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Evolution of the Cardiorespiratory System in Air-Breathing Fishes

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Abstract

Fishes have evolved a wide variety of air-breathing organs independently along different lineages. Of these air-breathing fishes, only some (e.g., mudskippers) venture onto land but the vast majority of them remain in water and use air as an oxygen source to different degrees. With the development of air-breathing capacity, the circulatory system of fishes has often been modified in various ways to accommodate blood to and from the newly developed air-breathing surface. However, most air-breathing fishes, except snakeheads and lungfishes, seem to lack the ability of separating O_2-rich effluent blood of the air-breathing organ from O_2-poor systemic venous blood during passage through the central cardiovascular system, although this has been inferred usually only from anatomical studies. Mudskippers attest to the fact that transition from aquatic to amphibious life is possible without restructuring the gross anatomy of the cardiorespiratory system, at least to some extent. Why then have some fish and ancestral vertebrates evolved the capacity of blood separation? The purpose of this paper is to review the current knowledge about the form and function of the cardiorespiratory system of selected species of air-breathing fishes (eel gobies, mudskippers, swamp eels, snakeheads and lungfishes, arranged in the order of higher specialization of the cardiorespiratory system) and discuss important issues relating to the topic.

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- swamp eel

1. Introduction

Transition from aquatic to terrestrial life during the Paleozoic era was one of the monumental steps in the evolution of vertebrates. Recent findings of new fossil records have substantially expanded our knowledge of osteological transformation during the fish-tetrapod transition that occurred in the Devonian period and inspired some new ideas about environmental settings in which the transition occurred (Clack 2002, 2007; Daeschler *et al*. 2006; Shubin *et al*. 2006; Clement and Long 2010; Laurin 2010; Niedźwiedzki *et al*. 2010). Nevertheless, those findings can give little insight into the question of how the cardiorespiratory system was transformed during the invasion of land by vertebrates. It is widely assumed that Devonian sarcopterygians that gave rise to tetrapods possessed the lungs and used them for aerial gas exchange while they were still inhabitants of the aquatic realm (Graham 1997; Liem *et al*. 2001). Subsequently, the lungs have become the major aerial gas exchange organ in tetrapods and birds (Cameron 1989; Nilsson 2010), with varying contributions of skin as a supplementary (in many amphibians) or in some exceptional cases, exclusive gas exchange surface (e.g., lungless salamanders, Boutilier 1990; Sheafor *et al*. 2000). In addition to the sarcopterygian-amphibian lineage, air breathing has independently arisen numerous times in the evolutionary history of bony fishes (Graham 1997). Many of the so-called ‘primitive’ bony fishes have a lung or lung-like gas bladder, such as polypterids, gars, bowfin and lungfishes. More advanced teleostean forms have developed a vast variety of air-breathing organs in different parts of the body, such as different segments of the alimentary canal (pharynx, esophagus, stomach or intestine), various types of outgrowth from the canal (e.g., respiratory swimbladders or suprabranchial chambers), specialized structures developing from the gills (e.g., labyrinthine organs) and the skin (Graham 1997).

The circulatory system of the Devonian vertebrates perhaps underwent considerable modifications in con-

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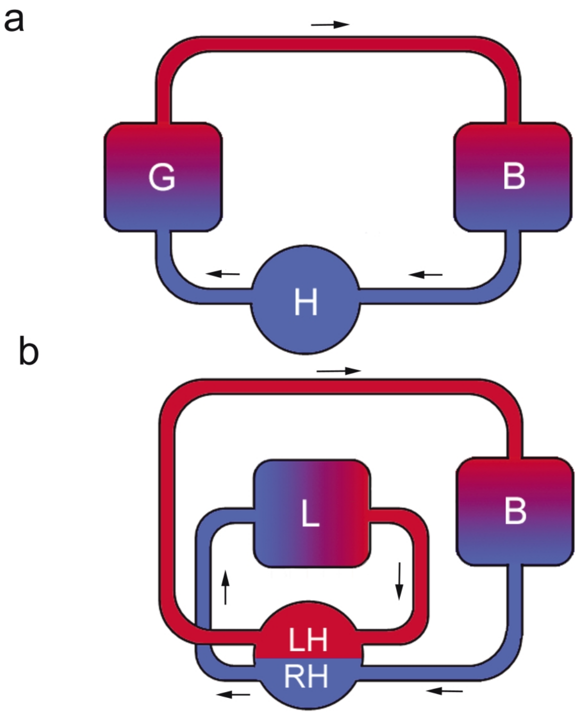

Fig. 1. Cardiovascular systems in fish (a) and adult birds and mammals (b). Red and blue vessels convey oxygen-rich arterialized blood and oxygen-poor venous blood, respectively. B, body; G, gills; H, heart; L, lungs; LH, left side of the heart; RH, right side of the heart. Arrows indicate directions of blood flow.

junction with the transition from aquatic to aerial mode of gas exchange (Liem *et al.* 2001), although this cannot be traced back from the fossil records but can only be inferred from comparison among extant animals. In all extant fishes, gills are placed upstream of and connected in series to the systemic bed in the vascular circuit (**Fig. 1a**). Fish hearts are undivided, consisting of a single atrium and a single ventricle that contain only systemic venous blood and impart kinetic energy only once during sequential perfusion of the gills and the systemic beds (Randall *et al.* 2002). This type of circulation is called a single circulatory system. On the other hand, in adult mammals and birds the pulmonary circuit is completely separated from the systemic circuit. The hearts of adult mammals and birds are completely divided into the right and left sides, in which the right atrium and ventricle deliver O_2-poor systemic venous blood to the pulmonary arteries with a relatively low pressure, whilst the left chambers eject O_2-rich pulmonary venous blood into a single systemic aorta with a much higher pressure to perfuse the systemic vascular bed (**Fig. 1b**, Randall *et al.* 2002). Thus, in adult mammals and birds the heart pressurizes the blood twice, once for pulmonary circulation and once again for systemic ejection, to complete a cycle through the body. This type of circulation is called a double or dual circulatory system. As the type and location of aerial respiratory organs varies widely among air-breathing fishes, so do their circulatory systems (Satchell 1976; Graham 1997). It is usually considered that bony fishes except dipnoans have not evolved any vascular specialization to allow the separation of two streams of blood through the central vascular pathways and therefore, the mixing of deoxygenated systemic venous blood and oxygenated effluent blood from the air-breathing organ is inevitable, although such a statement is in fact not always based on physiological determinations of blood gases and flow patterns on fully recovered, normally behaving animals.

Phylogenetic evolution from single circulation of fishes towards double circulation of tetrapods remains one of the central issues in zoology; however, it has not drawn as much interest in recent years as in a few decades ago. This short review summarizes currently available data on the anatomy and physiology of the cardiorespiratory system of selected species of air-breathing fishes that are relatively well investigated (eel gobies, mudskippers, swamp eels, snakeheads and lungfishes) and points out important knowledge gaps for future research directions in this field, with the hope that it will again stimulate the interest of comparative anatomists and physiologists. To achieve this goal, the first section will briefly illustrate the taxonomy and natural history of the selected species of air-breathing fishes to set the stage for subsequent discussion, the second section will deal with the anatomy of the cardiorespiratory system and the third section will explore the cardiorespiratory physiology of these fishes. Finally, the last section will discuss plausible scenarios for the evolution of the cardiorespiratory system in vertebrates and list selected topics, which are probably fruitful for future research in cardiorespiratory anatomy and physiology of air-breathing fishes.

2. Taxonomy and natural history of selected air-breathing fishes

2-1. Eel gobies

Eel gobies are cryptic, inconspicuous fish inhabiting the coastal and estuarine waters of the Indo-West Pacific region (Murdy 2011a). Eel gobies belong to the subfamily Amblyopinae (Gobiidae), which includes 23 species in 12 genera. Because of their cryptic nature, not much information is available on the natural history of eel gobies. Hora (1935) described that *Taenioides rubicundus* floated its head with gill chambers inflated at the surface of an aquarium containing foul water. Some 70 years later, the air-breathing capacity of another eel goby, *Odontamblyopus lacepedii*, was experimentally verified by Gonzales *et al.* (2006). Unlike syntopic mudskippers (*Beleophthalmus pectinirostris* and *Periophthalmus modestus*), *O.*

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lacepedii will not emerge from water during low tide, but remain inside burrows they excavate in mudflats. The burrows have two to seven openings on the mudflat surface and show a complex branching pattern, which probably varies with season (Gonzales *et al.* 2008a). Water filling the burrows is severely hypoxic (mean oxygen pressure (Po_2) 18 torr: 1 torr = 1 mmHg = 0.1333 kPa) as in mudskipper burrows and *O. lacepedii* was found to switch from water breathing to air breathing by gulping air shortly after the burrow openings emerged in low tide (Gonzales *et al.* 2006).

2-2. Mudskippers

Mudskippers typically refers to four genera of oxudercine gobies (Gobiidae: Oxudercinae) including *Boleophthalmus*, *Periophthalmodon*, *Periophthalmus*, and *Scartelaos*, but may also include other oxudercine gobies exhibiting an amphibious lifestyle (e.g., *Pseudapocryptes*). Murdy (2011b) recently reviewed the systematics of Oxudercinae. These fishes volitionally emerge from water and spend a significant portion of time on the mudflat surface (Clayton 1993; Ishimatsu and Gonzales 2011). Species of *Periophthalmus* and *Periophthalmodon* are usually more terrestrial, whilst *Boleophthalmus* and *Scartelaos* are more aquatic; neither of the latter two ventures far from water pools on an exposed mudflat during low tide and both appear to reside in their burrows during high tide, when the burrow openings are covered by the tide (Milward 1974; Zhang *et al.* 2000, 2003; Lee *et al.* 2005). Of the four mudskipper genera, species of *Periophthalmodon* and *Periophthalmus* are purely carnivorous (Sponder and Lauder 1981; Kruitwagen *et al.* 2007; Kutschera *et al.* 2008), whereas *Boleophthalmus* species are exclusively herbivorous (Yang *et al.* 2003). Less is known about the feeding habits of *Scartelaos*, but Milward (1974) reported them to be omnivorous. The distribution of mudskippers centers in the tropical Indo-Pacific. The known localities of *Boleophthalmus* and *Scartelaos* species are similar, ranging from the Persian Gulf throughout the East Asian countries and south to the tropical regions of Australia. The distribution of *Periophthalmodon* species is more restricted to Southeast Asia and mid-northern Australia. *Periophthalmus* species show the widest distribution among mudskippers, from the west coast of Africa to the Polynesian islands (Murdy 1989). Mudskippers have a high commercial value in some Asian countries (China, Korea and Vietnam) and are produced by aquaculture (Zhang *et al.* 1989; Bucholtz *et al.* 2009).

2-3. Swamp eels

Similar to oxudercine gobies, swamp eels show a wide spectrum of transition from aquatic to amphibious lifestyle within one taxonomic group. Swamp eels belong to the family Synbranchidae, which consists of four genera (*Macrotrema*, one species; *Ophisternon*, six; *Synbranchus*, two; *Monopterus*, eight: note that *Amphipnous* is now regarded as the subgenus of *Monopterus*, Nelson 2006). Liem (1987) reported that *Macrotrema caligans* is purely aquatic and exclusively water-breathing, *Ophisternon* and *Synbranchus* are facultative air-breathers and *Monopterus* is an obligate air-breathing fish, even though we are unaware of any publication on the respiratory physiology of *Macrotrema caligans* or *Ophisternon* species. Swamp eels are widely distributed in tropical and subtropical regions from West Africa, Asia, Australia, Mexico and Central and Southern America (Nelson 2006). These fishes often occur in swamps or marshy areas, where burrowing and amphibious habits are commonly displayed. Swamp eels lack paired fins and the dorsal and anal fins are reduced to skin folds, which are generally confluent with the much reduced caudal fin. Another peculiar feature of swamp eels is that their gill opening is confined to a single slit to the ventral body wall, except *Macrotrema*, in which the gill opening is more extensive and reaches to the lateral sides of the body (Rosen and Greenwood 1976). *Monopterus* and *Ophisternon* are consumed in Asian countries such as China, Indonesia and Vietnam and are produced by aquaculture (Sieu *et al.* 2009).

2-4. Snakeheads

Snakeheads are freshwater air-breathing fishes of the family Channidae, to which two genera (*Channa*, 26 species and *Parachanna*, three species) belong (Nelson 2006). Snakeheads inhabit stagnant freshwater ponds and creeks and their distribution is mainly limited to Asia (*Channa*) and Africa (*Parachanna*). Snakeheads are purely carnivorous, feeding on small fish, frogs, crayfish and other aquatic animals. *Channa argus* is the most northern species among the member of Channidae, inhabiting Northeast China, Siberia, and Korea (Matsubara 1963), but has been recently found to populate the United States and reproduce there (Landis and Lapointe 2010). Some snakeheads attain a body length of 1.2 m (Nelson 2006). Snakeheads have a high commercial value in Southeast Asian countries where the fish are produced by aquaculture (Poulsen *et al.* 2008).

2-5. Lungfishes

Lungfishes assume a key position in the research into the evolution from aquatic to terrestrial life in vertebrates. Together with the coelacanth, lungfishes are the only living sarcopterygian (lobe-finned) fishes and are probably the closest living relatives of the tetrapods (Blair and Hedges 2005; Hallström and Janke 2009; Shan and Gras 2011). The extant lungfishes include

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three genera, *Neoceratodus* (one species), *Protopterus* (four species) and *Lepidosiren* (one species) inhabiting freshwaters of Australia, Africa and South America, respectively (Jørgensen and Joss 2011). All lungfishes have a lung and use it for breathing air but the relative importance of aerial gas exchange in respiration varies between the genera (Burggren and Johansen 1986). Thus, Australian lungfish is a facultative air-breather practicing air breathing only during swimming activity or when the water is hypoxic. In contrast, African and South American lungfishes are obligate air-breathers and must breathe air even in normoxic water, although this has been questioned recently for *Protopterus aethiopicus* by Mlewa *et al.* (2007) on the basis of behavioral monitoring by radio telemetry. It remains to be seen whether the study site of Mlewa *et al.* (2007), where the water seems to be rarely hypoxic (Oduor *et al.* 2003), is typical for *P. aethiopicus*. There is no evidence for active emersion from the water for any lungfish species. On the other hand, it is well known that African and South American lungfishes both aestivate in mud during droughts. *Protopterus* spp. secrete mucus to form an underground cocoon (Mlewa *et al.* 2011), whereas *Lepidosiren* does not (de Almeida-Val *et al.* 2011). *Neoceratodus* does not aestivate (Kind 2011). African lungfishes are valued food fish and some major populations are under a threat of over-exploitation (Mlewa *et al.* 2011)

3. Anatomy of the cardiorespiratory system in air-breathing fishes

3-1. Eel gobies

The gills of *Odontamblyopus lacepedii* appear to be well-suited for aquatic respiration and lack any morphological specializations for aerial gas exchange; each gill arch contains two rows of gill filaments (holobranch, **Figs. 2a, b**), which in turn bear well-developed secondary lamellae on either side, as in ordinary water-breathing teleosts (**Fig. 2d**). The eel goby lacks a specialized accessory respiratory organ for air breathing but has developed a dense capillary network in the inner opercular epithelia (**Fig. 3**, Gonzales *et al.* 2008b). The cardiovascular anatomy of *O. lacepedii* basically retains the typical circulatory pattern of teleosts (**Fig. 1a**). A single ventral aorta emanates from the bulbus arteriosus, giving rise to four pairs of afferent branchial arteries, each of which subdivides into afferent filamental arteries. The afferent filamental arteries supply blood to secondary lamellae (**Figs. 2c, d**). After traversing the gills, oxygenated blood is collected into four pairs of the efferent branchial arteries to perfuse the systemic circuit. There is no vascular shunt connecting the afferent and efferent sides of the gills. Respiratory capillaries on the inner epithelia of the opercular cavity (**Figs. 3b, c**) are supplied from the effluent blood from the 1st and 2nd gill arches and drained by the anterior cardinal vein, which also drains venous blood from the head. The anterior cardinal vein unites with the posterior cardinal vein (returning venous blood from the trunk) to form the ductus Cuvieri, which opens into the sinus venosus together with the hepatic vein. Thus, O_2-rich blood from the air-breathing organ will probably mix with systemic venous blood before and during its passage through the heart.

3-2. Mudskippers

Similar to eel gobies, mudskippers lack specialized accessory respiratory organs (Graham 1976). Their gas exchange surface is composed of epithelia lining the buccal, pharyngeal and opercular cavities, the gills and the skin. The lack of specialized respiratory organs is not unique to mudskippers but prevalent among marine amphibious, air-breathing fish, such as blennies (Graham 1976). The suprabranchial chamber, which was reported for *Periophthalmodon schlosseri* by Yadav *et al.* (1990) and for another oxudercine *Pseudapocryptes lanceolatus* (=*Pseudapocryptes elongatus*, the latter is the valid scientific name of the species, see Ferraris 1995) by Yadav and Singh (1989), appears to be no more than a simple expansion of the buccopharyngeal cavity (Gonzales and Ishimatsu, unpublished observation) and, in structural complexity, is nothing like the suprabranchial chambers of the freshwater air-breathing fishes (e.g., *Channa* spp., Graham 1997 and see Subsection 3-4 Snakeheads).

Mudskippers retain the basic gill plan seen in purely aquatic teleosts, as in eel gobies. However, the gills of mudskippers show various degrees of development, from well-developed gills in more aquatic *Scartelaos* and *Boleophthalmus* to highly atrophied morphology, seen in *Periophthalmodon* and *Periophthalmus* species (Tamura and Moriyama 1976; Graham 1997; Graham *et al.* 2007). Gill filaments of *Periophthalmodon* and *Periophthalmus* are short, sparsely spaced and twisted (*Periophthalmus*) or frequently bifurcated (*Periophthalmodon*, Low *et al.* 1988; Gonzales *et al.* 2011). The secondary lamellae of *Periophthalmodon schlosseri* are covered by thick epithelia and frequently fused together (Schöttle 1931; Low *et al.* 1988; Wilson *et al.* 1999; Graham *et al.* 2007), to the extent that led Wilson *et al.* (1999) to conclude that the gill lamellae of the fish are generally ineffective for gas exchange.

It is generally held that the gross anatomy of the central vascular system in mudskippers is identical to that seen in typical teleosts. However, there are a few papers that have reported anomalous vascular connections in mudskippers. For instance, Schöttle (1931) reported that the ventral aorta of *Boleophthalmus viridis* (this scientific name is no longer valid, possibly *Scartelaos histophorus*) extends further anteriorly after giving off the first afferent branchial arteries and

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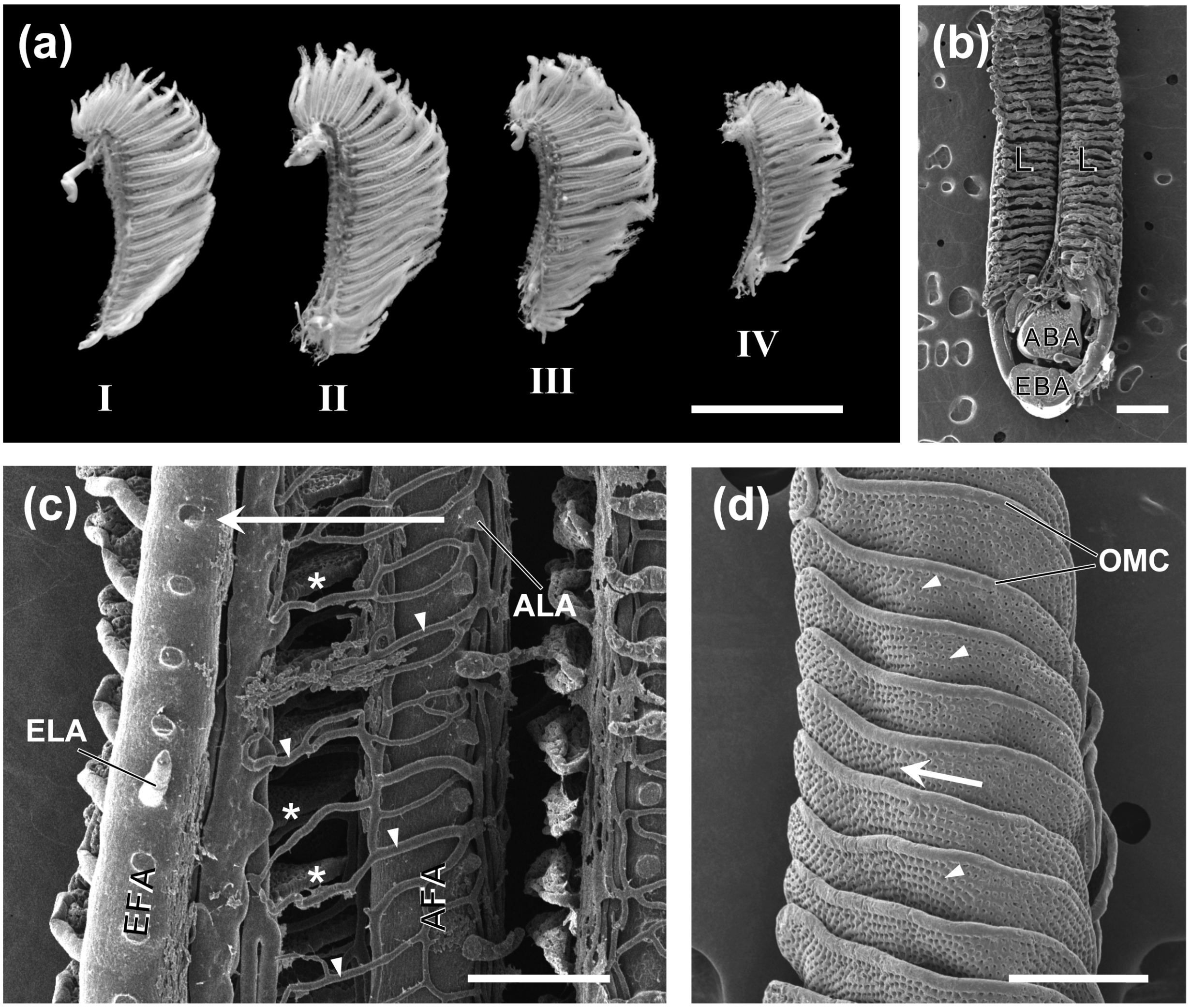

Fig. 2. Gill vasculature of *Odontamblyopus lacepedii*. Corrosion casts showing the four intact branchial arches (I–IV) of the left gills (a) and vascular details of the filaments and lamellae (b–d). A pair of filaments (holobranch) bears regularly spaced, well-developed lamellae (b). A network of vessels is apparent in the afferent but not efferent filament (c). Several lamellae have been removed from the single filament (hemibranch) to expose the intrafilamental circuit (arrowheads in c). The pillar cells (holes in the lamellae denoted by arrowheads in d) are arranged more or less in parallel with the outer marginal channel (OMC, d). Asterisks in (c) denote the discontinuous inner marginal channels (IMCs) of the lamellae. Arrows in (c and d) indicate the direction of blood flow. ABA afferent branchial artery; AFA afferent filamental artery; ALA afferent lamellar arteriole; EBA efferent branchial artery; EFA efferent filamental artery; ELA efferent lamellar areriole; L lamella. Scale bars: (a) 1 cm; (b) 500 μm; (c, d) 200 μm. With kind permission from Springer Science + Business Media: <*Environmental Biology of Fishes*, Respiratory vasculatures of the intertidal air-breathing eel goby, *Odontamblyopus lacepedii* (Gobiidae: Amblyopinae), **82**, 2008, 341–351, Gonzales *et al*., Fig. 3>.

bifurcates to become the hyomandibular arteries, which supply the respiratory surfaces of the buccopharyngeal and opercular cavities. Similarly, Niva *et al*. (1981) reported the presence of the bucco-opercular artery branching off from the first afferent branchial artery in *Boleophthalmus boddarti*, which is not shown in the Schöttle's figure for the same species. In addition, Das (1934) described that the first afferent branchial artery in *Pseudapocryptes elongatus* sends off the bucco-opercular artery at about one third of its origin from the ventral aorta.

In spite of the general retention of a teleostean circulatory pattern at a gross anatomical level, more subtle modifications are evident in the mudskipper's circulatory system at microscopic levels. The most obvious is rich vascularization of the inner epithelia of the bucco-opercular cavity and the skin. The capillary density in the inner epithelia of the bucco-opercular cavity is so high in *Periophthalmodon schlosseri* that a surface view of its vascular casts looks as if there is no

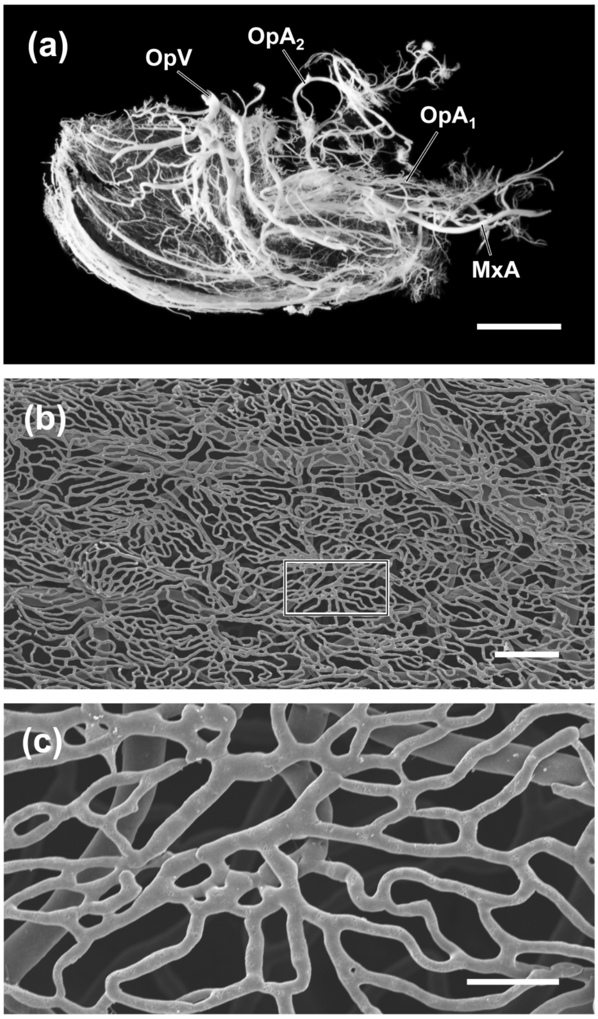

Fig. 3. Lateral view of the corrosion cast of the right operculum of *Odontamblyopus lacepedii*, anterior to the right (a). The inner epithelial surface of the operculum is overlaid by a dense network of capillary vessels (b). The boxed area in (b) is shown in a higher magnification SEM photograph in (c). MxA maxillary artery; OpA1 opercular artery from the anterior end of the 1st efferent branchial artery; OpA2 opercular artery from the distal end of the 1st efferent branchial artery; OpV opercular vein. Scale bars: (a) 5 mm; (b) 200 μm; (c) 50 μm. With kind permission from Springer Science + Business Media: <*Environmental Biology of Fishes*, Respiratory vasculatures of the intertidal air-breathing eel goby, *Odontamblyopus lacepedii* (Gobiidae: Amblyopinae), **82**, 2008, 341–351, Gonzales *et al.*, Fig. 4>.

space between the neighboring capillaries (**Fig. 4**). In addition, the entire length of the branchial arches and the leading, but not trailing, edge of the filaments are supplied by a dense network of capillary vessels (**Fig. 5**). **Figure 6** compares the respiratory capillary density of the tongue (a), palate (b) and inner operculum (c) in a purely water-breathing goby, *Acanthogobius hasta*, a facultative air-breathing eel goby, *Odontamblyopus lacepedii* and two species of mudskippers (*Periophthalmus modestus* and *Periophthalmodon schlosseri*, Gonzales *et al.* 2011). Increasing air-breathing capability is reflected by the progressively higher density of the capillaries in these epithelia, culminating in *Periophthalmodon schlosseri*, which has nearly the same capillary density on all these epithelia. Zhang (2000) examined the histology of the respiratory epithelia in oxudercine gobies and demonstrated rich vascularization of the inner epithelium of the operculum in *Boleophthalmus boddarti*, *B. dussumieri*, *B. pectinirostris*, *Oxuderces dentatus*, *Periophthalmodon septemradiatus*, *P. schlosseri*, *Periophthalmus chrysospilos*, *P. modestus*, *Pseudapocryptes elongatus*, and *Scartelaos histophorus*.

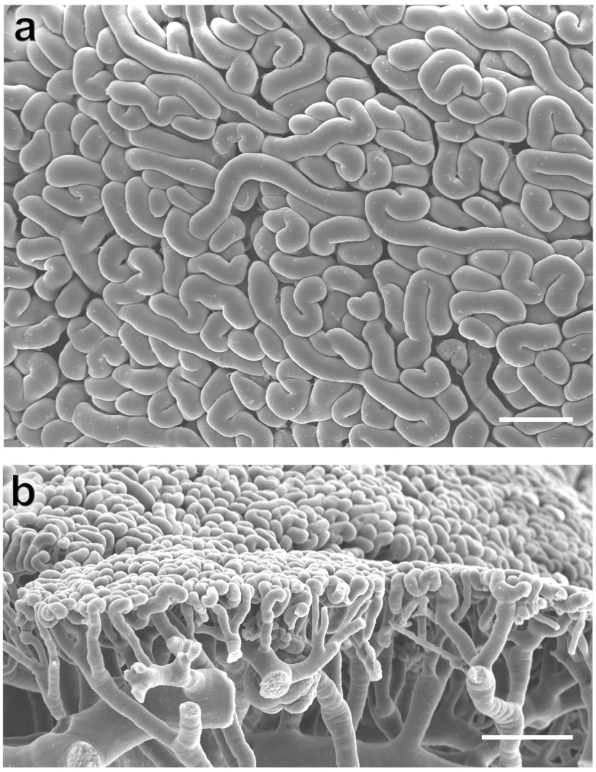

Fig. 4. Corrosion casts showing the surface (a) and transverse (b) views of the capillary network covering the inner operculum of *Periophthalmodon schlosseri*. In transverse view, the capillaries are shown connected to an underlying intermeshed network of arteries and veins. Each capillary forms a spiral coil as it emerges from the surface (b). Bars 20 μm (a), 50 μm (b). