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Ontogeny of Sexual Dimorphism in the Larynx of the Túngara Frog, *Physalaemus pustulosus*

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In the majority of frogs, males but not females produce vocalizations to attract mates. Sexual selection can influence the evolution of these vocalizations by modifying the frog's morphology. The larynx is the main organ responsible for sound production; thus, it constitutes a target of selection. The goal of this study was to determine qualitatively and quantitatively the sexual differences in the developmental sequence of larynges of *Physalaemus pustulosus (=Engystomops pustulosus).* We examined larynges of individuals ranging from recent metamorphs to adults. We provide quantitative measurements of laryngeal area and volume, vocal cords, and fibrous mass 1 (FM1), as well as qualitative descriptions of the developmental origin of vocal cords and FM1. Larynges of small male and female juveniles (less than 16 mm SVL) are similar, both externally and internally. The area of these larynges is no more than 1 mm²; no vocal cords or FM1 are present in either sex at this developmental stage. In individuals larger than 16 mm SVL, there is a marked divergence in relative size of the larynx between the sexes. The male larynx begins to exhibit a strong positive allometry until it reaches a plateau at 24 mm SVL. Also, at 16 mm SVL, the vocal cords and FM1 appear as distinctive outgrowths on the wall of the arytenoid cartilage in male larynges. Female growth rate of the larynx, however, is constant and exhibits negative allometry throughout ontogeny. Female vocal cords and FM1 are barely distinguishable as adults, and never attain the size and most likely neither the mobility as in the adult male larynx.

SEXUAL selection is responsible for the evolution of secondary sexual traits, which can result in extreme dimorphism between the sexes (Darwin, 1871). Many of these traits involve sexual signals, such as vocalizations, that serve to attract individuals of the opposite sex. Sexual dimorphism in acoustic signals has been reported in numerous animals, and the organs responsible for sound production have been well described in organisms such as arthropods (Ewing, 1989), fish (Courtenay, 1971; Fine, 1975; Bass and Clark, 2003), frogs (e.g., Trewavas, 1932; McClelland and Wilczynski, 1989; Ryan and Drewes, 1990; Boyd et al., 1999), birds (Greenewalt, 1968; Ames, 1971; Suthers, 2010), and mammals (e.g., Kahane, 1978; Lieberman, 1986; Fitch and Hauser, 1995; Frey and Riede, 2003; Riede and Titze, 2008).

Male and female frogs are able to produce calls (i.e., ''the sound resulting from a single expiratory cycle and involving a single contraction of the sides while nostrils are closed'', Schmidt, 1965:144). However, conspicuous advertisement calls are mostly restricted to males. Males produce advertisement calls to attract females to breeding sites, and females choose mates based mostly on the call features (Blair, 1958; Ryan and Rand, 2001; Gerhardt and Huber, 2002).

The larynx is the main organ responsible for sound production in anurans. The skeleton of a typical frog larynx consists of a pair of arytenoid cartilages supported by a ring of cartilage, the cricoid cartilage (Schneider, 1988; Fig. 1A–C). Most frog species produce calls by a force-pump expiratory mechanism, which consists of a system with three chambers: lungs, buccal cavity, and vocal sac, and three valves: nostrils, larynx, and vocal slits (Martin and Gans, 1972; Duellman and Trueb, 1986). To vocalize, frogs pump air into the lungs via the nostrils. As a result, pulmonary air pressure increases. Then the nostrils and mouth are closed, and pulmonary air pressure and/or contraction of body wall muscles pump the air through the larynx into the buccal cavity. At this point, the arytenoid cartilages of the larynx separate and open the glottis, allowing the airflow through the larynx. The air passing through larynx causes the vibration of vocal cords (strings of connective tissue; Duellman and Trueb, 1986) and arytenoid cartilages. The vibrations produced in the vocal cords and arytenoids are transmitted through the buccal cavity to the vocal sac (via the vocal slits). The vocal sac radiates and amplifies the sound (Martin and Gans, 1972; Duellman and Trueb, 1986; Schneider, 1988; Wells, 2007).

