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INVITED REVIEW

Pulmonary Smooth Muscle in Vertebrates: A Comparative Review of Structure and Function

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Synopsis Although the airways of vertebrates are diverse in shape, complexity, and function, they all contain visceral smooth muscle. The morphology, function, and innervation of this tissue in airways is reviewed in actinopterygians, lungfish, amphibians, non-avian reptiles, birds, and mammals. Smooth muscle was likely involved in tension regulation ancestrally, and may serve to assist lung emptying in fishes and aquatic amphibians, as well as maintain internal lung structure. In certain non-avian reptiles and anurans antagonistic smooth muscle fibers may contribute to intrapulmonary gas mixing. In mammals and birds, smooth muscle regulates airway caliber, and may be important in controlling the distribution of ventilation at rest and exercise, or during thermoregulatory and vocal hyperventilation. Airway smooth muscle is controlled by the autonomic nervous system: cranial cholinergic innervation generally causes excitation, cranial non-adrenergic, non-cholinergic innervation causes inhibition, and spinal adrenergic (SA) input causes species-specific, often heterogeneous contractions and relaxations.

Introduction

The airways and parenchymal tissues of vertebrate lungs are, nearly without exception, lined with smooth muscle controlled by the autonomic nervous system. The alteration and assignment of new functions for airway smooth muscle may have co-occurred with events in the evolution of the vertebrate lung, thus review and synthesis of what is known of this tissue should improve our knowledge of vertebrate pulmonary evolution (Fig. 1), perhaps shedding light on changes occurring when vertebrates left the water or developed new inspiratory and expiratory mechanisms. A comparative perspective on airway smooth muscle may also benefit researchers working on the pathophysiology of this tissue in humans. Although there are several recent reviews of airways smooth muscle in humans (Stephens 2001; Canning 2006; Doeing and Solway 2013), most descriptions of pulmonary smooth muscle in other taxa are presented as parts of focused physiological investigations. A comprehensive review of the structure, function, and innervation patterns of airway smooth muscle in vertebrates has never been conducted and is presented here. This review briefly outlines and then covers in detail what is known of the morphology, function, and control mechanisms of smooth muscle tissues lining the airways in vertebrates. After a brief overview, this paper will review knowledge of pulmonary smooth muscle in different groups of extant vertebrates, summarize innervation and control patterns, and end with a brief conclusion.

Brief overview of airway smooth muscle across vertebrates

The ancestral vertebrate gas-containing organ likely originated as an outgrowth of the ventral pharynx (Liem 1988) which may have evolved to provide an additional oxygenation mechanism in hypoxic water conditions (Randall et al. 1981), to provide for buoyancy (Liem 1988) or to help oxygenate the heart (Farmer, 1997). Gas-containing derivatives of the gut tube are called lungs if they are paired and of ventral origin, and swim bladders if they are unpaired and of dorsal origin (Graham 1997). The evolution of lungs and swim bladders is summarized in Fig. 1. Among actinopterygians, the buoyancy role

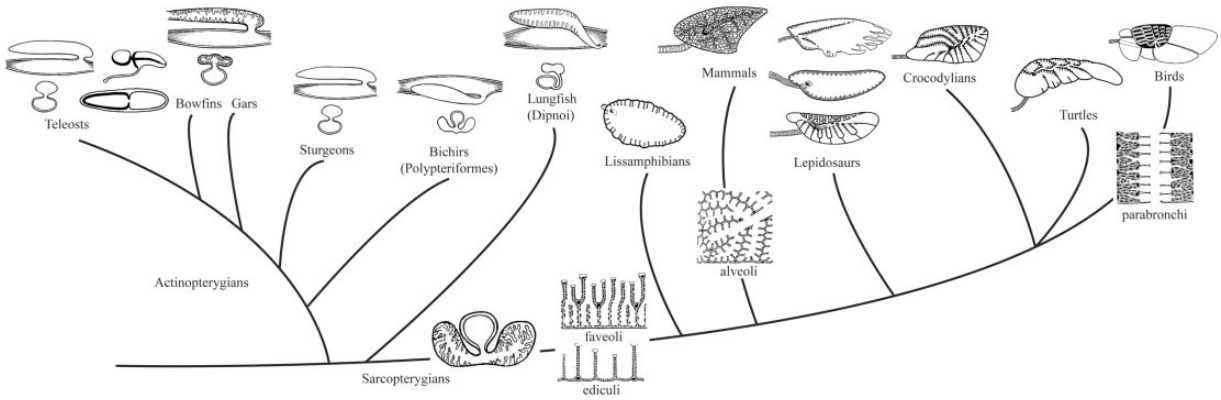

Fig. 1 Lung and swim bladder evolution in vertebrates. A dendrogram of major vertebrate groups with representative lung and swim bladders superimposed. Drawings adapted from Duncker (1978), Fänge (1983), and Romer and Parsons (1977). The gas-exchange tissue of non-avian reptiles and amphibians is organized into ediculi (single layer of chambers) or faveoli (multiple layers), which it forms blind-end alveoli and tubular parabronchi in birds. See text for details. Cladogram topology from Kardong (2014).

dominates, although bichirs (Polypteriformes) have lungs and the swim bladders of bowfins (*Amia*) and gars (Lepisosteiformes) retain respiratory epithelium (Graham 1997). Among teleosts, physoclists have lost the connection between pharynx and swim bladder while physostomes retain it.

All sarcopterygians have lungs, but only amniotes have a trachea and intrapulmonary bronchi (Liem 1988; Duncker 2004). Tetrapod lungs are characterized by internal elaboration that increases surface area. Lissamphibians and non-avian reptiles have gas-exchanging pockets termed ediculi when they form only one layer and faveoli when they form multiple layers, and the pockets are rimmed by thickened margins called trabeculae (Perry 1998). The gas exchanging airways of birds are organized into parallel tubes called parabronchi, and mammal airways end in blind sacs called alveoli (Maina 2002). Non-avian reptile lungs show varying amounts of heterogeneity in the distribution of gas-exchanging tissue, and birds have a system of compliant air sacs that ventilate an isovolumetric gas-exchanging region (Perry 1998).

Because the anatomy and function of the autonomic nervous system differs across different vertebrate groups, sympathetic and parasympathetic divisions will be abandoned, and innervation will be organized into cranial cholinergic (CC), cranial, non-adrenergic non-cholinergic (NANC), and spinal adrenergic (SA) nerve patterns. Investigations of autonomic innervation and their findings are summarized in Table 1. The function of autonomic innervation on airway smooth muscle and variation in autonomic nerve structure among vertebrates is discussed in the following sections after a brief outline.

Smooth muscle fibers regulate tone in blood vessels, the gut tube, and urinary tubes (Tortora and Nielsen 2014), were likely the major regulatory component of the earliest vertebrate gas organs. Because the lung is an outgrowth of the pharynx (Hislop 2002), smooth muscle almost certainly lined and regulated tension in the ancestral vertebrate lung. Further evidence for an ancestral smooth muscle layer in the lungs is provided by the universal prevalence and pattern of SA innervation of smooth muscle in the lungs and swim bladders of fishes. Smooth muscle plays a role in for expelling gases or controlling the resorption or release of gases into actinopterygian swim bladders.

Smooth muscle may be involved in deflation among lungfishes, and aquatic amphibians. In lissamphibians and non-avian reptiles, smooth muscle probably functions largely to maintain internal lung structures (Smith and Campbell 1976; Perry et al. 1989a). In non-avian reptiles, coordinated control of antagonistic muscle fibers may generate important intrapulmonary gas mixing through the bellowing action of lung faveoli (Perry et al. 1989a; Daniels et al. 1994). Smooth muscle could also play a role in deflation among lungfishes, and aquatic amphibians. Smooth muscle in birds and mammals is positioned to regulate airway caliber but may also play important roles in lung micromechanics. Parabronchial rigidity, which enables birds to have a very thin blood–gas barrier, may depend on the tension generated by smooth muscle fibers guarding the respiratory atria (Maina 2007), and sphincters guarding secondary and parabronchi may allow air to bypass gas exchange regions during vocal or thermoregulatory hyperventilation (King and Cowie 1969), preventing excessive water loss or respiratory alkalosis. In mammals, changes in airway smooth muscle tone may directs

Table 1 Summary of studies demonstrating innervation of airway smooth muscle in different species

