

## On the anuran muscle pulmonum proprius

Leandro Alcalde

**Abstract.** The present contribution provides a systematic survey on the variation of the musculus pulmonum proprius within Anura. Evidence is contrary to previous works that presented the muscle as an enigmatic feature present only in the family Pipidae. Although having different sites of origin, the m. pulmonum proprius was observed in all examined anurans. The muscle originates at the level of the transverse processes of the fourth vertebra in the neobatrachian families Bufonidae, Calyptocephalellidae, Ceratophryidae, Cycloramphidae, Dendrobatidae, Hylidae, Hylodidae, Leiuperidae, Leptodactylidae, Mantellidae, Microhylidae, Ptychadenidae and Ranidae. In *Bombina variegata* (Bombinatoridae) the origin of the muscle shifts caudally at the level of the transverse process of the sacral vertebra and, in Pipidae, it originates either at the ilium (*Xenopus*) or the femur (*Pipa*). Features of the m. pulmonum proprius do not vary in species with different modes of locomotion within Neobatrachia and there is no sexual dimorphism in this muscle. It is absent in the genera *Salamandra* (Caudata) and *Chthonerpeton* (Gymnophiona). Pending verification in Leiopelmatidae, the presence of m. pulmonum proprius may be considered as a previously unknown anuran synapomorphy.

**Keywords:** amphibian diaphragm, Anura, Lissamphibia, musculus pulmonum proprius.

Beddard (1895a, 1895b) coined the name musculus pulmonum proprius to describe a not previously described lung-inserted muscle, present in the Surinam toad (*Pipa*) and in the South African clawed frog (*Xenopus*). The author described variation in the origin of the muscle, which may occur at the ilium (*Xenopus*) or the femur (*Pipa*). In one of these works (Beddard, 1895b) the author introduced the term “diaphragm” to describe the association, at level of the root of the lung, between the m. pulmonum proprius with the fibers of the mm. sternohyoideus and transversus (described as m. obliquus internus). With respect to non pipid anurans, Beddard (1895b) found that the diaphragm of *Rana* is composed by fibers of the m. transversus only, making tacit the absence of m. pulmonum proprius in this genus. Ten years later, Keith (1905) introduced the hypothesis about the homology between the amphibian diaphragm and the cervical portion of the mammalian diaphragm. In reference to the composition of the amphibian diaphragm in non

pipid anurans Keith (1905) wrote these paragraphs (species names were updated following current taxonomic arrangements): “In *Pelophylax lessonae* and *Rana temporaria*, as may be seen in the very exact work of Gaupp (1896), and as shown in fig. 12, the anterior fibers of the transversalis come from the costal process of the 4th vertebra and end on the oesophagus side of the pericardium and the root of the lung between these insertions. This part of the transversalis covers the lung, and is already differentiated functionally from the rest of the transversalis. . .”, and “In *Bombina bombina* the fibers of the fourth segment spread outwards, and at their proximal end find an insertion to the pericardium”. These observations raise the following question: is the lung-inserted muscle of *Bombina*, *Pelophylax* and *Rana* homologous to the m. pulmonum proprius described by Beddard for *Pipa* and *Xenopus*? Few works dealing with the axial musculature in anurans were published and only some of them had studied the m. pulmonum proprius with either *Xenopus* or *Pipa* as model organism (Dunlap, 1960; de Jongh, 1972; Snapper, Tenney and McCann, 1974; De Troyer et al., 1981; Pickering, Campion and Jones, 2004; Uriona and Farmer, 2006; Kimura, 2010). Most of them have referenced

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Investigador Asistente CONICET, Instituto de Limnología Dr. R.A. Ringuelet, Avenida Calchaquí Km. 23, 5, Florencio Varela (CP1888), Buenos Aires, Argentina  
e-mail: alcalde@ilpla.edu.ar

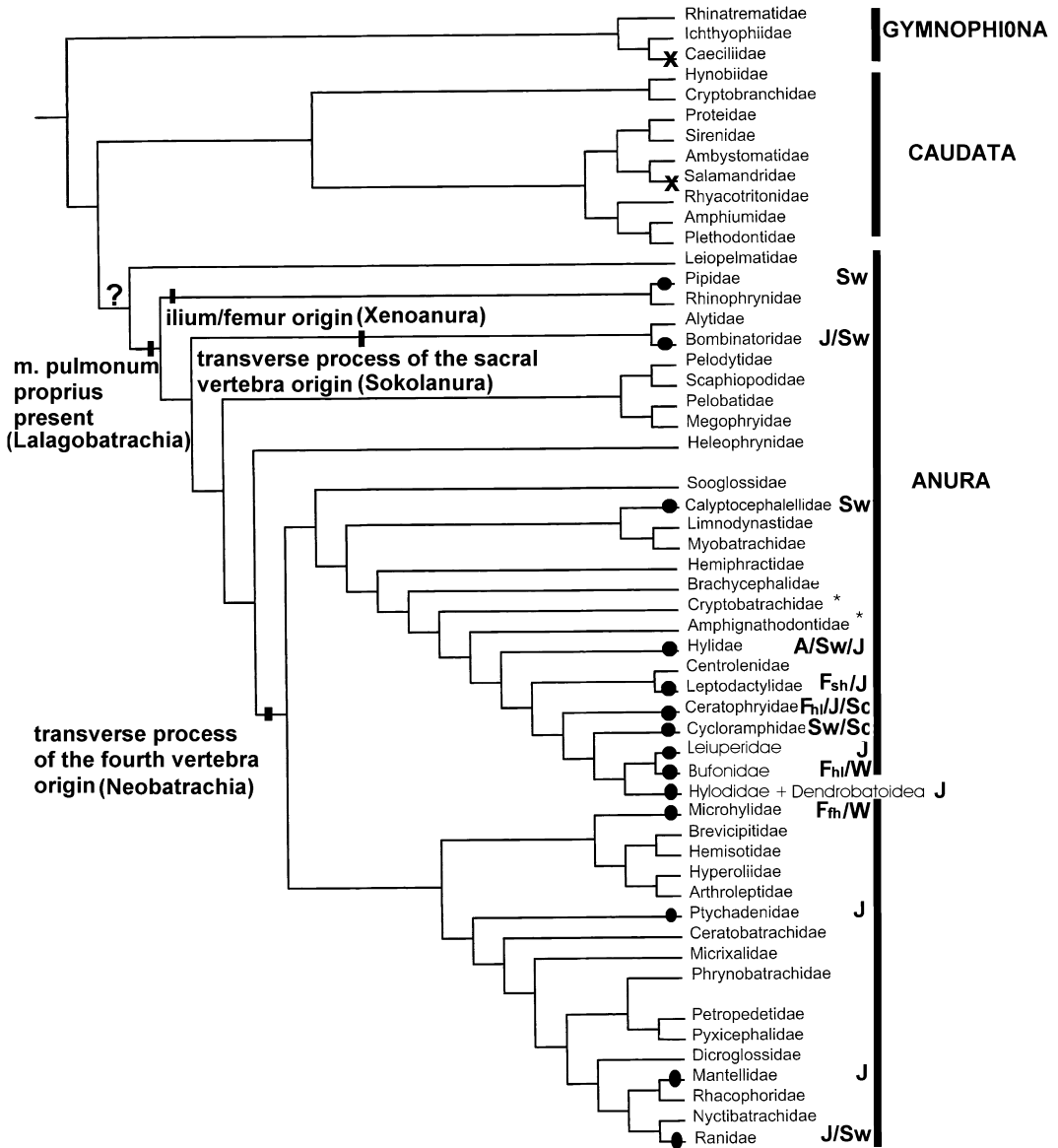
the work of Keith (1905) although without mentioning where the presence of *m. pulmonum proprius* is demonstrated for non pipid anurans. I consider that the systematic omission of such data may have been the pillar to consider this muscle as unique in *Pipa* and *Xenopus*. In addition, the probable accidental removal of *m. pulmonum proprius* may be the main reason to explain the overlooking of the muscle by most anatomists that worked with neobatrachians. In these anurans, this muscle is easy to cut it away during the extirpation of the viscera. Once cut, some of its fibers usually remain attached to the transverse process of the fourth vertebra together with the ones of the *m. rhomboideus posterior*, making a correct muscle identification difficult. A similar situation also occurs in *Bombina*.

