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# Histological based comparative study of vocal sac skin between four selected anuran species of Rajasthan

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**Abstract**

The sound generating system is unique in anurans and exhibits an immense morphological variation. The aim of proposed study of vocalization is to study biomechanics of this phenomenon and to trace adaptations of different sound production pattern by different species in anuran and to find out any evolutionary trend. The present study investigates the comparative study of ventral and vocal sac skin between *Duttaphrynus stomaticus, Euphlyctis cyanophlyctis, Fejervarya limnocharis* and *Microhyla ornata* were chosen. This Study is one of the first studies for the purpose of displaying the morphological and histological aspects of the vocal sac properties and their relationship to particular biomechanics of vocalization in anurans and also contributes in the field of amphibian reproductive biology.

**Key-words:** Biomechanics, vocalization, ventral Skin ,Vocal Sac skin , vocalization, calling behaviour

## Introduction

The anuran vocal sac is one of the most extraordinary inflatable structures found among vertebrates. Inflation of vocal sac characterizes advertisement calling behaviour in nearly all male frogs and toads. Prior to vocalization, anurans utilize a series of buccal pumping motions to inflate the lungs (De Jongh and Gans, 1969). Gross morphology of vocal sacs, associated muscles, and myo-integumental connections has been described in several species (Noble, 1931; Liu, 1935; Tyler,1971a,b; Duellman and Trueb, 1986), but acoustic and physiological functions of the vocal sac have not been fully elucidated. The abdominal muscular contractions that move this air during phonation are metabolically expensive (Bucher *et al.*, 1982) and elastic features of the vocal sac that might reduce such metabolic costs could be energetically advantageous. Ryan (1985) suggested that elasticity of the vocal sac may be used to store strain energy during call production and contribute subsequently to lung re-inflation between calls. Such elastic storage might reduce substantially the net costs of vocalization if the kinetic energy of air used in phonation is partially recovered rather than dissipated to the ambient atmosphere. The magnitude of this effect will depend on the character and ultimate resilience of elastic fibers within the vocal sac. The morphological

and physiological characteristic of the supra-laryngeal type vocal sac has been depicted in numerous species (Duellman and Trueb, 1986) yet have not been totally illustrated. During a call, pulmonary air is forced through the larynx into the buccal cavity and the vocal sac via contractions of the muscular body wall (Martin and Gans, 1972; Taigen and Wells, 1985; Wells and Taigen, 1989). In advertisement calls of the tungara frog *Physalaemus pustulosus,* air is shuttled between the lungs and an unusually large median subgular vocal sac (Dudley and Rand, 1991).In addition to such potential energetic savings, (Dudley and Rand, 1991) suggested that elastic recoil by the vocal sac also might permit lung re-inflation and thus call-repetition rates higher than otherwise attained by use of the much slower buccal pump. Acoustic signal produced by most anurans are accompanied by inflation of a conspicuous vocal sac. Among vertebrates, vocalization is highly developed in anurans and birds (Demirsoy, 1993). Vocal sac, thumb pad, and skin are described as secondary sexual characteristics (D'Islria *et al.,* 1982; Kao *et al*., 1994). Most male amphibians produce species -specific mating calls and this attracts only con-specific mates (Girgenrath and Marsh, 1997). The vocal sac is pigmented and obvious in numerous species (Greenberg, 1942; Duellman and Trueb, 1986;Ho¨dl and Ame´zquita, 2001), and in certain species can change color during the course of courtship (Wells, 1980), proposing that it might likewise assume a vital role in visual communication. In numerous frogs, vocal advertisement is accompanied by the inflation of a visually evident vocal sac. Recent evidence indicates that a pulsating sac increases the attractiveness of advertisement calls in tungaras (Rosenthal *et al*., 2004). The serotonin detected in various components and secretions of mucus and poison glands in *R. ridibunda* skin could function in the regulation of secretion in mucus glands, while triggering secretory material in the poison glands (Sengezer-Inceli,2008). The functional relationship between the structure of the vocal sac of male *Rana ridibunda* and prolactin, androgen and thyroxin hormones study has investigated (Sengezer-Inceli *et al*., 2008).

