The hyolaryngeal apparatus of three species of *Scinax* (Scinaxinae: Hylidae: Anura) and comparison with other Hylidae species

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The larynx is an important morphological structure for sound production in frogs. Recently, high levels of variation in calls and their relation to the morphology of frogs in the family Hylidae, in addition to molecular data, have resulted in significant changes in the taxonomy of this family. However, there are few studies that describe the structures and processes of the hyoid, cricoid or arytenoid in hylid frogs. We studied the hyolaryngeal anatomy of both males and females of three species of the hylid genus *Scinax* (*S. ruber*, *S. wandae* and *S. kennedyi*) using differential and enzymatic clearing and staining methods. We describe the laryngeal anatomy of these species and compare to those of other hylids. We found that *S. ruber* has the largest cartilage arytenoid of any species of the family Hylidae studied to date. We show the laryngeal differences between *Scinax* species and the interspecific variability in the shapes of the projections among the arytenoid in the males of those species. The taxonomic characters described here can play a key role in differentiating these species from other members of *Scinax,* contributing to the knowledge of the species examined, and helping to differentiate them from other species, and to contributing also the natural history and phylogenetic relationships within the genus.

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Abstract The larynx is an important morphological structure for sound production in frogs. Recently, high levels of variation in calls and their relation to the morphology of frogs in the family Hylidae, besides molecular data have resulted in significant changes in the taxonomy of this family. However, there are few studies that describe the structures and processes of the hyoid, cricoid, or arytenoid in hylid frogs. We studied the hyolaryngeal anatomy of both males and females of three species of the hylid genus *Scinax* (*S. ruber*, *S. wandae* and *S. kennedyi*) using clearing and double staining methods. We describe the laryngeal anatomy of these species and compare them with those of other hylids. We found that *S. ruber* has the largest arytenoid cartilage of any species of the family Hylidae studied to date. We show the laryngeal differences between *Scinax* species and the interspecific variability in the shapes of the projections among the arytenoid in the males of those species. The taxonomic characters described here can play a key role in differentiating these species from other members of *Scinax,* contributing to the knowledge of the species examined, and helping to differentiate them from other species, and to contributing also the natural history and phylogenetic relationships within the genus.

Keywords Arytenoid, cartilage, cricoid, larynx, morphology

Introduction

The hyolaryngeal apparatus is a complex structure comprising the cartilages and muscles of both the hyoid and larynx (Trewavas, 1933; Mason, 2006). In frogs, this structure is between the heart and head. The larynx comprise of a cricoid ring, and arytenoid cartilage. The hyoid is made of hyaline cartilage and a calcified cartilaginous rod. These structures are essential for call production in male frogs, and at the same time these structures are sexually dimorphic, fluctuating mainly in shape and size of the hyaline cartilages (Sassoon and Kelley, 1986; McClelland and Wilczynski, 1989; Yager, 1996; McClelland et al., 1997). Besides, the development of the esophageal cricoid process and the internal lumen in males (Ryan, 2001) serve to differentiate species (Rose, 2014), or males and females of the same species (Faivovich et al., 2002).

There are few descriptive studies of these structures, among them early works on the laryngeal anatomy in anurans, including studies of the skeleton (Frazier, 1924), and muscles (Trewavas, 1933; Wilder, 1892) based on either dissections or histological preparations (Blume, 1930). Likewise, there have been relatively few studies of anatomical variations in laryngeal structures among closely related species that show differences in advertisement calls (Eichelberg and Schneider, 1974).

Early works that do descriptions of the larynx in anurans includes Dugès (1834), Henle (1839), Wilder (1892), Ridewood (1897; 1900), Frazier (1924), Blume (1930) and Trewavas (1933). Faivovich, 2002 defined the esophageal process of the cricoid ring and the dorsal prominence of the arytenoid developed over the pharyngeal margin as a character, and described *Scinax* as a monophyletic group. Faivovich et al., 2005 defined the posterior part of the cricoid ring extensively elongated and curved as presumed morphological synapomorphies of *Scinax catharinae* group. Despite *Scinax* treefrogs (Hylidae) is a group species with high species richness (Ferrão et al., 2016), there are few studies in morphology (León, 1969; Gaudin, 1974) about this group.

Larynx and hyoid apparatus are movable parts. In the process of development, is regulated by genetic factors and environment (Hall, 2005; Shwartz *et al*., 2013) thus this is a source of information of development. The modifications of these structures in the adult of buccal apparatus imply ossification, increase in size, reduction, reabsorption and fusion or position changes of both cartilages and muscles (Rose, 2014). Changes of structures and skeletal joints in larval stages is greater than in the adult, the latter shows exclusive characters such as calling patterns and differentiation of the vocal sac apparatus (for example in *Pseudis* species, Goldberg *et al*., 2016). Despite these studies are focalized in specific structures, this information can help to understand whether behavior or physiology of Neotropical frogs in an integrative way. Additionally, the discovery of structures not described before can be relevant on frog systematics, studies of interspecific variation, and description of additional diagnostic characters for species-level taxonomy (Priti *et al*., 2016).

The anatomical structures of the larynx are widely recognized for the notorious presence of cartilaginous parts in the males of amphibians (Noble, 1931), so their study is important to distinguish sexually dimorphic larynges (laryngeal secondary sexual characters) and to recognize species. In this study, we present laryngeal components found in males of *Scinax ruber* previously not described (Faivovich, 2002) or only mentioned briefly (Kaplan, 1991;1999). It is important to note that although *S. ruber* was previously recognized as a junior synonym of *S. rubra* (Laurenti, 1768), both Blume (1930) and Trewavas (1933) provided descriptions of the hyolaryngeal anatomy of *Hyla rubra* Daudin 1802 from Therezopolis, Brazil. *Scinax ruber* is in the *Scinax ruber* clade (Faivovich et al., 2005) and has had several synonyms in its name, which makes it difficult to establish if the descriptions of the literature correspond to the species that were moved in different species such as *Scinax hayii* (Barbour, 1909) and *Hyla robertsimoni* (Donoso-Barros, 1966).