Some of the earliest detailed morphological descriptions of the frog larynx are in the genus *Xenopus* (e.g., Ridewood, 1898). *Xenopus* are fully aquatic and their calls are produced independent of the respiratory system (Yager, 1982, 1992; Yamaguchi et al., 2010). Click-like calls are produced by an implosion mechanism, which is the result of the rapid separation of a pair of hard cartilaginous disks (apparently part of the arytenoid cartilages) located at the anterior part of the larynx (Yager, 1982; Sassoon and Kelley, 1986). Not surprisingly, the most detailed developmental studies of the laryngeal apparatus are in this genus. Sexual differentiation in the larynges of *Xenopus laevis* occurs during the first six months after metamorphosis and consists of masculinization of male larynges (Sassoon and Kelley, 1986; Tobias et al., 1991a, 1991b). Even though *Xenopus* is one of the most well-studied anurans, they are far from typical frogs (e.g., *Xenopus* lack vocal cords, in Yager, 1992; see also Cannatella and Trueb, 1988), and thus differ from the standard model of sound production in most other frogs.

Given the lack of studies regarding the morphological development of the larynx in typical frogs, we investigated a model that represents most frog species. We study *Physalaemus pustulosus (=Engystomops pustulosus),* commonly known as the túngara frog, which is already a well-studied system for acoustic communication (Ryan, 1980, 1985, 2010). Thus, it provides a unique opportunity to relate the development of the larynx as a secondary sexual character with other aspects of this species. Our goal is to provide a fundamental

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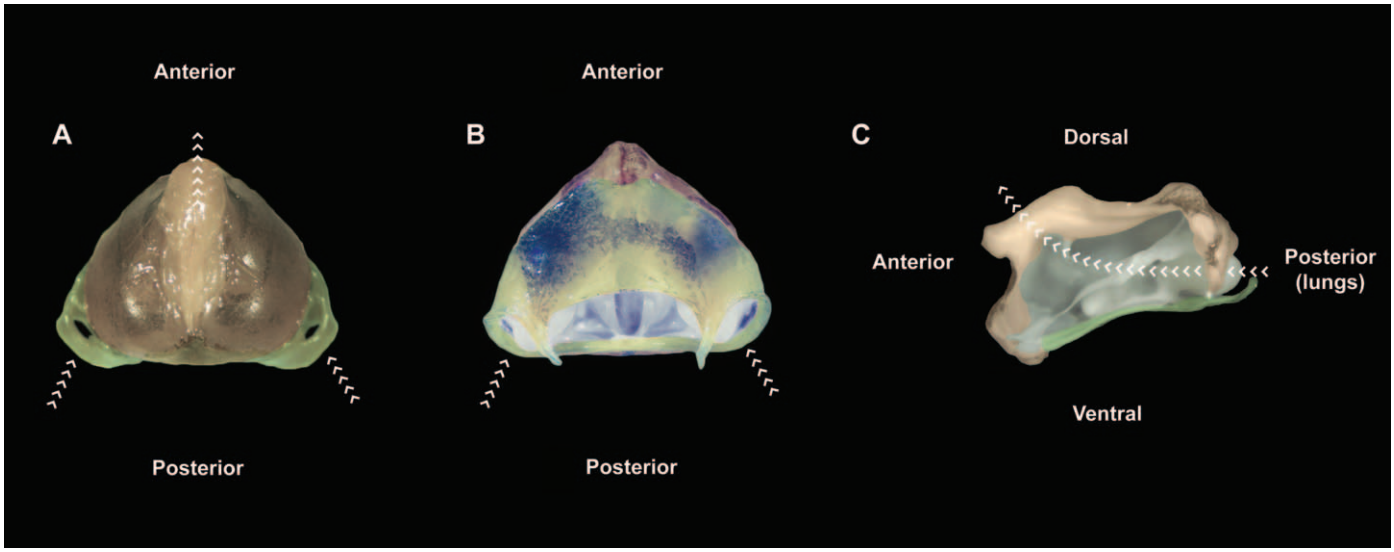

Fig. 1. Photographs of the larynx of an adult male of *Physalaemus pustulosus:* (A) dorsal, (B) ventral, and (C) mid-sagittal view. Arytenoid cartilages are noted in orange and cricoid cartilage in green. Arrows represent the direction of airflow.

understanding of the developmental changes of the laryngeal morphology in a frog species with a common mechanism of sound production, and describe the sexual differences in the ontogeny of the larynx. We determine, qualitatively and quantitatively, the sexual differences in the developmental sequence of larynges of *P. pustulosus* by examining the larynges of male and female frogs ranging from recently metamorphosed individuals to adults. We provide quantitative measurements of laryngeal area and volume, vocal cords, and fibrous mass 1 (FM1), and qualitative descriptions of the development of vocal cords and FM1. We discuss the importance of the laryngeal morphological changes in relation to physiological, behavioral, and reproductive traits of the frog.