The purposes of the present contribution are to describe the main features of the *m. pulmonum proprius* in several anuran families and to confirm if this muscle is present in the other extant lissamphibian orders, Caudata and Gymnophiona.

All studied materials are housed at the herpetological collections of the Colección Herpetológica del Instituto Nacional Malbran (CHINM, housed at the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”), Museo de La Plata (MLP), and Instituto de Limnología Dr. R.A. Ringuelet (ILPLA or NB-ILPLA). We studied 31 genera representing different anuran lineages and diverse modes of life. The length of the lungs was assessed considering their minimum extent (deflated state) and the anteroposterior extent of the pleuroperitoneal cavity was standardized at the maximum length using specimens in which the tips of the iliac shafts are at the level of the anterior end of the transverse process of the sacral vertebra. Locomotor types (arboreal, jumper, scansorial, swimmer and walker) were assessed by personal field observations (most cases), examination of the external features (dendrobatids, mantellids, ptychadenids, ranids) and bibliography (*Bombina*: Essner et al., 2010). Different types of burrowing behaviors were assessed according to Nomura, Rossa-Feres and Langeani (2009). Morphological terms are those proposed in the “Amphibian Anatomical Ontology” (<http://www.amphibianat.org/>). The following specimens were double stained and dissected (Taylor and Van Dyke, 1985; DSD) or only dissected (D) under a Wild stereomicroscope to assess the presence and characteristics of the *m. pulmonum proprius*: *Alsodes gargola* (Cycloramphidae, 1 DSD female – MLP 2891, 1 DSD male – MLP 2874), *Ameerega macero* (Dendrobatidae, 1 D male – MLP 893), *Atelognathus patagonicus* – aquatic

morphotype (Ceratophryidae, 1 DSD female MLP 3432, 1 DSD male – MLP 2796), *Batrachyla antartandica* (Ceratophryidae, 2 DSD females – CHINM 2542 and 2547), *Bombina variegata* (Bombinatoridae, 1 D female – MLP 766), *Boophis tephraeomystax* (Mantellidae, 1 D male – MLP 2672), *Calyptocephalella gayi* (Calyptocephalellidae, 1 DSD male – MLP 487), *Ceratophrys ornata* (Ceratophryidae, 1 DSD female – MLP 3643), *Rana temporaria* (Ranidae, 1 D male – MLP 154), *Rhinella fernandezae* (Bufonidae, 1 D female – ILPLA 066, 1 D male – ILPLA 046), *Crossodactylus schmidti* (Hylodidae, 1 D female – MLP 1362), *Cycloramphus asper* (Cycloramphidae, 1 D adult specimen, unknown sex – MLP 077), *Dermatonotus muelleri* (Microhylidae, 1 D male – MLP 648), *Eupsophus calcaratus* (Cycloramphidae, 1 DSD male – MLP 4011, 1 DSD female – MLP 2901), *Hylorina sylvatica* (Cycloramphidae, 1 DSD female – CHINM 1870), *Hypsiboas pulchellus* (Hylidae, 1 D female – NB-ILPLA 856), *Lepidobatrachus llanensis* (Ceratophryidae, 1 D female – MLP 1528), *Leptodactylus latinasus* (Leptodactylidae, 1 D female – NB-ILPLA 369, 1 D male – NB-ILPLA 829), *Leptodactylus latrans* (Leptodactylidae, 1 D male – ILPLA 021), *Limnomedusa macroglossa* (Cycloramphidae, 1 adult specimen, unknown sex – MLP 1410), *Lithobates catesbeianus* (Ranidae, 1 D female – MLP 4734), *Melanophryniscus atroluteus* (Bufonidae, 1 D female – MLP 332), *Nannophryne variegata* (Bufonidae, 1 D female – ILPLA 837, 1 D male – ILPLA 030), *Pelophylax* spp. (Ranidae, 1 D female – MLP 764), *Phyllomedusa sauvagei* (Hylidae, 1 D female – ILPLA 298), *Pipa* spp. (Pipidae, 1 D female – CHINM 7610), *Pleurodema borellii* (Leiuperidae, 2 DSD males – MLP 4791-92), *Pseudis paradoxa* (Hylidae, 1 adult specimen, unknown sex – ILPLA 028), *Ptychadena* spp. (Ptychadenidae, 1 D female – MLP 2673), *Rhinoderma darwini* (Cycloramphidae, 1 DSD male – MLP 487), and *Xenopus muelleri* (Pipidae, 1 D female – MLP 5234). In addition, we also dissected one *Salamandra atra* (Caudata, Salamandridae, 1 D female – MLP 766) and one *Chthonerpeton indistinctum* (Gymnophiona, Caeciliidae, 1 D specimen, unknown sex – MLP 5235). To contextualize the results, the phylogenetic hypotheses provided by Frost et al. (2006) and Grant et al. (2006) were chosen (see fig. 1).

