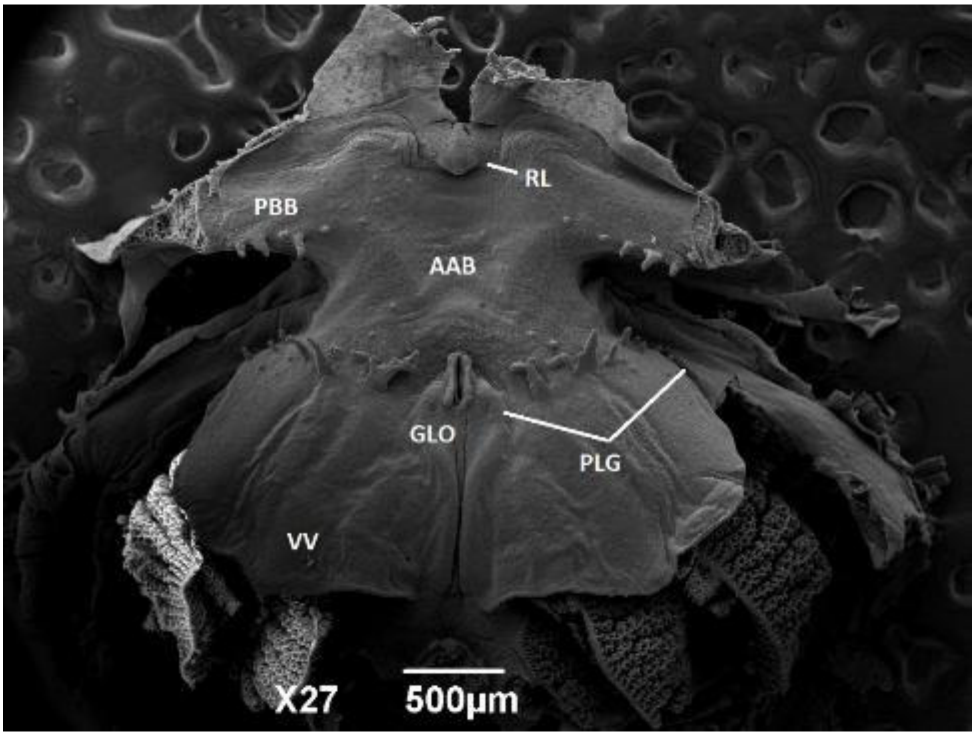

Figura 1: Assoalho bucal de *Elachistocleis bumbameuboi*. **AAB,** arena do assoalho bucal; **GLO,** glote; **PBB,** papila da bolsa bucal; **PLG,** papilas laterais da glote; **RL,** rudimento lingual; **VV,** velum ventral.

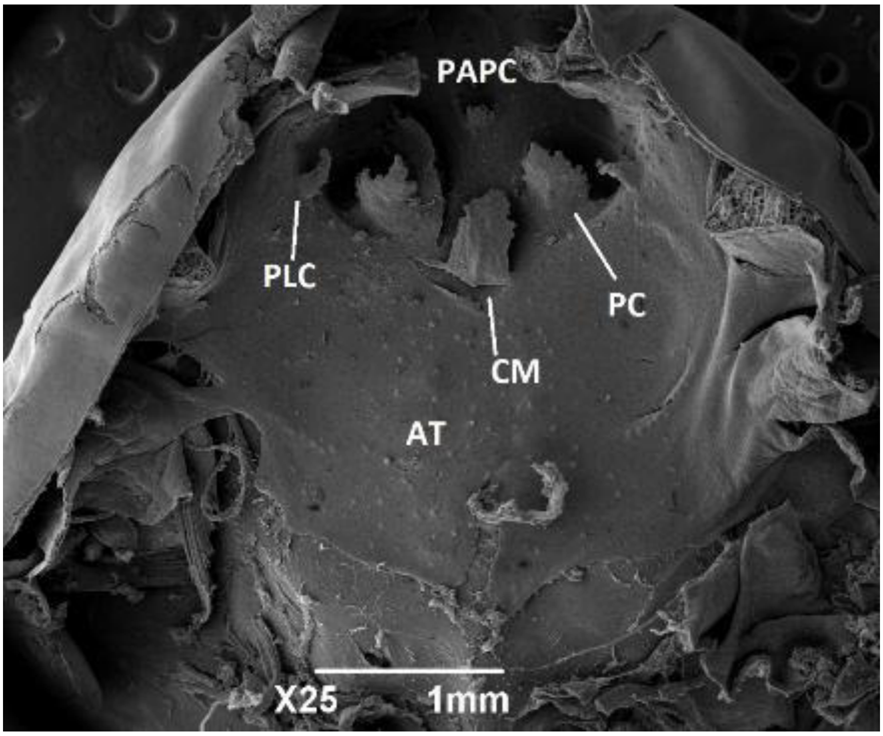

Figura 2: Teto bucal de *Elachistocleis bumbameuboi.* **AT,** arena do teto; **CM,** crista mediana; **PAPC,** papila da arena pré-coanal; **PC,** papila da coana; **PLC,** papila lateral da coana.

Caracterização do Condrocrânio e Aparelho Hiobranquial

O esqueleto larval dos anuros é basicamente formado pelo condrocrânio e o aparelho hiobranquial. O condrocrânio é uma caixa cartilaginosa que protege o cérebro e dá suporte aos órgãos e ao aparato bucal (Cannatella 1999). No seu início de desenvolvimento, o condrocrânio aparece como dois pares de elementos cartilaginosos: as paracordais e as trabéculas. As paracordais flanqueiam como varas longitudinais o final anterior da notocorda. As trabéculas se formam no mesmo plano, mas anteriormente às paracordais e são fusionadas anteromedialmente para formar a placa trabecular (Cannatella 1999; Sachssé 1996).

Região etmoidal

A região anterior do condrocrânio serve como suporte às cápsulas nasais e a mandíbula larvar. A placa etmoidal é derivada da fusão anterior mediana das trabéculas do crânio e dela dois cornos trabeculares se estendem ventralmente e anterolateralmente. Estes cornos são articulados ou fusionados a um par de cartilagens suprarostrais, que dão suporte à mandíbula superior (Figura 3).

Região orbital

A lateral do condrocrânio consiste na cartilagem orbital, que se estende da região posterior dos cornos trabeculares com a placa etmoidal e a região anterior das cartilagens paracordais. A cartilagem orbital que situa-se na parede lateral é fundida à base do crânio por três pilares: pilar proótico, pilar metótico e pilar antótico (Figura 5). Entre os pilares proótico e metótico situam-se o forame troclear e o forame ótico e, entre os pilares metóticos e antótico situa-se o forame oculomotor e, após o pilar antótico situa-se o forame proótico. Posteriormente, a cartilagem orbital se junta as cápsulas óticas (auditivas) *taenia tecti marginalis* (Sachssé 1996).

Caixa craniana e cápsulas óticas

O crânio é aberto dorsalmente via uma grande fenestra frontoparietal, que é delimitada lateramente pela *taenia tecti marginalis*, anteriormente pela placa etmoidal e posteriormente pela *tectum synoticum*. A fenestra frontoparietal pode ser dividida subdividida pela *taenia tecti transversalis*, que divide em fenestra parietal posteriormente e fenestra frontoparietal anteriormente. A *taenia tecti medialis*, se existir, parte da *taenia tecti transversalis* à *tectum synoticum* e subdivide a fenestra parietal em esquerda e direita (Cannatella 1999; Sachssé 1996).

A base do crânio é delimitada anteriormente pelo *planum trabeculare anticum* e posteriormente pelo *planum basale*. Em sua região central, há a presença da fenestra hipofisária. Lateralmente à fenestra hipofisária, há a presença do forame carótido, mais anterior, e do forame crânio-palatino, mais posterior (Figura 4).

As cápsulas óticas são estruturas subesféricas alocadas na região posterior da caixa craniana e correspondem às cartilagens que se formam ao redor do saco auditivo, correspondendo ao ouvido interno. As cápsulas óticas estão conectadas pela *tectum synoticum*. De cada cápsula ótica desenvolve uma crista achatada, a crista parótica, que se estende lateralmente da parede externa do canal semicircular lateral.

Palatoquadrado

O palatoquadrado é uma cartilagem longa e achatada que se liga sincondroticamente ao condrocrânio, normalmente, via quatro processos cartilaginosos: processo ascendente, processo basal, processo ótico e comissura quadrato-cranial. O palatoquadrado atua como suspensório da mandíbula. Sua porção anterior sustenta o processo articular e articula-se com a cartilagem de Meckel. Medianamente se funde à base do condrocrânio pela comissura quadrato-cranial que possui um processo quadrato-etmoidal. Lateralmente possui o processo muscular, que em sua região ventral pode sustentar um processo ventrolateral.

O palatoquadrado em sua região lateral mediana, desde o processo articular até a região posterior das cápsulas óticas possui o arco subocular. A grande lacuna que separa-o do condrocrânio constitui a fenestra sobocular. O arco subocular termina no processo ótico larval, que o conecta às cápsulas óticas. Na região posterior do palatoquadrado, pode existir um processo posterolateral, que pode sustentar um processo subótico em sua região ventral.

Maxilas superior e inferior

A maxila superior é formada pelas cartilagens suprarostrais, que sustentam o corpo central e as asas laterais. O corpo pode estar divido e as asas fusionadas ou não ao corpo. As asas apresentam um processo dorsal posterior. A suprarostral se conecta posteriormente ncondrótica, sindesmótica ou mista, à extremidade posterior dos cornos trabeculares (Sachssé 1996).

A maxila inferior é consistituida pela cartilagem de Meckel, que se articula posteriormente ao palatoquadrado e anteriormente é ligada a infrarostral sindesmoticamente ou sincondroticamente (Sachssé 1996). A cartilagem de Meckel possui o processo retroarticular, mais lateral e o processo dorsal.

Aparelho hiobranquial

O aparelho hiobranquial situa-se ventralmente ao condrocrânio e palatoquadrado e é derivado dos arcos viscerais, exceto do primeiro arco visceral que dá origem ao arco mandibular. Os ceratohiais, as copulas e placas hipobranquiais são os pistões de bombeamento bucal que transmite a água carregada de partículas alimentares para as branquiais e armadilhas de de comida branquiais (branchial food traps) (Figura 6).

Os ceratohiais articulam-se com o palatoquadrado por articulações hioquadradas sinoviais e entre si medianamente pela cópula II e pelo ligamento interhioide, cuja massa de cartilagem fibrosa em sua parte posterior, constitui a cópula I. Estas cópulas constituem as estruturas basihiobranquiais medais e a cópula II possui em sua extremidade posteroventral um processo urobranquial. Os ceratohiais possuem cinco processos: anterior, posterior, anterolateral, hioquadrado e lateral. O *par reuniens* é uma massa branca fibrosa e mediana de tecido que liga os ceratohiais medianamente e separa ass cópulas I e II.

Os ceratohiais, cópulas, ligamentos e parte das placas hipobranquiais, que sustentam os ceratobranquiais I a IV, constituem a maior parte do do esqueleto de suporte do assoalho bucal. Após a conexão das placas hipobranquiais, os ceratobranquiais se expandem dorsalmente em um processo posterolateral denominado espícula. As espículas sustentam o velum ventral.

Por fim, os cestos branquiais são formados, cada um, por placas hipobranquiais subtriangulares e quatro ceratobranquiais

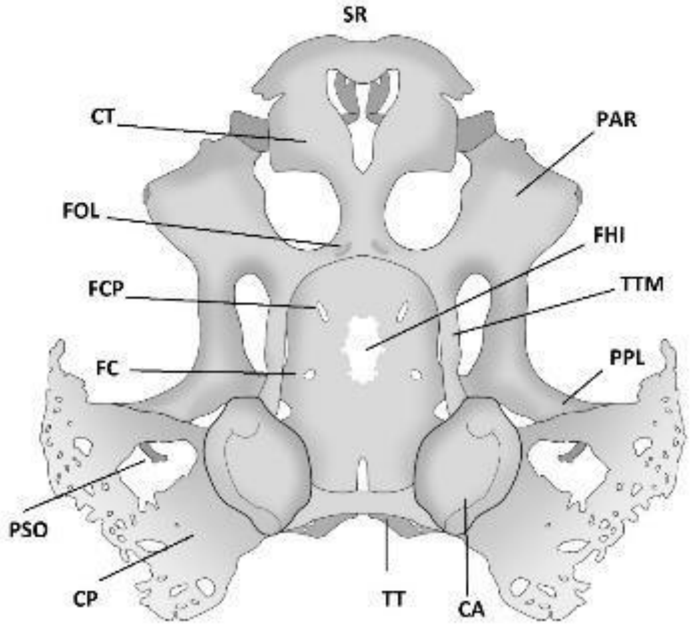

Figura 3: Vista dorsal do condrocrânio de *Elachistocleis bumbameuboi*. **CA,** cápsula auditiva; **CP,** crista parótica; **CT,** cornos trabeculares; **FC,** forame carótido; **FCP,** forame crâniopalatino; **FHI,** fenestra hipofisária; **FOL,** forame olfativo; **PAR,** processo articular; **PPL,** processo posterolateral; **PSO,** processo subótico; **SR,** suprarostral; **TT,** tectum synoticum; **TTM,** taenia tecti marginalis.

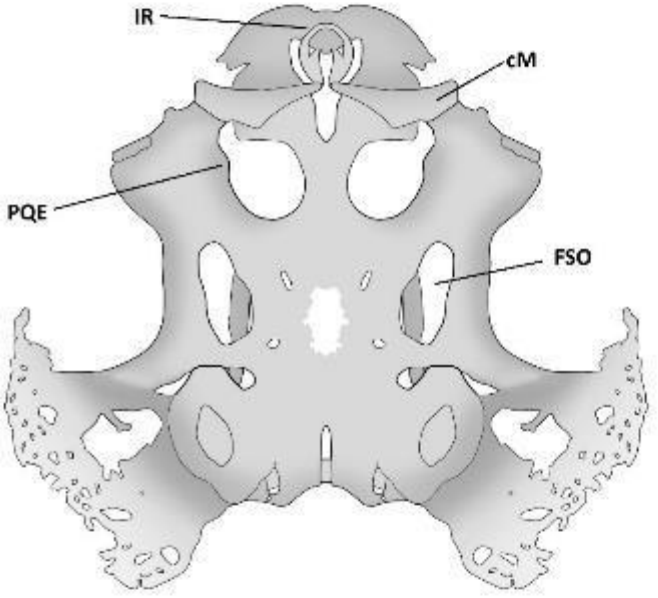

Figura 4: Vista ventral do condrocrânio de *Elachistocleis bumbameuboi*. **IR,** infrarostral; **cM,** cartilagem de Meckel; **FSO,** forame subocular; **PQE,** processo quadrato-etmoidal.

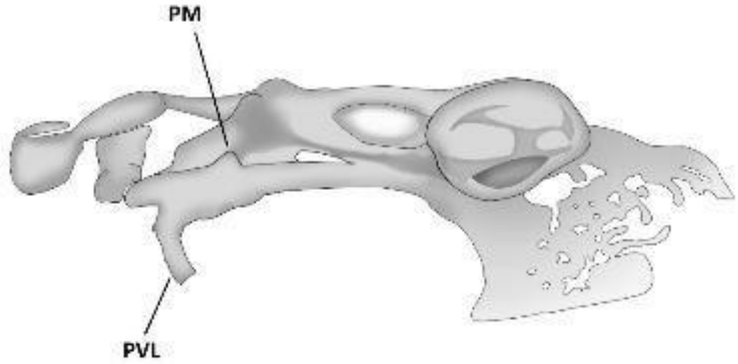

Figura 5: Vista lateral do condrocrânio de *Elachistocleis bumbameuboi*. **PM,** processo muscular; **PVL,** processo ventrolateral.