Here, we describe the hyolaryngeal anatomy of three species of *Scinax* Wagler, 1830, of the 14 that have been reported for Colombia (Acosta-Galvis, 2000; Frost, 2017; Acosta, 2017), of the *ruber* clade: *Scinax ruber*, *Scinax wandae* and *Scinax kennedyi.* Our study reveals extraordinary variations in the structure and size of the larynx in males not documented previously (Faivovich, 2002; Pombal and Gordo, 1991).

Material and methods

We focused our study on three species of the genus *Scinax* of the *Scinax ruber* clade (Faivovich et al., 2005). Males and females of *Scinax ruber* and *S. wandae* were disected. Females in *S. kennedyi* were not available. Nine specimens acquire from the collection of the Museo de Historia Natural "Lorenzo Uribe, S.J." of the Pontificia Universidad Javeriana (PUJ) were used (Table 1). Larynges were cleared and stained using the method of Dingerkus and Uhler (1977) with some modifications done by us, such as the proportion of ethanol and acetic acid (70:30), the quantity of Alcian Blue (75 mg) to 500 mL of total solution, and the staining time with Alcian Blue, increased to 72 hours. We captured digital images of the hyoid and laryngeal apparatuses using a stereomicroscope (Advanced Optical) and digital camera (Infinity 1 Lumenera Corporation) using white LED light. Anatomical drawings were done using a digitizing tablet (Bamboo Connect Pen) and Adobe Photoshop v. 5.1. For describing the anatomy of the hyolaryngeal structures, we used the anatomical nomenclature of Trewavas (1933) and additional names used by other authors (Henle, 1839; Wilder, 1892; Gaupp, 1896; Blume, 1930; Trueb and Cannatella, 1982; Ford, 1993; Haas, 1999; Maglia, 1999; Faivovich, 2002; Ponssa et al., 2010). Additional adult specimens were inspected from the collection of the California Academy of Sciences (CAS). We used a script in R to get a pruned tree with some species of Hylidae included in the Pyron and Wiens (2011) tree for comparison with *Scinax ruber*.

Results

Arytenoid

The arytenoid (= cartilago arytaenoidea *sensu* Haas, 1999) consists of two cartilaginous discs joined to form a single concave structure, forming an eight-shaped pattern in ventral view. The structures of the arytenoid (Ar) are oriented along the dorsoventral axis and connected with the posteromedial processes of the hyoid, though with the staining method used, the cartilage that connect these two structures was not displayed (Fig. 1). The insertion points of the arytenoid surface muscles (*sensu* Martin and Gans, 1972) are visible only in *S. ruber*. In ventral view on the anterior and posterior position of the arytenoid, there are ventral processes projecting from anterior to posterior into the arytenoid (ara); these processes are fused with the vocal cord (vc).

The arytenoid, in ventral view, is oval-shaped in *S. ruber* and *S. kennedyi*, while in *S. wandae* is nearly circular (Fig. 2A). In both *S. ruber* and *S. kennedyi*, the arytenoid is elongated in the lateral view (Fig. 2C). These cartilages in *S. wandae* are both concave and bell-shaped, corresponding to the convex contour of the outer surfaces. The size of the arytenoid in *S. ruber* is one-fifth of the total body length, bigger than in *S. wandae* and in *S. kennedyi.* This cartilage shows a medially depression in these three species, in both males and females (Fig. 2A). Also we observed two projections dorsomedially to the cardiac process (cpc) in both *S. ruber* and *S. kennedyi*. They are absent in *S. wandae* (Fig. 2A).

In the dorsomedially region of the arytenoid, there is a process (adp), described by McLachlan (1943) as a single short apex (= cartilago Santorini *sensu* Henle, 1839; = apical cartilage *sensu* Wilder, 1892; = cartilago apicalis *sensu* Blume, 1930; = dorsomedial prominence of the arytenoid *sensu* Trewavas, 1933; = prominentia apicalis *sensu* McLachlan, 1943; = prominence dorsomedial *sensu* Faivovich, 2002). The dorsomedial process of the arytenoid in *S. wandae* is more pronounced and shaped as an isosceles triangle with a slightly convex anterior margin, while that of *S. ruber* and *S. kennedyi* are not as long as in *S. wandae* (Fig 2C). In *Scinax ruber* this process is elongated and recurved like a small fold, and in *S. kennedyi* has a great bending inward in the rear of the arytenoid.

Cricoid

The cricoid (Cr) (= cartilago crico-trachealis *sensu* Gaupp, 1896; = cartilago cricoida *sensu* Haas, 1999) is oriented along the dorsoventral axis. It is an elliptical ring, with a projection extended anteroventrally. The largest central process of the cricoid (=articular process of cricoid *sensu* Trewavas, 1933; = cricoid ring *sensu* Maglia, 1999) was found in *S. ruber*, whereas in *S. wandae* it is the smallest, elongated and expanded distally.

In the cricoid, the esophageal process (epc) ventrally projects on the posteromedial processes (=Oesphageal process of the cricoid *sensu* Trewavas, 1933; = spina esophagea of the cricoid cartilage *sensu* McLachlan, 1943; = esophagic process *sensu* Faivovich 2002; = esophageal process *sensu* Ponssa et al. 2010). There are not projections from the cricoid in *S. kennedyi* not in *S. wandae;* the cricoid in both species has a wide blade adjacent to it. The shape of the posterior surface of the cricoid varies among the three species (Fig. 2B): it is elongated and slightly curved in both *Scinax ruber* and *S. kennedyi*, and in *S. ruber* is more slender than in *S. kennedyi*. In *S. wandae* this process is absent (Fig. 2C).

