Phyllomedusa 22(2):147–158, 2023

ISSN 1519-1397 (print) / ISSN 2316-9079 (online)
doi: http://dx.doi.org/10.11606/issn.2316-9079.v22i2p147-158

Comparative histology of the vocal sac in three species of hylid frogs with comments on its functional correlates

---

Natalia Ferreira Bueno,[1] Agustín J. Elias-Costa,[2,3] Délio Baêta,[4] and Evelise N. Fragoso-Moura[1]

[1] Universidade Federal de São Carlos, Departamento de Hidrobiologia. Rod. Washington Luís, Km 235, 13.565-905, São Carlos, SP, Brazil. E-mail: evelise@ufscar.br.

[2] Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" - CONICET, División Herpetología. Av. Ángel Gallardo 470, CABA (C1405DJR), Argentina.

[3] Museum für Naturkunde Berlin, Leibnitz Institute for Evolution and Biodiversity Science. Invalidenstrasse 43, Berlin 10115, Germany.

[4] Universidade Estadual Paulista, Departamento de Biodiversidade e Centro de Aquicultura. Av. 24A, 1515, 13.506-900, Rio Claro, SP, Brazil.

Abstract

Comparative histology of the vocal sac in three species of hylid frogs with comments on its functional correlates. The vocal sacs of frogs are elastic structures responsible for the circulation of air during vocalization, amplifying the sounds produced by these animals during multimodal communication. Vocal sacs present a wide array of morphologies among species and may be single, paired, or absent in adult males. Most studies on vocal sacs in Anura deal with their external morphology, and not with their internal structure, which has been explored in only a handful of species. The aim of this study was to assess vocal sac structure in three hylid species, *Dendropsophus haddadi, D. elegans,* and *Scinax fuscovarius,* using histological techniques. These species differ greatly in the degree of development and histological properties of the gular skin and submandibular musculature. In particular, elastic fibers are abundant in the thick *m. interhyoideus* and the relatively tight gular skin of *S. fuscovarius.* In contrast, in both species of *Dendropsophus* (although more evident in *D. elegans*), the *m. interhyoideus* is extremely thin and expanded, with a negligible number of elastic fibers that appear as a loose, pleated sheet when deflated. We analyzed videos of calling males of the three species and their close relatives, which show two different patterns of inflation/deflation. These patterns are strongly correlated with the histological properties of the vocal sac wall. The three species have different vocal sac shapes and rely differently on elasticity for vocal sac function.

Keywords: Animal histology, Anura, Elastic fibers, Morphology, Vocal slits, Vocalization.

Received 02 June 2023
Accepted 22 November 2023
Distributed December 2023

Resumo
Histologia comparativa do saco vocal de três espécies de hilídeos com comentários sobre suas correlações funcionais. Os sacos vocais dos anuros são estruturas elásticas responsáveis pela circulação do ar durante a vocalização, amplificando os sons produzidos por esses animais durante a comunicação multimodal. Os sacos vocais apresentam uma ampla gama de morfologias entre as espécies, podendo ser únicos, pareados ou ausentes em machos adultos. A maioria dos estudos sobre sacos vocais em Anura trata de sua morfologia externa, não incluindo dados sobre sua estrutura interna, que foi explorada em apenas algumas espécies. O objetivo deste estudo foi avaliar a estrutura do saco vocal de três espécies de hilídeos, *Dendropsophus haddadi, D. elegans* e *Scinax fuscovarius,* por meio de técnicas histológicas. Essas espécies diferem muito no grau de desenvolvimento e nas propriedades histológicas da pele gular e da musculatura submandibular. Em particular, as fibras elásticas são abundantes no músculo interioidal espesso e pele gular relativamente esticada de *S. fuscovarius.* Ao contrário, em ambas as espécies de *Dendropsophus* (embora mais evidente em *D. elegans*), o músculo interioidal é extremamente fino e expandido, com conteúdo não-significativo de fibras elásticas, aparecendo como uma folha solta e pregueada quando desinflado. Analisamos vídeos de vocalizações de machos das três espécies e parentes próximos, que mostram dois padrões bem diferentes de inflação/deflação. Além disso, estes estão fortemente correlacionados com as propriedades histológicas da parede do saco vocal, uma vez que as três espécies têm diferentes formatos de saco vocal e dependem diferentemente da elasticidade para a função do saco vocal.

Palavras-chave: Anura, Fendas vocais, Fibras elásticas, Histologia animal, Morfologia, Vocalização.

Introduction

Vocalization is an important feature in frog biology and plays a key role in reproduction, defense of territory, and conspecific recognition (Wells 2007). Vocal sacs are structures present only in adult males of most (but not all) species of frogs and toads and are key elements of their multimodal communication in both intra- and intersexual interactions (Boulenger 1882, Liu 1935, Wells 2007, Starnberger *et al.* 2014). Their extraordinary elasticity is often evident with the naked eye because they are capable of receiving enormous volumes of air and recovering their original shape at great speed. The function of this elasticity was only recently suggested by Ryan (1985), who reasoned that the vocal sac could store the strain energy of the air column during vocalization and recycle it for inflation of the lungs. During vocalization, the mouth and nostrils remain closed and the air stored in the lungs is cycled through the larynx to the oral cavity into the vocal sacs, and then back into the lungs (Gans 1973, Dudley and Rand 1991). In the absence of a diaphragm or rib cage, the lungs in anurans are filled by a mechanism called the buccal pump, which consists of actively pushing air from the oral cavity by depression and compression of the floor of the mouth (Gans 1973). If males vocalize with their mouths open, the air dissipates into the atmosphere, forcing them to repeat the cycle of lung filling, which is costly in both time and energy (Butcher *et al.* 1982, Prestwich 1994). Calling activity in frogs is among the most energetically expensive activities in ectotherm vertebrates (Taigen and Wells 1985). Storing air within an elastic cavity greatly reduces the energetic costs of vocalization because the column of air and the associated strain energy are passively reused (Dudley and Rand 1991).