Species	Cranial cholinergic (CC)	Cranial NANC	Spinal adrenergic (SA)
Actinopterygii			
Zebrafish			X_1 E_1
Goldfish			
Eel			E_5
Cod	E_6		X_6 C_6 R_6 I_6 E_6
Trout	X_7		
Wrasse	E_8		X_8 C_8 R_8 E_8 I_8
Polypterus bichir $^{\infty}$	X_2	X_2	C
Lepisosteus $^{\infty}$			X_{53}
Dipnoi			
Protopterus	V_9 $E_{3,4}$	V_{10} E_3	$I_{3,4}$
Lepidosiren	V_9	V_9	
Lissamphibia			
Rhinella marina	$V_{20?}$ C_{18} $E_{18,12}$	$V_{21,22}$ R_{12}	X_{17} $C_{12,13,11}$ $R_{11,13}$ $E_{18,13,12}$ $I_{18,12,13}$
Bufo vulgaris			E_{19} I_{19}
Necturus	V_{16}	R(v)I_{16}	I_{16}
Axolotyl	V_{16}	R(v)$_{16}$	I
Lepidosauria			
Tiliqua	X_{27} $E_{26,28,27}$ $C_{26,27}$ I_{27}	$R_{27,28}$	V_{26} I_{25} R_{28} C_{28}
Thamnophis	C_{23} E_{23}	R_{23}	I_{23} E_{23}
Testudinata			
Chelodina	E_{24}	L+$_{24}$	$X_{24,25}$ I_{24}
Aves			
Gallus	$E_{29,31}$ C_{29}	L_{31}	$I_{29,31}$ E_{31} X_{32}
Anas	E_{30}		
Mammalia			
Rat	E_{33}		E_{33}
Guinea pig	L+$_{48,49,51}$	L+$_{47,49}$ L$^{\S *}_{48,51}$	I_{33} L*$_{49}$ R_{49}
Rabbit	E_{34} C_{34}		C_{34} E_{34} I_{34}
Cat	C_{40}	R_{40} L$_{41}^{\dagger}$	L_{43}
Dog	E_{46} L+$_{46}$		$I^{\S}_{45}$ $R^{\S}_{44}$
Sheep	E_{35} L+$_{35}$	L_{35}	I_{35}
Cow (trachea)	L+$_{36}$ E_{36}	L_{37} I_{37}	
Baboon	E_{38}		E*$_{38}$ $I^{\S}_{38}$
Macaque (trachea)	E_{39} L+$_{39}$	L_{39}	$I^{\S}_{39}$
Human	L+$_{51}$ E_{50}	L_{51} E_{50}	0_{52}

Notes:
*, effect in trachea only;
§, only after pre-contraction;
†, effect not seen in trachea$_{42}$;
∞, innervation may also control striated muscle; X, nerves are present; V, non-classified cranial innervation; 0, innervation is probably not present; C, nerve stimulation causes contraction; R, nerve stimulation causes relaxation; L, local stimulation causes relaxation; L+, local stimulation causes contraction; I, pharmacological agent causes inhibition; E, pharmacological agent causes excitation. 1, Dumbarton et al. (2010); 2, Zaccone et al. (2007); 3, Johansen and Reite (1967); 4, Abrahamsson et al. (1979); 5, Lundin and Holmgren (1991); 6, Nilsson (1971); 7, Fahlen et al. (1965); 8, Fänge et al. (1976); 9, DeLaney et al. (1983); 10, Fishman et al. (1985); 11, Campbell and Duxson (1978); 12, Campbell (1971); 13, Holmgren and Campbell (1978); 14, Goldie et al. (1983); 15, Kobayashi and Yoda (1960); 16, Luckhardt and Carlson (1920); 17, McLean and Burnstock (1967b); 18, Wood and Burnstock (1967); 19, Shimada and Kobayasi (1966); 20, Campbell and McLean (1994); 21, Campbell et al. (1978); 22, Robinson et al. (1971); 23, Smith and Macintyre (1979); 24, Smith and Satchell (1987a); 25, Smith and Satchell (1987b); 26, McLean and Burnstock (1967a); 27, Burnstock and Wood (1967); 28, Berger (1973); 29, King and Cowie (1969); 30, Barnas et al. (1978); 31, Bhatla et al. (1980); 32, Bennet and Malmfors (1970); 33, Doidge and Satchell (1982); 34, Mustafa et al. (1982); 35, Sheller and Brigham (1982); 36, Cameron and Kirkpatrick (1977); 37, Cameron et al. (1983); 38, Middendorf and Russell (1980); 39, Olson et al. (1988); 40, Irvin et al. (1980); 41, Ito and Takeda (1982); 42, Don et al. (1988); 43, Altiere et al. (1984); 44, Cabezas et al. (1971); 45, Russell (1980); 46, Russell (1978); 47, Lundberg and Saria (1982); 48, Richardson and Bouchard (1975); 49, Szolcsányi and Barthó (1982); 50, Lundberg et al. (1983); 51, Taylor et al. (1984); 52, Undem and Potenzieri (2012); 53, Icardo et al. (2015).

air to reduce ventilation/perfusion inequality, protect the lungs from inhalant particles, and regulate alveolar volume at the expense of alveolar duct volume in terminal airways (Greaves et al. 1986). Smooth muscle development is extreme in marine mammals and may be related to the biomechanics or physiology of diving (Piscitelli et al. 2013).

Actinopterygii

Although the Actinopterygian swim bladder and lungs of other vertebrates likely evolved from a common ancestor with a primitive lung (Liem 1988), the diversification of the swim bladder in fishes is independent of lung evolution in tetrapods. Actinopterygian, physostomes maintain a connection called the pneumatic duct between the swim bladder and the foregut, whereas the swim bladder is anatomically isolated in physoclists (Fig. 1; Brainerd 2015). Among most physostomes, smooth muscle is present in part or all of the swim bladder wall (Alexander 1966; Smith and Croll 2011) or pneumatic duct. Active swim bladder deflation, called

the "Gasspuckreflex" or gas-spitting reflex, often involves contraction and relaxation of different smooth muscle tissues (Smith and Croll 2011; Zaccone et al. 2012a) and usually occurs before deep sounding, although some authors think this term should be reserved for gas loss due to decompression (Harvey et al. 1968). Some physostomes can also regulate swim bladder volume through secretion and reabsorption of gases and this is the sole mechanism of swim bladder inflation or deflation in physoclists (Smith and Croll 2011).

Smooth muscle is reported in tarpon (*Megalops*), especially in the non-respiratory portion of the bladder (Seymour et al. 2008). The lungs of some trahiras (*Hoplerythrinus*), obligate air-breathing Amazonian physostomes, have two well-defined layers of smooth muscle in the tunica media: a thin longitudinal internal layer and a thicker circular external layer, and a bundle of longitudinal smooth muscle fibers running the length of the swim bladder laterally (Cruz-Höfling et al. 1981). The lungs of arowanas (*Osteoglossum*), closely-related facultative air-breathers, are similar but lack the external longitudinal bundle (Cruz-Höfling et al. 1981).

In general, deflation of the swim bladder is mediated by output from the spinal autonomic nervous system (Nilsson 1972, 2009; Fänge 1983), although cranial autonomic innervation is also present (Zaccone et al. 2012b). Smooth muscle anatomy and innervation is comparatively well studied in the two-chambered swim bladder of the zebrafish (*Danio rerio*) (Fig. 1), which uses gas expulsion as the main mechanism of deflation. Smooth muscle in the zebrafish swim bladder consists of a sphincter between the bladder and esophagus and thick bands along the ventral surface of the anterior and along the sides of the posterior swim bladder chambers (which connects to the pneumatic duct) (Finney et al. 2006), such that relaxation of some muscle and contraction of others is necessary to raise the swim bladder pressure about 8 mm Hg higher than ambient an cause deflation (Robertson et al. 2008). The anterior chamber possesses a series of connective tissue folds which amplify the effect of smooth muscle contraction enabling 85% reductions in swim bladder volume in *in situ* studies (Dumbarton et al. 2010). *In vivo* pressure recordings and observation of *in vivo* and excised muscle contraction from noradrenaline stimulation (Dumbarton et al. 2010) show that the circumferential fibers in the anterior chamber contract after stimulation of β-adrenergic receptors to expel gas from the pneumatic duct (Dumbarton et al. 2010). Differences in adrenergic receptor type may make fine spinal autonomic control possible: Finney et al. (2006) detected choline acetyltransferase-immunoreactive somata only in the muscles of the esophagus, but tyrosine hydroxylase (HT) immunoreactive fibers were detected throughout the swim bladder itself. Furthermore, in the goldfish (*Carassius auratus*), HT and substance P immunoreactive fibers have been isolated by immunohistochemical methods in the craniodorsal part of the swim bladder, whereas the gas gland and pneumatic cells are reactive to vasointestinal peptide (VIP) (Zaccone et al. 2012b).