MATERIALS AND METHODS

Study system.—The túngara frog is a terrestrial Neotropical frog that has been the subject of numerous studies in sexual selection and communication (Ryan, 1980, 1985, 2010). A typical male advertisement call consists of a downward frequency sweep of about 300 milliseconds (ms) called the ''whine.'' Males usually produce only whines (simple calls) when calling alone, as the whine is sufficient to attract females. Males can also facultatively add one to seven short (ca. 35 ms) higher frequency suffixes or ''chucks'' at the end of the whine, forming a complex call, and they usually do so when competing acoustically with other males. Females prefer complex calls to simple calls, and calls with more chucks to fewer chucks (Rand and Ryan, 1981; Ryan, 1985; Akre et al., 2011). Comparative studies of the calls and larynges of *P. pustulosus* and some of its close relatives suggest that the whine is produced by the vibration of the vocal cords, whereas the chuck depends on the vibration of a pair of structures called fibrous masses 1 (FM1s; Drewry et al., 1982; Ryan and Drewes, 1990). Each FM1 (most apparent in adult male frogs) is an accretion of fibrous materials (Ryan and Drewes, 1990), probably connective tissue, connected to the arytenoid cartilage and vocal cord, and located in one of the bronchial passages of the larynx (Fig. 2C–E). The bronchial passages are openings formed by the posterior end of the cricoid ring and the bronchial processes of the cricoid, and serve as a point of attachment for the lungs to the larynx. Gridi-Papp et al. (2006) experimentally demonstrated that when the FM1 is surgically ablated, males are unable to produce the chucks. Also, another smaller fibrous mass, called fibrous mass 2 (FM2), is visible between the arytenoid wall and the vocal cord in adult males. However, the role of FM2 during call production is unclear (Drewry et al., 1982; Ryan and Drewes, 1990).

Túngara frogs reach metamorphosis about three weeks after hatching, at a size of approximately 12 mm snout–vent length (SVL). Males normally acquire the physiological ability to reproduce at one-half to two-thirds of the adult body size (Davidson and Hough, 1969), corresponding to a size of 15–20 mm SVL (mean adult body size is 30 mm SVL; Ryan, 1985). As with most anurans, males continue to grow after reaching sexual maturity. In fact, the smallest males found calling in the field are about 24 mm SVL (Ryan, 1985).

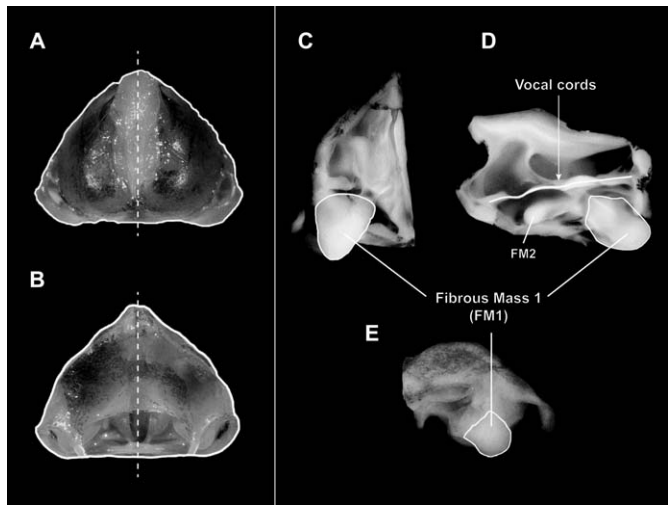

Fig. 2. Photographs of the larynx of an adult male of *Physalaemus pustulosus*. White lines show the measurements taken in this study: (A) dorsal area, (B) ventral area, (C) FM1 ventral area, (D) FM1 lateral area and vocal cords length, and (E) FM1 posterior area. A and B show the entire larynx and the dashed line in each corresponds to the mid-saggital cut. B, C, and D are half larynges after the mid-saggital cut.

Ryan and Drewes (1990) showed that adult males possess substantially larger larynges than conspecific adult females, and FM1s are absent in adult females. The only data available on development of the larynx come from the same study and consists of a sample of three juvenile males. Ryan and Drewes (1990) were first able to detect the presence of FM1s in a 13.4 mm male. FM1s were visible at intermediate size in a 16.8 mm male, and completely developed in an 18.7 mm male.