There is also some variation in the bronchial process (bpc), which is typically an elliptical lateral extension of the cricoid (*sensu* Trewavas, 1933) (= processus trachealis + processus pulmonalis *sensu* Gaupp, 1896; = tubercula hyoidea *sensu* Blume, 1930; = lateral arcs of the cricoid *sensu* McLachlan, 1943; = bronchial process *sensu* Maglia, 1999). This cartilage is curved ventrally towards the heart in both *S. ruber* and *S. kennedyi*, but is straight in *S. wandae* along the anteroposterior axis. The cartilago basalis (cb) (*sensu* McLachlan, 1943) (= sesamoids *sensu* Wilder, 1896) is present in *Scinax ruber*, but in *S. wandae* and *S. kennedyi* is absent (Fig. 1A). In the three species examined, there is a syndesmotic connection between the arytenoid and the cricoid cartilages.

Hyoid

The hyoid (Hd) (=Basilingual arch *sensu* Trewavas, 1933; = Corpus hyoidis *sensu* Haas, 1999) is a large cartilaginous structure of the hyolaringeal apparatus, located ventrally in the anterior body cavity, in the proximity of the heart (Fig. 1A). The hyoid typically exhibits three processes: a large and thin cartilage named the hyale (hy) (= cornu hyale *sensu* Haas, 1999) that project anterodorsally; posterolateral processes (plph), processus posterolateralis (*sensu* Haas, 1999), and ossified posteromedial processes (pmph) (= thyrohyal *sensu* Trewavas, 1933; = processus posteromedialis *sensu* Haas, 1999).

The posterolateral process of the hyoid, via the hyocricoid ligament, is joined to the bronchial process of the cricoid (Fig. 1), (= anterior process hyale *sensu* Trueb and Canatella, 1982). The hyoglossal sinus (hs) (= Hyolaryngeal sinus *sensu* Ford, 1993) and the hyale are shaped similarly in these three species. Neither hyo-arytenoid nor hyoglossal membrane were visualized with the staining technique we used (Fig. 1A).

In the hyale, there are the medial branches of anterior process of hyale (maph) (*sensu* Ford, 1993; = anterior process hyale *sensu* Trueb and Canatella, 1982) located anteriorly, the alary process (aph) (= anterolateral process *sensu* Ponssa et al. 2010) in lateral region of hyale, and the antero medial process of alary process (amap). These processes are known as the Gauppian processes (McLachlan, 1943).

The length of the hyoid is twice its middle width in both *S. ruber* and *S. kennedyi*, but in *S. wandae* its lenght twice the middle width. Neither *S. wandae* nor *S. ruber* have an anterior projection to the medial branch of the anterior process of the hyale (*sensu* Ford, 1993) (= cornu hyale *sensu* Haas, 1999). This process has an anterior projection medially as a bulge in *S. wandae* and *S. ruber*, whereas that in *S. kennedyi* has an additional foramen (Fig. 2A).

The posteromedial processes of the hyoid are elongated in *S. ruber* and *S. kennedyi,* but they are shorter in *S. wandae*, being ossified in all species. The posterolateral processes of the hyoid in *S. kennedyi* are the shortest of these three species, and the distal end of this process has a small bump in *S. ruber* and in *S. wandae*. *S. wandae* has a projection at the level of the anterior connection with the hyoid plate that the other two species do not have. The alary processes of *S. ruber* and *S. kennedyi* have one distal bulge, whereas *S. wandae* has two distal bulges (Fig. 2A).

Sexual dimorphism

We found striking sexual differences in the morphology of the larynx. The arytenoid and cricoid cartilages are bigger in males than in females, and these elements have fewer processes in females. Males of the three species of *Scinax* reveal different morphology and length of arytoid, cricoid and hyoid processes. The esophageal process is extended in males of *S. wandae* and *S. kennedyi*, but it is flat in *S. ruber* (Fig. 2A). The size of the larynx (cricoid, arytenoid and hyoid) in *S. ruber* is the largest among males of *Scinax*.

The structures of the hyoid and laryngeal apparatus among females of *S. wandae* and *S. ruber* did not show many differences, in contrast with males in the size and shape of the arytenoid cartilages. Between females, the size of the hyoid of *S. ruber* is larger than that of *S. wandae*, whereas the size of the cricoid and the arytenoid are the same. Besides, the variation in the shape of the distal part of the bronchial process of cricoid has a bulge, present in *S. wandae* but absent in *S. ruber*.

The size ratio of the larynx with respect to the body size in males was 0.194 in *S. wandae*, 0.228 in *S. kennedyi* and 0.241 in *S. ruber*, showing that *S. ruber* keeps the size of the larynx larger with respect to the other two species of *Scinax*. *Scinax ruber* not only has the biggest larynx among the males of this three species of *Scinax* but this size has not been reported in other hylid frogs (Fig 3 and Appendix 1). Size is one of the main characteristics that distinguish males from females regardless of the species.

Discussion

Nomenclatural problems

The genus *Scinax* is one of the most diverse genera within the family Hylidae, comprising 115 species (Frost, 2017) with considerable nomenclatural problems (Faivovich, 2002), including synonyms. Some *Scinax* species belonging to the Scinaxinae Subfamily of Hylidae are considered part of *Ololygon* (“*catharinae* clade”), and other species are classified as *Scinax* (“*rubra* clade”), depending on the biogeographical origin (Duellman et al., 2016).