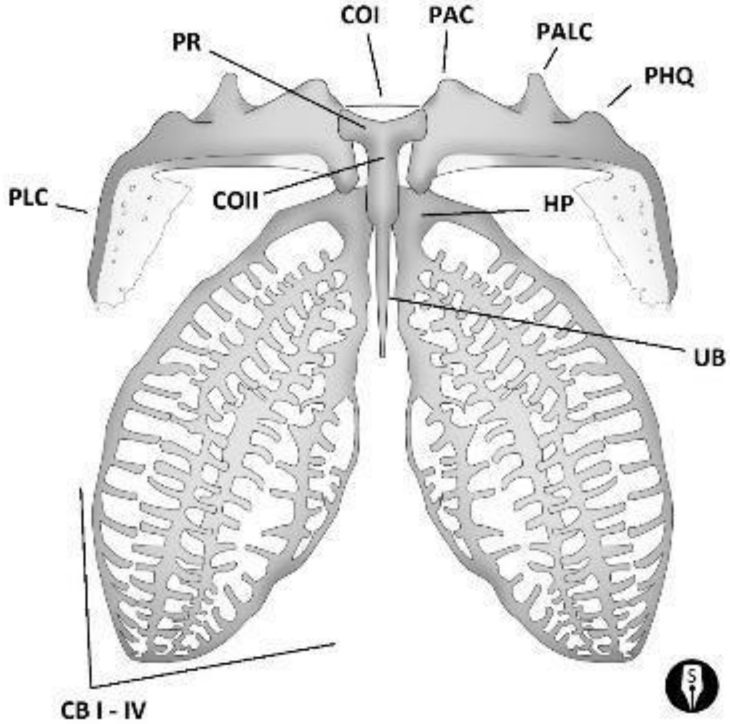

Figura 6: Aparelho hiobranquial de *Elachistocleis bumbameuboi* em vista ventral. **CB,** ceratobranquiais; **COI,** cópula I; **COII,** cópula II; **HP,** placa hipobranquial; **PAC,** processo anterior; **PALC,** processo anterolateral; **PHQ,** processo hioquadrado; **PLC,** processo lateral; **PR,** *pars reuniens*; **UB,** processo urobranquial.

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Capítulo I

O capítulo I compõe o artigo que será intitulado "Análise da morfologia externa, morfologia oral interna, condrocrânio e aparelho hiobranquial de larvas de *Elachistocleis* Parker, 1927 (Anura, Microhylidae)" e será submetido para publicação no período científico Herpetological Journal. Os critérios de redação e formatação seguem as normas deste periódico e estão disponíveis no ANEXO I.

A survey of the external morphology, internal oral morphology, chondrocranium and hyobranchial apparatus of *Elachistocleis* larvae Parker (Anura, Microhylidae)

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Abstract: In this study, the external morphology, internal oral morphology, chondrocranium and hyobranchial apparatus of *Elachistocleis bumbameuboi*, *E.* cf *piauiensis*, *E. cesarii* and *E. bicolor* are described and compared with each other and with other species of microhylids with description available in the literature. The general morphology of *Elachsitocleis* are conservative in many aspects. The differences are sutil and are found in the body shape, edge of the nares and snout, lateral line evident, presence of regular pustules in the buccal roof arena, posterolateral edge of the suprarostral cartilage, shape of the fenestra in the occipital region, hypophyseos fenestra visible or not, margin of the small process on the anterior region of the articular process, expansions in the ventrolateral process, shape and inclination of the subocular fenestra, subotic process single of slightly bifid, inclination of the anterolateral process. The features present in *Elachistocleis* genus support the similar morphological pattern of the species of Microhylidae.

Key-words: taxonomy, tadpoles, Microhylidae, cranial morphology, oral morphology.

Introduction

The genus *Elachistocleis* Parker is composed of 17 species distributed from south of Panama and Colombia, through Andes, south of Paraguay and southeastern of Bolivia, to central region of Argentina, central and southeastern of Brazil and Uruguay.

The study of the genus based on external morphology of the larvae has the descriptive works of *E. surinamensis*, *E. bicolor*, *E. erythrogaster*, *E. panamensis*, *E. pearsei*, *E. cesarii* and *E. haroi*.

The internal oral morphology and the chondrocranium of larvae of the genus have been poorly studied, with *E. bicolor* and *E. panamensis* as the only species described and compared based on this characters. Thus, we describe and analyse comparatively larvae of four species of the genus *Elachistocleis* with emphasis on the external morphology, internal oral morphology, chondrocranium and hyobranquial apparatus.

Materials and Methods

We analysed specimens from the following zoological collections: HUFMA (Universidade Federal do Maranhão), UESPI (Universidade Federal do Piauí), AAGARDA (Universidade Federal do Rio Grande do Norte), DZSJRP (Universidade Estadual Paulista) and UNNEC (Fac. de Ciencias Exactas y Naturales y Agrimensura da Universidad Nacional del Nordeste na Argentina). The following species were analysed in this study: *Elachistocleis bumbameuboi* (Caramaschi), *Elachistocleis* cf. *piauiensis* (Caramaschi & Jim), *Elachistocleis cesarii* (Miranda-Ribeiro), *Elachistocleis bicolor* (Guérin-Méneville).

We used the following methods to avoid mistakes in species identity: tadpoles from the same lots of original descriptions; identification confirmed from the original descriptions; tadpoles collected from the same ponds where adults in reproductive activity were monitored and observed the occurence of a single species of *Elachistocleis*. Tadpoles developmental stages were determined according to Gosner and all them were at stages 31-39. Addicional information about the material examined is presented in Appendix 1. Specimens were analysed with the program Leica Application Suite 4.0.0 under the stereoscopic microscope Leica DFC295. To the internal oral morphology, specimens were dissected and stained with 3% methyline blue solution. Descriptions follow Wassersug, Candioti and measurements from the main structures follow Weber & Camaraschi with modifications. To evidence structures of the chondrocranium and hyobranquial apparatus, specimens were cleared and stained for cartilage using the technique from Potthoff with modifications. Descriptions follow Cannatella, Haas and Candioti. Illustrations of the extenal morphology, internal oral morphology, chondrocranium and hyobranchial apparatus werer produced on Adobe Illustrator CS5 from photographs obtained under stereoscopic microscope with image analyzer.

Results

External morphology and coloration pattern

The tadpole of *Elachistocleis* has a rounded body in dorsal view and a globular/depressed body in lateral view (Tabela 1, Fig.1,2). However, there is a intra and interespecific variation in the body shape. Specimens of *E. cesarii* from the northeastern part of Brazil and *E. bicolor* from Brazil have a visible rounded body (Fig.1E,G), while the other species have a rounded/ovoid body, including *E. cesarii* from the southeastern part of Brazil, although every species has at least one specimen with a ovoid body with a narrower posterior region of the body than the anterior region .

In lateral view all species have a globular/triangular body. The mouth is terminal, small with emargination absent; rows of marginate papillae are absent. Two oral flaps, separated by a medial notch in the mouth, have irregular margin, mas in *E. bicolor* is predominantly smooth (Fig.2G), while other species the oral flaps margin has a undulate or slighlty papillar variation, although *E. cesarii* has a more papillar-jagged margin. (Fig.2H). Moreover, the papillar form is most evident on the limits of the oral flaps to all species analyzed. Lacking keratinized mouthparts and superior and inferior jaws. The snout shape in dorsal view, from the beginning of the infrarostral to the median region of the suprarostral, presents a variation observed intra and interespecies; also, there is a slightly concave depression in the median region. In dorsal view, *E. bumbameuboi* has a slightly pointed than rounded snout (Fig.1A); in lateral view, the snout is truncated to all species. Eyes small and lateral. Nares absent. Ventral spiracle, long and wide, median with sinistral inclination, opening posterodorsally directed and located in the posterior region of the body; inner wall fused to the vent tube and with same size as outer wall; posterior wall shorter than inferior wall. Vent tube long, fused to the ventral fin, sinstral, located between ventral fin and spiracle, posterior wall shorter than inferior wall, posterodorsal opening. Tail muscle thin in dorsal view; with gradual tapering in lateral view and rounded tip. Dorsal and ventral fins convex, with variable but mostly same height, with ascending shape at the first portion and descending at the third portion. Dorsal fin low, emerging on the body-tail junction with smooth inclination in a smooth wide arc, parallel to the tail muscle; in *E. Cesarii* the emerging fin on the body-tail junction is more pronounced and the fin shape in wide arc is undulated (Fig.2D); ventral fin low, parallel to the tail muscle in a smooth wide arc not as much as dorsal fin; lateral line not evident in *E.* cf. *piauiensis*, *E. bicolor* from Argentina and most individuals of *E. bumbameuboi*; in *E. bicolor* from the south part of

Brazil, neuromasts were evident in most of individuals, while *E. cesarii* were less evident, but visible.

Coloration in preservative: There is a great variation regarding the coloration in preservative of the species. The coloration is presented by the agglomeration of dark dots in larger quantities in dorsal region. *E. cesasrii* and *E. bicolor* from Brazil have a orange brown coloration, while *E. bicolor* from Argentina has a dark green coloration; *E. bumbameuboi* has a dark brown coloration and *E.* cf. *piauiensis* is the only species with a clear dark coloration. The dorsal region is characterized by the agglomeration of dots and some individuals of all species analyzed can have a median stripe depigmented with variable visibility. In some individuals of *E. bicolor* the median stripe is dark. The lateral region is presented by a coloration gradient, which the superior region is dark and the inferior region is clearer by having fewer dots; moreover, there are light or depigmented blotches in various sizes extending from the snout to posterior region of the body. In the anterior region of the ventral view there are dark dots and the posterior region is observed light or depigmented blotches with variable number. In most of individuals in all species presents blotches in different sizes and shapes extending ventrolaterally in the body forming a median stripe; this stripe is descontinuous or not. The posterior region of the ventral region is nearly transparent, mainly in *E.* cf. *piauiensis*, but in *E. cesarii* and *E. bicolor* from Brazil, this tranparency is not clearly discernible. Regarding the muscle tail, the blotch pattern observed ventrolaterally in the ventral region ends in a depigmented stripe on the anterior third of the tail. All species have a layer of connective tissue on the anterior third of fins in circular form decreasing to the end of the anterior third of the tail. This layer of tissue is clear and is formed by several dots irregularly distributed; in *E. cesarii* and *E. bicolor* from Brazil is easily discernible and well developed. Dorsal fin has blotches formed by dots likewise the body; the number of blotches is variable intra and interspecific, with *E. cesarii* and *E. bicolor* having several blotches.

Internal Oral Morphology

Because of great similarity among species regargind the internal oral morphology, we choose to present a general illustration of the oral cavity, pointing differences between species analyzed.

Ventral aspect (Table 2, Fig.3): The buccal floor is trapezoid, wider in the posterior region. The lower lip is rounded, bending at the posterior region. Anterior buccal region from the beginning of the tongue anlage is a narrow cavity formed by the bending of laterall walls of the lower lip toward the midline region of the buccal floor. On the internal walls of the lower lip there are pustules and small papillae (3 to 8 pustules and 1 papilla in *E. bumbameuboi*; 7 to 11 pustules and no papilla in *E.* cf. *piauiensis*; 3 to 6 pustules and one papilla on each side of the infrarostral in *E. cesarii*; 4 to 6 pustules and on papilla on eache side of median region of the Meckel cartilage in *E. bicolor*). In the median region between the walls of the infrarostral cartilage, one developed papilla dorsoposteriorly directed can be seen, but not observed in individuals of *E.* cf. *piauiensis*. Posteriorly, buccal floor is wider. The tongue anlage is triangular or rounded with the top projecting backwards. All median region of the buccal floor, from the tongue anlage to the glottis is a wide, shallow and smooth depression. It is limited posteriorly by a row of long transverse and curved fingerlike papillae (6 to 12 papillae in *E. bumbameuboi*; 8 to 12 papillae in *E.* cf. *piauiensis*; 8 to 16 papillae in *E. cesarii*; 10 to 14 papillae in *E. bicolor*) on each side of the glottis. This papillae have irregular size and shape and some of them are bifurcated or bifid. (Fig.3A); pustules and papillae are scarce among the row of papillae and the anterior region. The lateral limit of the buccal floor is marked by well developed buccal pockets slightly oblique; the buccal pockets are wide, extending beyond the lateral limit of the ventral velum and are imperforated (Fig.3A). The anterior wall of each buccal pocket has 1 to 3 papillae posteriorly directed; this papillae are irregular, wider than high and present or not pustules on eache side; usually this papillae are connected by the base. The glottis is located in the anterior region of the ventral velum, at the median third of the buccal floor; it is long and narrow, visible with little prominent glottal lips; laterally, two long conduits extend to the end of the ventral velum, involving the glottis and dividing it in right and left portion. The ventral velum is long (corresponding to 2/5 of the buccal floor length) and trapezoid form, extending backwards on its sides; it is thin, parcially attached to the filter plates and with the bottom edge free and dorsally curved. The velar surface is irregular and with wide, smooth projections. The glandular zone is indistinct.

Dorsal aspect (Table 2, Fig. 3): Trapezoid form as the buccal floor. Prenarial arena is wide, smooth surface except for the presence on medioposterior region of a papilla with irregular margin and rounded base in most of individuals. In *E. bumbaemuboi* and *E.* cf. *piauiensis* is possible to observe 2 to 3 rounded projections in the external area of this papilla and after can be observed pustules on each side; in *E. bicolor* there are 3 pustules on each side of the papilla slightly visible, while other species the presence of one pustule may occur (Fig.3B). Posteriorly to the prenarial arena papilla can present a pustule, absent in *E. bicolor*, but in *E.* cf. *piauiensis* there are two pustules.The coanae are circular depressions, Posteriormente à papila da arena pré-coanal, é possível haver a presença de uma pústula, ausente para *E. bicolor* e para *E.* cf. *piauiensis* pode haver duas pústulas. As coanas são duas depressões circulares, slightly wider than long; from the posterior margin of each choana, a large, flat and multifid narial wall papilla arises; this papilla is square or slightly triangular, wide base and concave front face (Fig.3B); centripetal margin of the papilla arises laterally, next to the anterior region of the choanae and extend upwardly surrounding the choanae to the posterior region; having free edge, with multiple conical projections (9 to 15 in *E. bumbameuboi*; 14 to 17 in *E.* cf. *piauiensis*; 11 to 13 in *E. cesarii* and 8 to 14 in *E. bicolor*), anteriorly oriented, covering almost entirely nasal depression. Around each choana, pustules are scarce, except in one individual of *E. bicolor*, that presents pustules on the narial wall papilla. The postnarial arena is almost absent with a median ridge located right behind the choanae. Median ridge developed, trinagular, with a concave anterior face and irregular margin with conical projections (14 to 15 in *E. bumbameuboi*; 13 to 17 in *E.* cf. *piauiensis*; 12 to 14 in *E. cesarii* and 13 to 16 in *E. bicolor*) anteriorly oriented. The narial lateral papillae are tall, triangular; it can be bifurcated and are lateroventrally located to the choanae; have irregular margin with 2 to 6 projections followed by on to three pustules around the posterior edge of each choana. The buccal roof arena is defined by numerous small pustules irregularly distributed and concentrated in the median region after the median ridge; *E. bicolor* presents two pars of regular, distributed pustules after the median ridge, slightly bigger than other pustules surrounding the buccal roof arena; in *E. bumbameuboi* and less discernible in *E.* cf. *piauiensis*, a lateral line of pustules is visible on the buccal roof arena and are obliquely directed. The dorsal velum is regularly long on the lateral region, which curl up in the anteroventral direction reaching the ventral portion of the ventral velum. No pustule included in the text can be identified in the Figure.

Chondrocranium

Upper jaws and ethmoid region

Chondrocranium wider than long.(Table 3, Fig.4). The suprarostral cartilage has corpus and alae fused in a single structure with a curved anterior margin and posterior margin bearing a median projection in a “V” shape. In *E. cesarii*, this projection is more pronounced (Fig.4G). On the posterior margin of alae not connected with the trabecular horns, there is projection similar to a “W” shape in all species, except *E. bicolor* (Fig.4E). The trabecular horns are short, horizontals and distally wider; they are similar in width between species, but broader and robust in *E. cesarii* (Fig.4G). The trabecular horns internal walls diverge producing a “V” design, while the external walls are opposed, becoming approximately parallel in a salient angle. Ethmoidal plate thin and narrow. Prenasal process absent.

Braincase

The braicase is open dorsally and has a broad frontoparietal fenestra delimited anteriorly by the ethmoidal plate, laterally by *taenia tecti marginalis* and posteriorly by *tectum synoticum* and internal margin of the otic capsule. The orbital cartilages are thin and poorly condrified (Fig. 4,5).

In lateral view, a broad fenestra from the median region to the anterior region of the otic capsule corresponds to the junction of the optic, oculomotor, trochlear and prootic foramina; it has a ventral margin slightly wavy in all species, but in *E. cesarii* is more pronounced (Fig.5G).

The cranial floor has a hypophyseos fenestra is occluded and poorly chondrified to *E. cesarii*, *E.* cf. *piauiensis* and some individuals of *E. bumbameuboi*; almost completely occluded to *E. bicolor* and slightly oval and irregular margin to most individuals of *E. bumbameuboi*. It is delimited anteriorly by *planum trabeculare* *anticum* and posteriorly by *planum basale.* The carotid and craniopalatine foramina are visible, the latter elongate and larger than the rounded former; *E. cesarii* has this foramina smaller in comparison to the body proportion (Fig.4H). In the posterior region, the notochord canal corresponds to 47% in *E. bumbameuboi*, 54% in *E.* cf. *piauiensis*, 60% in *E. bicolor* and 79% in *E. cesarii* of the chondrocranium length.

Otooccipital region

The otic capsules are slightly square, corresponding to 30% of the chondrocranium length and are connected by *tectum synoticum*, that has width equal to *taenia tecti marginalis*; both are large. The crista parotica are well developed and expanded in all species; it has margins slightly chondrified and digitiform; anteriorly are connected with the *processus lateralis posterior*. The fenestra ovalis are is located ventrolaterally on each otic capsule.

Palatoquadrate

The palatoquadrate is parallel to the braincase and are attached via three points: the quadratocranial comissure, the ascending process, attached to the cranial floor, and the larval otic process. In the anterior region, the articular process is short, wide, and has three small anterior processes (muscular process, quadratoethmoid process and a small process on the anterior region). The small process on the anterior region is short and has sligtly rounded margin; *E.* cf. *piauiensis* has this process shorter and rounder (Fig.4C). The quadratocranial comissure is wide and robust and the quadratoethmoid process is present and not pronounced, specially in *E. bicolor* (Fig.4E). The muscular process is scarcely developed and has a long ventrolateral process and with irregular shape. In one individual of *E. bumbameuboi*, the ventrolateral process presented a small digitiform expansion similar to the crista parotica.

The ascending process is low, short, robust and not covered by the otic capsules. The subocular fenestra is narrow, oval and covered parcially by the orbital cartilage, except in *E. bicolor*, which has a rounder shape (Fig.4E). The subocular fenestra is parallel to the longitudinal axis of the braincase, but has a inclination in the anterior region more pronounced in *E. cesarii* (Fig.4C).

The subocular bar is slightly narrow and in the most posterior part presents a long, rectangular, flat processus lateralis posterior that has expansions in the posterior region connected to the crista parotica expansions and bears a single subotic process of variable shape in all species, except in *E. bumbameuboi* and *E. bicolor*, that presents a least one individual with a slighty bifid subotic process (Fig.4B).

Lower jaws

The Meckel's cartilage has slightly triangular shape, wide and has a retroarticular and dorsal process; its anterior edge is tapered on the connection with the infrarostral cartilages. The infrarostral cartilages are fused in a "U" shape; laterally, it has a laminar shape with the inner face having a pointed projection and the outer face, on the connection with the Meckel's cartilage, having an arrow-shape.

Hyobranchial apparatus

The Copula I presents as a very thin, cartilaginous bar. The ceratohyal have five process: anterior process, anterolateral process, lateral process, hyoquadrate process and posterior process (Fig. 5). The ceratohyals are elongate and connected by *par reuniens*, which is wider than Copula II. The Copula II is short, robust and bears a thin and long urobranchial process; the Copula II is fused to the hypobranchial plates. The anterior process is rounded. The anterolateral process is thin and tall, slightly less condrified than other process and has a rounded margin and a inclinantion towards the anterior process, in *E. bicolor*, this inclination is more pronounced (Fig. 5F). The hyoquadrate process is robust and rounded.

The lateral process is long and tapered, with a inclination towards the ceratobranchials and bears a posterior expansion that parcially covers the ceratobranchial I. The hipobranchial plates are fused with each other and with the Copula II. The ceratobranchials are fused to the hipobranchial plates and constitute a large branchial basket. Three large and long spicules are observed in the dorsal region of the hyobranchial apparatus.

DISCUSSION

External morphology and color pattern

The morphology of larvae of the genus *Elachistocleis* here analyzed is highly conservative and is similar to results found in previous studies. Besides, many features are shared with other larvae the family Microhylidae.

The contradiction about the lack of knowledge of the intrageneric variation of larvae of anurans in the literature leads to observational errors regarding the characterization of particular character. Thus, and considering that many of the characters previously described for species of *Elachistocleis* had been mainly based on a anuran larval literature stil incipient, the standardization of the morphology is necessary.

Pereyra *et al.* described the new species *E. haroi* to Argentina and found that the only character of the tadpole that differed from known species were the shape of the dermal flaps. In their study, Pereyra *et al.* observed that the lack of clarity in the description of the character “dermal flaps” in previous descriptions of *E. bicolor* may lead to observational erros that can only be corrected accessing the intrageneric variation.

The results for this character here analyzed show that the variation between species, although is not clearly evident, regarding the character “mostly not jagged” described to *E. bicolor* by Rossa-Feres *et al.* was the same observed to the individuals here analyzed. Besides, the description of Magalhães *et al.* to this character to *E. cesarii* does not match with the individuals analysed in our study, that show a pattern “serrated-papillar” stronger than other species.

Based on this informations, the differences observed about the external morphology in this study are: I) body shape in dorsal view; II) edge of the dermal flaps; III) edge of the snout in dorsal view; IV) angle in which the dorsal fin emerges in the body; V) lateral line visible or not.

Regarding the body shape, due to the lack of a descritive pattern to larvae of Microhylidae, we don’t discuss the differences in the literature, since this character may be closely associated with personal perspective of the authors. Still, for this study te body morphology was quite similar and, although we have observed certain pattern in most of individuals, we verified that the variation in morphology, mainly in dorsal view, is clear, intra and interespecific speaking.

Thus, individuals of *E. cesarii* from the northeastern part of Brazil has a globular body stronger than other species, although individuals from the southeastern part of the country showed a oval body in addition to the oval body presented by *E. bumbameuboi* and *E.* cf. *piauiensis*, while *E. bicolor* has a oval body to most of individuals from Argentina and a globular body to individuals from Brazil. This difference between individuals of *E. cesarii*, regarding tadpoles collected in northeastern and southeastern parts of Brazil, and *E. bicolor*, regarding tadpoles from Argentina and Brazil, may be a possibility of distinct populations of species that have been assigned with the same name and requires further investigation. Intraespecific variations in the body observed in this study are relatively the same for all species and, although we don't analyze tadpoles in early stages for all species, specimens of *E. bumbameuboi* at stages 27 to 31 maintain the same body variation presented by later stages as well.

The coloration of the body is another character used in the description of tadpoles. We know that the coloration in preservative is influenced by the method of fixation as well as the type of material used to preserve specimens (formaldehyde 10% or a mixture of formaldehyde and alcohol). Thereby, the loss of colour *in vivo* caused by formaldehyde interfere in the detailed description of a species. Despite this, we verified that *E.* cf. *piauiensis* has a lighter coloration, followed by *E. bumbameuboi*, while *E. cesarii* and *E. bicolor* have a darker skin, with *E. cesarii* presenting a dark brown pigmentation and coppery tons, while *E. bicolor* from Brazil has a brown coloration similar to *E. cesarii* or a dark green coloration presented by individuals from Argentina.

The coloration of species previously described as dark brown is similar to individuals analyzes in our study. *Elachistocleis cesarii* described by Magalhães *et al.* has the same color as specimens from our study, besides having the same coloor as *E. panamensis*, that is described as having a reddish brown body. While *E. surinamensis* and *E. ovalis* have a dark body similar to individuals in vivo of *E. haroi*.

Lavilla recognized the existence of a layer of thick connective tissue on the first half of the muscle tail and fins in *D. muelleri* observed well developed in tadpoles of *E. cesarii* and *E. bicolor*, although we observed on other species either. The posterior border of this layer of tissue is marked by the end of the depigmented median stripe on the muscle tail and, as described by Lavilla, of uncertain homology.

Elachistocleis bicolor and *E. cesarii* have neuromasts forming the lateral line visible. The presence of a lateral line only is not observed in *E.* cf. *piauiensis*, but in *E. bumbameuboi* is slightly visible.

Internal oral morphology

As described by many authors, the internal oral morphology of tadpoles of Microhylidae is relatively uniform and the features observed in *Elachistocleis* in this study is similar to the species previously described: *Dermatonotus muelleri*, *Ramanella obscura*, *Kaloura rugifera*, *K. borealis*, *Chiasmocleis leucosticta*, *Microhyla rubra*.

Only *E. bicolor* and *E. panamensis* have the buccopharyngeal morphology described to the genus. The buccopharyngeal cavity is highly uniform in numerous species, as pointed out by Candioti: unperforated choanae with a large papillar projection emerging from the posterior margin, prepocket papillae, lingual and lateral ridge papilla absent, buccal roof and floor almost naked, with a tall papillae bilaterally disposed on both sides of the glottis.

Among individuals analyzed, we observed that the variation between the main structures of buccal roof and floor are not enough the clearly separate species, except for the presence of a par of organized pustules in *E. bicolor*, after the median ridge. D'Heursel & Baldissera Junior observed an increase in number of buccal floor arena papillae by stage 40 in *E. bicolor*, with several papillae becoming pustulated and multifurcate. Therefore, it is difficult to observe differences regarding the presence of absence or even the morphology of papillae and pustules among species. Furthermore, a certain intraespecific variation is observed, especially in the morphology of narial lateral papillae, prepocket papillae and even the number of buccal floor arena papillae.

The three papillae found in th velar margin of the ventral velum in *E. bicolor* by Echeverría & Lavilla, were not observed in any of the species analyzed here, including *E.bicolor*. All species have a smooth velar margin. Besides, the two pars of pustules after the median ridge exhibiting a regular distribution in *E. bicolor* match the description of Echeverría & Lavilla, but were not found in other species. Furthermore, a few individuals of *E. bumbameuboi* and *E.* cf. *piauiensis* have a lateral line of pustules on the buccal roof arena and are obliquely directed not observed in other species. Finally, the presence of small conical pustules on the buccal roof arena to *E. bicolor* by Candioti were not observed in the species analyzed here.

Chondrocranium and Hyobranchial Apparatus

The chondrocranium and hyobranchial apparatus are highly conservative and similar in many aspects to other species of Microhylidae previously described.

Only 12 microhilids of the New World have the chondrocranium described in detail, as pointed out by Trueb *et al.*, who describes the tadpole of *Gastrophryne carolinensis* and compared to 10 chondrocrania described at the time. In addition to his work, Bowatte & Meegaskumbura describes in detail the chondrocranium of *Ramanella obscura*. Along with this species, three other species from the Old World have been described – *Uperodon systoma* and *Microhyla ornata* and *Kaloula pulchra*.

Based in this study, the differences found between species of *Elachistocleis* are sutil, but visible and are listed in the following order: I) A more pronounced median projection of the posterior margin of the suprarostral corpus; II) Posterior margin of the alae of the suprarostral cartilage in "W" shape or smooth; III) Ventral margin of the broad fenestra in the lateral of the braincase slightly wavy or visibly wavy; IV) Presence of a hypophyseos fenestra; V) Margin of the small process on the anterior region of the articular process; VI) presence of digitiform expansions on the ventrolateral process; VII) Shape and inclination of the subocular fenestra; VIII) Subotic process bifid or single. In the hyobrachial apparatus, the only sutil diferrence observed is a pronounced inclination of the anterolateral process in *E. bicolor*.

Candioti, discussing the reduction of the crista parotica in *E. panamensis*, describes it as a laminar, cartilaginous and parcially fenestrated, extending between the otic capsule and the posterior region of the palatoquadrate. Also, this character were not observed in *Chiasmocleis leucosticta*, *M. ornata* and *U. systoma*, but is observed in *D. muelleri*, *G. carolinensis*, *K. Pulchra* and *O. pyburni*.

The lack of a crista parotica in *E. bicolor* described by Candioti was not observed in the species analyzed in our study, that exhibit a well developed crista parotica connected to the posterior margin of the processus lateralis posterior. The presence of absence of this character is discussed by Candioti, which comparing tadpoles of *E. bicolor* analyzed personally and with other previously descriptions, declares as a manifestation of intraespecific variability and, since the tadpoles are in comparable stages, excludes ontogenetic differences.

Finally, all species analyzed present a ventrolateral process, with irregular shape and size with one individual of *E. bumbameuboi* at stage 36 presenting digitiform expansions similar to the crista parotica. Haas considers the presence of this process as a synapomorphysm from american microhylids. Its absence is observed in some species from Old World, as *M. ornata* and *Uperodon systoma*, *Phrynomantis palmata, Kaloura Pulchra* and *Paradoxophyla bifasciatus*. However, its absence is also observed in some species of the New World as *D. muelleri*, *E. panamensis*, *G. carolinensis*, *Hamptophryne boliviana*. Candioti argues then the absence of the ventrolateral process in *Dermatonotus* would be a reversion.

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APÊNDICE I

Espécimes analisados

E. bumbameuboi - HUFMA 2073, n = 14, Bacabeira, MARANHÃO, Brasil; HUFMA 2074, n = 7, São José de Ribamar, MARANHÃO, Brasil.

E. cf. *piauiensis* - UESPI 305, n= 5. Ilha Grande, Piauí, Brasil.(Universidade Federal do Piauí),

E. cesarii. – AAGARDA 3734, n=12, Macaíba, Rio Grande do Norte, Brasil; DZSJRP 2370.1, n= 2, Taubaté, São Paulo, Brasil; DZSJRP 1088.5, n=4, Vitória Brasil, São Paulo, Brasil.