## Material and Methods

In this study, four accidentally dead or road kills adult male frogs *Duttaphrynus stomaticus* (Family-Bufonidae), *Euphlyctis cyanophlyctis*(Family - Dicroglossidae), *Fejervarya limnocharis*(Family – Dicroglossidae) and *Microhyla ornata*(Family - Microhylidae) were used. The ventral and vocal sacs were taken and fixed in Bouin's fluid for 24 h for morphological studies. After dehydration, sample was embedded in wax and 4-6 μm thick sections were prepared via microtome. The sections were stained with haematoxylin-eosin and Domagk (1948) method also used.

## Result

The skin of anuran like all other vertebrates consists of an outer layered epidermis and an underlying layer is dermis, which contain blood vessels nerve fibers, pigment cells and at least two types of gland (mucous and poison**)** surrounded by connective tissue rich in collegenous fibers. Epidermis is thin and made up of stratified squamous epithelium. Multilayered epidermis is differentiated into two layers (a**)** Outermost layer is stratum corneum. Keratinocytes formed the keratinized squamous stratified epithelium and (b**)** Inner layer called as stratum germinative consisting of single layered. Beneath the epidermis is thin basement membrane and then dermis. Just below the epidermis, the dermis was subdivided into a stratum

spongiosum and a stratum compactum. Mixed and granular glands and chromatophores were located in the stratum spongiosum and myoepithelial cells surrounded their secretory portions. A series of alternating layers of bundles of collagenous fibers characterized the stratum compactum dermis. The vocal sac of *Duttaphrynus stomaticus* have internally localized in the mouth (subgular type) and contained two different layers namely an internal and an external sheath. The vocal sac of *Euphlyctis cyanophlyctis* have completely separated paired lateral vocal sacs. *The vocal sac of Fejervarya limnocharis* have incompletely separated paired subgular vocal sacs and The vocal sac of *Microhyla ornata* have single external median subgular mono vocal sac.Amphibians inhabit a wide variety of habitats and various niches of one particular habitat. Due to the environmental conditions of their habitat and variety of functions performed by these, their skin undergoes various modifications some of these are reflected in cellular organization of ventral skin (Table 1). In concluded that the epidermis of ventral skin is thickest in *Duttaphrynus stomaticus* and thinnest in *Fejervarya limnocharis.* The dermis of ventral skin is composed of two layers outer one stratum spongiosum and stratum compactum .The dermis of ventral skin is thickest in *Duttaphrynus stomaticus* and thinnest in *Fejervarya limnocharis.* The mucous and serous glands are larger and well developed in *Duttaphrynus stomaticus.* The mucous and serous glands are numerous in *Microhyla ornata. Duttaphrynus stomaticus* has internal single or median subgular vocal sac. Both sheaths of the vocal sacs of *Duttaphrynus stomaticus* include elements contributing to the expansion and shrinkage movements. The external sheath of the vocal sac contacts with the environment and is accepted as an extension of the skin. A layer formed by elastic fibers is present in the dermis of this sheath. The internal sheath being an extension of the oral mucosa is composed of epithelial and muscle layers. The elastic fibers are present among the muscle bundle in this layer. The muscles and elastic fibers present in both layers are the supporting structure of the changes in the shape which is occur dependent on the functions of the vocal sac. The large mucous glands are present in external sheath. The mucous secreted keeps the skin moist and also permits respiratory gaseous exchange through richly vascular skin thus compensating for the poor development of lungs. In the external sheaths, the thickness of the stratum corneum is greatest where the skin is exposed to maximal friction. Its cells accumulate a keratin protein. Since keratin is tough and insoluble in water, the keratinized stratum corneum provides protection against mechanical injuries fungal and bacterial attacks and loss of body moisture. In the internal sheath, the areolar connective tissue is present. The internal sheath is black due to presence of chromatophores. Epidermis of vocal sac skin is thickest in *Duttaphrynus stomaticus* and thinnest in *Euphlyctis cyanophlyctius*. Dermis of vocal sac skin is thickest in *Duttaphrynus stomaticus* and thinnest in *Euphlyctis cyanophlyctius*. Numerous mucous glands and chromatophores are seen imbedded in stratum spongiosum. Mucous glands are larger in size and numbers in *Duttaphrynus stomaticus* comparison to that of is *Euphlyctis cyanophlyctis Microhyla ornata* and *Fejervarya limnocharis*. The mucous secreted keeps skin moist and also permits respiratory gaseous exchange through richly vascular skin thus compensating for poor development of lungs. In internal sheath, areolar connective tissue is present (Table 2). The presence of elastic fibers in both the vocal sac and body wall of male frog strongly suggests that elastic recoil is important for both inhalatory and exhalatory movements during calling. This conclusion is reinforced by a pronounced sexual dimorphism for this character; elastic fibers are much less