Blume (1930) and Trewavas (1933) both made descriptions of larynx of *Hyla rubra* (Daudin, 1802), but in fact this name corresponds to different species: *Scinax alter* (Lutz, 1973), *Scinax duartei* (Lutz, 1951), *Scinax funereus* (Cope, 1874) or *Scinax x-signatus* (Spix, 1824), then there is no certainty to know the species that they described. *Scinax ruber* (Laurenti, 1768) was previously known as *Hyla rubra* Peters, 1872, *Hyla rubra hübneri* Melin, 1941 and *Scinax rubra* Duellman and Wiens, 1992. Rivero (1969) mentioned *Hyla rubra*, as a junior synonym of *Hyla rubra* Laurenti, 1768. Lutz (1973) described the *Hyla rubra* group and included in this group the subspecies *Hyla rubra rubra* Daudin, 1802, *Hyla rubra huebneri* Melin, 1941, and *Hyla rubra altera* which was replaced by *Hyla rubra orientalis* Lutz, 1968 (Frost, 2017). On the other hand, *Scinax alter* Alves and Carvalho-e-Silva, 2002 has two synonyms, *Hyla rubra altera* Lutz, 1973 and *Scinax altera* Pombal et al. 1995.

Likewise, *Scinax funereus* (Cope, 1874) was previously known as *Scinax rubra* Duellman and Wiens, 1992 and *Scinax ruber* Köhler and Böhme, 1996 (Frost, 2017). While Duellman and Wiens (1993) proposed *Hyla robersimoni* Donoso-Barros, 1966 as a junior synonym of *Scinax ruber*, De la Riva et al. (2000) suggested that the *Scinax ruber* species are composite (Frost et al., 2006). Fouquet et al. (2007) suggested that the frog complex *Scinax ruber* include multiple species that represent different clades, forming with molecular data the group *Scinax boesemani, Scinax cruentommus* and *Scinax x-signatus* (Frost, 2017). The authors described different species under the same name, especially with *Scinax ruber*. There are species described with the homonym *Hyla rubra* Daudin, 1802, and the names for referring to the "*Hyla rubra*" group Fouquette and Delahoussaye, 1977. Rivero (1969) concluded that the use of

Scinax ruber has generated confusion because there is homonymy between *Scinax ruber* and other *Scinax* species with the name of *Hyla rubra*.

The *ruber* group is relatively well-known in the family Hylidae from molecular phylogenetic analysis (Faivovich et al., 2005), studies of the ecology, and diets of tadpoles (Alcalde and Blotto, 2006; Altig, 2006; Altig et al., 2007). Pombal et al. (1995) transferred all the species of the *Scinax x-signata* group to the *Scinax rubra* species group, based on the existence of similarities in morphology and vocalizations. They suggested, furthermore, that morphological variation, vocalizations, and reproductive environment indicate the presence of at least two distinct genera under the current definition of *Scinax*; Langone and Cardoso (1997) held the same conclusion. Although taxonomic arrangements of frog species have molecular support (Faivovich et al., 2005; Pyron and Wiens, 2011) some studies have already noted the relationship between morphology and ethology. This information will help to clarify taxonomic conflicts of the species known as *Scinax ruber*.

Morphology of larynx

Considering just morphology, Kaplan (1999) compared species of the genus *Hyla*, including *Scinax ruber* (former *Scinax rubra*), describing briefly these species and finding a basal cartilage structure that he named sesamoid. We describe that structure as a diagnostic character that can help distinguish species of *Scinax*. Neither Faivovich (2002) nor Faivovich et al. (2005) described these cartilages.

S. ruber and *S. kennedyi* have an esophagic process, but it is not present in *S. wandae*. Equally, it was evident that the posterior part of the cricoid varied in the three species, being elongated and slightly curved in *Scinax ruber* and *S. kennedyi*, but it is slender in *S. ruber* than in *S. kennedyi*. Faivovich (2002) described the posterior part of the cricoid ring as a projection (= esophageal process) oriented toward the heart. On the other hand, *S. perereca* and *S. littoralis* have the dorsal prominence of arytenoid developed over the pharyngeal margin in males, but this character is not found in *S. fuscomarginata*.

Alcalde and Roset (2003) showed that the developmental degree of the lateral process of the ceratohyal has an association with the feeding mechanism of the larvae. This association allows to differentiate *Scinax* species from *Hyla* species, because of the absence of this lateral process is characteristic of *Hyla* species. Of the seventeen characters evaluated by Alcalde and Roset (2003) to differentiate *Hypsiboas raniceps* (former *Hyla raniceps* Cope, 1862), *Scinax granulatus* Peters, 1871 and *Scinax squalirostris* Lutz, 1925, they found seven that were taken from the larynx in the male adults. It has not been elucidated whether the lateral process is synapomorphic for the *Scinax* genus or just for the clade *rubra*. McDiarmid and Altig (1999) highlight the need to target and focus efforts in the direction of overcoming the limitations that currently exist about the poor knowledge we have of the larynx morphological structure.

Sound production

Although we could not demonstrate a correlation between hyolarygeal forms with the production of sound, it is important to emphasize that this correlation was found by other authors. Ryan and Drewes (1990), working on sister species of *Physalaemus pustulosus* group, found a direct relationship between the size of the cartilaginous structures of the larynx and fibers mass production of sounds; they also reported the ontogenetic and sexual dimorphism patterns among the species examined. Selection forces allowing calls depend largely on the understanding of the morphological structure changes of one species to another, the relation to production of sounds, and behavior influence of the females in the choice of partners. Boul and Ryan (2004) related the morphology and physiology of *Physalaemus petersi* suggesting that the evolution of complex calls might have an associated underlying morphology.

Gridi-Papp (2014), inducing calls artificially in his laboratory using larynx structures of arboreal species, found high frequencies compared with those in the nature, concluding that the calls are not only limited to the air pass through the larynx. We can deduce that these calls may be influenced by other factors such as temperature. Several mating calls especially in hylids have been studied, but the relationship with the morphological structures has been poorly inquired. Unfortunately, the lack of records of vocalizations of *S. ruber*, *S. kennedyi* and *S. wandae* did not allow us to determine whether these structures of the larynx are related to the production of sound.