This hypothesis was supported by the finding of a layer of abundant elastic fibers in the structures associated with vocalization (Jaramillo *et al.* 1997). The elastic fibers are abundant in the trunk muscles responsible for forcing air into the buccal cavity and, in turn, the vocal sac and the lungs, which expand enormously during the process. This discovery expanded the traditional concept that vocalization was based on muscular action. Since then, studies aimed at characterizing this system have been scarce (Savitsky *et al.* 2000, 2002, Targino *et al.* 2019, Elias-Costa *et al.* 2021), and the taxonomic distribution of the abundance of elastic fibers among different species and their spatial organization are unknown.

Anatomically, vocal sacs consist of three elements: the gular skin, the superficial submandibular musculature composed of the *m. intermandibularis* and *m. interhyoideus,* and an internal mucosa derived from the evagination of the buccal floor (Noble 1931, Inger and Greenberg 1956, Tyler 1971, 1974). Several studies described the anatomy of submandibular muscles and vocal sac structures in a wide variety of anuran families (Duellman 1956, 2001, Inger 1956, Liem 1970, Trueb 1971, Trueb and Tyler 1974, Tyler 1971, 1972, 1974, Drewes 1984, Burton 1998, Faivovich *et al.* 2011, Elias-Costa and Faivovich 2019, Elias-Costa *et al.* 2021). Only a few of these studies included histological techniques to assess the diversity in tissue composition (Jaramillo *et al.* 1997, Elias-Costa *et al.* 2017, 2021, Targino *et al.* 2019, Moura *et al.* 2021). In particular, Targino *et al.* (2019) compared the histological properties of the vocal sac wall in several microhylids and studied their evolution in the family, including the functional implications of the differences in the content of elastic fibers among species.

Hylidae, composed of 1050 described species to date, is the most diverse family of anurans (Frost 2023). Vocal sac structure in this family has been studied by several authors (e.g., Liu 1935, Duellman 2001, Tyler 1971, 1974, Faivovich 2002, Faivovich *et al.* 2011, Elias-Costa *et al.* 2021, Moura *et al.* 2021, Araujo-Vieira *et al.* 2023). Despite these extensive surveys, the histology of the vocal sac wall remains mostly unexplored. The two papers that referred to histological properties of the vocal sac wall did not discuss differences among species in light of vocalization dynamics (Elias-Costa *et al.* 2021, Moura *et al.* 2021).

The present study provides novel insights into the comparative histology of the vocal sac of three species of hylids. We considered differences among species in a morphofunctional context to hypothesize about the possible implications of the observed patterns in the dynamics of inflation of the vocal sac.

Materials and Methods

Taxon Sampling

We studied eleven specimens of three species of Hylidae found in Brazil: *Dendropsophus elegans* (Wied-Neuwied, 1824) (CFBH 36849*, 36851, and 36853), *Dendropsophus haddadi* (Bastos and Pombal, 1996) (CFBH 33203*, 36825, 36829, and 36832), and *Scinax fuscovarius* (Lutz, 1925) (CFBH 19629*, 40349, 40351, and 40354). All specimens were adult males with well-developed vocal sacs. Specimens indicated with an asterisk were only examined externally to assess gross morphology. Collection acronyms follow Sabaj *et al.* (2022).

Histological Procedures

Samples were obtained from collection specimens fixed in formalin and stored in 70% ethanol. The entire buccal floor, tongue, associated musculature, and the gular skin were separated from the mandible using a scalpel and submitted to standard histological processing. Samples were embedded in histological paraffin and cut at 6 µm-thick sections with a Microm HM340E semi-automatic microtome. Sections were stained with a standard Hematoxylin-Eosin stain for general observation of the tissuesand nitric orcein in order to reveal the presence of elastic fibers (Humason 1972, Bancroft and Gamble 2008). Inspection of the slides was performed using light microscopy; images were recorded with an Olympus BX61 microscope and cellSens Dimension software.

Qualitative Assessments of Vocal Sac Inflation Dynamics

To hypothesize if the histological differences observed among species have a functional correlation, we qualitatively studied videos of calling males of the three species. We observed inflation of the vocal sac during vocalization, paying attention to how the column of air is moved between the lungs and the vocal sac, and whether resting volumes of air in the vocal sac remained in between calls. To provide a wider context for our discussion, we studied videos of other closely related species (sources listed in Appendix 1).

Results

Comparative Histology

From observations and analyses of histological sections, we identified and described the structures present in the vocal sacs of the three species of hylids. Anatomically, their structure resembles that described for most anuran species: an internal mucosa derived from the buccal floor is externally enveloped by a thin lamina of muscle fibers, the *m. intermandibularis,* anteriorly, and the *m. interhyoideus,* posteriorly. This muscle layer is ventrally covered by the gular skin, and separated by a space, the submandibular and pectoral lymphatic sacs. In this region of the body, this space is divided by a thin membrane of connective tissue, the postmandibular lymphatic septum, which connects the *m. interhyoideus* and the gular skin. Elastic fibers are dispersed throughout the extension of the vocal sac wall in the three species. They form a mesh mostly concentrated around the external layer of muscle fibers and the internal layer of the dermis, but with scattered elastic fibers interspersed inside all tissues. The relative degree of development, abundance, and distribution of elastic fibers differed among the three species.