well developed in the female. Expression of elastic fibers in the vocal sac suggests that this structure plays a predominant role in lung re-inflation. Elastic tissues also could prevent overstretching during vocalization, and may help to minimize the muscular effort necessary to hold the vocal sac against the throat when not calling. The extent of energetic saving associated with elastic storage in the vocal sac is unclear because no data are at present available on the time-dependent elastic characteristics of anuran vocal sacs. Variation in vocal sac morphology presumably corresponds to acoustic diversity and associated elastic demands during call production, but at present this relationship is unclear. Vocalization is one of the essential parts of the social behavior in anurans. The muscle and elastic fibers present in both layers are the supporting structure of the changes in the shape which is occur dependent on the functions of the vocal sac. The present study is one of the first studies for the purpose of displaying morphological and histological aspects of median subgular located vocal sac. Present study also contributes to the field of amphibian reproductive biology and biomechanics of vocalization.

## Discussion

Results obtained indicate that vocal sac appears to act as a radiator and amplifier rather than a resonator while applying resonance tubes rules. With mouth closed, vocal sac was inflated and acoustic energy was concentrated in advertisement call. Similar results were obtained by Martin (1972**)**; Capranica and Moffat (1983**)**; Rand and Dudley (1993**)**. Results obtained indicate that presence of pigment serves to protect organs from effects of radiant energy. Therefore, dark colour of vocal sac acts as thermoregulator in the adult frog. Most studies described that elastic fibers in vocal sac would be stretched during phonation and would push air back into lungs after call (McAlister, 1961; Bucher *et al.*, 1982; Dudley and Rand, 1991; Jaramillo *et al.*, 1997**)**. This process might be assisted by contraction of muscular layer in vocal sac (Martin and Gans, 1972**)**. Vocal sac are significant in anuran systematic because they can exhibit significant variation between related pecies (Tyler,1971).Vocal sacs, while highly variable in size and shape, are present in virtually all male anurans that produce advertisement calls. Similar results were obtained by Duellman and Trueb (1985). The vocal sac isn't an acoustic cavity resonator, in spite of the fact that it might serve to direct the call towards the receiver or as a reservoir of mechanical vitality amid calling (Rand and Dudley, 1993). Histological analysis of the vocal sac and body wall in the leptodactylid frog *Physalaemus pustulosus* suggests that both muscle and elastic fibers are important in call production (Jaramillo *et al.*, 1997). As recent, review suggests that elastic properties of vocal sac and lung re-inflation without spending mechanical energy. Similar results were obtained by Ryan (1985b**)**; Jaramillo *et al.*, (1997**)**; Gridi-Papp (2003**)**. Ventral epidermal is thinnest in *Duttaphrynus stomaticus. Microhyla ornata* has more epidermal layers than *Duttaphrynus stomaticus.*Among Dicroglossidae ventral epidermis is thickest in *Euphlyctis cyanophlyctis* as compare to *Fejervarya limnocharis* . Similar results were obtained by Brizzi *et al.*, ( 2003).The vocal sac is a visually conspicuous attribute of most male frogs, but its role in visual communication has only been demonstrated recently in diurnally displaying frogs (Cummings *et al*., 2008). Similar results were obtained by Elias-Costa *et al*., (2017) concluded that the unique arrangement of submandibular muscles and vocal sac structure in Crossodactylus, Hylodes, and Megaelosia allows us to identify the three morphological synapomorphies for the family Hylodidae. In conclusion Anuran calling is

produced by their laryngeal apparatus. However, the vocal sac mediates the calling procedure as an expandable pouch that executes as a sound resonator and radiator. These structures show morphological and physiological changes amid an annual reproductive cycle.