From the point of view of frog physiology, Ryan (2001) compared S*cinax ruber* with *S. boulengeri* finding that *S. ruber* has energy expenditure greater than *S.*

boulengeri. He discovered that it also implies a higher percentage of external musculature, which can be related to the skeletal structures. Ryan (2001) also described behavioral and physiological differences between *Scinax squalirostris* and *S. boulengeri,* finding that the first one has more glycogen and lipids storage in the trunk muscles running faster than the second one. Glycogen spent by *Scinax ruber* and *S. boulengeri* is also related to the rate of calling, because of a large accumulation of air in the larynx in short periods of time implies greater energy expenditure (Ryan, 2001). Future research relating anatomy, physiology, and ethology would allow learning more about these species.

Differentiation between males and females

Males in anurans have larger larynges than females producing calls in different social contexts of frogs, which can be affected by ambient sounds of the natural habitat (Preininger et al., 2016). The larynx size is maybe also related to both the body size and sex (Wells, 1977) and to the metabolism, so that the sizes can be influenced by the utilization of energy to produce calls (McClelland et al., 1996). Although we did not have enough specimens to understand the cause that male sizes of the larynx of *S. wandae* and *S. kennedyi* are smaller than this in *S. ruber*, it is possible that the main cause is the own size of each species. Faivovich (2002) showed the hyoid and laryngeal apparatus measures of *Scinax fuscomarginata*, *S. perereca* and *S. littoralis*, stressing the fact that *Scinax ruber* has the largest larynx of the genus.

Considering that we found differences in the size and shape of the cricoid and arytenoid cartilages between males and females, it is necessary to know the development of these structures, because it is controlled with androgens that maybe produce changes of anatomic dimorphisms in the larynx (Boyd et al., 1999). We believed that these anatomical structures could help to infer relationships among species. For example, Chacko (1965) studied the larynx in *Hoplobatrachus tigerinus* (former *Rana tigrina*) and *Duttaphrynus melanostictus* (former *Bufo melanostictus*), finding a big structure (arytenoid) in *B. melanostictus* that distinguish it from of other species of the genus of Bufonidae. On the other hand, it is possible that if there was a unique biogeographical origin, we would understand whether phylogeny, morphology, and ethology of these species are related.

Sizes in Hylidae

The structures of the hyoid apparatus and the larynx are difficult to access when the organism cannot be dissected, as in the case of collection museum samples submerged in glycerol. However, it was possible for us to access these structures, and we were able to make drawings for comparisons. In the case of 21 species of the CAS, it was possible to obtain information from 14 species of the Hylidae family that had not previously been reported and neither been described, as shown in Figure 3.

In reviewing the descriptions of larynges of hylids of several authors, we note that many studies do not provide a scale bar (Blume, 1930; Trewavas, 1933). This limits our comparisons from previous studies, preventing us to do some statistical analysis of sizes and proportions. Trewavas (1933) mentioned that Blume (1930) showed that the arytenoid of the larynx in males of *Hyla arborea*, *H. faber*, *H. pickeringii*, *Chorophilus feriarum* and *Phyllomedusa moschata* is very large, but did not show measurements for precise comparison.

Our observations showed that *Scinax ruber* has the largest arytenoid among the *Scinax* species observed, including those specimens reviewed at the CAS and those species reported by other authors (Parker, 1881; Wilder, 1896; Blume, 1930; Trewavas, 1933; Duellman and Trueb, 1966; Schneider, 1970; Eichelberg and Schneider, 1974; Haas, 1999; Kaplan, 1999; Faivovich, 2002; Maglia et al., 2007; Fears, 2010; Havens, 2010; Shearman and Maglia, 2015); (See Appendix 1).

There are great differences in the form and size of the cartilages among species. For example: males of *Gastrotheca riobambae* have bigger sizes of the cricoid than *Scinax ruber*, and the size of the arytenoid in males of *Acris gryllus* (Acridinae) is similar to the size found in *Scinax ruber* (Figure 3). *Diaglena spatulata* has the biggest hyoid, but at the time of visualizing both the arytenoid and cricoid in our species of *Scinax*, we could not compare these structures because they were not present or not stained. The same happened in the case of *Hyla meridionalis* which leads to a loss of information when the specimens of these species were observed. Although arytenoid size is closely related to body size and age, the relationship between the total size of the hyoid and that of the larynx has not drastic changes in the size in hylids, leading to the conclusion that the larynx size found in *Scinax ruber* is not proportional to size body and is very large with respect to other hylid species.

Species of Pelodryadidae and Phyllomedusidae show the smallest cricoid and arytenoid, compared with other species of the Hylidae, namely with *Scinax ruber* and *Gastrotheca riobambae* (Hemiphractidae). There are other characters differentiating the larynx of *Scinax ruber* from that of other hylids: *G. riobambae* has a group of foramina in the cricoid; Acridinae species (Hylidae) have an oval larynx, *Smilisca dentata, Diaglena spatulata, Hyla* meridionalis and *Plectrohyla glandulosa* (Hylinae) have an ossification between the postero medial processes of hyoid, making a continuous leaf of the hyoid plate, finally *Trachycephalus typhonius* and *Osteocephalus taurinus* (Lophyhylinae) have a different form of larynx.

We could differentiate among the species of the genus *Dryophytes, Pseudacris, Litoria,* and *Agalychnis* by the presence/absence and form of processes in the hyoid. *Pseudacris crucifer, Pseudacris regilla* and *Hyla meridionalis* have the longest hyoid postero medial processes. *Litoria aurea* is one of the species getting an extended cartilage at the ends of the alary process of the hyoid. *Trachycephalus typhonius, Hyla meridionalis, Plectrohyla glandulosa, Pseudaris crucifer, Litoria peronii* and *Agalychnis dacnicolor* do not have an alary process or an anteromedial process of the alary process of the hyoid; this character can distinguish them from other Hylid species.