E. bicolor. – DZSJRP 1266.1, n=6, Teodoro Sampaio, São Paulo, Brasil; DZSJRP 828.2, n=5, Fazenda Rio Grande, Paraná, Brasil; DZSJRP 279.2, n=1, Nova Aliança, São Paulo, Brasil; DZSJRP 66.1, n=1, Nova Aliança, São Paulo, Brasil; DZSJRP 59.1, n=4, Nova Aliança, São Paulo, Brasil. (Universidade Estadual Paulista); UNNEC 12217, n= 10, Corrientes, Capital, Argentina.

LEGENDAS

Fig 1. Morfologia externa de *Elachistocleis*. *Elachistocleis bumbameuboi* (A) dorsal, (B), ventral, estágio 36; *E.* cf. *piauiensis* (C) dorsal, (D), ventral, estágio 36; *E. bicolor* (E) dorsal, (F), ventral, estágio 35; *E. cesarii* (G) dorsal, (H) ventral, estágio 36.

Fig 2. Morfologia em vista lateral e da borda das abas dermais de *Elachistocleis*. *Elachistocleis bumbameuboi* (A) lateral, (E) aba dermal; *E.* cf. *piauiensis* (B) lateral, (F) aba dermal; *E. bicolor* (C) lateral, (G) aba dermal; *E. cesarii* (D) lateral, (H) aba dermal.

Fig 3. Morfologia oral interna de *Elachistocleis* com as variações de determinadas estruturas. (A) assoalho bucal, (B) teto bucal. AAB, arena do assoalho bucal; GLO, glote; LI, lábio inferior; PLB, papila da bolsa bucal; PLG, papilas laterais da glote; RL, rudimento lingual; VV, velum ventral; AT, arena do teto; CM, crista mediana; PAPC, papila da arena pré-coanal; PC, papila da coana; PLC, papila lateral da coana.

Fig 4. Condrocrânio em vista dorsal e ventral de *Elachistocleis*. *Elachistocleis bumbameuboi* (A) dorsal, (B) ventral, estágio 36; *E.* cf. *piauiensis*, (C) dorsal, (D) ventral, estágio 36; *E. bicolor* (E) dorsal, (F) ventral, estágio 35; *E. cesarii* (G) dorsal, (H) ventral, estágio 35. CA, cápsula auditiva; cM, cartilagem Meckel; CP, crista parótica; CT, cornos trabeculares; FC, forame carótido; FCP, forame crâniopalatino; FHI, fenestra hipofisária; FOL, forame olfativo; FSO, forame subótico; NT, notocorda; PAR, processo articular; PPL, processo posterolateral; PSO, processo subótico; ST, suprarostral; TT, tectum synoticum; TTM, taenia tecti marginalis.

Fig 5. Condrocrânio em vista lateral e aparelho hiobranquial em vista ventral de *Elachistocleis*. *Elachistocleis bumbameuboi* (A) lateral, (B) aparelho hiobranquial; *E.* cf. *piauiensis* (C) lateral, (D) aparelho hiobranquial; *E. bicolor* (E) lateral, (F) aparelho hiobranquial; *E. cesarii* (G) lateral, (H) aparelho hiobranquial. PM, processo muscular; PVL, processo ventrolateral; CB, ceratobranquiais; COI, cópula I; COII, cópula II; HP, placa hipobranquial; PAC, processo anterior; PALC, processo anterolateral; PHQ, processo hioquadrado; PLC, processo lateral; PR, *pars reuniens*; UB, processo urobranquial.

Tabela 1. Média das medidas morfométricas da morfologia externa de *Elachistocleis*. CT, Comprimento Total; CC, Comprimento do Corpo; LC, Largura do Corpo; LMC, Largura da Musculatura Caudal; AC, Altura do Corpo; AND, Altura da Nadadeira Dorsal; AMC, Altura da Musculatura Caudal; ANV, Altura da Nadadeira Ventral; DIO, Distância Interorbital; DOF, Distância Olho-Focinho; DMO, Diâmetro do Olho; CE, Comprimento do Espiráculo; LE, Largura do espíraculo.

Tabela 2. Média das medidas morfométricas da morfologia oral interna de *Elachistocleis*. CA, comprimento do assoalho; CVV, comprimento do velum ventral; CCM, comprimento da crista mediana; CPBB, comprimento da papila da bolsa bucal; CPCC, comprimento da papila da coana; CPLCC, comprimento da papila lateral da coana; CPPC, comprimento da papila da área pré-coanal; CT, comprimento do teto; DCC, distância entre as coanas; LA, largura do assoalho; LT, largura do teto; LCM, largura da crista mediana; LPBB, largura da papila da bolsa bucal; LPCC, largura da papila da coana; LPPC, largura da papila da área pré-coanal; LVV, largura do velum ventral.

Tabela 3. Média das medidas morfométricas do condrocrânio de *Elachistocleis*. CA, comprimento da cápsula auditiva; CC, comprimento do condrocrânio; COT, comprimento do corno trabecular; LC, largura do condrocrânio; NT, comprimento do canal da notocorda; PSO, comprimento do processo subótico; PVE, comprimento do processo ventrolateral.

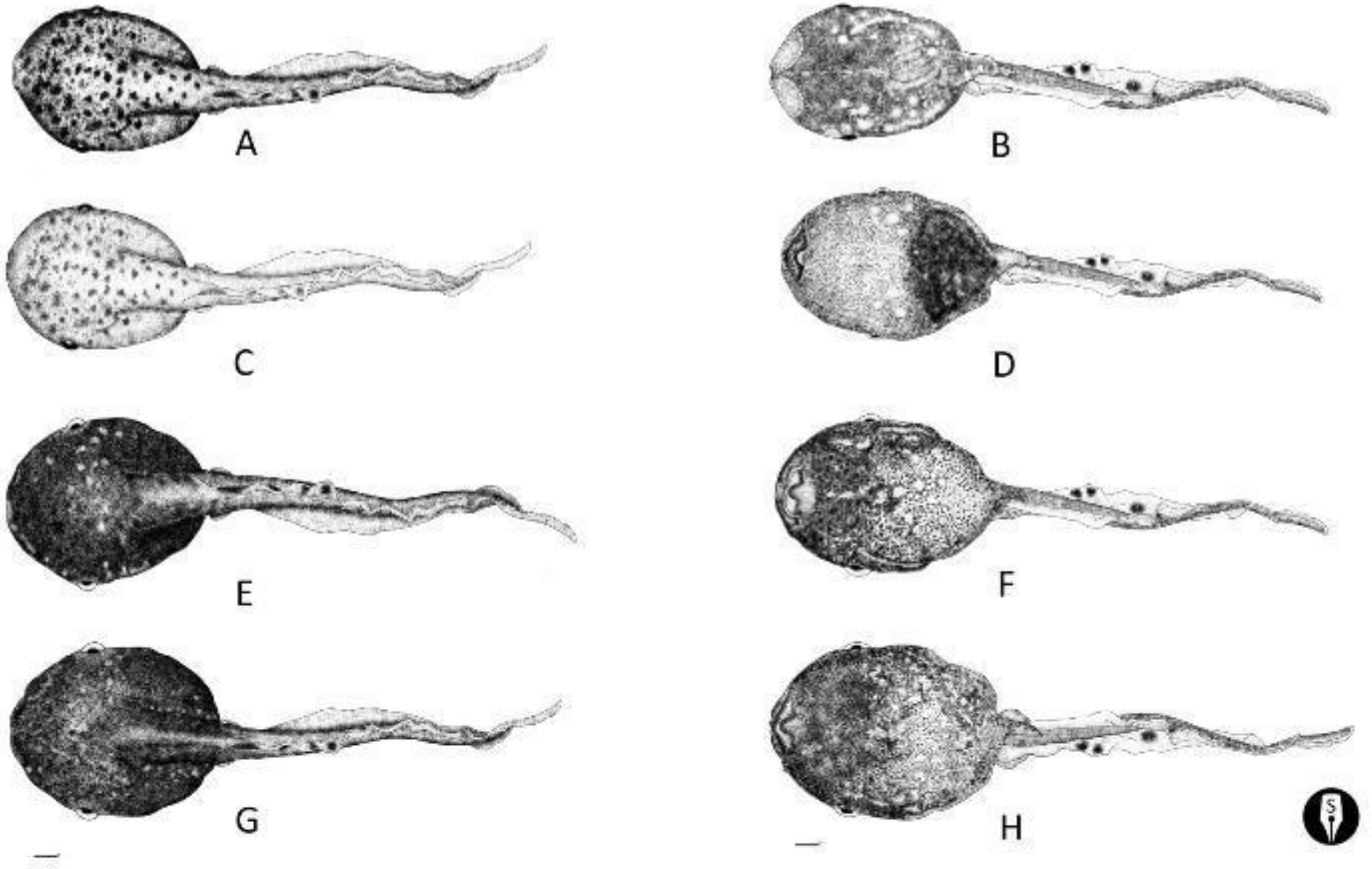

Fig 1.

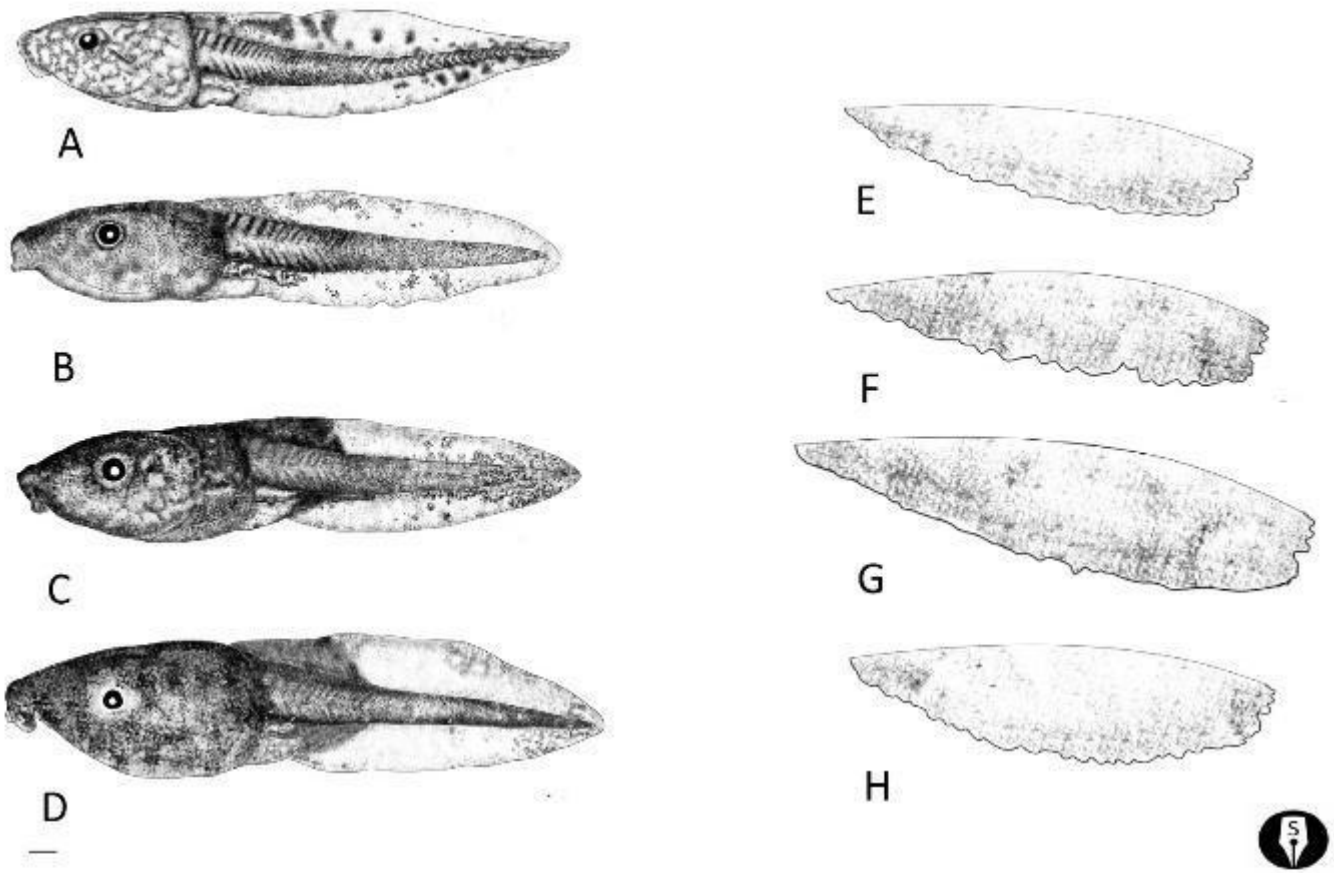

Fig 2.

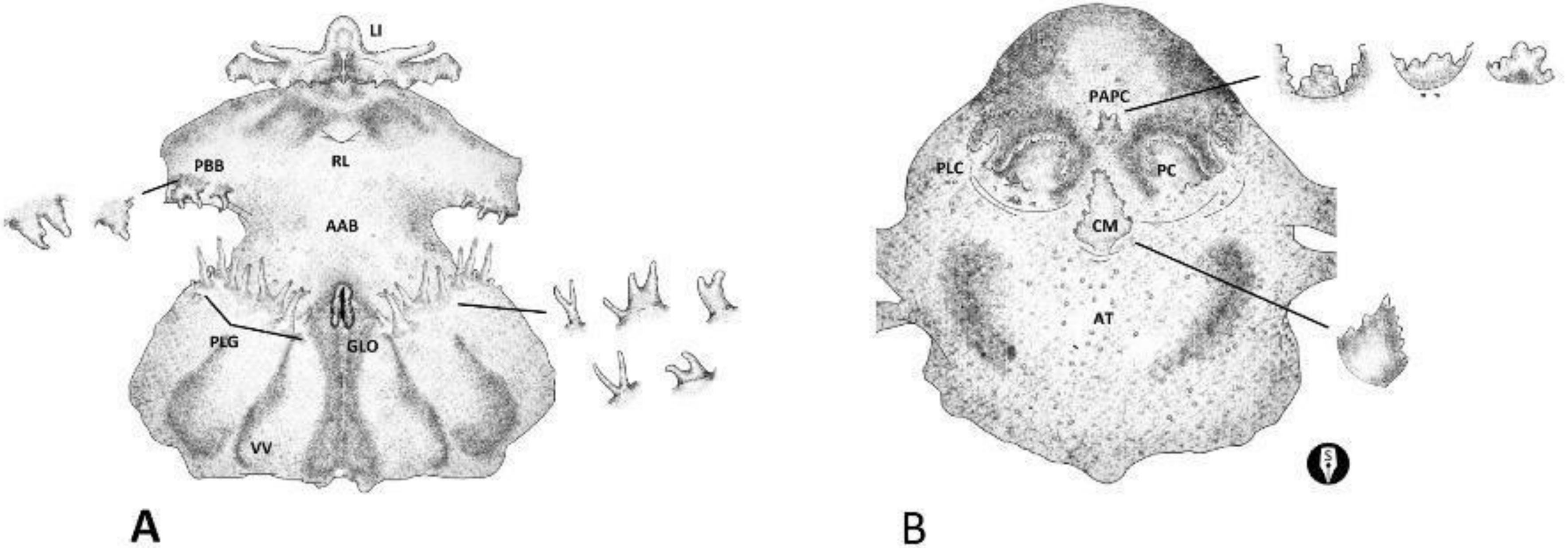

Fig 3.