Even though direct expulsion of gas is not possible in physoclists, smooth muscle contraction and relaxation still regulates the amount of air in their swim bladders. Often, the absorptive epithelium is isolated from the main gas chamber by an oval constriction, commonly called a diaphragm. In toadfish, cod, and perch (Fänge 1983), the caliber of this oval constriction is determined by antagonistic circumferential and radial smooth muscle fibers. A functionally homologous structure exists in the wrasse (*Ctenolabrus*) (Smith and Croll 2011). Autonomic regulation of these structures is adrenergic, with α-receptors regulating the opening radial muscles and β-receptors regulating the closure circumferential muscles (Nilsson 2009). Swim bladder smooth muscle may have other functions other than expelling gases: early reports on sea horse physiology reported changes in swim bladder geometry with body position (Peters 1951) which may be mediated by smooth muscle.

Many actinopterygian fishes have striated muscle in their lungs or swim bladders. Striated muscle is found in the lungs of bichirs (Brainerd 1994a), and the swim bladders of *Lepisosteus*, *Amia* (*Notopterus*), and trahiras (*Erythrinus*) (Company and Rahn 1971; Crawford 1971; Liem 1989). There is no functional evidence that muscle helps to deflate the swim bladder *Lepisosteus*, but the striated muscle is positioned to collapse the bladder and is innervated by SA nerves. It is also surrounded by neuroepithelial bodies containing VIP that are positive to serotonin (Zaccone et al. 2012a; Icardo et al. 2015), consistent with SA control. The original addition of striated muscles in fish gas organs may have served to increase the speed or force of exhalation, provide active control of the gas organ for hydrostatic purposes, or to decouple ventilation from hormonal control. Other expiratory mechanisms are noted in physostomes: striated muscle is implicated in exhalation in *Notopterus*, *Lepisosteus*, and *Polypterus*, and *Arapaima* probably powers exhalation through buccal expansion (Greenwood and Liem 1984).

Lungfish (Dipnoi)

Smooth muscle has been reported in all three families of lungfish. In the Australian lungfish (*Neoceratodus*), Grigg (1965) reported smooth muscle in the transverse (primary) and reticulated (secondary) septa of the unpaired lung. In both septa, a layer of smooth muscle is sandwiched between layers of connective tissue (Grigg 1965). This smooth muscle takes the form of thickened pillars in the dorsoventrally oriented transverse septa, and forms thickened bands at the edges of the secondary septa. Smooth muscle structure similar to that of *Neoceratodus* has also been described in the African lungfish (*Protopterus*), the African lungfish (Klika 1967; Maina and Maloiy 1985), and sections of the paired lungs of the south American lungfish (*Lepidosiren*) stain positive for smooth muscle (Bishop and Foxon 1968).

Sarcopterygian fishes primarily use a two-stroke buccal pump, with inspiration powered by buccal floor muscles (Brainerd 1994b). Based on its anatomical position, Grigg (1965) concluded that smooth muscle, along with elastic tissues and hydrostatic pressure, was responsible for exhalation in *Neoceratodus*. Lung smooth muscle contraction likely maintains intrapulmonary pressure above atmospheric through the lung emptying phase (Johansen et al. 1967). Based on Grigg's work and radiological recordings, Bishop and Foxon (1968) hypothesized a similar role for smooth muscle in *Lepidosiren*. Studies of ventilation in *Protopterus* hypothesized that lung emptying was passive (McMahon 1969) however the smooth muscle has been shown to contract dramatically with acetylcholine (Johansen and Reite 1967), leading Johansen and Reite (1967) to hypothesize an expiratory function for smooth muscle in this taxon. Brainerd et al. (1993) found no increase in pleuroperitoneal pressure during exhalation in *Protopterus* suggesting that hypaxial muscle contraction has no role in lung emptying, but pulmonary smooth muscle lies inside the lung could assist lung emptying while increasing only intrapulmonary and not pleuroperitoneal pressure. Lung emptying is correlated with electromyographic recordings from the abdominal muscles, however, in aestivating *Protopterus aethiopicus* on land where lung pressure increases before exhalation (DeLaney and Fishman 1977). DeLaney and Fishman (1977) hypothesize that the abdominal contractions serve to replace hydrostatic pressure which normally assists lung emptying.

Lissamphibia

Smooth muscle is present in the middle layer of lissamphibian lungs, between the inner and outer epithelia. It is usually thin, consisting of only occasional cells in European tree frogs (*Hyla arborea*) but is very thick in *Amphiuma*, where it can be 50–180 µm in the lung wall and 1000 µm in the septa (Goniakowska-Witalifiska 1995). The lungs of caudates usually contain a great deal of smooth muscle (Czopek 1962) and have variable amounts of parenchymal elaboration. Cryptobranchids have large, poorly vascularized lungs with very little smooth muscle and do most gas exchange across the skin (Guimond and Hutchison 1976). Hydrostatic pressure and active contraction of the transversus abdominus musculature contribute to exhalation in this group (Brainerd 1999). In contrast, the aquatic salamanders of Sirenoidea rely on the lungs for gas exchange which have large amounts of smooth muscle (Czopek 1962). In *Amphiuma tridactylum*, smooth muscle is organized in the walls of primary septa, and in thick rings at the openings to secondary septa in the anterior lung, and in the wall of the lung in the middle and especially posterior sections (Stark-Vancs et al. 1984). Contraction of these muscles contracts the primary and secondary septa, pulling the lung walls together reducing the sizes of the septal openings. Treatment with acetylcholine causes lung collapse, especially in the posterior lung (Stark-Vancs et al. 1984; Fig. 2A).

In both genera of aquatic salamanders, *Siren* and *Amphiuma*, radiographic studies (Guimond and Hutchison 1974; Martin and Hutchison 1979) reveal that the lung fully collapses during deflation. Deflation and re-inflation occur evenly with the posterior section collapsing first and re-inflating last, suggesting that the smooth muscle in the posterior lung wall is active in exhalation (Martin and Hutchison 1979). The transversus abdominus is active during the second half of deflation, contributing to expiration (Brainerd and Dumka 1995; Brainerd and Monroy 1998; Brainerd 1999). Hydrostatic pressure may also contribute to deflation (Guimond and Hutchison 1973; Martin and Hutchison 1979). In contrast, Simons et al. (2000) argue that smooth muscle probably does not play a major role in exhalation in adult tiger salamanders (*Ambystoma tigrinum*) because exhalation is much faster than in *Amphiuma*.

Caecilians have smooth muscle in the external lung wall and in the septal walls (Maina and Maloiy 1988; Kuehne and Junqueira 2000). Smooth muscle may be important for exhalation in the two central American

species: *Dermophis mexicanus* (Carrier and Wake 1995; Bennett et al. 1999) and *Typhlonectes natans*, where pressure in the pseudo-trachea exceeds ambient pressure during exhalation (Prabha et al. 2000).

In frogs (Anurans), the degree of lung septation varies. Primary, secondary, and tertiary septa invested with smooth muscle divide the lungs of the cane toad (*Rhinella marina*) into nested partitions along the lung wall (Smith and Campbell 1976). Smooth muscle forms thick bundles at the open edge of each order of partition, smaller strips that run around the partitions at regular intervals, occasional longitudinal bundles that run perpendicular to the other strips and is scattered throughout the lung wall (Smith and Campbell 1976). Smith and Campbell (1976) hypothesize that smooth muscle contraction in the lung of *R. marina* functions to maintain the internal lung structure by closing the trabeculae (Fig. 3A), pulling the septa internally away from the lung wall, which is rigid due to intrapulmonary pressure. In contrast, Lawry (1999) hypothesized that contraction of septal smooth muscles would serve to collapse the septa, which are held rigid by hydrostatic pressure in blood vessels. Under this model, active pumping of pulmonary air could be accomplished by varying pulmonary blood pressure and visceral smooth muscle tone in the pulmonary septa (Lawry 1999).

Control of smooth muscle is best studied in *R. marina*, where cranial and sacral pathways join together into a vagosympathetic trunk that innervates the lung (Campbell et al. 1978). CC cause contraction of visceral lung muscle, and cranial NANC fibers cause smooth lung relaxation, using either NO, VIP, or ATP as a transmitter (Campbell and McLean 1994). SA postganglionic nerves, also running through the vagosympathetic trunk, cause contraction of septal edge musculature and relaxation of smooth muscles in the lung wall (Holmgren and Campbell 1978). An immunohistochemical study of nerve fibers before and after vagotomy in the same species reported 50% NANC, 25% cholinergic, and 25% adrenergic fibers (Campbell et al. 1978) with only the NANC fibers persisting after denervation, indicating that their cell bodies are intrapulmonary. The vagosympathetic trunk reaches muscle bundles through small myelinated and non-myelinated fibers at the septal margins. Distally, each muscle cell is innervated by two pairs of axons, one cholinergic and another adrenergic (Campbell et al. 1978).