Besides that it is clear that the larynx and hyoid apparatus, and their associated muscles, have a huge influence in anuran advertisement calls (Hauser, 1996; Fabrezi *et al*., 2016), they would help to recognize species in adult stages. The comparisons of the hyolaryngeal elements will help to propose phylogenetic relationships between divergent taxa (Mangiamele et al., 2016).

Conclusions

All these comparisons show that the existence of morpho-ecological information integrated on the larynx would provide data on taxonomic characters that distinguish one species from another when these are cryptic or sinmorphic, or their names have been presented as synonyms. The anatomical structure of the larynx can contribute to distinguish species with taxonomic problems and one species from another too. This kind of studies offers evidence that it can be useful to resolve interspecific relationships within *Scinax* to avoid confusions among hylids. Finally, we can conclude that the integration of morphology of structures like these along with molecular data will provide new insights on Hylid taxonomy and systematics.

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Table 1(on next page)

TABLE 1

Specimens used in this study

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Specie	**Sex**	**ID museum**	**Location**	**Altitude (m)**	**Geographic coordinates**
Scinax ruber	Male	MUJ 4545	Casanare Department Aguazul Municipality	300	5º9’376’’ N 72º34’58.8’’
		MUJ 6018	Meta Department San Martín Municipality	470	3º39’14’’ N - 73º27’1.1’’ W
		MUJ 9037	Caldas Department La Dorada Municipality	490	5º22’2.9’’ N 74º47’36.7’’ W
	Female	MUJ 4053	Meta Department Villavicencio Municipality	1125	4º8’14’’ N - 73º40’31’’ W
		MUJ 4188	Huila Department Neiva Municipality	780	2º 57' 14" N - 75º12’37’’ W
Scinax wandae	Male	MUJ 2564	Meta Department Puerto López Municipality	155	4º18’31.9’’ N 72º4’47’’ W
	Female	MUJ 2562			
Scinax kennedyi	Male	MUJ 7782	Meta Department Puerto López Municipality	180	4º16’56.4’’ N 72º32’18.5’’ W
		MUJ 7784			

MUJ = Museum of Natural History “Lorenzo Uribe, S.J.” of Pontificia Universidad Javeriana

Figure 1

FIGURE 1. Hyoid and larynx apparatus of *Scinax ruber* (MUJ 4545, male)

A. Ventral view. B. Lateral view: Ar, arytenoid; ara, arytenoid process; cpc, cardiac process; pv, pulvinar vocale; vc, vocal cord; Cr, cricoid; bpc, bronquial process; cb, cartilage basalis; epc, esophageal process; Hd, hyoid; amap, antero medial process of alary process; aph, alary process; hs, hyoglossal sinus; hy, hyale; maph, medial branch of anterior process; plph, postero lateral process; pmph, postero medial process. Scale bar = 1 mm. Red, ossified; blue, chondrificated