Dissections and measurements.—We obtained 31 male and female túngara frogs of different sizes from a breeding colony maintained at the University of Texas at Austin, and one additional female (Texas Natural History Collection, TNHC 24071) from the Herpetology Collection of the Texas Natural Science Center. All frogs (including the museum specimen) originated from central Panama. The colony frogs were euthanized with 20% benzocaine, fixed in 10% formalin, and preserved in 70% ethanol. We dissected and examined 14 females (12.6–28.0 mm SVL) and 18 males (12.1–28.7 mm SVL). The sex of the individuals was determined by the presence/absence of vocal sacs (adult and subadult individuals), gonad differentiation (all individuals), or gamete differentiation (juveniles). To examine the larynges, we followed the general procedure used by Boul and Ryan (2004). We removed the lower mandible of the frog and gently excised the hyolaryngeal apparatus. All connective tissue and muscles surrounding the larynx were removed to expose the cricoid cartilage, arytenoid cartilage, and bronchial passages. We took digital photographs of different parts of the larynx using a SPOT microscopy camera (2.2.1) attached to an Olympus SZH microscope with a scale of 0.2 or 0.3 mm. To maximize the depth of field, we photographed each section at different focal planes and combined the photos in Adobe Photoshop CS5.1. All area measurements were performed in ImageJ (Rasband, 1997–2012).

We measured the dorsal area (Fig. 2A), ventral area (Fig. 2B), and volume of the entire larynx, and used R software (R Development Core Team, 2012) to find the best fitting curve for growth rate in each case. Also, we made a mid-sagittal section to record the presence/absence of FM1s and vocal cords. If FM1s were present, the total area of one of them was estimated by summing three area measurements (from ventral, posterior, and mid-sagittal views; Fig. 2C–E). We recorded the presence/absence of vocal cords, described the attachment of one of them to the inner wall of the arytenoid, and measured its length (Fig. 2D). Measurements of FM1 and vocal cord were made on the right side of the larynx, except in specimen TNHC 85848, in which the right side was damaged; thus, measurements of its FM1 correspond to the left side. The number of individuals used for each measurement varies because very small larynges (females and juvenile males) quickly desiccate (in a couple of minutes) under the microscope lights. If the larynx itself and/or its corresponding structure (e.g., vocal cords) were not intact, we did not use them for the measurements.

Volumes of larynges were measured by water displacement according to Archimedes Principle. We used an Eppendorf tube (0.6 mL) or a fine pipette tip (sealed at one side end), depending on the size of the larynx, as volumetric containers. For the larger larynges, we filled and marked an Eppendorf tube with 200 μL of water. We removed the larynx that had been in ethanol and placed it in water for several minutes, we soaked up any excess water from the larynx and then immediately submerged it inside the tube. The quantity of volume displaced was recorded by carefully removing the displaced liquid using a 10 μL micropipette. For smaller larynges, we used a sealed pipette tip filled with 10 μL of water instead of the Eppendorf tube, and the same procedure was performed. Two naive observers conducted the procedures independently, producing two datasets. The final volume corresponded to the average of both datasets. The deviation between observers was calculated by transforming the difference between them into a percentage. The percentage of inter-observer deviation for small larynges (those with volumes between 0.5 and 1.5 mm^3) was 25.7%, and the percentage of deviation for medium and large larynges (those with volumes of 6 mm^3 and more) was 7.8%.

RESULTS

Larynges of small juvenile males and females (12–16 mm SVL) are similar both externally and internally (Fig. 3A, D; Appendix 1). In these individuals, the larynx is a small structure with an area of no more than 1 mm^2, with no vocal cords or FM1s present in either sex. At 16 mm SVL there is a rapid and dramatic divergence between the sexes in the scaling of the larynx to body size. The male larynx begins to exhibit strong positive allometry until reaching a plateau at 24 mm SVL. The female larynx, however, maintains the growth rate of small juveniles, which results in a negative allometric growth throughout its ontogeny (Fig. 4A–C). Overall, the female larynx increased in area by approximately 4-fold from 12.6 to 28.0 mm SVL, whereas the male larynx increased approximately 24-fold across a similar body size range (Figs. 3, 4).