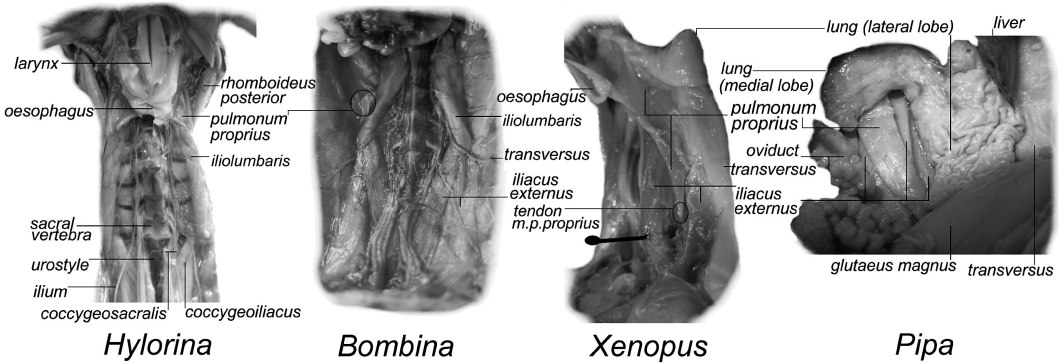
In the two pipids studied herein, *Pipa* spp. and *Xenopus muelleri*, the features of the *m. pulmonum proprius* fit the general description given by Beddard (1895a, 1895b). Both genera have well-developed lungs that reach the caudal end of the pleuroperitoneal cavity, and the lungs of *Pipa* are bilobed due to an additional medial lobe shorter than the lateral (fig. 2). The *m. pulmonum proprius* of *Pipa* originates in the femur, runs ventral to the three-headed *m. iliacus externus* and inserts at the dorsum of the oesophagus and dorsal root of the lung by two poorly-marked medial and lateral venters (figs 2 and 3). The medial venter inserts in the root of the me-