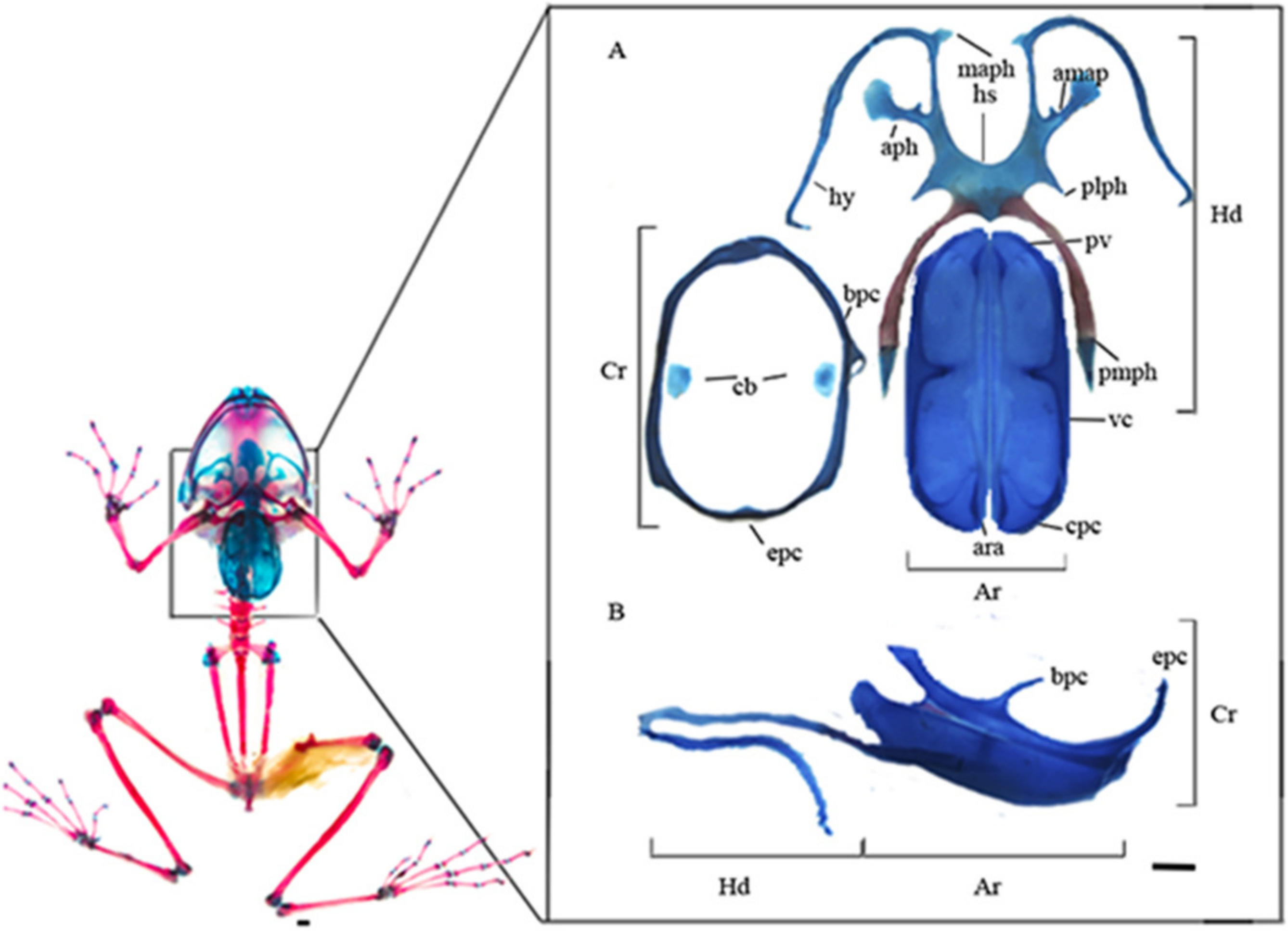

Figure 2

FIGURE 2. Hyoid and laryngeal apparatuses of species of *Scinax*

A. Ventral view, B. Frontal view, C. Lateral view. Males of *Scinax ruber* (MUJ 9037), *S. wandae* (MUJ 2564), *S. kennedyi* (MUJ 7782). Females of *Scinax ruber* (MUJ 4188), *S. wandae* (MUJ 2562). bpc, bronquial process of cricoid; epc, esophageal process of cricoid; adp, aretynoid dorsomedial process. Scale bars = 1 mm. Red, ossified; blue, chondrificated.

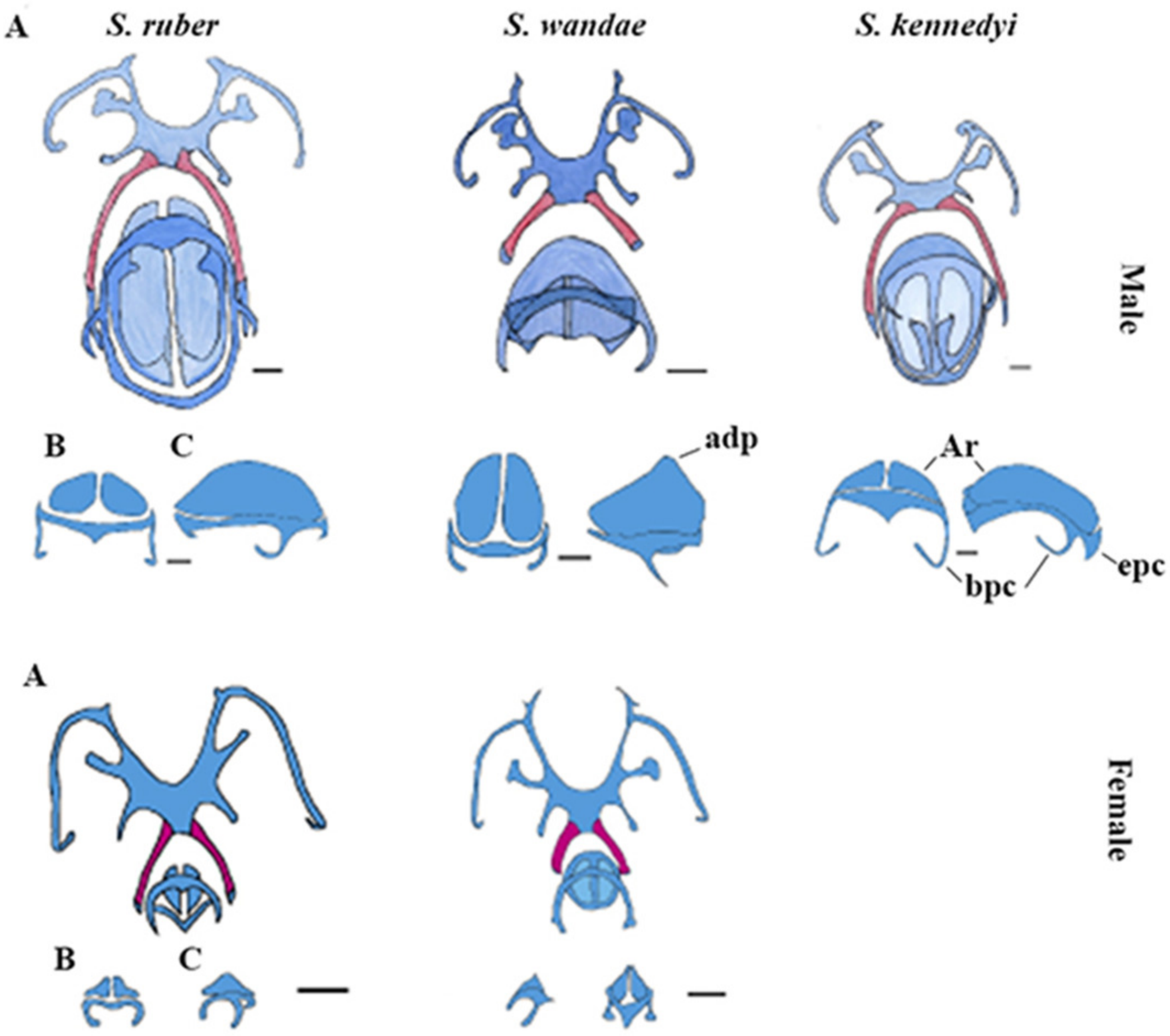

Figure 3

FIGURE 3. Comparison of hyoid and larynx of some species (ventral view) with *Scinax ruber* Material of California Academy of Sciences used