Developmental origin of vocal cords.—The drastic increase in male larynx size at 16 mm SVL also coincided with the formation of vocal cords. At this point in development the vocal cords begin to appear as distinctive outgrowths on the medial side of the wall of each arytenoid cartilage. By the time males reach 19.5 mm SVL, the outgrowths (one on each side) separate from the arytenoid cartilage, and the vocal cords become distinguishable (Fig. 3E). Each vocal cord has a completely free medial edge; its posterior part is thick and dense, and clearly attached to the wall of the arytenoid cartilage, whereas the anterior part is thinner and looser with a slight thickening visible at the medial free edge. A very thin membrane attaches the anterior part of the vocal cords to the arytenoid cartilage. At 24 mm SVL the male vocal cords are well defined, as the membrane that attaches the vocal cords to the arytenoid cartilage becomes even thinner in the anterior part (Fig. 3F). In females, a visible outgrowth of vocal cords appears on the wall of the arytenoid cartilage at 20 mm SVL. Overall, the female larynx does not exhibit major changes during its ontogeny (Fig. 3 A–C). In contrast to males, the attachment of the vocal cords to the wall of the arytenoid cartilage remains very thick, even in adult females. As a result, juvenile males greater than 19.5 mm SVL and adult males possess conspicuous and very movable (to the touch) vocal cords, whereas females do not (Appendix 1).

Developmental origin of fibrous mass.—Each FM1 is first visible as a vertically oriented outgrowth (if viewed posteriorly) on the arytenoid cartilage in a 16.0 mm juvenile male. The FM1 originates from the wall of the arytenoid cartilage; its position is more lateral and posterior than the vocal cords.

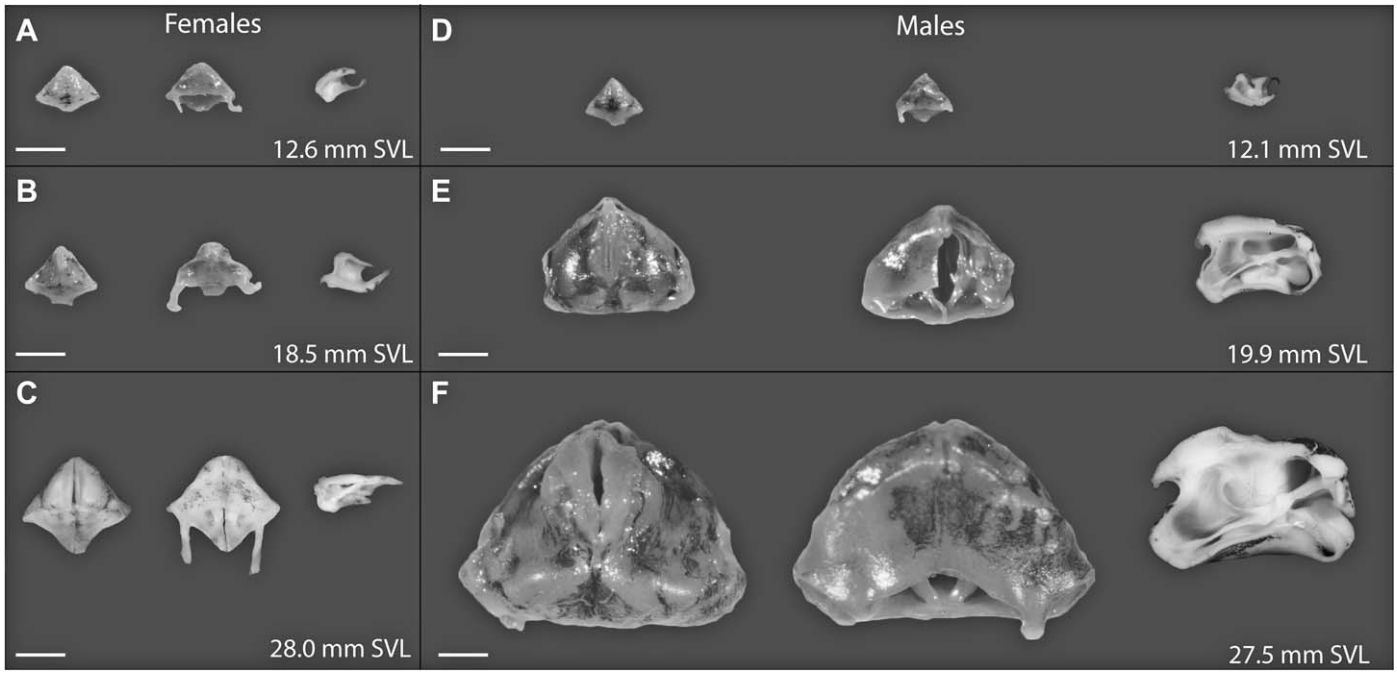

Fig. 3. Dorsal, ventral, and mid-sagittal view of a female (A–C) and male (E–F) larynx at different developmental sizes. Scale bars = 1 mm.