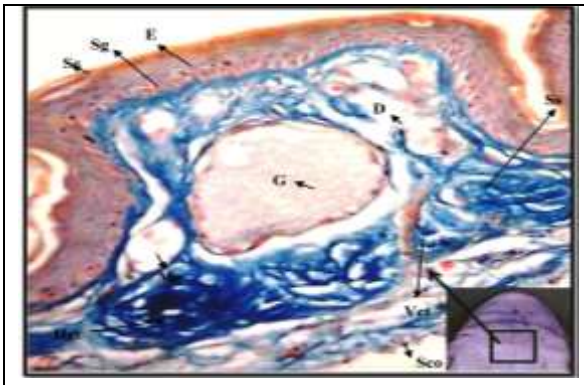


**Fig.1 Ventral skin of *Duttaphrynus stomaticus* X 20**

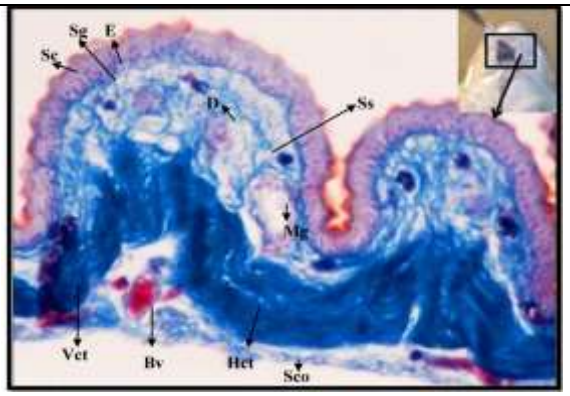


**Fig.2 The external sheath of the vocal sac skin of *Duttaphrynus stomaticus.* X 20**

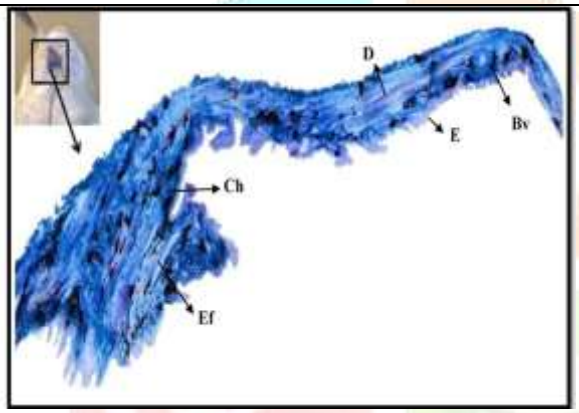


**Fig.3 The internal sheath of the vocal sac skin of *Duttaphrynus stomaticus* . X 10**

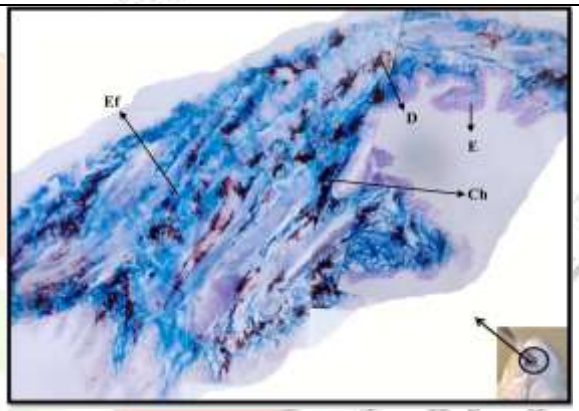


**Fig.4 The internal sheath of the vocal sac skin of *Duttaphrynus stomaticus* . X 20**

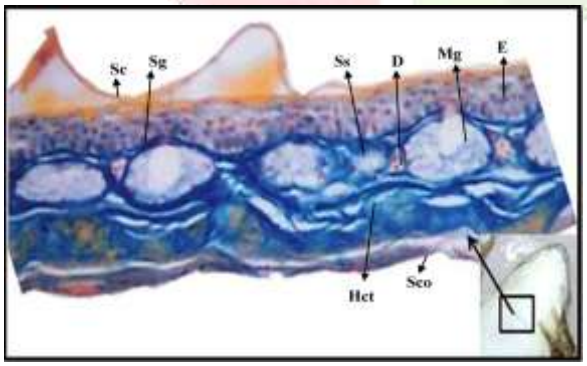


**Fig. 5 Ventral skin of *Euphlyctis cyanophlyctis* X 20**

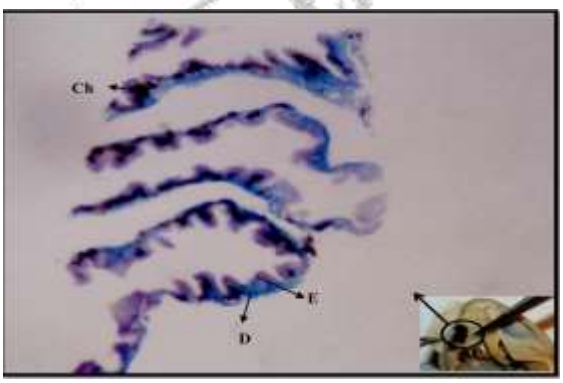


**Fig. 6 Vocal sac skin of *Euphlyctis cyanophlyctis.* X 4**

**Here :E** -Epidermis,, **D**- Dermis,,**Sc-** Stratum corneum,,**Ss**-Stratum spongiosum,,**Sg**-Stratum germinative,,**Sco**-Stratum compactum,,**Ch** - Chromatophores,,**Mg-** Mucous gland, **Hct** - Horizontal connective tissue fibres and **Vct** -Vertical connective tissue fibres.