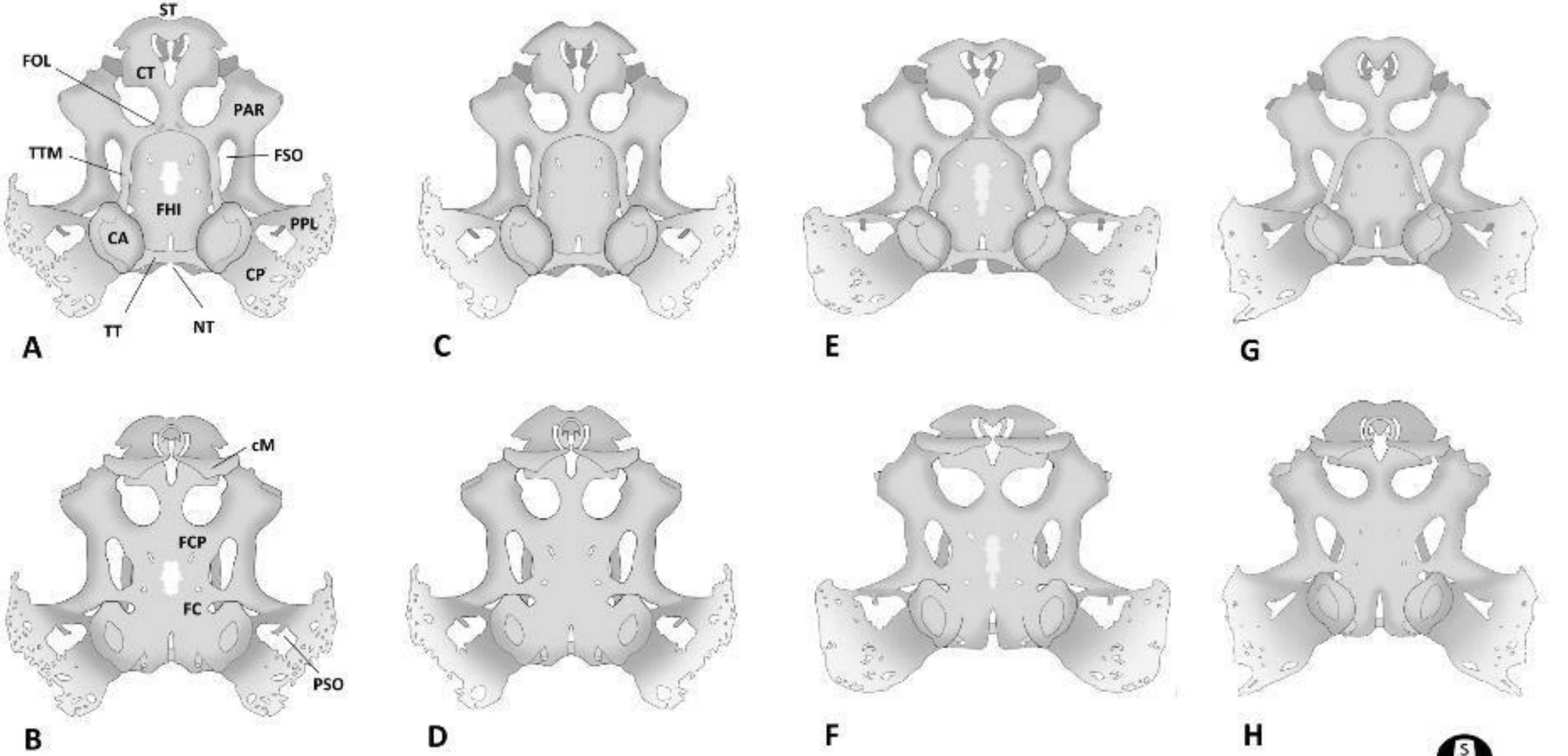

Fig 4.

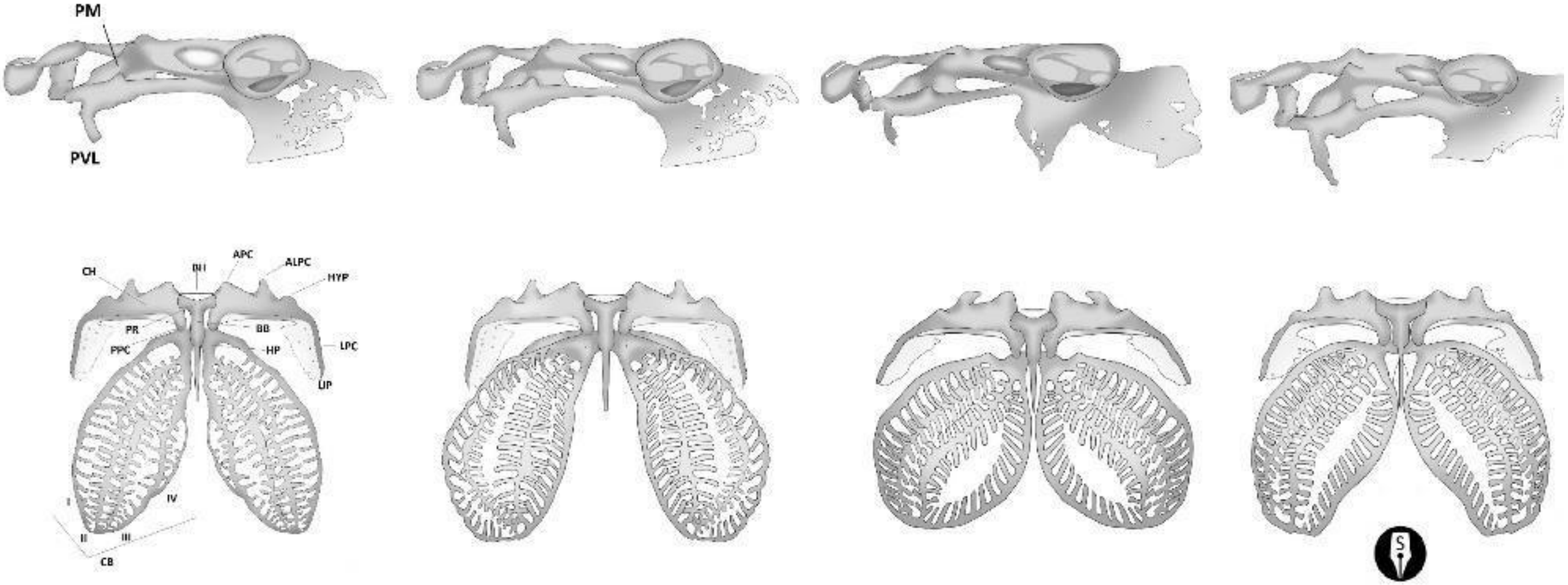

Fig 5.

Tabela 1.

Espécie	CT	CC	LC	LMC	AC	AND	AMC	ANV	DIO	DOF	DMO	CE	LE
E. bumbameuboi	21,10	6,60	4,95	1,46	3,15	1,18	2,20	1,10	4,32	2,22	0,76	1,47	0,73
E. piauiensis	23,00	6,99	5,05	1,50	3,07	1,25	2,15	1,20	4,03	2,26	0,72	1,53	0,66
E. cesarii	22,96	7,39	5,87	1,41	3,78	1,63	1,95	1,57	5,13	2,45	0,68	1,64	0,72
E. bicolor	24,83	7,24	5,49	1,63	3,64	1,36	2,09	1,41	4,95	2,54	0,66	1,56	0,88

Tabela 2.

Espécie	**CA**	**LA**	**CVV**	**LVV**	**CT**	**LT**	**DCC**	**CPCC**	**LPCC**	**CPLCC**	**CCM**	**LCM**	**CPBB**	**LPBB**	**CPPC**	**LPPC**
E.bumbameuboi	3,03	2,64	1,26	3,05	3,64	2,30	0,33	0,44	0,34	0,24	0,46	0,30	0,18	0,23	0,10	0,13
E. cf. *piauiensis*	3,30	2,51	1,43	3,33	4,09	2,64	0,26	0,51	0,58	0,34	0,64	0,44	0,14	0,44	0,14	0,16
E. cesarii	3,17	2,57	1,34	3,19	3,87	2,47	0,30	0,48	0,46	0,29	0,55	0,37	0,16	0,34	0,14	0,13
E. bicolor	3,05	1,93	1,50	2,72	4,22	2,73	0,33	0,44	0,39	0,27	0,51	0,34	0,17	0,20	0,10	0,12

Tabela 3.

Espécie	**CC**	**COT**	**NT**	**CA**	**PVE**	**PSO**	**LC**
E.bumbameuboi	3,93	0,85	0,49	1,18	0,80	0,38	4,64
*E.*cf. *piauiensis*	3,77	0,82	0,54	1,16	0,58	0,32	4,71
E. cesarii	4,39	1,06	0,79	1,20	1,16	0,26	5,35
E. bicolor	4,00	1,09	0,60	1,15	0,66	0,33	4,84

Capítulo II

O capítulo II compõe o artigo que será intitulado “Descrição do girino de *Elachistocleis bumbameuboi* Caramaschi 2010 (Anura, Microhylidae)” e será submetido para publicação no período científico Zootaxa. Os critérios de redação e formatação seguem as normas deste periódico e estão disponíveis no ANEXO II.

The tadpole of *Elachistocleis bumbameuboi* Caramaschi 2010 (Anura, Microhylidae)

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Abstract: The tadpole of *Elachistocleis bumbameuboi* is described. The external morphology, internal oral morphology, chondrocranium and hyobranchial apparatus are analyzed and compared. The external morphology, such as the body shape, absence of keratinized structures and ventral spiracle resembles that of other species of the genus. Some differences include the edge shape of the dermal flaps and the body coloration. The internal oral morphology is very similar to that of other microhylids, with minimal differences that are related to the presence of papillae in the lower lip, the smooth edge of the ventral velum and pustules in the prenarial arena. The chondrocranium is characterized by a well-developed crista parotica, and the hyobranchial apparatus is similar to that in other species of the genus.

Keywords: amphibian, skeleton, oral apparatus, taxonomy.

Introduction

The genus *Elachistocleis* Parker 1927 currently includes 17 species (Frost 2015): *Elachistocleis bicolor* (Guérin-Méneville), *Elachistocleis bumbameuboi* Caramaschi, *Elachistocleis carvalhoi* Caramaschi, *Elachistocleis cesarii* (Miranda-Ribeiro), *Elachistocleis erythrogaster* Kwet and Di-Bernardo, *Elachistocleis haroi* Pereyra *et al.*, *Elachistocleis helianneae* Caramaschi, *Elachistocleis magnus* Toledo, *Elachistocleis matogrosso* Caramaschi, *Elachistocleis muiraquitan* Nunes-de-Almeida and Toledo, *Elachistocleis ovalis* (Schneider), *Elachistocleis panamensis* (Dunn *et al.*), *Elachistocleis pearsei* (Ruthven), *Elachistocleis piauiensis* Caramaschi and Jim, *Elachistocleis skotogaster* Lavilla *et al.*, *Elachistocleis surinamensis* (Daudin), and *Elachistocleis surumu* Caramaschi.

In the genus, only *E. surinamensis* (Kenny 1969), *E. bicolor* (Williams & Gudynas 1987; Rossa-Feres & Nomura 2006), *E. erythrogaster* (Kwet & Di-Bernardo 1998), *E. panamensis* (Candioti 2006), *E. pearsei* (Lynch 2006), *E. cesarii* (Magalhães *et al.* 2012) and *E. haroi* (Pereyra *et al.* 2013) have the tadpole with the external morphology described. *Elachistocleis bicolor* and *E. panamensis* are the only species in which the internal oral morphology (Candioti 2006; 2007; D'Heursel & Baldissera Junior 1999; Echeverría & Lavilla 200) and chondrocranium (Candioti 2006; 2007; Lavilla & Langone 1995; Haas 2003) of the tadpole have been described. Because the use of larval characters in taxonomic studies can assist in solving description problems, in this article, we describe the external morphology, internal oral morphology, the chondrocranium and the hyobranchial apparatus of the tadpole of *E. bumbameuboi*, comparing the results with other species currently described.

Material and methods

The tadpoles were collected during fieldwork in the municipalities of São José de Ribamar and Bacabeira, Maranhão State, Brazil, from April to May 2013 from temporary ponds with herbaceous vegetation; these ponds were observed during the reproductive period. During this period, a single species of the microhylid *Elachistocleis* was active. Four adult *Elachistocleis* frogs from Bacabeira (HUFMA 1735, 1736, 1738–1740) and five from São José de Ribamar (HUFMA 1741–1745) were collected at the same site as the tadpoles to confirm species identification. The tadpoles were anesthetized with 5% xylocaine and preserved in 10% formalin. Adults were anesthetized with 5% xylocaine, fixed with 10% formalin and preserved in 70% alcohol. The voucher specimens are on deposit in the Coleção de Herpetologia of the Universidade Federal do Maranhão HUFMA 2073; HUFMA 2074).

We took morphometric measurements from 12 specimens, with three at stage 31, one at stage 32, three at stage 34, three at stage 35 and two at stage 36 (Gosner 1960), with the program Leica Application Suite 4.0.0 under a Leica DFC295 stereoscopic microscope with an attached camera. Measurements are shown in Table 1 in Appendix I. Descriptions and measurements follow Altig & McDiarmid (1999). The following measurements were taken: total length (TL), body length (BL), body width (BW), tail muscle width (TMW), body height (BH), dorsal fin height (DFH), tail muscle height (TMH), ventral fin height (VFH), interorbital distance (IOD), eye-snout distance (ESD), eye diameter (ED), spiracle length (SL) and spiracle width (SW).

For the internal oral morphology, four tadpoles (Stages 30, 34, 36 and 37) were dissected; the description follows Wassersug (1976) and Candioti (2007), and the measurements follow Weber & Camaraschi (2006), with modifications. Measurements (Table 2, Appendix I) were taken with the program Leica Application Suite 4.0.0 under the stereoscopic microscope (Leica DFC295). An additional sample at stage 36 was photo documented under a scanning electron microscope (Jeol JSM-630EO), accessed in the Centro de Pesquisa Gonçalo Moniz da FIOCRUZ, Bahia. The specimen was processed according to the following procedures: a) Wash – two washes of 10 minutes each with a buffer solution of sodium cacodylate 0.1 M, pH 6,8; b) Post-fix – place in solution of osmium tetroxide in buffer solution 1% sodium cacodylate 0.1 M for 30 minutes; Wash – three washes of 5 minutes each in a buffer solution sodium cacodylate 0.1 M; c) Dehydration - subjected to a series of increasing ethanol (30%, 50%, 70%, 90% and 100%) baths of 15 minutes each; d) Drying – dry to critical point in CO2; e) Assembly – mount on aluminum stubs under carbon ribbon; and f) Metallization – coated with gold by sputtering in a Denton Vacuum IV for approximately 15 minutes.

To verify the structures of the chondrocranium and hyobranchial apparatus, five specimens (one at stage 34, one at stage 35, two at stage 36 and one at stage 37) were cleared and stained using the technique of Potthoff (1984) and were described according to Haas (2001; 2003), De Beer (1937) and Candioti (2007).