Early experiments on mudpuppies (*Necturus*) found a marked inhibition of lung tone from the infusion of adrenaline and pituitrin (5-HT and vasopressin) (Luckhardt and Carlson 1920), consistent with SA and NANC inhibitory innervation. Further experiments further point to cranial inhibitory innervation: vagotomy also causes contraction of the lung in *Necturus*, which can be temporarily reversed by stimulation of the severed vagal nerve (Luckhardt and Carlson 1920). Vagotomy also causes lung contraction in the axolotl (*Ambystoma mexicanum*) and is inhibited by adrenaline (Luckhardt and Carlson 1920).

Although hypaxial exhalation is probably a synapomorphy of living tetrapods, lissamphibians display a diversity of pulmonary smooth muscle functions including exhalation in some Caudata and Gymnophiona (caecilians), and maintenance of internal lung structure in Anura. It is possible that contraction of the transversus abdominus muscle first acted in concert with smooth muscle contraction to effect lung emptying, eventually becoming the dominant exhalation mechanism in amniotes. In this case, perhaps smooth muscle and hypaxial musculature contribute differentially to exhalation depending on habitat and lung morphology in amphibians. The lengthy lungs of *Siren* may require smooth muscle contraction to empty completely, and perhaps smooth muscle contraction is more important for effective lung emptying in animals without well-developed ribs.

Non-avian reptiles

In general, the gas-exchanging parenchyma of reptile lungs consists of distal chambers, termed ediculae or faveoli, separated by first, second, and third order septa and rimmed by thick openings called trabeculae (Perry 1998) (Fig. 3). Thick smooth muscle bundles or myoelastic bundles are always found in the trabeculae (Fig. 3A, C). Modulation of smooth muscle contraction has been hypothesized to maintain internal lung structure at various degrees of inflation (Perry and Duncker 1978), or even contribute to intrapulmonary gas mixing (Daniels et al. 1994). Smooth muscle has been found in the trabecular rings of red-eared slider turtles (*Trachemys scripta*) (Perry 1978), loggerhead turtles (*Caretta caretta*) (Perry et al. 1989b), Australian snake-necked tortoises (*Chelodina longicollis*) (Smith and Satchell 1987a), tegus (*Tupinambis teguixin*) (Perry et al. 1989a), skinks (*Tiliqua rugosa*) (Burnstock and Wood 1967; McLean and Burnstock 1967a), Nile crocodiles (*Crocodylus niloticus*) (Perry 1988), geckos (*Gekko gecko*) (Perry and Duncker 1978). In *C. longicollis*, fewer smooth muscle fibers were observed in the thin-walled posterior lung (Smith and Satchell

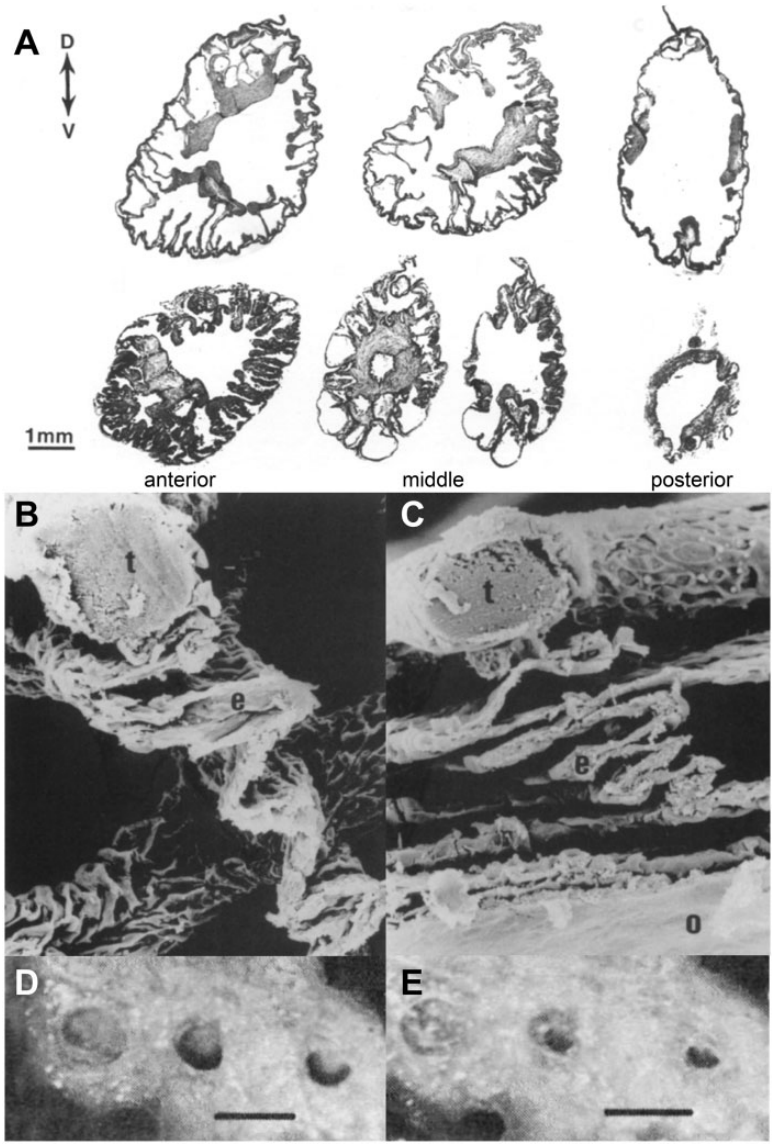

Fig. 2 Smooth muscle and lung contraction in selected species. **A**) The effect of acetylcholine-induced contraction on the lung of *Amphiuma tridactylum*. Sections of an inflated lung before (top) and after application of acetylcholine (below) show contraction of the lung surface and septum. From (Stark-Vancs et al. 1984). **B**) Lung inflation (left) and **C**) deflation (right) are accompanied by collapse of the interedicular septa (**E**) in central netted dragons (*Ctenophorus nuchalis*) from Daniels et al. (1994). **D**) Parabronchial cross-sectional area before and after (E) spontaneous smooth muscle contraction in goose (*Anser anser*) lungs. From Barnas et al. (1978).

1987a), compared with the anterior lung, where the parenchyma is thicker.

In addition, many non-avian reptiles display antagonistic smooth muscle groups in the walls of ediculae or faveoli (Fig. 3D, G), with the trabecular rim muscles positioned to hold the internal lung structure rigid and open, and perpendicular muscles in the walls of the trabecular septa positioned to collapse the septa (Perry 1988). Such muscular arrangement has been documented in *T. teguixin* (Perry et al. 1989a), *C. niloticus* (Perry 1988), the bull snake (*Pituophis sayi*) (Wallach 1998), and garter snake (*Thamnophis sirtalis*) (Pohunková and Hughes 1985). In the tegu, septal wall fibers run parallel (Fig. 3D) to the septal edge, but perpendicular (Fig. 3G) in the crocodile. Septal smooth musculature is observed in the black mamba snake (*Dendroaspis polylepis*) (Maina 1989), which probably also has trabecular rim smooth muscle. In contrast, no smooth muscle is found in the septal walls of the unicameral lungs of *T. rugosa* (Burnstock and Wood 1967). In species with antagonistic muscle groups, the ratio of trabecular to septal smooth muscle seems to increase with body size (Perry 1998), perhaps indicating that relatively greater force is required to distort larger trabeculae.