In *Scinax fuscovarius,* the gular skin of fixed specimens is relatively tight with only a few large folds (Figure 1). The vocal sac mucosa occupies the space underlying the posterior half of the *m. intermandibularis* and the entire *m. interhyoideus.* This muscle is thick and scarcely folded. The anterior third of the *m. interhyoideus* is similar in thickness as the *m. intermandibularis,* and the limit between them cannot be easily seen in parasagittal sections. Large numbers of elastic fibers were identified both in the *m. interhyoideus* and the vocal sac internal mucosa. They form trabeculae intertwined among muscle fibers and bundles concentrated in the exterior layer of the *m. interhyoideus* and the internal layer of the dermis. The *m. interhyoideus* only contacts the gular skin through the well-developed postmandibular lymphatic septum. In its most posterior portion, the vocal sac mucosa is extremely folded showing an irregular pattern on the internal surface of the vocal sac. This excess tissue suggests that the vocal sac is highly distensible during vocalization but contracted when at rest.

Dendropsophus elegans (Figure 2) shows a different morphofunctional pattern. The *mm. intermandibularis* and *interhyoideus* are extremely thin and expanded. In a relaxed position, they are loose and highly folded. Most of the vocal sac cavity is supported by the *m. interhyoideus,* which contacts the gular skin mostly through the postmandibular lymphatic septum but also through several smaller, punctual bridges of connective tissue. The vocal sac is less elastic than in *S. fuscovarius,* a condition evident by the loose and pleated aspect of the muscle, which does not revert to its original position, the smaller amount of elastic fibers in histological sections (only a very thin layer in the external layer of the *m. interhyoideus* and the internal layer of the dermis), and the smooth aspect of the internal mucosa, which lacks excess tissue.

Dendropsophus haddadi (Figure 3) shows an intermediate condition between the two other species. The *m. interhyoideus* is very thin and

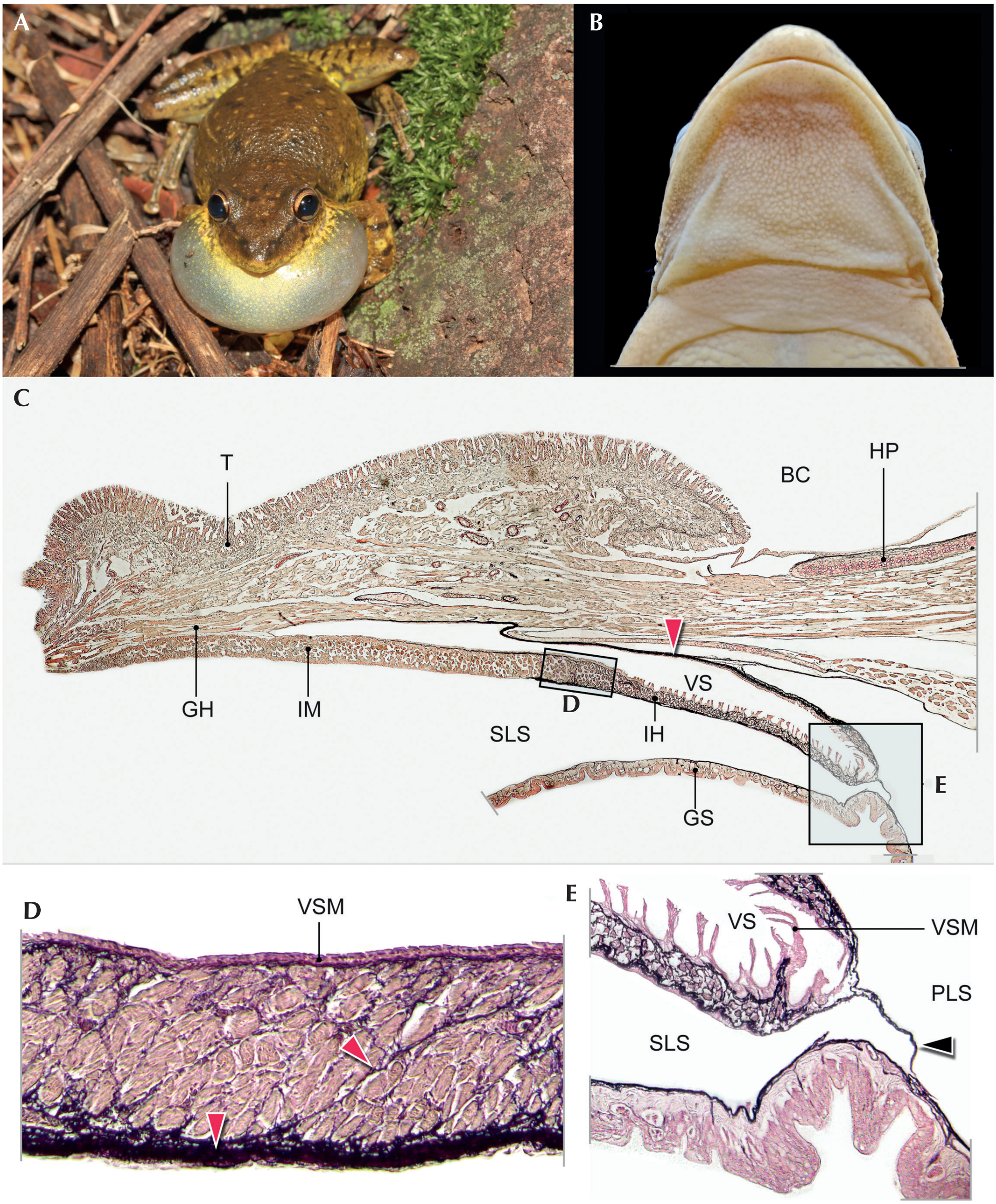

Figure 1. Vocal sac of *Scinax fuscovarius.* (**A**) Vocalizing male. (**B**) Ventral view of fixed specimen (CFBH 19629). (**C**) Sagittal section (40×) of vocal sac stained with nitric orcein (elastic fibers in black). (**D–E**) Details (400×) of regions indicated in (C). BC: buccal cavity, HP: hyoid plate, IH: *m. interhyoideus,* IM: *m. intermandibularis,* SLS: submandibular lymphatic sac, PLS: pectoral lymphatic sac, GS: gular skin, GH: *mm. geniohyoidei,* T: tongue, VSM: vocal sac mucosa, black arrow: postmandibular lymphatic septum, red arrow: bundles of elastic fibers. Photo (A) by Diego Baldo.

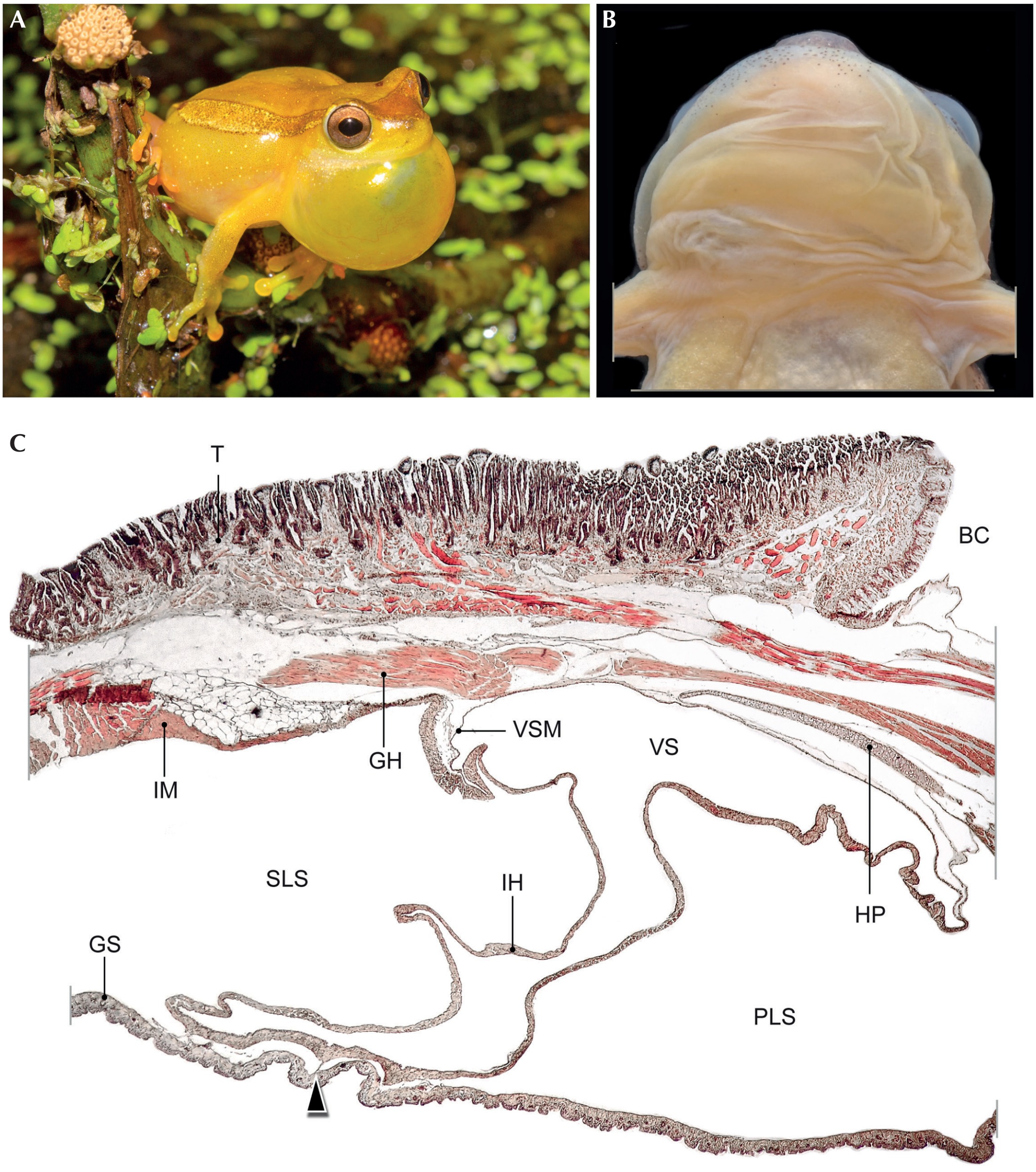

Figure 2. Vocal sac of *Dendropsophus elegans*. (**A**) Vocalizing male. (**B**) Ventral view of fixed specimen (CFBH 36849). (**C**) Sagittal section (40×) of vocal sac stained with Masson's trichrome. BC: buccal cavity, GH: *mm. geniohyoidei,* GS: gular skin, HP: hyoid plate, IH: *m. interhyoideus,* IM: *m. intermandibularis,* PLS: pectoral lymphatic sac, SLS: submandibular lymphatic sac, T: tongue, VS: vocal sac cavity, VSM: vocal sac mucosa, black arrow: postmandibular lymphatic septum. Photo (A) by Ricardo Marques.