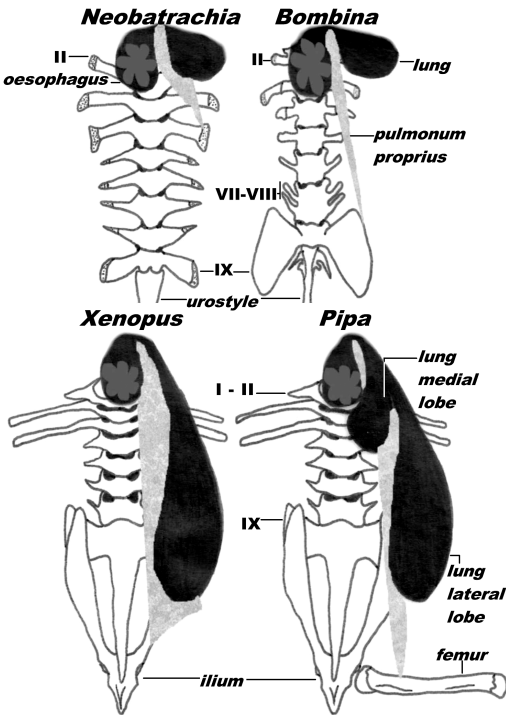
**Figure 1.** Phylogenetic tree showing the relationships of the main groups of Lissamphibia as presented by Frost et al. (2006) with modifications on certain terminals according Grant et al. (2006). Asterisks denote families currently included within Hemibractidae. Black dots mark families with *m. pulmonum proprius* present. The x denotes absence of the muscle. The modes of locomotion and the burrowing behaviors were mapped in the cladogram at the level of each family. References: A: arboreal; F(fh): fossorial (forelimb head - first burrowers); F(hl): fossorial (hindlimb - first burrowers); F(sh): fossorial (snout head - first burrowers); J: jumpers; Sc: scansorial; Sw: swimmers; W: walkers.

dial lung lobe by a short ligament and presents fleshy insertion in the dorsolateral face of the oesophagus. The lateral venter has a fleshy insertion at the root of the lateral lung lobe. The origin in the femur is tapered by the *m. glutaeus magnus* (fig. 2).

The *m. pulmonum proprius* of *Xenopus* originates along the midposterior lateral face of the ilium. Most fibers run anteriorly across the cavity to insert at the root of the lung and dorsum of the oesophagus whereas the most lateral ones spread laterally in a diffuse manner to in-



**Figure 2.** Main features of the m. pulmonum proprius and related structures in *Hylorina*: Neobatrachia: Cycloramphidae (ventral view), *Bombina*: Sokolanura: Bombinatoridae (ventral view), *Xenopus* and *Pipa*: Xenoanura: Pipidae (both in ventrolateral view from the right side of the animal). In *Bombina*, the m. pulmonum proprius was accidentally removed in the right side of the animal and partially cut in the left side. In *Xenopus*, the heads of the m. iliacus externus were separated to visualize the tendon at the origin of the m. pulmonum proprius. Photos are not to scale.



**Figure 3.** Origin and insertion of m. pulmonum proprius in: Neobatrachia, *Bombina*, *Pipa* and *Xenopus*. The roman numbers indicate number of vertebrae. Diagrams are not to scale.

sert at the m. transversus (figs 2 and 3). In the ilium, the origin of the m. pulmonum proprius is overlaid by the central and the lateral heads of the m. iliacus externus. Once the m. iliacus externus is removed, two kinds of bone at-

tachment for the m. pulmonum proprius become evident: a short and strong tendon (for the posteriormost fibers) or a fleshy origin (for the anteriormost ones) (fig. 2). The m. pulmonum proprius in both pipids receives innervations from the spinal nerves that merge between the fourth and fifth vertebrae.

In *Bombina variegata* (Bombinatoridae), the short and rounded lungs never reach the posterior end of the pleuroperitoneal cavity as do in the pipids examined herein. The m. pulmonum proprius of *Bombina* represents an intermediate situation between pipids and neobatrachians (see below). The m. pulmonum proprius of *Bombina* originates at the lateral end of the transverse process of the sacral vertebra and inserts in the dorsal root of the lung and dorsum of the oesophagus (figs 2 and 3). The innervation is the same as in pipids.