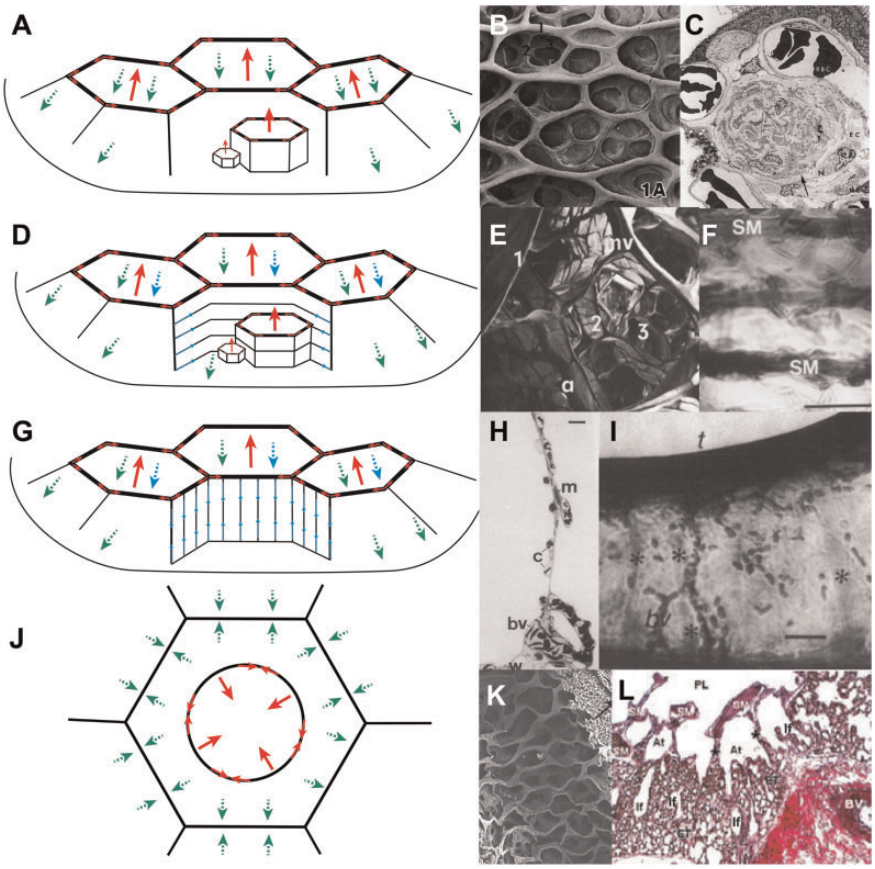

Fig. 3 Septal structure and smooth muscle in vertebrate lungs. **A**) Contraction of smooth muscle rings at the openings of lung trabecular (red arrows) would act to suspend the internal parenchymal network, acting against the surface tension of the lung wall (green arrows). This is the situation in rattlesnakes (*Crotalus viridis*) and blue-tongued skinks (*T. rugosa*). **B**) Scanning electron micrograph (SEM) of gas exchange tissue in the respiratory section of the lung *C. viridis*. **C**) Section across the trabecular ring in *C. viridis* with arrow pointing to the large bundle of smooth muscle. **D**) Contraction of additional longitudinal rings of interfaveolar or septal smooth muscle would act to collapse the septa. Condition in the cane toad (*R. marina*) (Smith and Campbell 1976) and tegu (*T. teguixin*) (Klemm et al. 1979; Perry et al. 1989b). **E**) Light micrograph of the lung walls in *R. marina* showing multiple levels of faveoli and smooth muscle bundles in the septal walls and trabeculae. **F**) Side view of an interfaveolar septum in a tegu lung showing longitudinal smooth muscle bands. **G**) Contraction of rings of interfaveolar or septal smooth muscle that lie perpendicular to the long axis of the faveoli would act to directly shorten each faveolus. Condition in Nile crocodiles (*C. niloticus*) (Perry 1988), Tokay geckos (*G. gecko*) (Perry et al. 1994), and green lizards (*Lacerta*) (Meban 1978). **H**) Transverse section through the interfaveolar septum of a gecko lung showing perpendicular smooth muscle bundles (m). **I**) Side view of an interedicular septum in a crocodile lung showing perpendicular smooth muscle bundles (*). **J**) In the parabronchi of birds, contraction of smooth muscle in the atrial rims opposes radial tension of collagen fibers in the parabronchial septum to hold the parabronchial structure in place. **K**) Smooth muscle rings guarding the entrance to atria in the parabronchi. **L**) Cross section of a parabronchus showing smooth muscle (SM) bundles lining the openings to atria (At). B and C from Luchtel and Kardong (1981), E from Smith and Campbell (1976), F from Perry et al. (1989b), H from Perry et al. (1994), I from Perry (1988), J modified from Maina et al. (2010b), K from Maina (2007), and L from Maina et al. (2010b).

Peristaltic waves of smooth muscle contraction have been observed in the lungs *T. scripta* (Carlson and Luckhardt 1920), *T. teguixin* (Scheid et al. 1977), and *G. gecko* (Perry and Duncker 1978), and have been hypothesized to function in intrapulmonary gas mixing (Donnelly and Woolcock 1977; Klemm et al. 1979; Hlastala et al. 1985; Perry et al. 1989a). It is also possible that trabecular smooth muscle tone changes during ventilation such that the internal lung lumen diameter remains constant as the lung wall expands, allowing each falveolus to function as a bellows during ventilation (Daniels et al. 1994; Fig. 2B, C). Trabecular rim and septal smooth muscle have been quantified in a few species of non-avian reptiles (Table 2). Stimulation of smooth muscle elicited much greater responses in the faveolar section of the lung of several species of *Thamnophis* (Smith and Macintyre 1979) than the saccular region, implicating either a structural or gas mixing function for the tissue in this taxon.

Few studies on the innervation of visceral smooth muscle in non-avian reptiles have been conducted,

Table 2 Quantitative data on smooth muscle in non-avian reptiles

Species	Lung tissue volumem L/kg body mass % of total lung volume	Trabecular smooth musclem L/kg body mass % of lung tissue	Septal smooth musclem L/kg body mass % of lung tissue	Trabecular/ septal SM
Varanus exanthematicus$_1$	9.12 (9%)	0.71 (8%)	0.09 (1%)	7.9 (89%)
Tupinambus teguixin (*nigropunctatus*)	3.39 (14.6%)	0.25 (7.3%)	0.25 (7.3%)	1.0 (50%)
Gekko gekko$_2$	2.3 (19%)	0.4 (17%)	0.05 (2%)	8.0 (89%)
Crocodylus niloticus$_3$	4.14 (11%)	0.3 (7%)	0.18 (4%)	1.7 (63%)

Source: Data from 1, Perry (1998); 2, Perry (1983); 3, Perry et al. (1994).

but results are consistent. In general, CC innervation is excitatory, cranial NANC innervation is inhibitory, while adrenergic α-receptors mediate contraction and β-receptors mediate inhibition (Berger 1973; Berger and Burnstock 1979). Typical responses are seen in a study of isolated lung strips from *C. longicollis*, where transmural stimulation or direct application of acetylcholine caused a rapid contraction of lung tissue, which was abolished by hyoscine (a muscarinic receptor antagonist), demonstrating cholinergic excitatory input mediated through muscarinic receptors (Smith and Satchell 1987b). Contraction caused by transmural stimulation persisted despite the presence of hyosine, implicating excitatory NANC or SA fibers in addition to the CC pathway (Smith and Satchell 1987a), while noradrenaline caused a β-receptor-mediated relaxation blocked by propranolol (Smith and Satchell 1987b). In whole-lung preparations of *Thamnophis*, vagal nerve stimulation at high frequency (20 Hz) caused contraction followed relaxations, while low frequency stimulation (2 Hz) caused relaxation only (Smith and Macintyre 1979). Atropine or hyoscine abolished the contraction, but not the relaxation, pointing to CC-mediated excitation and cranial NANC-mediated inhibition of airway smooth muscle in this group. Furthermore, ACh administration caused lung contraction that was prevented by atropine or hyoscine (Smith and Macintyre 1979). Although stimulation of the spinal efferent did not cause lung contraction, direct application of noradrenaline causes contraction followed by relaxation. The contraction and relaxation are blocked by α- and β-blockers and agonists, respectively (Smith and Macintyre 1979).

The rate of periodic smooth muscle contractions increases with asphyxia in turtles (Carlson and Luckhardt 1920) and increased CO_2 concentration while contractions are abolished in the absence of CO_2 (Scheid et al. 1977), supporting a gas mixing role for the tissue in non-avian reptiles. Intrapulmonary CO_2 receptors may influence smooth muscle: they have been documented throughout the lungs of *T. teguixin*, can respond at 1.3 Hz to CO_2, and the relationship between CO_2 and contraction is maintained after vagotomy (Scheid et al. 1977). Smooth muscle contraction also generates lung mechanoreceptor output (Scheid et al. 1977). Integration and control may occur in intrapulmonary ganglia, identified in the septal walls in *C. longicollis* (Smith and Satchell 1987a), the base of the lung of pond sliders (*Chrysemys picta*), *T. rugosa*, *Thamnophis* (Jones 1912; Burnstock and Wood 1967; McLean and Burnstock 1967b).