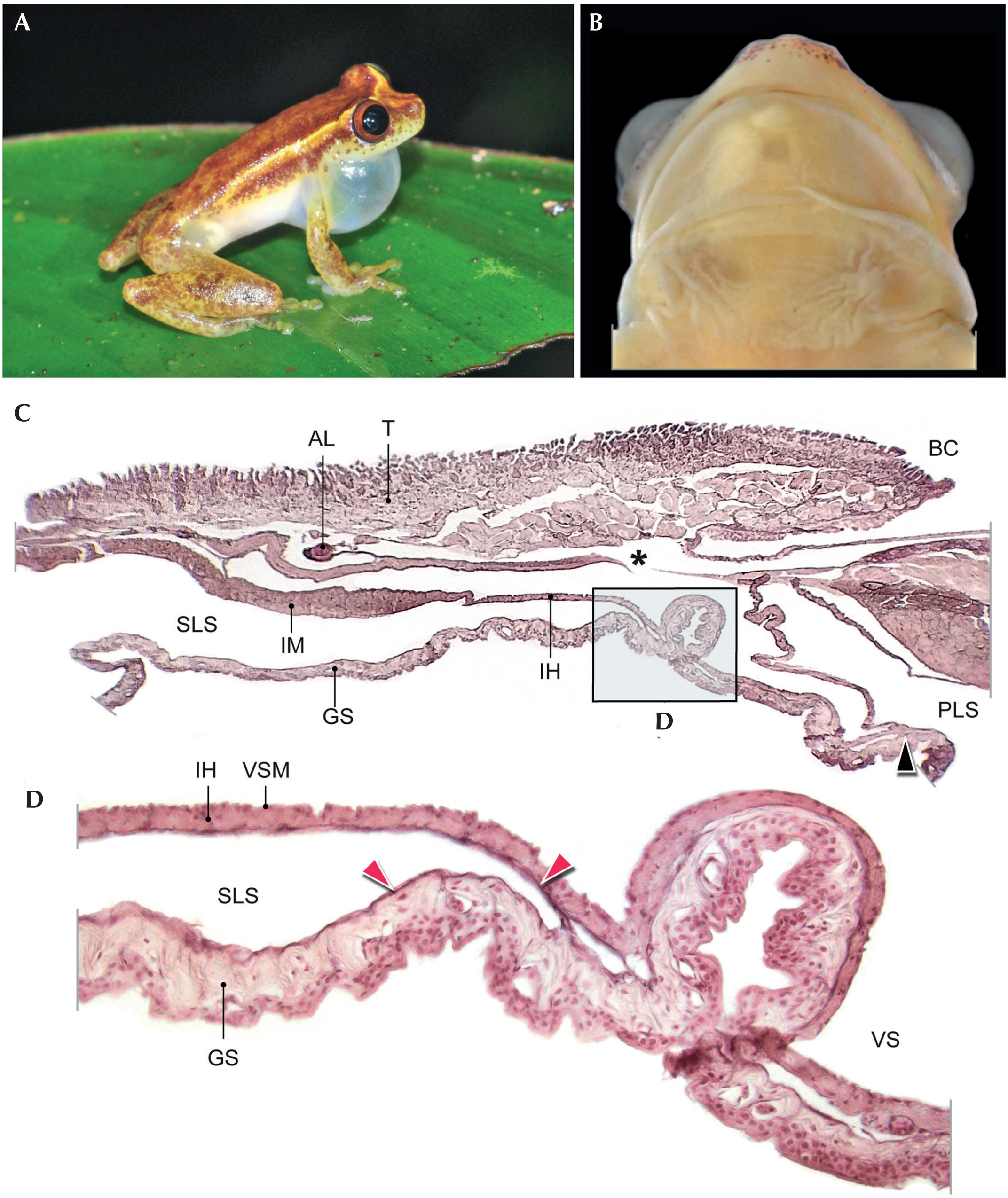

Figure 3. Vocal sac of *Dendropsophus haddadi*. (**A**) Vocalizing male. (**B**) Ventral view of fixed specimen (CFBH 19629). (**C**) Parasagittal section (40×) of vocal sac stained with nitric orcein (elastic fibers in dark red). (**D**) Details (400×) of region indicated in (C). AL: anterolateral process of hyoid (hyale), BC: buccal cavity, HP: hyoid plate, IH: *m. interhyoideus*, IM: *m. intermandibularis*, SLS: submandibular lymphatic sac, PLS: pectoral lymphatic sac, GS: gular skin, GH: *mm. geniohyoidei*, T: tongue, VSM: vocal sac mucosa, asterisk: vocal slit, black arrow: postmandibular lymphatic septum, red arrows: bundles of elastic fibers. Photo (A) by Peter Janzen.

expanded in a relaxed position, but to a lesser degree than *D. elegans,* indicating a relatively smaller vocal sac. Unlike the two previous species, the vocal sac does not extend anteriorly, but is restricted to the throat and pectoral regions. This condition is evident in ventral view, where only the skin of the posterior half of the gular region is expanded and pleated. Likewise, as observed in the sagittal sections, the *m. intermandibularis* is considerably thicker than the *m. interhyoideus,* which likely accounts for the smaller expansion of the former during vocalization. Because expansion of the vocal sac follows the direction of less resistance, inflation occurs posteriorly. The number of elastic fibers is similar to that of *D. elegans* and considerably smaller than that of *S. fuscovarius,* with very few fibers in the external layer of the *m. interhyoideus* and the internal layer of the dermis.

Qualitative Description of Vocal Sac Inflation

When analyzing videos of vocalizing males of the three species (obtained from public, online sources; see Appendix I), we observed different dynamics of vocal sac inflation. In *S. fuscovarius,* males completely inflate and deflate their vocal sacs in each cycle of sound emission (Pattern A). Deflation is almost automatic, since the air rapidly exits the vocal sac into the lungs after each burst. The vocal sac is almost completely deflated after each cycle, and air is mostly stored in the lungs. This pattern was also observed in several other species of *Scinax* (see Appendix I), although not in *S. fuscomarginatus* (Lutz, 1925) and *S. madeirae* (Bokermann, 1964). In contrast, in both species of *Dendropsophus,* the vocal sac remains almost fully inflated even in between calls (Pattern B). The amount of air used for sound production seems to be relatively small compared to the volume stored in the lungs and the vocal sac. Deflation is subtle and relatively slow. This pattern was observed in all *Dendropsophus* studied as well as in other hylids (Appendix I).

Discussion

Vocal sacs in anurans show an astounding morphological diversity, which is even greater if the internal structure is considered. This variation occurs in shape, size, color, and diverse modifications of the gular skin (Wells 2007, Köhler *et al.* 2017). This morphological diversity has been extensively surveyed both in taxonomic and systematic studies (e.g., Boulenger 1882, Liem 1980, Drewes 1984, Duellman 2001, Faivovich 2002), as well as in contributions specifically exploring this structure (e.g., Liu 1935, Tyler 1971, 1972, 1974, Tyler and Duellman 1995). Only a handful of papers have been dedicated to the histology of the vocal sac wall and its functional implications in different species (Jaramillo *et al.* 1997, Elias-Costa *et al.* 2017, Targino *et al.* 2019, Moura *et al.* 2021).

In the present study, we explored the fine structure of the vocal sac wall in three species of hylids. We identified several features in *S. fuscovarius* that suggest that males of this species rely on elasticity for vocal sac function. These features include the large amount of highly concentrated elastic fibers, forming bundles and networks present in extensions of the *m. interhyoideus* and the gular skin, the folded surface of the internal mucosa, and the relatively tight aspect of the muscles and gular skin in fixed specimens. This arrangement implies a much more energetically efficient process, since the air and strain energy used in sound production are passively recycled and redirected to the lungs to be used in the next vocalization (Dudley and Rand 1991, Jaramillo *et al.* 1997).

In both species of *Dendropsophus,* although more clearly in *D. elegans,* the vocal sac wall is loose and pleated when deflated. Elastic fibers were found in smaller numbers and were infrequent in the tissues of the gular region. The *m. interhyoideus* is extremely thin and very loose in the gular region, both in live and fixed specimens. These two species differ in the relative size and position of their vocal sacs, which are larger and occupy the entire gular region in *D. elegans,* and smaller and restricted to the throat in *D. haddadi.* These elements suggest that these species do not depend as strongly on elasticity for vocal sac function.

By qualitative evaluation of videos of calling males of these three species, it is evident that they manage the column of air differently during vocalization. While the vocal sac in *S. fuscovarius* is rapidly deflated after each note, instantly refilling the lungs, the vocal sac in both species of *Dendropsophus* remains partially inflated in between calls. Most likely this arrangement is derived from the differing content of elastic fibers, which greatly affects the relationship of the *m. interhyoideus* and the gular skin to internal air pressure (Dudley and Rand 1991). As suggested by studies in the Túngara frog, *Engystomops pustulosus* (Cope, 1864), the abundance of elastic fibers observed in the vocal sac wall are most likely correlated with their abundance in the lungs and the trunk muscles, which receive the force of the column from the vocal sac (Jaramillo *et al.* 1997).

These two patterns of vocal sac inflation/deflation may represent two extremes of a continuum. Some species are hard to assign to one pattern or another since the volume of the vocal sac greatly varies during vocalization, but a considerable volume of air is retained after sound emission [e.g., *Dendropsophus minutus* (Peters, 1872), *Scinax squalirostris* (Lutz, 1925)]. Future studies using quantitative methodologies will prove useful in fully understanding this phenomenon. In the species we studied, a clear correlation exists between the inflation dynamics and the histology of the vocal sac wall, a pattern that can be easily extrapolated to all frogs and toads.

Conclusions

Anura currently contains more than 7600 described species; however, the histology of the vocal sac is only known for a handful of them. Herein, we reported the fine structure of the vocal sac and compared species with diverse shapes and functional patterns. We hope this contribution will inspire further studies that quantify the content of elastic fibers and a statistically supported comparison with acoustic parameters of the vocalization.

Acknowledgments

We thank Célio F. B. Haddad (UNESP) for allowing access to the specimens under his care and Jurity Antônia Machado Milan and Claudinei Arcanjo de Oliveira for technical support and laboratory help. Diego Baldo, Ricardo Morais, and Peter Janzen kindly permitted the use of their photos of *Scinax fuscovarius, Dendropsophus elegans,* and *Dendropsophus haddadi*. CONICET awarded a postdoctoral fellowship to AJEC.

References

Araujo-Vieira, K., A. C. C. Lourenço, J. V. A. Lacerda, M. L. Lyra, B. L. Blotto, S. R. Ron, D. Baldo, M. O. Pereyra, Á. M. Suárez-Mayorga, D. Baêta, R. B. Ferreira, C. L. Barrio-Amorós, C. Borteiro, R. A. Brandão, C. A. Brasileiro, M. A. Donnelly, M. J. M. Dubeux, J. Köhler, F. Kolenc, F. S. F. Leite, N. M. Maciel, I. Nunes, V. G. D. Orrico, P. L. V. Peloso, T. L. Pezzuti, S. Reichle, F. J. M. Rojas-Runjaic, H. R. Silva, M. J. Sturaro, J. A. Langone, P. C. A. Garcia, M. T. Rodrigues, D. R. Frost, W. C. Wheeler, T. Grant, J. P. Pombal Jr., C. F. B. Haddad, and J. Faivovich. 2023. Treefrog diversity in the Neotropics: phylogenetic relationships of Scinaxini (Anura: Hylidae: Hylinae). *South American Journal of Herpetology 27 (Special Issue):* 1–143.

Bancroft, J. D. and M. Gamble (eds.). 2008. *Theory and Practice of Histological Techniques.* 5th Edition. Philadelphia. Churchill Livingstone, Elsevier Ltd. 725 pp.

Boulenger, G. A. 1882. *Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum.* 2nd Edition. London. Taylor and Francis. 528 pp.

Burton, T. C. 1998. Variation in the hand and superficial throat musculature of neotropical leptodactylid frogs. *Herpetologica 54:* 53–72.

Butcher, T. L., M. J. Ryan, and G. A. Bartholomew. 1982. Oxygen consumption during resting, calling, and nest building in the frog *Physalaemus pustulosus. Physiological Zoology 55:* 10–22.

Drewes, R. C. 1984. A phylogenetic analysis of the Hyperoliidae (Anura): Treefrogs of Africa, Madagascar and the Seychelles Islands. *Occasional Papers of the California Academy of Sciences 139:* 1–70.

Dudley, R. and A. S. Rand. 1991. Sound production and vocal sac inflation in the túngara frog, *Physalaemus pustulosus* (Leptodactylidae). *Copeia 1991:* 460–470.

Duellman, W. E. 1956. The frogs of the hylid genus *Phrynohyas* Fitzinger, 1843. *Miscellaneous Publications of the Museum of Zoology, University of Michigan 96:* 1–60.

Duellman, W. E. 2001. *Hylid Frogs of Middle America.* 2nd Edition. Ithaca. Society for the Study of Amphibians and Reptiles, Natural History Museum of the University of Kansas. 1158 pp.

Elias-Costa, A. J. and J. Faivovich. 2019. Convergence to the tiniest detail: vocal sac structure in torrent-dwelling frogs. *Biological Journal of the Linnean Society 128:* 390–402.

Elias-Costa, A. J., K. Araujo-Vieira, and J. Faivovich. 2021. Evolution of the strikingly diverse submandibular muscles in Anura. *Cladistics 37:* 489–517.

Elias-Costa, A. J., R. Montesinos, T. Grant, and J. Faivovich. 2017. The vocal sac of Hylodidae (Amphibia, Anura): Phylogenetic and functional implications of a unique morphology. *Journal of Morphology 278:* 1506–1516

Faivovich, J. 2002. A cladistic analysis of *Scinax* (Anura: Hylidae). *Cladistics 18:* 367–393.

Faivovich, J., D. Baêta, F. Vera Candioti, C. F. B. Haddad, and M. J. Tyler. 2011. The submandibular musculature of Phyllomedusinae (Anura: Hylidae): a reappraisal. *Journal of Morphology 272:* 354–62.

Frost, D. R. 2023. Amphibian Species of the World: an Online Reference. Version 6.2. Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. Captured on 01 November 2023.

Gans, C. 1973. Sound production in the Salientia: mechanism and evolution of the emitter. *American Zoologist 13:* 1179–1194.

Humason, G. L. 1972. *Animal Tissue Techniques.* 3rd Edition. New York. W. H. Freeman and Company. 641 pp.

Inger, R. F. 1956. Morphology and development of the vocal sac apparatus in the African frog *Rana (Ptychadena) porosissima* Steindachner. *Journal of Morphology 99:* 57–72.

Inger, R. F. and B. Greenberg. 1956. Morphology and seasonal development of sex characters in two sympatric African toads. *Journal of Morphology 99:* 549–574.

Jaramillo, C., A. S. Rand, R. Ibañezz, and R. Dudley. 1997. Elastic structuresin the vocalization apparatus of the tungara frog *Physalaemus pustulosus* (Leptodactylidae). *Journal of Morphology 233:* 287–295.

Liem, S. S. 1970. The morphology, systematics, and evolution of the Old World treefrogs (Rhacophoridae and Hyperoliidae). *Fieldiana, Zoology 57:* 1–145.

Liu, C. 1935. Types of vocal sac in the Salientia. *Proceedings of the Boston Society of Natural History 41:* 19–40.

Köhler, J., M. Jansen, A. Rodriguez, P. J. R. Kok, L. F. Toledo, M. Emmrich, F. Glaw, C. F. B. Haddad, M.-O. Rödel, and M. Vences. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa 4251:* 1–124.

Moura, M. R. and W. Jetz. 2021. Shortfalls and opportunities in terrestrial vertebrate species discovery. *Nature Ecology and Evolution 5:* 631–639.

Noble, G. K. 1931. *The Biology of the Amphibia.* New York and London. McGraw-Hill. 577 pp.

Prestwich, K. 1994. The energetics of acoustic signaling in anurans and insects. *American Zoologist 34:* 625–643.

Ryan, M. J. 1985. *The Túngara Frog. A Study in Sexual Selection and Communication.* Chicago. University of Chicago Press. 230 pp.

Sabaj, M. H. 2022. Codes for Natural History collections in Ichthyology and Herpetology (online supplement). Version 9.0 (2022). Electronical Database accessible at https://asih.org, American Society of Ichthyologists and Herpetologists, Washington, DC.