In a 19.5 mm male, FM1 is clearly visible as a small round mass attached to the arytenoid and at this time it is also connected to the posterior part of the vocal cord (Fig. 3E). In adult males, the membranous connections of FM1 to the wall of the arytenoid cartilage and vocal cord are still present. These connections, however, are thinner, and seem to provide more degrees of freedom of movement to the FM1. At this point in development, each FM1 is suspended in one of the bronchial passages, obstructing much of the airflow that passes from the lungs to the larynx through this passage (see also Drewry et al., 1982; Boul and Ryan, 2004).

Overall, our observations suggest the FM1s begin to form when males attain a body size of 16 mm SVL, and are fully developed and connected to the vocal cords (visible as two round masses) when males reach 19 mm SVL. These observations suggest that the initiation of FM1s growth coincides with the onset of sexual size dimorphism in the larynx (Fig. 4). Additionally, FM1 measurements also show an ontogenetic pattern similar to the larynx itself. In females, FM1s were visible as very small masses only in the largest female (28.0 mm); smaller females never develop visible signs of the FM1s or outgrowths similar to those of males (Appendix 1).

DISCUSSION

Ryan and Drewes (1990) described the sexual dimorphism in the larynx of adults of *Physalaemus pustulosus*, and found that female larynges are much smaller than those of males. However, the ontogeny of sexual differentiation had not been investigated. Here we have described the ontogenetic changes in male and female larynges and found that sexual differentiation begins in juveniles at 16 mm SVL. At this size, males undergo a substantial increase in larynx growth rate and FM1s and vocal cords begin to form. The larynges of females barely increase in size, and never develop conspicuous vocal cords or FM1s. Also, vocal cords and FM1s of adult females do not possess the mobility that those structures attain in adult male larynges.

Sexual selection can lead to extreme positive allometry in male morphological traits (Green, 1992; Petrie, 1992; Kodric-Brown et al., 2006). In *P. pustulosus*, there is positive allometric growth in male larynx size (Fig. 4) and the structures related to the production of advertisement calls: vocal cords (involved in the production of the whine) and FM1s (involved in the production of the chuck). This differentiation is not surprising as the larynx is a secondary sexual trait under strong sexual selection. Such allometry has also been reported in other frogs. In *Hyla arborea,* female larynx size is one-third to one-half the size of the male larynx (laryngeal height: Schneider, 1970), half the size in *Rana catesbeiana* (laryngeal length and width: Boyd et al., 1999), and about one-third the size in *Rana pipiens* (volume: McClelland and Wilczynski, 1989). In the túngara frog, if we consider only the length of the arytenoid, the male larynx is two to three times larger than the adult female larynx. However, if laryngeal area and volume are taken into account, the male larynx area is seven to eight times greater than the female larynx, and the male larynx volume is 15–30 times greater than the female larynx.

Not only is the larynx of adult females smaller, but also the critical components of sound production (vocal cords and FM1s; Appendix 1) are markedly under-developed, and resemble a larynx of 16 mm SVL juvenile males. Given these observations, we suggest that the vocal cords of only the largest female (28.0 mm) may be able to vibrate. Schmidt (1972) suggested that females produce more attenuated release calls because their vocal cords may be less flexible. We think that the vocal cords in females of *P. pustulosus* may not have much mobility due to their thick attachment to the wall of the arytenoid cartilage. Interestingly, the smallest female found amplexed in the field, from a sample of 261 females during 2005 in central Panama, was 27.0 mm SVL (MJ Ryan, unpubl. data). Thus the slight change in attachment of vocal cords (which may be related to the ability of females to produce release calls) in adult female larynges seems to coincide with the onset of reproduction.