In the simple lung of *T. rugosa*, which has only one layer of smooth muscle, the response to vagal stimulation depends on smooth muscle tone: at high tone vagal stimulation generates a small contraction and large inhibition, whereas a large contraction and smaller inhibition are generated at low tone (Burnstock and Wood 1967). In this species, SA efferents join the vagus nerve, similar to the condition in *R. marina*. During sustained stimulation, the muscles started a slow relaxation after about 30 s (Burnstock and Wood 1967), indicating that some level of intrinsic control is present. α-receptors mediate excitatory SA innervation and β-receptors mediate inhibitory innervation (Berger 1973). Compared with *R. marina*, immunohistochemical staining techniques indicate that SA fibers innervate less fibers more densely, although large spatial variation is seen in both species (McLean and Burnstock 1967a, 1967b).

Aves

Smooth muscle is present throughout the avian lung and forms a uniform sheet lining the primary bronchus (King and Cowie 1969; Cook and King 1970). A network of spiral fiber bundles also encase the tertiary bronchi (parabronchi), and secondary bronchi, where they are thicker (King and Cowie 1969). Massive muscular sphincters surround the openings to the parabronchi and atria, which are in turn lined with more irregular smooth muscle bundles (King and Cowie 1969; Maina et al. 2010a). Muscle fibers in the primary bronchus run parallel to each other

and the long axis of the bronchus and muscle fibers and nerves are more densely packed than in the parabronchi (Cook and King 1970).

Although the innervation of airway smooth muscle in birds remains poorly studied, a few investigations yield general trends. King and Cowie (1969) demonstrated CC contractions from vagal stimulation that can be blocked with atropine and cause reductions in atrial calibers, and often complete atrial collapse. Application of catecholamines and adrenaline cause local airway dilation and constriction, respectively (King and Cowie 1969), although simple application of atropine is not enough to cause bronchodilation. The resting tone in bird bronchi seems therefore to be intrinsic and not dependent on CC input. Immunohistochemical investigations found few SA nerves in the trachea, many in the bronchi (most densely in the primary bronchus), and only occasional nerves in the air sacs (Bennet and Malmfors 1970; Bennett 1971). Local electrical stimulation causes relaxation in chickens (*Gallus gallus*), or contraction after the tissue is already relaxed with adrenergic drugs (Bhatla et al. 1980). The inhibitory response to electrical stimulation is unaffected by β-blockers (Bhatla et al. 1980), suggesting a cranial NANC inhibitory innervation.

King and Cowie (1969) hypothesized that control of smooth muscle in birds could cause intrapulmonary gas mixing or prevent a respiratory alkalosis during thermoregulatory hyperventilation by shunting lung gases away from the parabronchi, but Barnas et al. (1978) found no consistent response of smooth muscle tone or bronchial caliber from changes in CO_2 or O_2 concentration. Smooth muscle responses may have been blocked in this study, however, by the use of sodium pentobarbital for anesthetic, a known smooth muscle depressant (Altura and Altura 1975). ACh causes strong rhythmic contractions in the bronchi of *G. gallus* (King and Cowie 1969) that could facilitate pulmonary gas flow if they occur naturally.

In geese anesthetized with sodium pentobarbitol, Barnas et al. (1978) observed spontaneous increases in airway resistance due to contraction of the smooth muscle at the openings of parabronchi (Fig. 2D, E). Similar contractions could be elicited from mechanical or vagal stimulation (Barnas et al. 1978). Although the contractions caused by vagal stimulation were abolished by atropine, spontaneously-occurring contractions or contractions caused by mechanical stimulation could only be blocked with a drug cocktail (Barnas et al. 1978), suggesting that local control of airway smooth muscle probably contributes to spontaneous contraction.

Smooth muscle contraction may be critical for structural strength and maintenance of rigidity in the avian lung (Fig. 3J). In the tension-integrity (tensegrity) model, the inward tensing force provided by the atrial smooth muscle delimiting the lumen of the parabronchi acts against outward tension forces provided by elastic and collagen fibers rendering the entire parabronchial structure adaptively rigid (Maina 2007; Maina et al. 2010b). Contraction of parabronchial smooth muscle has also been hypothesized to collapse parabronchi during diving in penguins, preventing barotrauma (Ponganis et al. 2015).

Finally, bronchial smooth muscle may also contribute to pulmonary fluid mechanics in the bird lung. Inspiratory aerodynamic valving in the primary bronchus, thought to be a major determinant of unidirectional airflow (Banzett et al. 1987), is contingent on airway caliber in the segmentum accelerans and may therefore be regulated by contraction of smooth muscle (Wang et al. 1988) in this region. The segmentum accelerans dilates with elevated CO_2 levels (Wang et al. 1992), suggesting active control by smooth muscle.

Mammalia

In mammals, bronchial smooth muscle develops from the wrapping of mesenchyme around the growing ectoderm airways and is present in all of the adult conducting airways. It is not present in the gas-exchanging alveoli, but small smooth muscle fibers may penetrate as far as the free edge of alveolar walls toward the alveolar duct (Weibel 1984). In healthy human lungs, much more smooth muscle relative to airway diameter is present in the terminal airways [smooth muscle layer: bronchi diameter ratio is 0.0049 in segmental bronchi, 0.0084 in bronchobronchiolar border, 0.0118 in the membranous bronchioles, and 0.0215 in the terminal bronchioles (Ebina et al. 1990)], implying a much greater capacity to generate internal pressure in the terminal airways because luminal airway pressure is proportional to wall tension over airway radius. This allometry may indicate the importance of distal bronchial constriction.

One function of terminal bronchial smooth muscle may be to regulate alveolar expansion. An increase in smooth muscle tension at the level of the alveolar duct will constrict the alveolar entrance rings, increasing septal surface area and alveolar volume at the expense of alveolar duct volume (Greaves et al. 1986). Increased surface tension will counteract the effects of smooth muscle such that contraction of smooth muscle in the alveolar ducts and alveolar

surface tension may thus interact to regulate alveolar volume and surface area (Greaves et al. 1986). Analysis of feline airways marked with tantalum beads and frozen lungs show that alveolar and alveolar duct volume are proportional at high and medium volumes (Storey and Staub 1962), but alveolar volume decreases less than alveolar duct volume at the lowest lung volumes (Klingele and Staub 1970). At extremely low lung volumes, collapse of airways occurs before total collapse of alveoli (atelectasis), which is advantageous because it is substantially more difficult to re-inflate a lung after atelectasis than airway collapse (Greaves et al. 1986). Critical opening pressures are approximately 4 cm H_2O for airways, but at least 15–25 cm H_2O for alveoli (Greaves et al. 1986). The smooth muscle of the terminal bronchi might be important for keeping the alveoli inflated at very low lung volumes, reducing the work in re-inflation.

Smooth muscle may also regulate the distribution of ventilation by modulating airway caliber. Because the force of gravity pulls down on the lungs and pulmonary blood, the average alveolar size is greater in the apex than the base of the lung in humans at functional residual capacity (Glazier et al. 1966; Milic-Emili et al. 1966), although the difference is usually abolished at total lung capacity (Glenny and Robertson 2011). The base of the lung should therefore receive higher ventilatory flows than the apex because volume of the alveoli in the base of the lung will change in most during ventilation (Glenny and Robertson 2011). Early studies confirmed this hypothesis but found little evidence of gravitational gradients in the prone posture (Amis et al. 1984; Orphanidou et al. 1986). Later work on humans and animals using higher resolution imaging techniques found limited gravitational gradients, but largely only examined prone and supine postures (Hoffman and Chon 2005; Glenny and Robertson 2011). Because gravity acts to pool blood at the bottom of the lung, ventilation–perfusion matching may be driven largely passively by gravity or by similarity in the design of airway and blood vessel branching patterns (Glenny and Robertson 2011). Smooth muscle could attenuate the effect of gravity-induced ventilation heterogeneity by constricting airways in the base of the lung, such that more air would flow to the apex than otherwise. Constriction of the apical bronchi, conversely, may be advantageous in upright humans if the gravitationally-induced perfusion heterogeneity is greater than the ventilation heterogeneity. During exercise, the effect of gravity on ventilation distribution is reduced, but ventilation/perfusion inequality remains roughly constant (Melsom et al. 1999). Local control of pulmonary vascular resistance probably maintains ventilation–perfusion matching (Glenny and Robertson 2011), but modulation of local airway resistance from the action of airway smooth muscle may also be responsible.