Savitzky, A. H., A. S. Rand, and B. A. Savitzky. 2002. Organization of the elastic fibers in the vocal sac musculature of neotropical frogs. Abstracts of Papers Presented at the Joint 18th Annual Meeting of the American Elasmobranch Society, 80th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 50th Annual Meeting of the Herpetologists' League, and 45th Annual Meeting of the Society for the Study of Amphibians and Reptiles: 266.

Savitzky, A. H., K. A. Roberts, and A. S. Rand. 2000. Organization of elastic fibers in the vocal sac of frogs. Abstracts of Papers Presented at the Society for Integrative and Comparative Biology.

Starnberger, I., D. Preininger, and W. Hödl. 2014. The anuran vocal sac: a tool for multimodal signalling. *Animal Behaviour 97:* 281–288.

Taigen, T. L. and K. D. Wells. 1985. Energetics of vocalization by an anuran amphibian (Hyla versicolor). *Journal of Comparative Physiology B 155:* 163–170.

Targino, M., A. J. Elias-Costa, C. Taboada, and J. Faivovich. 2019. Novel morphological structures in frogs: vocal sac diversity and evolution in Microhylidae (Amphibia: Anura). *Zoological Journal of the Linnean Society 187:* 479–493.

Trueb, L. 1971. Phylogenetic relationships of certain neotropical toads with the description of a new genus (Anura: Bufonidae). *Contributions in Science, Los Angeles County Museum 216:* 1–40.

Trueb, L. and H. J. Tyler. 1974. Systematics and evolution of the Greater Antillean hylid frogs. *Occasional Papers, Museum of Natural History, University of Kansas 24:* 1–60.

Tyler, M. J. 1971. Observations on anuran myo-integumental attachments associated with the vocal sac apparatus. *Journal of Natural History 5:* 225–231.

Tyler, M. J. 1972. Superficial mandibular musculature, vocal sacs and the phylogeny of Australo-Papuan leptodactylid frogs. *Records of South Australian Museum 16:* 1–20.

Tyler, M. J. 1974. Superficial mandibular musculature and vocal sac structure in the Anura. Ms. Thesis. Department of Anatomy and Histology, University of Adelaide.

Tyler, M. J. and W. E. Duellman. 1995. Superficial mandibular musculature and vocal sac structure in hemiphractine hylid frogs. *Journal of Morphology 224:* 65–71.

Wells, K. D. 2007. *The Ecology and Behavior of Amphibians.* Chicago and London. University of Chicago Press. 1400 pp.

Editor: Vanessa K. Verdade

Appendix I. *Sources of online videos of vocalizing males.*

Pattern A:
Studied species:
Scinax fuscovarius: https://youtu.be/ndhVNhEvuqM, https://youtu.be/HxifDT3KKoo

Other closely-related species:
Aplastodiscus eugenioi: https://youtu.be/jean8fJCz00
Dendropsophus seniculus: https://youtu.be/g3NDSM6-0BU
Scinax duartei: https://youtu.be/CQ84iu_CYz8
Scinax elaeochroa: https://youtube.com/shorts/c5Ghawc0lX4?feature=share
Scinax eurydice: https://youtu.be/ZtwfqsCOL54
Scinax fuscovarius: https://youtu.be/ndhVNhEvuqM, https://youtu.be/HxifDT3KKoo
Scinax garbei: https://youtu.be/riAWAc9K1fE
Scinax granulatus: https://youtu.be/ZxrllNOPNRE
Scinax nasicus: https://youtu.be/CMt0eiPgrfA
Scinax nebulosus: https://youtu.be/IN4sW150QkQ
Scinax perereca: https://youtube.com/shorts/pEZvsl6J4Wg?feature=share
Scinax ruber: https://youtu.be/TIeKpSg42zo
Scinax squalirostris: https://youtu.be/xAFn8m4eWoc
Scinax sugillatus: https://youtu.be/Jky7jd-2c4g
Scinax tymbamirim: https://youtu.be/LuoXxgP6nY4

Pattern B:
Studied species:
Dendropsophus elegans: https://youtu.be/E-npc9AQdGc
Dendropsophus haddadi: https://youtu.be/UMkSAoQNiDo

Other closely-related species:
Boana pulchella: https://youtu.be/iz51sqVszfw
Dendropsophus acreanus: https://youtu.be/ZxZeKEsf4eM
Dendropsophus ebraccatus: https://youtu.be/fB4ElgW2tNA
Dendropsophus jimi: https://youtu.be/PeFWnguisjQ
Dendropsophus leali: https://youtu.be/M4D-NUS6__k
Dendropsophus microps: https://youtu.be/3vjOrVr0Z1E
Dendropsophus nanus: https://youtu.be/VoFt_7blF7w
Dendropsophus rubicundulus: https://youtu.be/od469uVL9U8
Dendropsophus sarayacuensis: https://youtu.be/_3Tk95S16C4
Julianus uruguayus: https://youtu.be/xt4SKlXsQc4
Ololygon berthae: https://youtu.be/n0FALJsXpJA
Ololygon perpusillus: https://youtu.be/oMGNWW93v30
Scinax fuscomarginatus: https://youtu.be/WFvCg1g1_Vo
Scinax madeirae: https://youtu.be/ZNdPddxUv3g
Sphaenorhynchus caramaschii: https://youtu.be/zR43uQI3FTY

Species not assigned to any pattern (mixed):
Dendropsophus minutus: https://youtu.be/CwIqeujoeQM
Scinax squalirostris: https://youtu.be/xAFn8m4eWoc