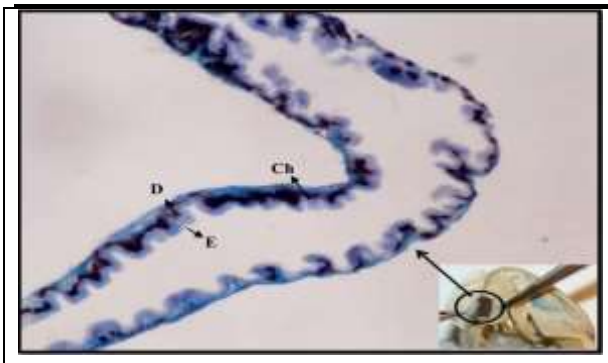


**Fig.7 Vocal sac skin of *Euphlyctis cyanophlyctis.* X 10**

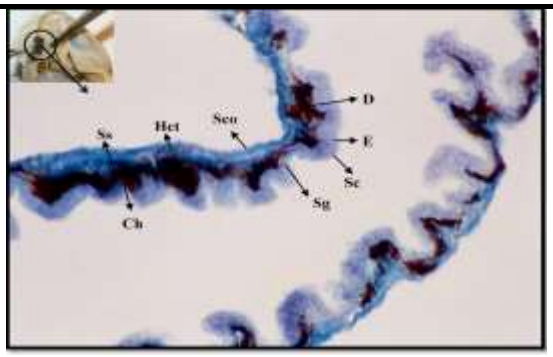


**Fig.8 Vocal sac skin of *Euphlyctis cyanophlyctis.* X 20**

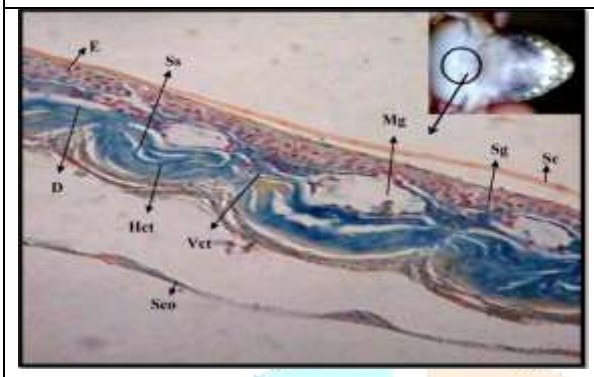


**Fig.9 The ventral skin sac skin of *Fejerverya limnocharis*. X 20**

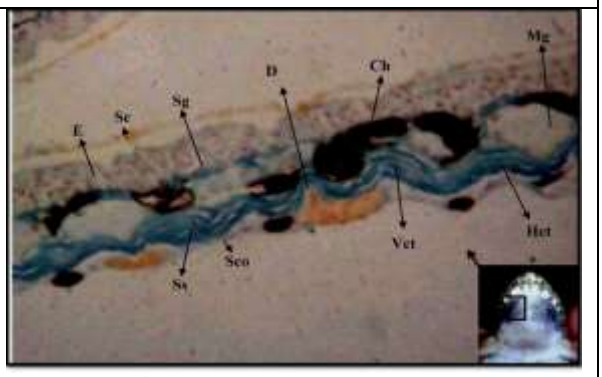


**Fig.10 The vocal sac skin *Fejerverya limnocharis.* X 20**

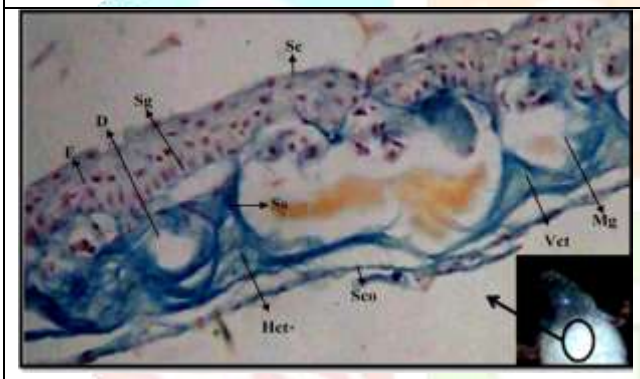


**Fig.11 The ventral skin of *Microhyla ornata.* X 20**

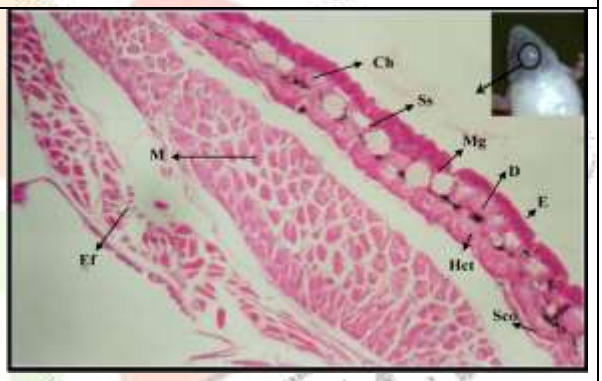


**Fig.12 The vocal sac skin of *Microhyla ornata* showing all vocal sac layers. X 4**

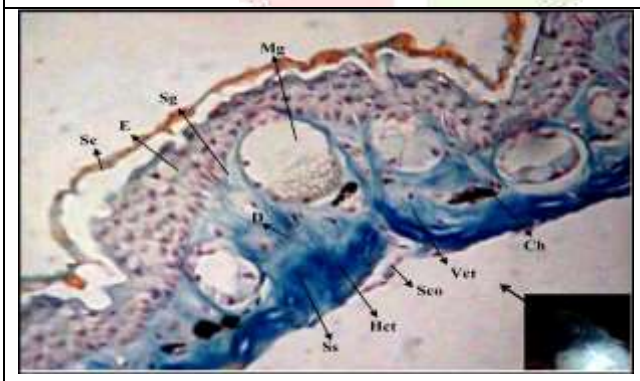


**Fig.13 The vocal sac skin of *Microhyla ornata.* X 10**

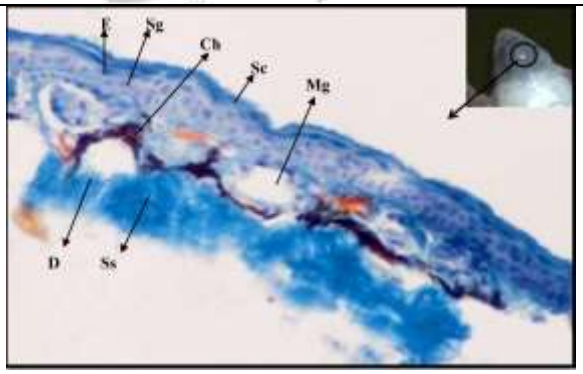


**Fig.14 The vocal sac skin of *Microhyla ornata.* X 20**

**Table 1**

Comparison of the ventral skin of four anuran species

| Skin layers | Name of Sub layers | *Duttaphrynus stomaticus* | *Euphlyctis cyanophlyctis* | *Fejervarya limnocharis* | *Microhyla ornata* |
|---|---|---|---|---|---|
| Epidermis | Stratum Corneum | ++++ | ++ | + | +++ |
| | Stratum Germinative | ++++ | + | ++ | +++ |
| Dermis | Stratum Spongiosum | ++++ | + | ++ | +++ |
| | Stratum compactum | ++++ | ++ | + | +++ |
| | Mucous gland | + | Circular Numerous placed touching each other just below epidermis imbedded in stratum spongiosum | Flask shaped multi-cellular alveolar, Numerous placed touching each other just below epidermis imbedded in stratum spongiosum | Circular Numerous placed touching each other just below epidermis. |
| | Horizontial Connective tissues | Fibers are arranged giving a general appearance of mat or net like.Fibers are loosely arranged. These are wavy.Inter-fibril spaces are large. | Fibers are arranged in sheets or layers. General appearance is wavy. Fibers are tightly held. Inter- fibril space is narrow | Fibers are arranged loosely in fibrous pools. General appreance is fibrous These are irregular. Inter-fibril space is narrow in some places and wider in some places . | Fibers are lying loosely in bundles or pools. These are wavy. Inter-fibril spaces is more |
| | Vertical Connective tissues | ++++ | + | + | + |
| | Blood vessels | + | + | + | + |
| | Nerve fibers | + | + | + | + |
| | Lymph space | + | + | + | + |
| | Alveolar spongy | ++ | + | + | ++ |