The lungs of diving mammals are often reinforced with cartilage and smooth muscle (Kooyman 1973) (Fig. 4). Pinnipeds generally have less reinforcement than cetaceans (Denison and Kooyman 1973), and phocids have more smooth muscle in the smaller airways than otariids (Gray et al. 2006), although the final few millimeters of pinniped terminal airways are without cartilaginous reinforcement, and the alveoli are organized in lobules protected by thin stroma (Denison and Kooyman 1973). Smooth muscle reinforcement may be related to diving depth, as the deeper-diving Weddell seal (*Leptonychotes weddellii*) has greater amounts of smooth muscle than the crabeater seal (*Lobodon carcinophaga*) (Welsch and Drescher 1982). In the eared seals (Otariidae), cartilage lines the terminal airways all the way to the alveolar sacs, which are surrounded by thick stroma (20–30 μm), whereas terminal bronchi of sea otters and walruses have thick stroma and alveoli with and without cartilaginous ducts (Denison and Kooyman 1973). Compared with dog lungs, sea lion lungs collapse more completely at pressure (Denison et al. 1971) because cartilage maintains airway patency even at low pressures, allowing all of the air to escape the alveoli before conducting airways collapse. Most earless seals (phocids) exhale before deep dives (Piscitelli et al. 2013) and contraction of smooth muscle in terminal airways devoid of cartilage may enhance lung emptying (Welsch and Drescher 1982). Contraction of smooth muscle in phocid terminal airways may also help to re-inflate collapsed alveoli after a dive, a function provided by the thicker elastic stroma in other pinnipeds.

In cetaceans, terminal bronchiole anatomy is highly derived (Fig. 4). Delphinids replace longitudinal smooth muscle with a layer of elastic tissue (Wislocki 1948). In airways about 2 mm or smaller in diameter, smooth muscle organizes into discrete myoelastic sphincters (MES) between adjacent links of hyaline cartilage both of which run between two layers of antagonistic elastic tissue (Piscitelli et al. 2013). Contraction of the MES system in delphinids can separate the terminal bronchi into 25–40 independent chambers that may allow precise control of air movement (Wislocki 1942; Fanning and Harrison 1974; Piscitelli et al. 2013; Fig. 4B). Smooth muscle does not form MESs in baleen whales (Mysticetes)

and instead runs in a longitudinal layer through highly elastic terminal airways devoid of cartilaginous reinforcement (Piscitelli et al. 2013). Toothed whales (Odontocetes) have cartilage rings and MESs in the penultimate bronchi, and MSEs only in the terminal bronchi and alveolar ducts (Wislocki 1929; Fanning and Harrison 1974; Piscitelli et al. 2013). Functional hypotheses for MESs include: air trapping in the alveoli to continue gas exchange at depth, enhancement of rapid exhalation, protection of alveoli from barotrauma, prevention of eversion or prolapse of alveoli into the terminal bronchi, and the protection of intercartilaginous tissue from barotrauma (Piscitelli et al. 2013).

Innervation and control of pulmonary smooth muscle tissue is comparatively well studied in mammals. The right and left vagus nerves carry cholinergic and NANC input from nerves in the rostral pole of the nucleus ambiguus (the external formation) or the rostral portion of the dorsal motor nucleus (Undem and Potenzieri 2012). Preganglionic nerves leading to airways receive mainly glutamate-driven excitatory input from the nucleus tractus solitarius, pons, hypothalamus, and amygdala (Haxhiu et al. 2005). Integration, relay, and reflex mediation occurs in cranial autonomic ganglia along extra and intrapulmonary airways, which occur between nerve trunk branches of the vagus in the European ferret (*Mustela putorius*) and cat (*Felis catus*) (Dey et al. 1981; Undem and Potenzieri 2012). Although morphology and distribution of ganglia is species-specific, they are usually largest in the trachea and main-stem bronchi but are also dispersed into the intrapulmonary nerve plexuses (Undem and Potenzieri 2012).

Airway smooth muscle receives both excitatory and inhibitory cranial innervation; normal rhythmical contraction of the muscle is abolished after vagotomy (Jammes and Mei 1979; Undem and Potenzieri 2012). The majority of effects of CC stimulation are mediated by muscarinic cholinergic receptors: the M3 subtype mediates contraction (Eglen et al. 1996) while the role of M2 subtypes is unclear, but may tune the response of smooth muscle to the presence of additional transmitters and peptides and function in negative feedback (Undem and Potenzieri 2012). Inhibitory cranial innervation is largely of the NANC variety in mammals and wholly NANC in humans (Undem and Potenzieri 2012). NANC and CC innervation are both carried by the vagus nerve but use distinct pathways and ganglia and arise from distinct preganglionic nerves (Undem and Potenzieri 2012). In guinea pigs, ganglia for cholinergic contraction are located in the airway, whereas the NANC inhibitory ganglia are in the esophageal plexus (Canning and Undem 1993). NANC innervation of the bovine trachea is mimicked by VIP in tracheal strips (Cameron et al. 1983).

Smooth airway innervation of spinal origin is not as well studied and varies by species in mammals: stimulation of intrinsic bronchial adrenergic nerves causes smooth muscle relaxation or contraction in guinea pigs (*Cavia porcellus*), cats, and dogs (*Canis familiaris*), but not in humans, monkeys, rats (Doidge and Satchell 1982), or rabbits (Mustafa et al. 1982; Undem and Potenzieri 2012). Mammals with functional SA control express β1-adrenoreceptors and β2-adrenoreceptors while species without functional control largely express β2-adrenoreceptors. An SA inhibitory pathway may be important in mammals that frequently use panting for thermoregulation. Hyperventilation causes bronchoconstriction in many mammals (Koyama et al. 1992; Nogami et al. 1998; Suzuki and Freed 2000), and mammals that pant may need to attenuate this response to maintain a high lung compliance to reduce the work of panting, or to maintain adequate levels of gas exchange during hyperventilation. Sheep (*Ovis aries*) and dogs (Rahardja et al. 2011), but not guinea pigs (Richards 1970), rely extensively on panting for thermoregulation. The SA inhibitory response is confined to the trachea in guinea pigs (Doidge and Satchell 1982), however, and may have a different function in this species.

Spinal origin nerves dominate autonomic control system of the airway vasculature and can also regulate smooth muscle in both systems through epinephrine release from the adrenal medulla (Barnes 1986). GABA-mediated spinal preganglionic neurons (in the lateral horn of the thoracic spinal cord) receive input from various parts of the central nervous system and project to paravertebral ganglia and the stellate ganglion (Undem and Potenzieri 2012). In baboons, although α-adrenergic receptors could not be located in intrapulmonary airways, β-receptor adrenergic inhibition of contraction caused by histamines is found throughout the lung (Middendorf and Russell 1980). SA inhibition is demonstrated in the Macaque trachea only after contraction with phentolamine (an α-receptor agonist) (Olson et al. 1988).

There is evidence for heterogeneity in the innervation of mammal lungs—some of the lung are more often more affected by certain pathways than others. In sheep lungs, greater doses of catecholamines are needed to elicit relaxation in bronchi than the trachea (Sheller and Brigham 1982). In addition,