Results

Description: (Figs. 1A, B, C, D) Total length approximately 21,10 mm (Table 1; Stage 31/36). Body longer than wide, ovoid in dorsal view and globular/depressed in lateral view. The mouth is terminal, small, with emargination absent; lacking keratinized mouthparts and papillae; two dermal flaps in front of the mouth, undulate and irregular edge, slightly papillar. Snout rounded with truncated tip in dorsal and lateral view. Eyes are small, directed and positioned laterally. Nares are absent. A single, long and wide spiracle is located midventrally at the end of the body, corresponding to 22% of the body length, with sinistral inclination and the opening directed posterodorsally. Internal and external wall of the spiracle of the same length; the internal wall is fused to the vent tube; superior wall shorter than the inferior wall. Vent tube long, sinistral and fused to the ventral fin and the spiracle, opening posterodorsally. Tail long, corresponding to 68% of the total length. Tail muscle developed, corresponding to 70% of the body height; with gradual tapering in lateral view and rounded tip. Dorsal and ventral fins convex, with approximately the same height, ascending shape at the first portion and descending at the third portion. Dorsal fin low and corresponding to 53% of the tail muscle; emerging on the body-tail junction with smooth inclination in a wide arc; ventral fin low and corresponding to 50% of the tail muscle; smooth inclination in a wide arc, but less than dorsal fin. Lateral line not evident in most individuals.

Coloration: In life, dorsal region ranges from yellowish or greenish brown, with several dark dots, presenting or not as reddish orange blotches on the dorsolateral region or with dots irregularly distributed throughout the dorsal region; some individuals present a yellowish white medial stripe on the dorsal region. Ventral region light, with anterior region presenting several dark dots, and posterior region presenting white blotches with different sizes and shapes, extending ventrolaterally to the body; some individuals have a white medial stripe, continuous or not. With medial stripe on the anterior third of the tail muscle, translucent fins and dark, white or reddish orange pigmentation. Eyes are dark with gold blotches. (Figure 1).

In preservative, body ranges from dark to light brown with depigmented blotches in ventral and lateral region. Translucent fins and scarce blotches scattered throughout the tail and with same coloration as the body.

Variation: One individual at stage 31 and one at stage 34 presented rounded or elongated shape in dorsal view. The snout shape can be rounded or slightly pointed. Moreover, there are many variations in the coloration in preservative; some tadpoles are lighter than others, with slightly translucent posterior regions in ventral view, and the viscera are partially visible.

Internal Oral Morphology

Ventral aspect (Figure 2A): Trapezoid buccal floor, wider in the posterior region. There are five to seven small, rounded pustules irregularly distributed on the lower lip. In the external region of the lower lip, there is a slightly developed papilla wider than high, and in the middle region of the "U" shape of the lower lip, there is a papilla directed posterodorsally. The tongue anlage is triangular or rounded, directed posteriorly. The middle region of the buccal floor forms a wider, shallow and smooth cavity. The buccal floor arena is wider than long and limited posteriorly by a transverse and curved row of six to twelve long papillae on each side of the glottis. These papillae have irregular size and form, and one individual at stage 36 presented bifurcated papilla. Pustules and papillae are scarce between the row of papillae laterally and anteriorly. The outer limits of the buccal floor arena form an oblique plane with buccal pockets. The buccal pockets are elongated, extending beyond the lateral limits of the ventral velum and are unperforated. There is one prepocket papilla on each buccal pocket wider than high, compressed, directed posteriorly and with irregular margins with 3 to 5 projections. There are pustules and small papillae on each side of the prepocket papilla. The glottis arises from the center of the buccal floor, anteriorly to the ventral velum. The glottis is narrow, elongated and visible; on each side of the glottis there are two elongated conduits extending to the end of the ventral velum; these are involved and divide the velum into right and left portions. The ventral velum is long (corresponding to 2/5 of the buccal floor length) and trapezoid, has a thin structure and is partially attached to the filter plates. The posterior margin of the velum is free and curved dorsally. Secretory pits are only visible under an electron microscope.

Dorsal aspect (Figure 2B): Trapezoid buccal roof, similar to the buccal floor. The prenarial arena is quadrangular, wide and has a smooth surface. On the posterior region, in the middle of the prenarial arena, there is a papilla with 2 or 3 rounded and internal projections and 2 or 3 external projections. Anteriorly to this papilla, one to two small, rounded pustules can be seen. Rounded choanae, slightly wider than long. On the posterior margin of each choana, a long, large and flat papilla arises, and pustules are scarce. This papilla is quadrangular or slightly triangular and has a large base with concave anterior face, covering the choana almost completely, and has 9 to 15 conical projections. The postnarial arena is short. The median ridge is developed, triangular, has a concave anterior face and fifteen conical projections directed anteriorly. The lateral papillae of each choana are elongated, bifurcated or not and have 2 to 6 projections. The buccal roof arena is defined by the presence of several small pustules, irregularly distributed. There is a lateral row of pustules discernible on the buccal floor arena, directed obliquely. The dorsal velum is moderately long and curved anteroventrally.

Chondrocranium and Hyobranchial Apparatus

The chondrocranium of a larva represents 17% of the body length (Figure 3A, B). The maximum width is at the level of the posterolateral process of the palatoquadrate. The suprarostral cartilage has a corpus and alae fused in a single structure with a curved anterior margin and a posterior margin bearing a medial projection. The suprarostral cartilage is fused to the anterior margin of the trabecular horns, and only the lateral margin is free. The trabecular horns correspond to 20% of the total length of the chondrocranium and are short, flat, horizontal and distally wider, with the internal margins, diverging and joining in the posterior margin to form a “V”; the external margins are opposed, turning almost parallel in a salient angle. Ethmoidal plate is thin and narrow, and the nasal septum is absent (Figure 3A). The pila prootica of the orbital cartilage is lateral to the olfactory foramina, extending from the anterior region of the quadratocranial commissure with the ethmoidal plate to the otic capsule via the taenia tecti marginalis. Within the lateral walls of the chondrocranium, in the central area of the posterior region with the otic capsule, there is a large foramen that joins the optic, oculomotor, trochlear and prootic foramina (Figure 3C). The cranial floor is thin, and the carotid and craniopalatine foramina are well defined. The craniopalatine foramina are elongated and wider than the rounded carotid foramina. The basicranial fenestra is visible and with irregular margins, but in some individuals is almost completely closed (Figure 3B). The notochordal canal extends 16% of the chondrocranium length. The chondrocranium is open dorsally and the frontoparietal fenestra is lined by the taenia tecti marginalis (tectum transversum and taenia tecti medialis absent). The otic capsules are slightly quadrangular and correspond to 30% of the total length of the chondrocranium. The fenestra ovalis is located ventrolaterally on each otic capsule. Dorsally, the otic capsules are joined by the tectum synoticum, which is slightly wider dorsally than the taenia tecti marginalis. The crista parotica is laminated with papillar-like expansions and joins with the posterolateral process of the subocular bar.

In the palatoquadrate, the articular process is short, wide, and bears three small processes (muscular process, quadratoethmoid process and antorbital process). The muscular process is scarcely developed and has a long ventrolateral process. The quadratocranial commissure is wide and robust. The quadratoethmoid process is scarcely visible. The subocular fenestra is narrow, oval and partially covered by the orbital cartilage in dorsal view. The processus pseudopterygoideus is absent. The subocular bar is slightly narrow and in the posterior region has a long, flat and rectangular posterolateral process with a processus suboticus, bifid or not, and the posterolateral process has expansions similar to the crista parotica. The palatoquadrate attaches to the braincase via three points: the quadratocranial commissure, the ascending process in the cranial floor and the larval otic process. The ascending process is short, robust and not covered by the otic capsule. The lower jaw includes the Meckel's and the infrarostral cartilages. Meckel's cartilage is triangular and has a retroarticular and dorsal process and a thin extremity in the articulation with the infrarostral cartilage. The infrarostral cartilage is fused in a "U" shape; laterally, it has a laminar shape with an internal face with a pointed projection and the external face flat in the articulation with Meckel's cartilage.

In the hyobranchial skeleton, the ceratohyals are elongate, and each has a rounded anterior process. The anterolateral process is tall, thin and medially directed. The posterior process is tall, thin, narrow and partially concealed by the hypobranchial plate. The hyoquadrate process is robust and rounded, and the lateral process has a posterior expansion partially covering the ceratobranchial I. The basihyal is very thin and the *pars reuniens* is continuous with the ceratohyals and fused with the basibranchial. The basibranchial is thin and the posterior region is thickened and fused with the hypobranchial plates; the urobranchial is thin, very long (about 2 times longer than the basibranchial) and is tapered distally. The hypobranchial plates are fused with each other and with the basibranchial. The ceratobranchials are fused to the hypobranquial plates and constitute a large branchial basket. Dorsally, three spicules are differentiated and well developed (Figure 3D).

Discussion

External morphology

The tadpole of *E. bumbameuboi* morphologically resembles the tadpoles of *E. erythrogaster* (Kwet & Di-Bernardo 1998)*,* *E. cesarii* (Magalhães *et al.* 2012), *E. haroi* (Pereyra *et al.* 2013), *E. bicolor* (Williams & Gudynas 1987; Rossa-Feres & Nomura 2006), *E. surinamensis* (Kenny 1969), *E. panamensis* (Candioti 2006) and *E. pearsei* (Lynch 2006).

As noted by Pereyra *et al.* (2013), some type of observational errors of certain characters in Neotropical tadpoles, such as the morphology of the dermal flaps in *Elachistocleis*, can only be corrected when the intrageneric tadpole variation is known. Pereyra *et al.* (2013) observed that the interspecific morphology is very similar in *Elachistocleis*, including a lack of significant differences in body shape. Furthermore, when comparing the tadpole of *E. haroi* with other species of the genus, the main diagnostic feature was based on the configuration of the edges of the dermal flaps. Laufer *et al.* (2013) studied the ontogeny of the dermal flaps in *E bicolor* and *E. haroi* at stages 27 to 38 and observed certain ontogenetic variation in the configuration of the dermal flaps in contrast with the "short and semi-circular" oral flaps described by Magalhães *et al.* (2012) for *E. cesarii*. Hence, in this work we compared only the configuration of the dermal flap edges.

In this study, the undulated and irregular edge of the dermal flaps in *E. bumbameuboi* contrast with the smooth dermal flap edges of *E. ovalis* (Kenny 1969) and the mostly non-jagged dermal flap edges of *E. bicolor* (Rossa-Feres & Nomura 2006), which Williams & Gudynas (1987) have described as jagged.

Although the coloration was not used as a diagnostic feature by Pereyra *et al.* (2013), and because we agree this character can be highly influenced by how tadpoles are fixed and preserved, with the original coloration being lost during the preservation of the specimens, *E. cesarii* is described herein, in life. The body ranged from dark to light brown on the dorsal region and pale and slightly transparent on the ventral region, with the tail having the same coloration as the body. *Elachistocleis haroi*, larvae, *in vivo,* were mostly black with smoothly pigmented tail fins.

Internal oral morphology

The internal oral anatomy in *Elachistocleis* is very conservative. *Elachistocleis bumbameuboi* shares most of the features presented by other described Microhylidae species including *Microhyla rubra*, *Dermatonotus muelleri*, *Chiasmocleis leucosticta*, *Chaperina fusca*, *Micryletta steinegeri* and *Ramanella obscura* (Bowatte & Meegaskumbura 2011; Echeverría & Lavilla 2000; Langone *et al.* 2007; Inger 1985; Chou & Lin 1997) and has great similarity with *E. bicolor* (D’Heursel & Baldissera Junior 1999; Echeverría & Lavilla 2000; Candioti 2007) and *E. panamensis* (Candioti 2006, 2007), two species of the genus currently being described.

Elachistocleis bumbameuboi has, in the posterior region of the lower lip, besides small pustules, a slightly developed papilla, laterally, in the external region, and a developed papilla in the medioventral region, directed posterodorsally, unlike *E. bicolor* and *E. panamensis*, which only have small pustules (Candioti 2006; Candioti 2007), and *E. bicolor,* which was described by D’Heursel & Baldissera Junior (1999) as having two papillae on each side of the lower lip, directed posteriorly.

The buccal floor arena in *E. bumbameuboi* is predominantly smooth, with scarce pustules and some individuals having a slightly developed papilla, contrasting with *E. bicolor*, which has a buccal floor arena devoid of any structure (Candioti 2007; Echeverría & Lavilla (2000). The number and form of papillae on each side of the glottis were not compared because these characters are most likely related to the larval stage and can be highly variable, as described by D’Heursel & Baldissera Junior (1999), who observed an increase in the number of papillae, with several becoming pustulated and multifurcated at stage 40 in *E. bicolor*. Echeverría & Lavilla (2000) described the velar margin in *E. bicolor* as having three papillae, the inner being the largest, whereas *E. bumbameuboi* and *E. panamensis* have a smooth posterior surface of the velum, devoid of any protuberance.

The characters observed on the buccal roof in *E. bumbameuboi* that differ from those in *E. bicolor* include the presence of bifurcation on the lateral papilla of the choana in some individuals; this bifurcation is absent in *E. bicolor* (Candioti 2007; Echeverría & Lavilla 2000; D'Heursel & Baldissera Junior 1999). The presence of small pustules associated with the papillae in the prenarial arena and the absence of small papillae on the buccal roof arena, although similar according to D’Heursel & Baldissera Junior (1999), are interpreted as absent and present, respectively, according to Candioti

(2007), and Echeverría & Lavilla (2000). *Elachistocleis panamensis* (Candioti 2006), similar to *E. bicolor*, has papillae on the buccal roof arena and has no pustules close to the papillae in the prenarial arena.

The internal oral morphology between *E. bumbameuboi* and *Dermatonotus muelleri* described by Echeverría & Lavilla (2000), and Candioti (2007), include the following similarities: almost flattened surfaces; circular and unperforated choanae with a large projection emerging from the posterior margin; absence of lingual and infralabial papillae; absence of a papillae posterior to the median ridge; presence of prepocket papillae; buccal roof and floor almost naked, with tall papillae attached on each side of the glottis; and exposed glottis located between a divided, well-developed ventral velum.

Some differences in the buccal floor include the following: absence of a small papilla on the medioposterior region in Meckel's cartilage in *D. muelleri*; irregular margin of the ventral velum in *D. muelleri* (Echeverría & Lavilla 2000) different from the smooth margin of the ventral velum in *E. bumbameuboi*; and prepocket papillae, as described by Candioti (2007), different from *E. bumbameuboi*, which is very wide and has irregular margin. In the buccal floor, *E. bumbameuboi* has a papilla on the prenarial arena similar to *D. muelleri*, but apparently, *D. muelleri* has several pustules around the prenarial papilla, whereas in *E. bumbameuboi,* the presence or not of pustules is only observed posteriorly to the papillae. In *D. muelleri*, there are two pairs of pustules behind the median ridge, not observed in *E. bumbameuboi*.

Chondrocranium and hyobranchial apparatus

The features of *E. bumbameuboi* tadpoles that are commonly observed in the skeletal structure in microhylid larvae are as follows: 1) suprarostral cartilage with corpus and alae fused in a single structure; 2) frontoparietal fenestra with *tectum transversum* and *taenia tecti medialis* absent; and 3) presence of a subotic process on the palatoquadrate. The characters observed in this work are similar to other species of Microhylidae described: *Hamptophryne boliviana* (De Sá & Trueb 1991), *D. Muelleri* (Lavilla 1992; Candioti 2007), *C. Leucosticta* (Langone *et al.* 2007), *Gastrophryne carolinensis* (Haas 2003; Trueb *et al.* 2011), and *R. obscura* (Bowatte & Meegaskumbura 2011).