**Note:** + Less Developed
+ + developed
++ + Well developed
+ + + + Much well developed
-Absent
(+**)** show thickness of the cells

**Table 2**

Comparative study of vocal sac skin of four anuran species

| **Name of Sub layers** | *Duttaphrynus stomaticus* | | *Euphlyctis cyanophlyctis* | *Fejervarya limnocharis* | *Microhyla ornata* |
|---|---|---|---|---|---|
| | External | Internal | | | |

| | | | | | |
|---|---|---|---|---|---|
| Stratum Corneum | ++++ | + | + | ++ | +++ |
| Stratum Germinative | ++++ | ++++ | + | ++ | +++ |
| Basement Membrane | + | + | + | + | + |
| Stratun Spongiosum | + | + | + | + | + |
| Stratum compactum | ++++ | + | + | + | +++ |
| Mucous glands | +++ | + | + | +++ | ++ |
| Chromatophores | - | + | ++++ | ++ | +++ |
| Horizontial connective tissues | + | ++++ | + | + + | +++ |
| Vertical connective tissues | ++ | - | + | + | + |
| Blood vessels | + | + | + | + | + |
| Nerve fibers | + | + | + | + | + |
| Lymph space | + | + | + | + | + |
| Alveolar spongy | + | ++ | + | + | ++++ |

**Note:** + Less Developed
+ + developed
++ + Well developed
+ + + + Much well developed
-Absent
(+**)** show thickness of the cells

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**References:**

1. Brizzi, R., Delfino, G.and Jantra, S. (2003) An overview of breeding glands. In reproductive biology and phylogeny of Anura. B.G.M. Jamieson (ed.).Science Publishers, Inc., Enfield, New Hampshire.253-317
2. Bucher, T.L., Ryan, M.L. and Bartholomew,G.A. (1982). Oxygen consumption during resting, calling and nest building in the frog,*Physalaemus pustulosus.* Physiol. Zool. 55: 10-22.

3. Capranica R.R. and Moffat, A. J.M. (1983) Neurobehavioral correlates of sound communication in anurans: In J.P. Ewert, R.R. Capranica, and D.J. Ingle, eds. Advances in Vertebrate Neuroethology. Plenum, New York, NA 70 ASI series A, Vol. 56, 701-730
4. Cumming, M.E., Bernal, X.E., Reynaga, R., Rand, A.S. and Ryan, M.J. (2008). Visual sensitivity to a conspicuous male cue varies by reproduction state in *Physalaemus pustulosus* female. The Journal of Experimental Biology 211, 1203-1210.
5. D'Istria M., Picilli A., Basile C., Delrio G. and Chieffi G. (1982). Morphological and biochemical variations in the skin of *Rana esculenta* during the annual cycle. *General and Comparative Endocrinology*. 48: 20-24.
6. De Jongh, H.J. and Gans, C. (1969). On the mechanism of respiration in the bullfrog,*Rana catesbeiana*: A reassessment. J.Morphol. 127: 259 – 290.
7. Demirsoy A. (1993). *Yaşamın Temel Kuralları*, Omurgalılar / Anamniyota, Cilt III-Kısım I, 2. Baskı. Ankara, Meteksan A.Ş. 558 pp
8. Domagk, L. (1948). Kernectrot Aniline –Blue, Orange G. In Microskopische Technik. BRomeis, Oldenberg, Munchen. PP 346
9. Dudley, R., and Rand, A.S. (1991). Sound production and vocal sac inflation in the tungara frog, *Physalaemus pustulosus* (Leptodactylidae). Copeia : 460 – 470.
10. Duellman, W.E. and Trueb, L. (1986). Biology of Amphibians. McGraw-Hill. New York
11. Elias-Costa, A. J. ; Montesinos, R. Grant, T. and Faivovich,F.(2107) .The vocal sac of Hylodidae (Amphibia, Anura): Phylogenetic and functional implications of a unique morphology.Journal of Morphology. 2017;1–11.
12. Girgenrath M. and Marsh R.L. (1997). In vivo performance of trunk muscles in tree frogs during calling. The Journal of Experimental Biology. 200: 3101-3108.
13. Greenberg, B. (1942). Some effects of testosterone on the sexual pigmentation and other sex characters of the cricket frog (*Acris gryllus*). Journal of Experimental Zoology, 91, 435–446.
14. Gridi-Papp, M. (2003) Mechanism, behavior, and evolution of calling in four North American Treefrogs. Ph.D. Diss. University Of Texas, Austin.
15. Ho¨dl, W. and Ame´zquita, A. (2001). Visual signaling in anuran amphibians. In: Anuran Communication (Ed. by Ryan, M. J.), pp.121-141. Smithsonian Institution Press.
16. Jaramillo, C., Rand, A. S., Ibáñez, R. and Dudley, R. (1997) Elastic structures in the vocalization apparatus of the tungara frog, *Physalaemus pustulosus* (Leptodactylidae):. J. Morphol., 233: 287–295
17. Kao Y. H., Alexander P. S., Yang V. V. C. and Yu J.Y.L. (1994). Annual patterns of nuptial pad and vocal sac development in the male Chinese bullfrog (*Rana rugosa* Wiegmann). Zoological Studies, 33 (2): 153-159.
18. Kelley D.B. (2004). Vocal communication in frogs. Current Opinion in Neurobiology. 14:751-757
19. Liu, C.C. (1935). Types of vocal sacs in the Salientia. Proc. Bost. Soc. Nat. Hist. 41: 19-40.
20. Martin, W.F., and Gans, C. (1972). Muscular control of the vocal tract during release signaling in the toad *Bufo valliceps*. J.Morphol. 137: 1 – 28.
21. McAlister, W. H. (1961): The mechanics of sound production in North American *Bufo*. Copeia, 86-95