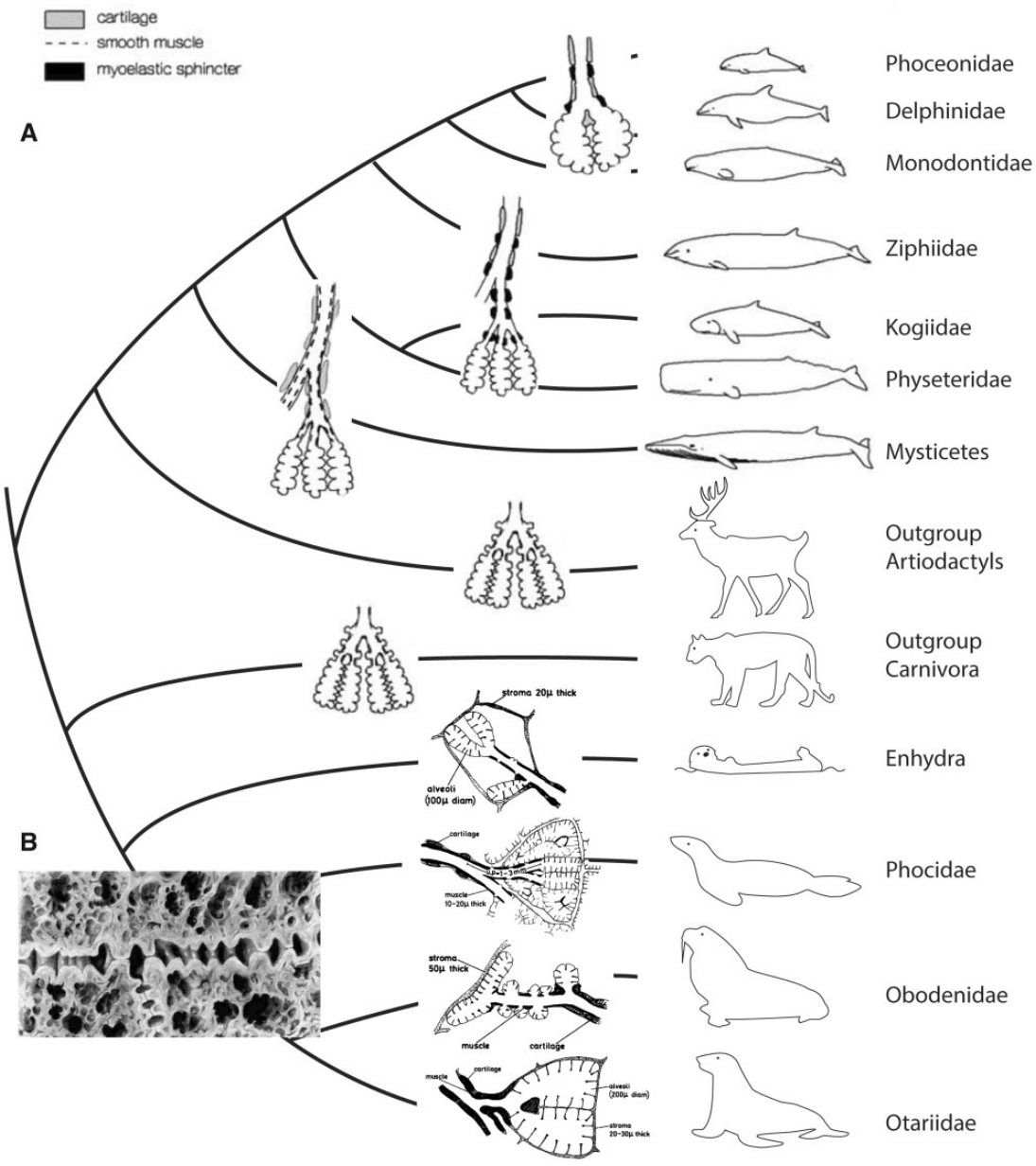

Fig. 4 Pulmonary smooth muscle adaptations along a cladogram of marine and semi-aquatic mammals. **A**) Airway cartilage and MSE reinforcement on a cladogram of Cetacea and marine carnivores. **B**) Extreme development of MSE in the bottlenose dolphin may enable segmentation of gas passages into 15–40 isolated chambers. Cladogram topology from Flynn et al. (2005) and Price et al. (2005).

muscle strips from the trachea, bronchi, and parenchyma contract with ACh, but tetrodotoxin (which blocks sodium channels in nerve cell membranes) only blocks contraction in the trachea and bronchi (Sheller and Brigham 1982). This led Sheller and Brigham (1982) to conclude that the parenchyma of sheep has no functional innervation and ACh caused smooth muscle contraction directly. Further study indicates similar response to ACh in first through fourth-order bronchi in sheep lungs, but contraction of smaller bronchi is more dependent on modulation from airway epithelium (Hatziefthimiou et al. 2009). Heterogeneity in receptor threshold may reflect tuning to the local receptor environment instead of functional heterogeneity of airway smooth muscle. For example, intrapulmonary and tracheal muscle strips from baboons responded similarly to ACh (Middendorf and Russell 1980). NANC innervation relaxes smooth muscle in the intrapulmonary airways (Irvin et al. 1980) and distal trachea (Ito and Takeda 1982) of the cat but not in the cervical trachea (Don et al. 1988). SA innervation is also heterogeneous: a study of airway innervation in five mammal species by Doidge and Satchell

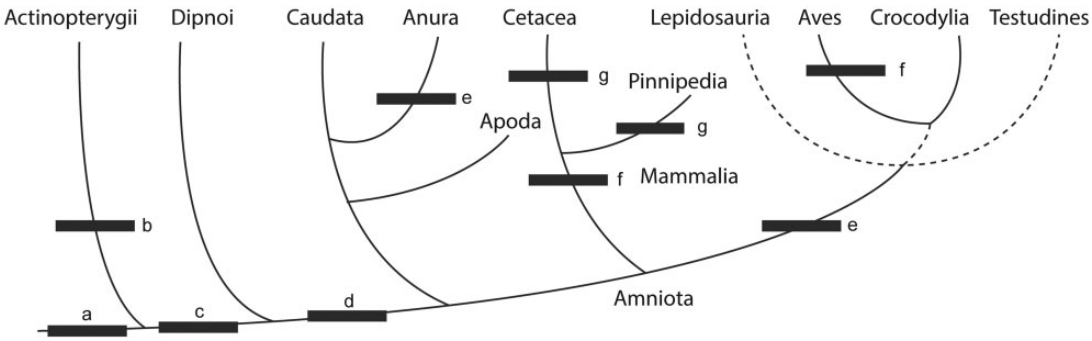

Fig. 5 Hypothesized events in pulmonary smooth muscle evolution superimposed on a vertebrate cladogram. **A**) Ancestral smooth muscle function of regulating tension and perhaps aiding exhalation and body orientation in the water column. **B**) Smooth muscle also redistributes air in the swim bladder and regulates the opening of the pneumatic duct in physostomes. **C**) Smooth muscle contraction maintains internal lung structure (see Fig. 2). **D**) Hypaxial muscle contraction supplements lung emptying. **E**) Antagonistic smooth muscle groups facilitate intrapulmonary gas mixing (see Figs. 2, 3B). **F**) Smooth muscle regulates airway caliber. **G**) Smooth muscle contributes to heavy airway reinforcement. Cladogram topology from Kardong (2014).

(1982) demonstrated tracheal SA inhibition in the guinea pig only, but bronchial inhibition in all species studied excluding rats (guinea pigs, rabbits, monkeys, humans).

Smooth muscle innervation

The innervation of airway smooth muscles also provides information about its evolution (Table 1). CC output is excitatory in all species studied, with ACh causing tissue contraction. This pattern is likely basal to vertebrates and controlled the ancestral air-filled organ. In the simplest scenario, CC input was the only external autonomic control, and relaxation of smooth muscle occurred intrinsically or at the cessation of cholinergic stimulation. This scenario is supported by the lack of cranial NANC innervation in actinopterygians, where SA innervation often stimulates contraction and relaxation of opposing muscle groups.

The main condition in tetrapods is for NANC nerves to mediate inhibition. Known exceptions are *Chelodina* turtles, with a purportedly excitatory NANC innervation (Smith and Satchell 1987a), guinea pigs, where cranial NANC pathways can cause tracheal contraction (Richardson and Bouchard 1975; Taylor et al. 1984), and dogs, where cranial NANC inhibitory pathways have not been demonstrated. Airway smooth muscle in dogs and sheep responds comparatively uniformly with relaxation to SA stimulation (Cabezas et al. 1971; Russell 1980; Sheller and Brigham 1982)—SA stimulation could serve to antagonize reflex bronchoconstriction resulting from hyperventilation in thermoregulatory panting.

SA pathways seem to represent a parallel control system for airway smooth muscle. While cranial nerves provide the main inhibition and excitation of smooth muscle throughout the lung or swim bladder, SA fibers seem to target specific regions of visceral smooth muscle. Heterogeneity of SA stimulation is demonstrated in the mucosa of swim bladders and *R. marina*, where SA nerves cause contraction of septal rim musculature and relaxation of lung wall musculature (Campbell and McLean 1994). Under this paradigm, studies of SA innervation of reptile lungs are needed to determine if antagonistic smooth muscle groups are differentially activated by SA innervation. In addition, study of the innervation of smooth muscle in marine mammals is needed. MESs are probably controlled by SA innervation, while cranial autonomic innervation controls smooth muscle tone throughout the lung.

Concluding remarks

Lung story short, airway smooth muscle has adapted from an ancestral role regulating hollow-organ tension (Fig. 5A) to take on new roles reflecting the morphological and physiological diversity of vertebrate respiratory structures. In actinopterygians, smooth muscle is involved in deflation and regulating gas absorption and secretion in the swim bladder (Fig. 5B). Smooth muscle contraction suspends the intrapulmonary structure (Fig. 5C) as well as potentially contributing to intrapulmonary mixing in many amphibians and non-avian reptiles (Fig. 5E) and is the primary regulator of airway caliber and ventilation distribution in birds and mammal (Fig. 5F) lungs. CC innervation is excitatory, cranial NANC innervation is largely inhibitory, and SA innervation causes species-specific heterogeneous responses. Further study of smooth muscle function may shed further light on vertebrate lung evolution

and the importance of lung micromechanics on pulmonary function.

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