However, some features of the chondrocranium and hyobranchial apparatus differ from two previously described species of the genus: *E. bicolor* (Candioti 2007; Lavilla & Langone 1995; Haas 2003) and *E. panamensis* (Candioti 2006; Candioti 2007). The presence of a basicranial fenestra is observed in *E. bumbameuboi* and *E. bicolor* (Lavilla & Langone 1995), but Candioti (2007) describes the basicranial fenestra as being entirely closed in *E. bicolor* and *E. panamensis*.

Candioti (2006) affirms that the presence of a crista parotica developed in *E. bicolor* is a manifestation of interspecific variability because, differently from Haas (2003), Candioti (2007), and Lavilla & Langone (1995), this character is not observed. *Elachistocleis bumbameuboi* has a well-developed crista parotica with papilla-like expansions connected to the posterior region of the posterolateral process of the palatoquadrate. Its absence was also observed in *E. panamensis* (Candioti 2006).

Haas (2003) affirms the presence of a ventrolateral process as a synapomorphy of American microhylids. In *E. panamensis* this character is reduced or absent, and Candioti (2006) argues its absence could be a regression. An individual of *E. bumbameuboi,* at stage 35, presented a small expansion similar to the crista parotica on the ventrolateral process, which was not observed on the tadpoles of *E. bicolor* and may also be related to a case of interspecific variability.

This interspecific variability was observed in *E. bicolor* with regard to the muscular process as well, which was reported as present by Candioti (2007) but absent by Lavilla & Langone (1995), and with regard to the presence of a quadratoethmoid process, present in Lavilla & Langone (1995) and absent in Candioti (2007). The shape of the subotic process also presented great variation, with *E. bumbameuboi* (single or bifid) being similar to *E. bicolor*. Candioti (2007) describes this process as bifid, although the illustration of Lavilla & Langone (1995) clearly shows the process as single and subcylindrical.

The differences among *E. bumbameuboi*, *E. bicolor* and *E. panamensis* in the hyobranchial apparatus are very subtle. *Elachistocleis bumbameuboi* has a basihyal process appearing as a thin and cartilaginous bar, whereas Lavilla & Langone (1995) observed a rounded projection on the posterior region in *E. bicolor*.

With regard to *G. carolinensis* (Haas 2003; Trueb *et al.* 2011) and *D. muelleri*, the two species described as closely related to *Elachistocleis*, the skeleton features are very similar. However, neither *G. carolinensis* nor *D. muelleri* presents a ventrolateral process. Furthermore, *D. muelleri* presents, according to Lavilla (1992), a small triangular process arising from the buccal floor on the lateral side of the subocular fenestra, and this is absent in *E. bumbameuboi*.

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Appendix I

Table 1.

Stage	TL	BL	BW	TMW	BH	DFH	TMH	VFH	IOD	ESD	ED	SL	SW
31	18,95	5,25	4,03	1,19	2,44	0,91	1,96	1,00	3,59	1,60	0,63	1,01	0,59
31	19,54	5,65	4,25	1,13	2,63	1,01	1,87	1,01	3,67	1,85	0,65	1,19	0,71
31	18,89	5,28	4,11	1,14	2,66	1,01	1,90	1,02	3,64	1,77	0,63	1,08	0,55
32	19,92	6,33	4,52	1,16	3,16	1,17	1,78	1,11	3,97	2,25	0,64	1,17	0,74
34	21,11	5,84	4,49	1,32	2,70	1,01	2,01	1,08	4,01	1,89	0,64	1,41	0,57
34	20,54	5,72	4,49	1,55	2,48	1,03	2,10	1,09	3,85	1,83	0,68	1,22	0,55
34	17,79	5,72	4,26	1,20	2,61	1,02	1,89	1,11	3,76	1,76	0,64	1,12	0,65
35	23,59	8,39	6,52	2,01	4,16	1,55	2,83	1,08	5,57	3,19	1,05	2,04	0,94
35	21,62	8,34	5,99	1,76	4,07	1,60	2,72	1,26	5,37	2,71	0,99	1,89	0,93
35	24,21	6,94	5,01	1,56	3,21	1,05	2,19	1,16	4,47	2,60	0,79	1,58	0,68
36	23,22	8,71	6,50	1,97	4,32	1,58	2,77	1,35	5,57	2,75	1,05	2,35	0,82
36	23,77	7,04	5,17	1,55	3,33	1,17	2,34	1,01	4,43	2,43	0,78	1,55	1,00
Mean	21,10	6,60	4,95	1,46	3,15	1,18	2,20	1,10	4,32	2,22	0,76	1,47	0,73

Table 2.

Stage	**BFL**	**BFW**	**VVL**	**VVW**	**BRL**	**BRW**	**DBC**	**NWPL**	**NWPW**	**NLPL**	**MRL**	**MRW**	**PPPL**	**PPPW**
30	2,48	1,62	0,96	2,45	2,96	1,98	0,28	0,51	0,35	0,17	0,56	0,25	0,21	0,16
34	2,35	2,06	1,21	2,76	2,75	1,74	0,36	0,35	0,32	0,24	0,34	0,28	0,20	0,16
36	3,91	3,47	1,53	3,59	4,11	2,84	0,34	0,35	0,30	0,30	0,52	0,32	0,16	0,42
37	3,40	3,40	1,33	3,40	4,74	2,66	0,33	0,57	0,39	0,27	0,40	0,33	0,16	0,19
Mean	3,03	2,64	1,26	3,05	3,64	2,30	0,33	0,44	0,34	0,24	0,46	0,30	0,18	0,23

Appendix II

Specimens analyzed:

E. bumbameuboi – HUFMA 1735, n = 1, Bacabeira, MARANHÃO, Brasil.

E. bumbameuboi – HUFMA 1738, n = 1, Bacabeira, MARANHÃO, Brasil.

E. bumbameuboi – HUFMA 1739, n = 1, Bacabeira, MARANHÃO, Brasil.

E. bumbameuboi – HUFMA 1740, n = 1, Bacabeira, MARANHÃO, Brasil.

E. bumbameuboi – HUFMA 1741, n = 1, São José de Ribamar, MARANHÃO, Brasil.

E. bumbameuboi – HUFMA 1742, n = 1, São José de Ribamar, MARANHÃO, Brasil.

E. bumbameuboi – HUFMA 1743, n = 1, São José de Ribamar, MARANHÃO, Brasil.

E. bumbameuboi – HUFMA 1744, n = 1, São José de Ribamar, MARANHÃO, Brasil.

E. bumbameuboi – HUFMA 1745, n = 1, São José de Ribamar, MARANHÃO, Brasil.

E. bumbameuboi - HUFMA 2073, n = 14, Bacabeira, MARANHÃO, Brasil.

E. bumbameuboi - HUFMA 2074, n = 7, São José de Ribamar, MARANHÃO, Brasil.

Legends:

Figure 1: Dorsal view (A), ventral view (B), lateral view (C) and detail of the dermal flap edge (D) of the external morphology of *Elachistocleis bumbameuboi*, stage 36.

Figure 2: Scanning electron micrographs of the buccal floor (A) and buccal roof (B) of the internal oral mophology of *Elachistocleis bumbameuboi.* BFA, buccal floor arena; BFAP, buccal floor arena papilla; BP, buccal pocket; BRA, buccal roof arena; C, choana; G, glottis; MR, median ridge; NLP, narial lateral papilla; NWP, narial wall papilla; PNA, prenarial arena; PNAP, prenarial arena papilla; PPP, prepocket papilla; TA, tongue anlage; VV, ventral velum.

Figure 3: Chondrocranium in dorsal view (A), ventral view (B), lateral view (C) and hyobranquial apparatus in ventral view (D) of *E. bumbameuboi*, stage 36. ALPC, anterolateral process of ceratohyal; APC, anterior process of ceratohyal; ARP, articular process; ASP, ascending process; BB, basibranchial; BF, basicranial fenestra; BH, basihyal; CB(I-IV), ceratobranchial; CF, carotid foramen; CH, ceratohyal; CP, crista parotica; CPF, craniopalatine foramen; HP, hypobranchial plate; HYP, hyoquadrate process; IC, infrarostral cartilage; LOP, larval otic process; LPC, lateral process of ceratohyal; MC, Meckel's cartilage; MP, muscular process; OC, otic capsule; OF, olfactory foramina; PLP, posterolateral process; PPC, posterior process of ceratohyal; PR, *pars reuniens*; PS, *processus suboticus*; QEP, quadratoethmoid process; SB, subocular bar; SF, subocular fenestra; SC, suprarostral cartilage; TH, trabecular horns; TS, *tectum synoticum*; TTM, *taenia tecti marginalis*; UP, urobranchail process; VP, ventrolateral process.

Table 1: Morphometric measurements of the external morphology (in mm) to 12 individuals of *Elachistocleis bumbameuboi*. BH – Body height; BL – Body length; BW – Body width; DFH – Dorsal fin height; ED – Eye diameter; ESD – Eye-snout distance; IOD – Interorbital distance; SL – Spiracle length; SW – Spiracle width; TL – Total length; TMH – Tail muscle height; TMW – Tail muscle width; VFH – Ventral vin height.

Table 2: Morphometric measurements of the internal oral morphology (in mm) to 5 individuals of *Elachistocleis bumbameuboi*. BFL – Buccal floor length; BFW – Buccal floor width; BRL – Buccal roof length; BRW – Buccal roof width; DBC – Distance

between the choanae; MRL – Median ridge length; MRW – Median ridge width; NLPL – Narial lateral papilla length; NWPL – Narial wall papilla length; NWPW – Narial wall papilla width; PPPL – Prepocket papilla length; PPPW – Prepocket papilla width; VVL – Ventral velum length; VVW – Ventral velum width.

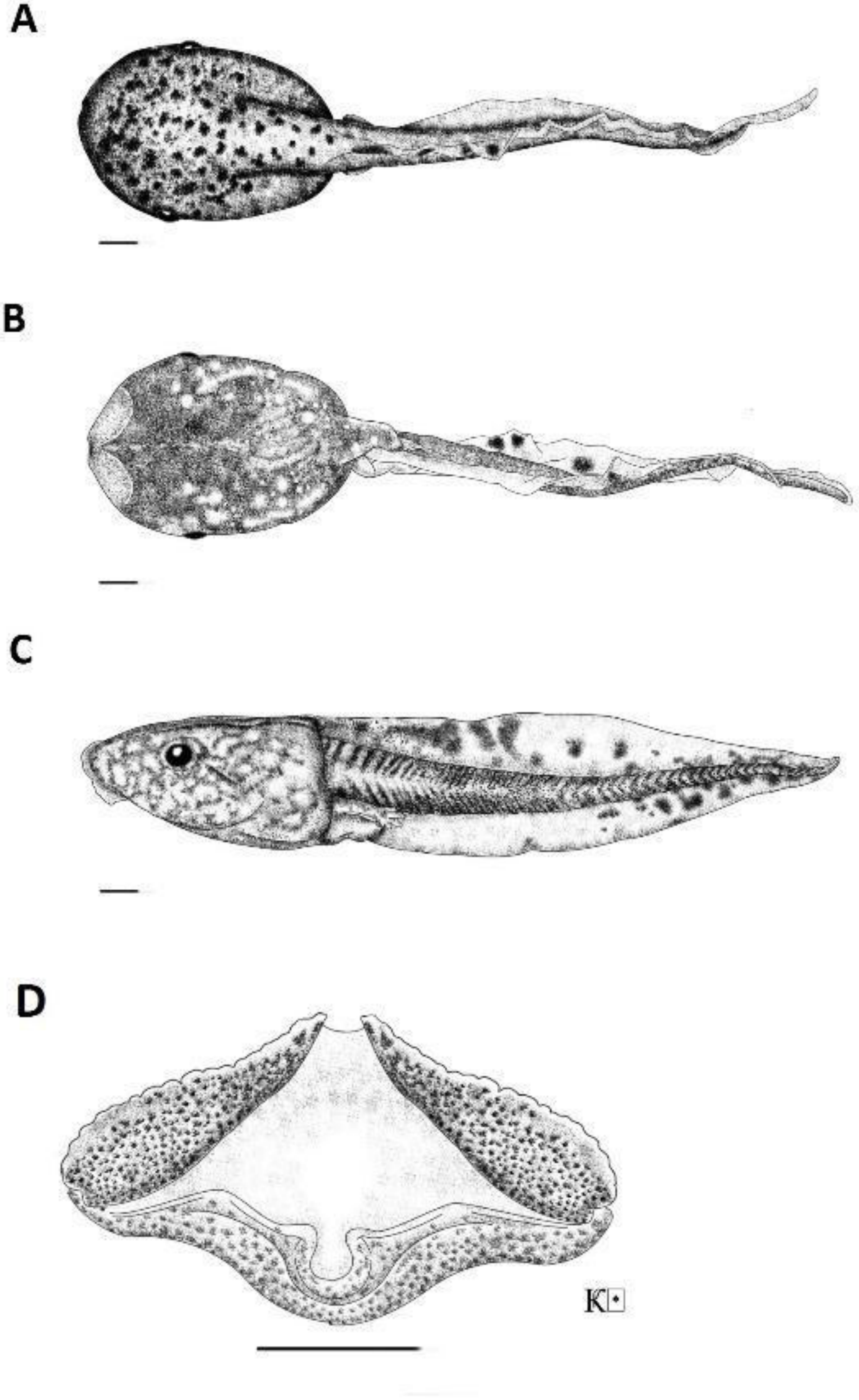


Figure 1:

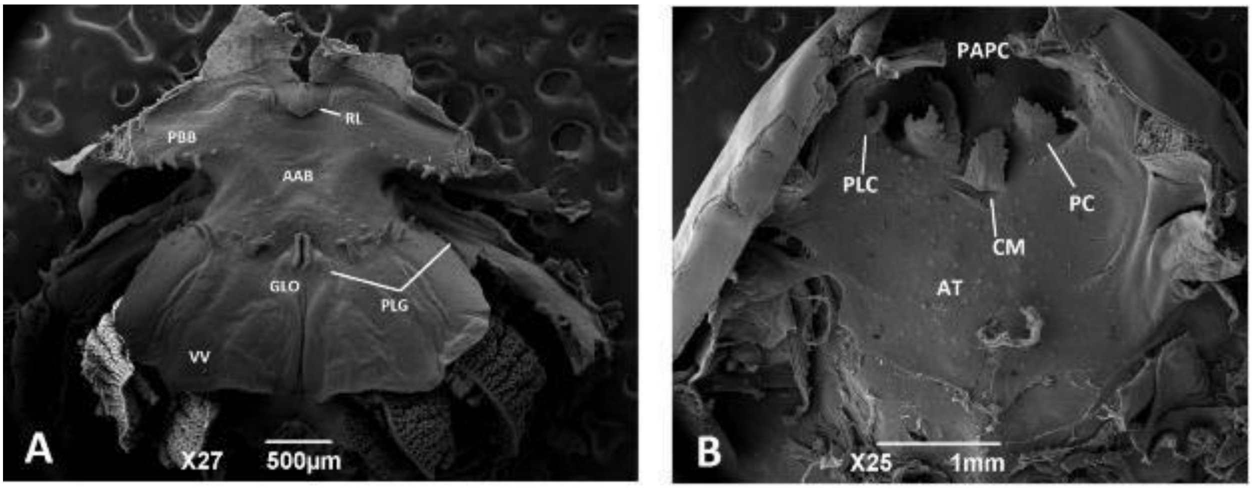
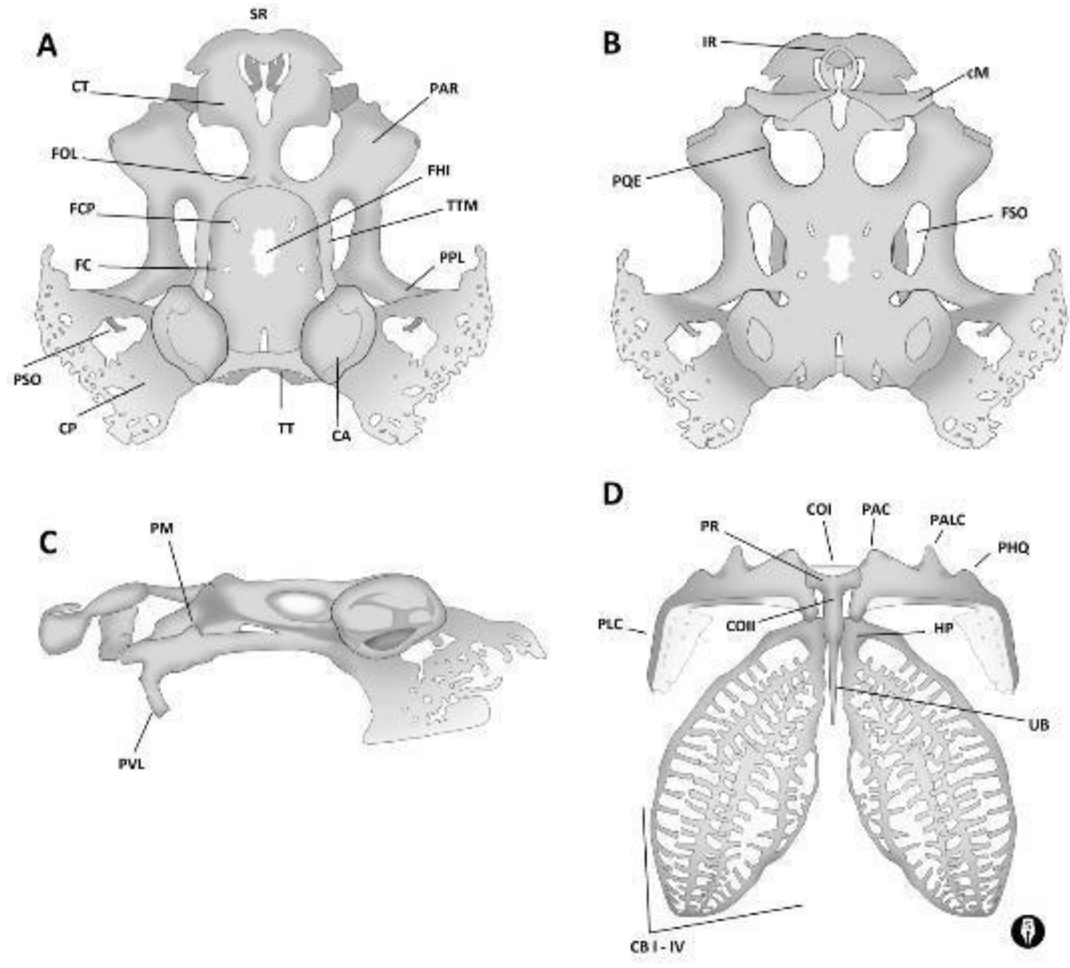


Figure 2:


A
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PAC
PALC
PR
PHQ
PLC
COII
HP
UB
CB I - IV

Figure 3:

Conclusões gerais

- A morfologia externa, morfologia oral interna, condrocrânio e aparelho hiobranquial das larvas de *Elachistocleis* são conservativos na maioria dos aspectos observados.

- A análise dos girinos mostrou que as características larvais encontradas em *Elachistocleis* são semelhantes às características encontradas por outras espécies da família Microhylidae.

- As características da morfologia externa que diferenciaram entre as espécies do gênero *Elachistocleis* foram: o formato do corpo em vista dorsal; borda das abas dermais; borda do focinho em vista dorsal; ângulo de emergência da nadadeira dorsal no corpo; linha lateral visível ou não.

- As características da morfologia oral interna foram muito conservativas e bastante variáveis intra e interespecificamente. *Elachistocleis bicolor* possui um par de pústulas distribuídas regularmente atrás da crista mediana que nenhuma das outras espécies possui e *E. bumbameuboi* e *E.* cf. *piauiensis* possui em poucos indivíduos uma linha de pústulas na lateral do teto bucal.

- As características do condrocrânio e aparelho hiobranquial que demosntraram utilidade para distinguir algumas espécies foram: projeção mais acentuada da margem anterior central do corpo da suprarostral; borda posterior das asas da suprarostral em forma de W ou não; borda ventral da fenestra da região occipital levemente ondulada ou claramente ondulada; presença de uma fenestra hipofisária; borda do processo afilado do processo articular do palatoquadrado; presença de expansões digitiformes no processo ventrolateral; formato e inclinação da fenestra subocular; processo subótico único ou levemente bífido; inclinação do processo anterolateral somente em *E. bicolor.*

- As comparações da morfologia larvar para o gênero *Elachistocleis* não evidenciaram características exclusivas em comparação com outras espécies da família Microhylidae.

- O estudo encontrou as mesmas características encontradas em descrições anteriores, principalmente quanto a morfologia oral interna e condrocrânio de *E. bicolor* e quanto à morfologia externa das demais espécies de *Elachistocleis*.

Anexo I

Normas para submissão de artigos no periódico Herpetological Journal (disponível em http://www.thebhs.org/pubs_journal.html, acessado em 01 de dezembro de 2014).

Instructions to authors

1) The Herpetological Journal publishes a range of features concerned with reptile and amphibian biology. These include: Full Papers (no length limit); Reviews and Mini-reviews (generally solicited by a member of the editorial board); Short Notes; and controversies, under Forum (details available from the Editor). Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance. Authors should bear in mind that the Herpetological Journal is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history.

2) All submissions and illustrations should initially be sent by e-mail to the Scientific Editor as a single MS Word or PDF document, with a final size of 2 MB or less, containing the text and figures. At this stage, figures do not need to be at high resolution as long as the information contained in them can be clearly seen by the reviewers. Please inform the Scientific Editor that you intend to submit before sending any files. If submission by e-mail is not possible, please contact the Scientific Editor for advice. All papers will be subject to peer review by at least two referees. Authors are invited to suggest the names of up to three suitable reviewers for their manuscript, although the Editor reserves the right to use alternative referees. Papers will be judged on the basis of the reports supplied by referees, scientific rigour and the degree of general interest in the subject matter. The Editor's decision will be final. 3) Authors should consult a recent edition of the Journal for general guidance. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the Oxford English Dictionary. Papers should be double-spaced with wide margins all round. The usual rules of zoological nomenclature apply.

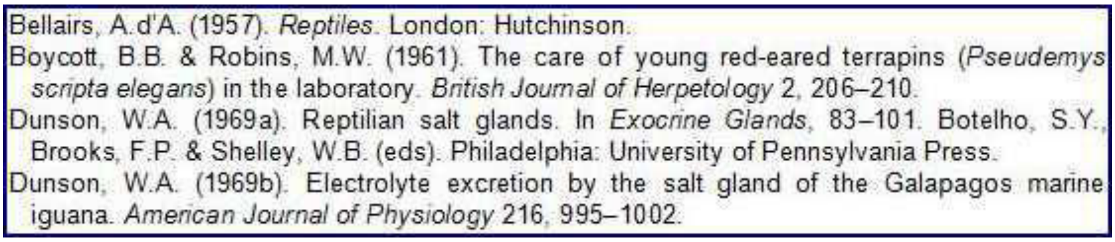
4) For all papers, the title page should contain only the following: title of paper; name(s) and brief addresses of the author(s); a running title of five words or less; and the name and full address of the corresponding author with (if available) an e-mail address. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Keywords, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. The first subhead will be centred in capitals, the second shouldered in bold lower case, and the third run on in italics. Footnotes are not permitted. Short Notes (generally less than six manuscript pages and accompanied by a single data set, i.e.

one table or one figure) should be produced as continuous text, preceded by an abstract of no more than 100 words.

5) Tables are numbered in arabic numerals, e.g. Table 1; they should be as simple as possible and typed double-spaced on separate sheets with a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided, as should tables that split over more than one page or that need to be set in landscape format.

6) Line drawings and photographs are numbered in sequence in arabic numerals, e.g. Fig. 1. Colour photographs can only be included at cost to the author (contact the Managing Editor for a quotation). If an illustration has more than one part, each should be identified as (a), (b), etc. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

7) References in the text should be given as in the following examples:



Bellairs, A.d'A. (1957). *Reptiles*. London: Hutchinson.

Boycott, B.B. & Robins, M.W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* 2, 206–210.

Dunson, W.A. (1969a). Reptilian salt glands. In *Exocrine Glands*, 83–101. Botelho, S.Y., Brooks, F.P. & Shelley, W.B. (eds). Philadelphia: University of Pennsylvania Press.

Dunson, W.A. (1969b). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *American Journal of Physiology* 216, 995–1002.

“Smith (1964) stated –”; “- as observed by Smith & Jones (1963).” “- as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)”. For three or more authors, the first author’s surname followed by “et al.” should be used (Smith et al., 1972). In the list of references, the full title of the journal should be given. Articles “submitted” or “in prep.” may not be cited in the text or references. The following examples will serve to illustrate the style and presentation used by the Journal.

8) The Journal is typeset direct from the author’s electronic text, so final acceptance of a paper will depend upon the production by the author of PC-compatible computer file(s) ready for the press. At this stage, figures should be supplied separately as highquality iles and NOT embedded in the text file. Preferred formats are MS Word (text) and MS xcel, Bitmap, TIFF, Windows Metafiles (.wmf, .emf) or JPEG files (graphics). Authors should note that figures are normally reduced to a single column width and should bear this in mind when drawing figures, e.g. ensuring that font sizes will be legible once reduced. Minimum resolution required is 1800 pixels wide for reproduction at single column width and 3600 pixels wide for reproduction at twocolumn width.

9) Proofs are prepared as PDF files and corrections should be returned to the Managing ditor by return of e-mail. Alterations should be kept to the correction of errors; more

xtensive alterations will be charged to the author. 10) A PDF file of the paper is rovided free of charge. Note that paper reprints are no longer supplied.

11) All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of work. Likewise, work that has involved the collection of endangered species or disturbance to their habitat(s) will require full justification.

Anexo II

Normas para submissão de artigos no periótico Zootaxa (disponível em http://www.mapress.com/zootaxa/support/author.html#Preparation of manuscripts, acessado em 01 de dezembro de 2014).

Preparation of manuscripts

1) *General.* All papers must be in English. Authors whose native language is not English are encouraged to have their manuscripts read by a native English-speaking colleague before submission. Nomenclature must be in agreement with the *International Code of Zoological Nomenclature* (4th edition 1999), which came into force on 1 January 2000. Author(s) of species name must be provided when the scientific name of any animal species is first mentioned (the year of publication needs not be given; if you give it, then provide a full reference of this in the reference list). Authors of plant species names need not be given. Metric systems should be used. If possible, use the common font New Times Roman and use as little formatting as possible (use only **bold** and *italics* where necessary and indentions of paragraphs except the first). Special symbols (e.g. male or female sign) should be avoided because they are likely to be altered when files are read on different machines (Mac versus PC with different language systems). You can code them as m# and f#, which can be replaced during page setting. The style of each author is generally respected but they must follow the following general guidelines.

2) The **title** should be concise and informative. The higher taxa containing the taxa dealt with in the paper should be indicated in parentheses: e.g. A taxonomic revision of the genus *Aus* (Order: family).

3) The **name(s) of all authors** of the paper must be given and should be typed in the upper case (e.g. ADAM SMITH, BRIAN SMITH & CAROL SMITH). The address of each author should be given in *italics* each starting a separate line. E-mail address(es) should be provided if available.

4) The **abstract** should be concise and informative. Any new names or new combinations proposed in the paper should be mentioned. Abstracts in other languages may also be included in addition to English abstract. The abstract should be followed by a list of **key words** that are not present in the title. Abstract and key words are not needed in short correspondence.

5) The arrangement of the **main text** varies with different types of papers (a taxonomic revision, an analysis of characters and phylogeny, a catalogue etc.), but should usually start with an **introduction** and end with a list of **references**. References should be cited in the text as Smith (1999), Smith and Smith (2000) or Smith *et al.* 2001 (3 or more authors), or alternatively in a parenthesis (Smith 2000; Smith & Smith 2000; Smith *et*

al. 2001). All literature cited in the text must be listed in the references in the following format (see asample page here in PDF).

A) **Journal paper**:

Smith, A. (1999) Title of the paper. *Title of the journal in full*, volume number, page range.

B) **Book chapter**:

Smith, A. & Smith, B. (2000) Title of the Chapter. *In*: Smith, A, Smith, B. & Smith, C. (Eds), *Title of Book*. Publisher name and location, pp. x–y.

C) **Book**:

Smith, A., Smith, B. & Smith, C. (2001) *Title of Book*. Publisher name and location, xyz pp.

D) **Internet resources**

Author (2002) Title of website, database or other resources, Publisher name and location (if indicated), number of pages (if known). Available from: http://xxx.xxx.xxx/ (Date of access). Dissertations resulting from graduate studies and non-serial proceedings of conferences/symposia are to be treated as books and cited as such. Papers not cited must not be listed in the references. Please note that:

(1) **journal titles must be written in full (not abbreviated)**

(2) **journal titles and volume numbers are followed by a ","**

(3) **page ranges are connected by "n dash", not hyphen "-", which is used to connect two words.**

For websites, it is important to include the last date when you see that site, as it can be moved or deleted from that address in the future. On the use of dashes: (1) Hyphens are used to link words such as personal names, some prefixes and compound adjectives (the last of which vary depending on the style manual in use). (2) En-dash or en-rule (the length of an ‘n’) is used to link spans. In the context of our journal that means numerals mainly, most frequently size, dates and page numbers (e.g. 1977–1981; figs 5–7) and also geographic or name associations (Murray–Darling River; a Federal–State agreement). (3) Em-dash or em-rule (the length of an ‘m’) are used far more infrequently, and are used for breaks in the text or subject, often used much as we used parentheses. In contrast to parentheses an emdash can be used alone; e.g. What could these results mean—that Niel had discovered the meaning of life? En-dashes and em-dashes should not be spaced.

6) Legends of **illustrations** should be listed after the list of references. Small illustrations should be grouped into plates. When preparing illustrations, authors should bear in mind that the journal has a matter size of 25 cm by 17 cm and is printed on A4 paper. For species illustration, line drawings are preferred, although good quality B&W or colour photographs are also acceptable. See a guide here for detailed information on preparing plates for publication.

7) **Tables**, if any, should be given at the end of the manuscript. Please use the table function in your word processor to build tables so that the cells, rows and columns can remain aligned when font size and width of the table are changed. Please do not use Tab key or space bar to type tables.

8) **Keys** are not easy to typeset. In a typical dichotomous key, each lead of a couplet should be typed simply as a paragraph as in the box below:

1 Seven setae present on tarsus I ; four setae present on tibia I; leg I longer than the body; legs black in color ... Genus A

- Six setae present on tarsus I; three setae present on tibia I; leg I shorter than the body; legs brown in color ... 2

2 Leg II longer than leg I ... Genus B

- Leg II shorter than leg I ... Genus C