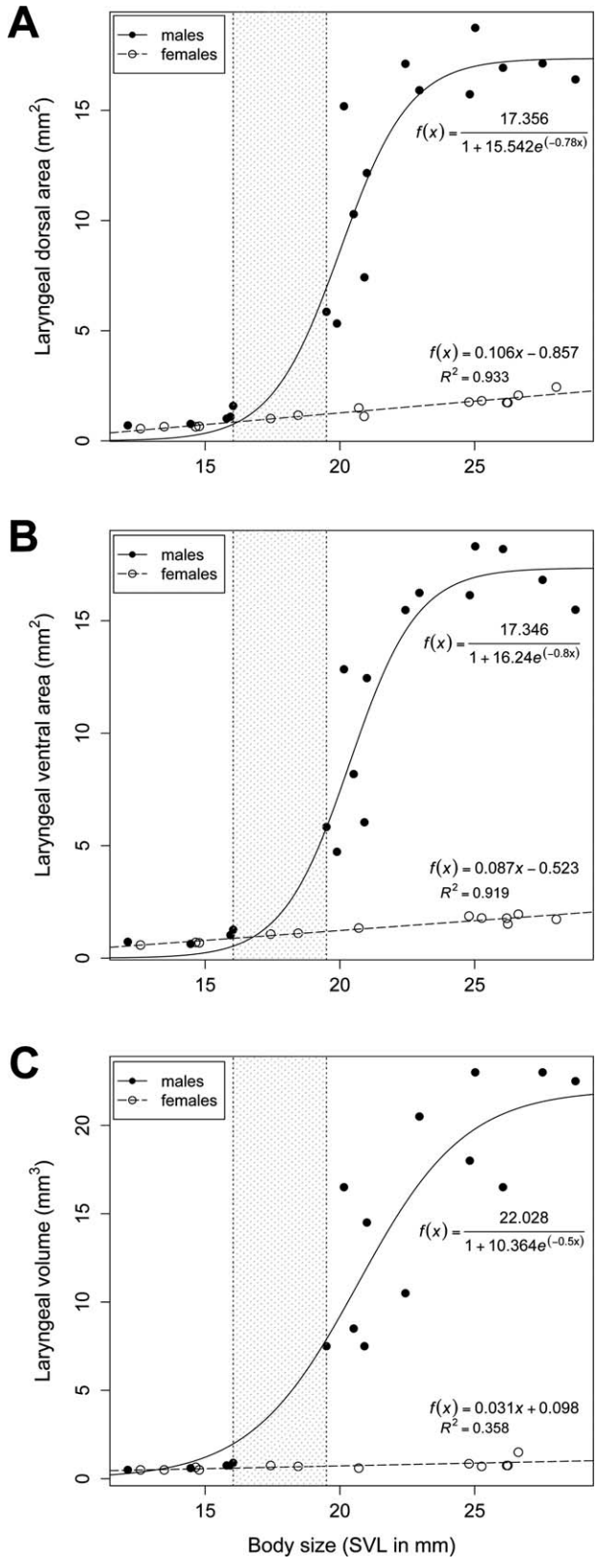

Fig. 4. Larynx growth relative to body size in *Physalaemus pustulosus:* (A) laryngeal dorsal area, (B) laryngeal ventral area, and (C) laryngeal volume. Shaded area corresponds to the time window in which strong positive allometry begins in males.

Morphological differentiation during ontogeny may be coupled with behavioral and physiological changes. In this study, the size of the larynx (area and volume) seems to stabilize in growth in individuals larger than 22.4 mm SVL. It is possible that at this size males have developed a larynx capable of producing calls. Interestingly, the smallest males found calling in the field (Central Panama) are approximately 24 mm SVL (Ryan, 1985). Thus, it seems that males initiate calling behavior just after the vocal apparatus is fully developed. Another indication that morphological changes are coupled with behavioral changes is evident in the study performed by Baugh and Ryan (2010). They conducted two-choice phonotaxis experiments with juvenile frogs, in which the frogs had the option to approach a speaker broadcasting a conspecific call or a silent speaker. Males and females significantly increase their association time with conspecifics when frogs reach 51 days postmetamorphosis, an age that corresponds to an average size of 15 mm SVL. This size is very close to the point at which morphological divergence between male and female larynges commences.

Calling behavior might be coupled with physiological changes as well. Male gonads are completely developed and males are known to acquire the physiological ability to reproduce between 15–20 mm SVL (Davidson and Hough, 1969; Ryan, 1985). Our findings show that most morphological changes that lead to sexual dimorphism in the male vocal apparatus occur in the same time window in which they acquire the ability to reproduce (i.e., 16–22 mm SVL). Some studies have suggested that the sexual differentiation of the male vocal apparatus is mediated by androgens (e.g., Boyd et al., 1999), which most likely have organizational effects as masculinizing hormones. This may also be the case for *P. pustulosus,* but further studies using androgens on frogs at body size of 16 mm SVL are necessary to test this hypothesis.

Masculinization of male larynges in *Xenopus laevis* consists of changes in the shape, tension, fiber type, and recruitment of muscles surrounding the male larynx (Sassoon and Kelley, 1986; Tobias et al., 1991a, 1991b). In *Xenopus*, female larynges increase in mass and size, but do not change in shape like those of males. In *P. pustulosus,* masculinization of the male larynx may also be the cause for the observed sexual differences. We did not measure the muscle characteristics in this study; however, we did observe that they were less developed in adult females. One major finding is the striking sexual difference in growth rate of the larynx during the development of *P. pustulosus*.

FM1 was previously described as an accretion of fibrous material connected to the wall of the arytenoid and vocal cord (Drewry et al., 1982; Ryan and Drewes, 1990), but its developmental origin was unknown. Our recent observations suggest that FM1s grow one from each of the medial walls of the arytenoid cartilages. FM1s do not develop from the vocal cords but instead their origin is independent. However, the FM1s do appear at the same time as the vocal cords during development, and each FM1 connects to its corresponding vocal cord during the development of the larynx. The independent origin of the FM1 might account for the fact that it vibrates independently of the vocal cords, allowing individuals to produce the distinctive ''chuck.''

In summary, we have provided insights into the developmental pattern of the larynx of the Neotropical frog *P. pustulosus*. We have revealed a time window in which the sexual differentiation of the larynx takes place. Our findings contribute to the general knowledge of developmental differences in sound-producing organs, which have been poorly studied. We expect the same pattern to be repeatable in other frog species.

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APPENDIX 1

Measurements performed on the larynges of males (M) and females (F) of *Physalaemus pustulosus.*

TNHC number	Sex	SVL (mm)	Dorsal area (mm^2)	Ventral area (mm^2)	Volume (mm^3)	Vocal cords	Vocal cord length (mm)	FM 1	Sum of 3 FM1 areas (ventral, lateral, and posterior)
85834	F	12.6	0.56	0.58	0.5	Absent	n/a	Absent	n/a
85835	F	13.5	0.65	–	0.50	Absent	n/a	Absent	n/a
85839	F	14.7	0.64	0.70	0.65	Absent	n/a	Absent	n/a
85856	F	14.8	0.66	0.68	0.50	Absent	n/a	Absent	n/a
85857	F	17.4	1.01	1.07	0.75	Absent	n/a	Absent	n/a
85838	F	18.5	1.16	1.11	0.70	Absent	n/a	Absent	n/a
85837	F	20.7	1.49	1.34	0.60	Wall thickened	0.63	Absent	n/a
85847	F	20.9	1.11	–	–	Wall thickened	–	Absent	n/a
85833	F	24.8	1.76	1.87	0.85	Wall thickened	0.86	Absent	n/a
85861	F	25.3	1.82	1.78	0.70	Wall thickened	0.89	Absent	n/a
85852	F	26.2	1.74	1.78	0.75	Wall thickened	0.86	Absent	n/a
85851	F	26.2	1.73	1.52	0.75	Wall thickened	0.84	Absent	n/a
85853	F	26.6	2.07	1.95	1.50	Absent	n/a	Absent	n/a
24071	F	28.0	2.44	1.73	–	Wall thickened	1.00	Small outgrowth	n/a
85840	M	12.1	0.70	0.73	0.50	Absent	n/a	Absent	n/a
85860	M	14.5	0.77	0.63	0.60	Absent	n/a	Absent	n/a
85862	M	15.8	1.01	–	0.75	Absent	n/a	Absent	n/a
85859	M	15.9	1.10	1.02	0.75	Absent	n/a	Absent	n/a
85836	M	16.0	1.59	1.26	0.90	Wall thickened	0.64	Small outgrowth	n/a
85845	M	19.5	5.86	5.83	7.50	Well defined	1.75	Present	0.65
85843	M	19.9	5.33	4.73	–	Well defined	1.80	Present	0.49
85846	M	20.2	15.19	12.84	16.50	Well defined	2.59	Present	2.33
85841	M	20.5	10.29	8.18	8.50	Well defined	2.11	Present	0.81
85842	M	20.9	7.42	6.04	7.50	Well defined	1.67	Present	0.59
85844	M	21.0	12.16	12.45	14.50	Well defined	2.48	Present	1.57
85848	M	22.4	17.11	15.48	10.50	Well defined	–	Present	1.56
85858	M	23.0	15.91	16.24	20.50	Well defined	2.92	Present	1.35
85849	M	24.8	15.73	16.14	18.00	Well defined	2.50	Present	2.20
85855	M	25.0	18.74	18.31	23.00	Well defined	3.05	Present	–
85850	M	26.1	16.93	18.19	16.50	Well defined	2.30	Present	1.87
85854	M	27.5	17.13	16.82	23.00	Well defined	3.01	Present	1.35
85832	M	28.7	16.40	15.49	22.50	Well defined	3.07	Present	1.68