Acris gryllus (CAS 63520 male); *Agalychnis callidryas* (CAS 141854 male, CAS 142163 male); *Agalychnis dacnicolor* (CAS 15574 male); *Diaglena spatulata* (CAS 142491 male); *Dryopsophus gilleni* (CAS 121272 male); *Dryophytes arenicolor* (CAS 10547 male); *Dryophytes japonicus* (CAS 135836 male); *Gastrotheca riobambae* (CAS 93885 female, CAS 152048 male); *Hyla meridionalis* (CAS 138799 male); *Litoria arfakiana* (CAS 107541 male); *Litoria aurea* (CAS 159026 male); *Litoria modica* (CAS 106280 male); *Litoria peronii* (CAS 83209 male); *Osteocephalus taurinus* (CAS 12353); *Plectrohyla glandulosa* (CAS 122617 male); *Pseudacris crucifer* (CAS 09996 male); *Pseudacris regilla* (CAS 93959 male, CAS 93960 male, CAS 93963 male, CAS 156577 male, CAS 156578 male); *Pseudacris triseriata* (CAS18400 male); *Smilisca dentata* (CAS 135055 male, CAS 135067 male); *Tialocohyla smithii* (CAS 140910 male, CAS 140912 male); *Trachycephalus typhonius* (CAS 12306 male). Red, ossified; blue, chondrificated.

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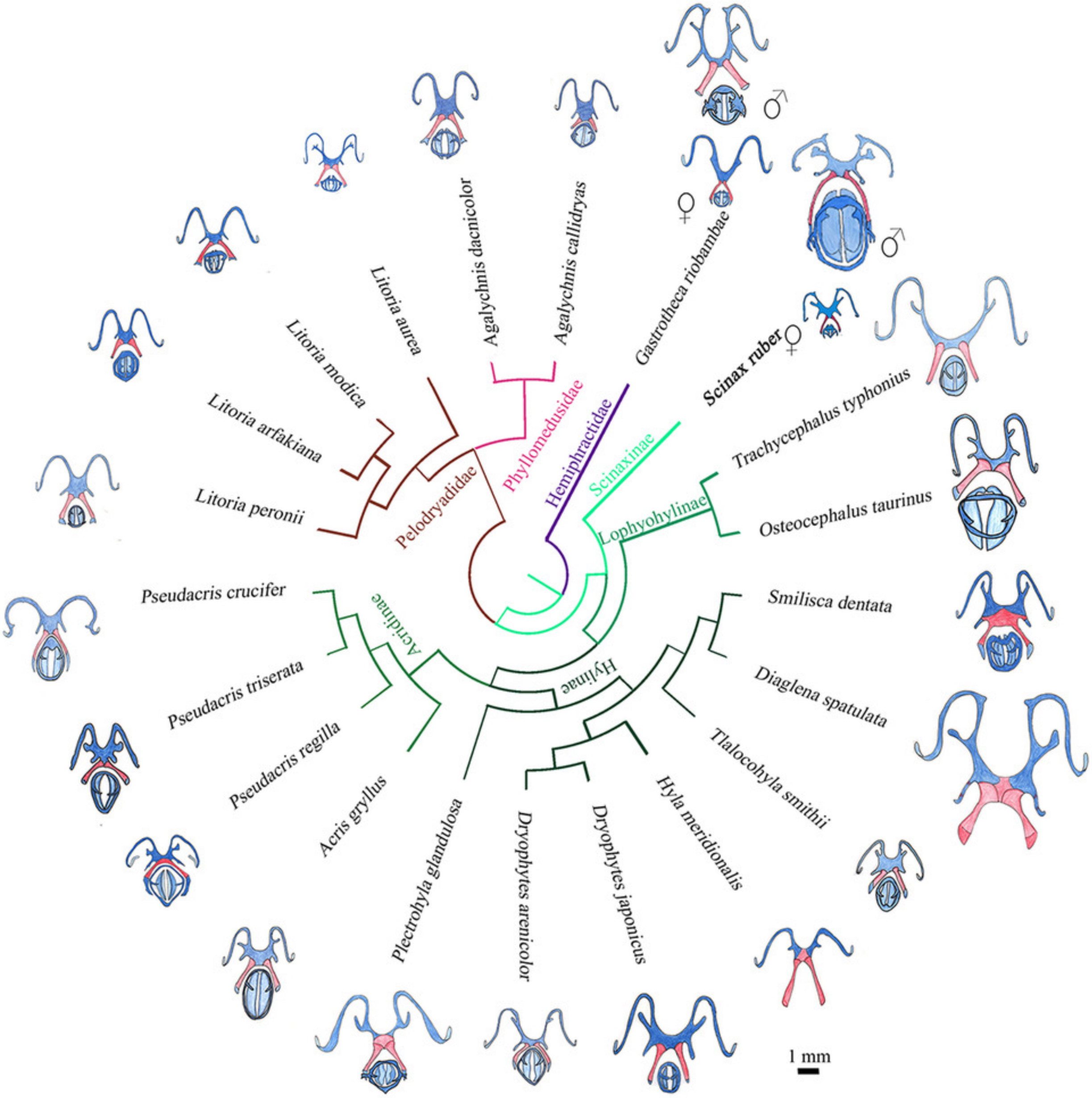
Litoria aurea
Agalychnis dacnicolor
Agalychnis callidryas
Gastrotheca riobambae
♂
♀
♂
Scinax ruber
♀
Litoria modica
Litoria arfakiana
Litoria peronii
Pelodryadidae
Phyllomedusidae
Hemiphractidae
Scinaxinae
Lophyohylinae
Trachycephalus typhonius
Osteocephalus taurinus
Pseudacris crucifer
Acridinae
Hylinae
Smilisca dentata
Diaglena spatulata
Pseudacris triserata
Pseudacris regilla
Acris gryllus
Plectrohyla glandulosa
Dryophytes arenicolor
Dryophytes japonicus
Hyla meridionalis
Tlalocohyla smithii
1 mm