In the neobatrachians studied here (Bufonidae, Calyptocephalellidae, Ceratophryidae, Cycloramphidae, Dendrobatidae, Hylidae, Hydrolidae, Leiuperidae, Leptodactylidae, Mantellidae, Microhylidae, Ptychadenidae, and Ranidae) the m. pulmonum proprius runs between the transverse process of the fourth vertebra and the dorsal root of the lung and dorsum of the oesophagus (figs 2 and 3). In these families the muscle have the same origin as the m. rhomboideus posterior that runs between the trans-

verse process of the fourth vertebra and the medial face of the suprascapula (fig. 2). The m. pulmonum proprius of the Neobatrachia receives the same nerve supply as in Bombinatoridae and Pipidae. The observation of both sexes of some species (e.g., *R. fernandezae* and *L. latrans*) reveals that the m. pulmonum proprius lacks features associated with sexual dimorphism.

With respect to the “amphibian diaphragm”, the m. pulmonum proprius of *Bombina*, *X. muelleri* and *Pipa* does not receive fibers of the m. transversus as occurs in the neobatrachian frogs. No muscles similar to m. pulmonum proprius were found in the specimens of Caudata (*Salamandra atra*) and Gymnophiona (*Chthonerpeton indistinctum*).

Although pending verification in Leiopelmatidae, the m. pulmonum proprius appears as a previously unknown anuran synapomorphy that, interestingly, presents four different sites of origin that supports natural groups within Anura (fig. 1): (a) the m. pulmonum proprius is present, at least, at the level of the common ancestor of the Lalagobatrachia clade, (b) Xenoanura (*Pipa* and *Xenopus*) presents the most caudally shifted origin of the m. pulmonum proprius, (c) the primitive condition within Sokolanura (*Bombina*) seems to be the m. pulmonum proprius with origin on the transverse process of the sacral vertebra, and (d) Neobatrachia presents the most cranial origin of the muscle, which occurs at level of the transverse process of the fourth vertebra.

As suggested above, all evolutionary discussions about the m. pulmonum proprius are largely conditioned by verification of the muscle in Leiopelmatidae. The situation in both leiopelmatid genera is crucial to establish the polarity of the character, but whichever the primitive state was, the condition present in Pipidae seems to be gained via a caudal shift of the muscle origin as an adaptation to the fully-aquatic life. In fact, the origins of many other axial and pectoral muscles are caudally shifted in the genera *Pipa* and *Xenopus* (e.g., mm. latissimus dorsi and rectus abdominis; see

Grobbelaar, 1924). Prikryl et al. (2009) suggested that such caudal shift in these set of muscles is presumably playing a role in swimming. Such swimming hypothesis should be considered with extreme caution since many of the non pipid anurans studied herein are swimmers (see fig. 1) and, when compared with pipids, they have a different origin of the m. pulmonum proprius. Finally, the knowledge of the condition in the non aquatic family Rhinophrynidae is necessary to support the “swimming” hypothesis about the evolution of the m. pulmonus proprius in Xenoanura.

With respect to the function of the m. pulmonum proprius, most studies dealing with this aspect used *Xenopus* as the model organism (Snapper et al., 1974; Pickering et al., 2004). Two main functions have been suggested for the m. pulmonum proprius: respiratory (Snapper et al., 1974) and gastrointestinal (Pickering et al., 2004). Although the latter authors have demonstrated that m. pulmonum proprius acts as a lower oesophageal sphincter, they did not rule out other physiological roles such as preventing aerophagy or retracting the stomach after vomiting. At the light of the present knowledge, the above mentioned functional hypotheses should be repeated considering a larger sample of anurans instead of only the fully-aquatic pipids.

To conclude, I would like to remark that this evidence allows reconsidering the m. pulmonum proprius as a synapomorphic anuran trait instead of an enigmatic feature present only in the family Pipidae. To a better understanding of the evolution of this interesting muscle the question arose as whether it is present in the family Leiopelmatidae and in the genus *Rhinophrynus*, the only non-aquatic Xenoanura.

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