22. Noble, G.K. (1931). The Biology of the Amphibia. McGraw – Hill. New York
23. Rand, A.S. and Dudley, R. (1993). Frogs in helium: The anuran vocal sac is not a cavity resonator. Physiol. Zool. 66: 793-806.
24. Rosenthal, G.G., Rand, A.S. and Ryan, M.J. (2004). The vocal sac as a visual cue in anuran communication: an experimental analysis using video playback. Anim. Behav.2004, 68: 55-58.
25. Ryan, M.J. (1985). The Tungara Frog: A Study of Sexual Selection and communication. University of Chicago Press. Chicago
26. Sengezer-Inceli M., Süren S., Murathanoğlu O. and Kaptan, E. (2004) Immunohistochemical detection of serotonin in the skin of frogs (*Rana ridibunda*) kept at different temperatures. Bratislava Biologia.59(2): 273-281.
27. Sengezer-Inceli, M., Sancar-Bas, S., and Kaptan, E. (2008). Vocal sac of *Rana ridibunda*: A morphology and Immunohistochemical Study. IUFS J. Biol.67 (2):135-144.
28. Suthers R.A., Narins P.M., Lin W.Y.,Schnitzler, H.U., Denzinger A., Xu C. H. and Freng, A.S. (2006). Voices of the dead: complex nonlinear vocal signals from the larynx of an ultrasonic frog. The Journal of Experimental Biology. 209: 4984-4993.
29. Taigen, T.L., and Wells, K.D. (1985). Energetic of Vocalization by an anuran amphibian (*Hyla versicolor*). J. Comp. Physiol. B 155: 163 – 170.
30. Tyler, M.J. (1971a) .The phylogenetic significance of vocal sac structure in hylid frogs. Univ. Kansas Publ. Mus. Nat. Hist. 19:319 – 360.
31. Tyler, M.J. (1971b). Observations on anuran myo-integumental attachements associated with vocal sac apparatus. J. Nat. Hist. 5:225-231.
32. Washington, D.C.: Jaramillo C. (1997). Elastic structures in the vocalization apparatus of the Tugara Frog *Physalaemus pustulosus* (Leptodactylidae).Journal of Morphology. 233: 287-295.
33. Wells, K.D. (1980). Social behavior and communication of a dendrobatid frog (*Colostethus trinitatis*). Herpetologica, 36,189–199.
34. Wells, K.D. and Taigen, T.L. (1989). Calling energetic of a neotropical tree frog, *Hyla microcephala*. Behav. Ecol. Sociobiol.25: 13 – 22.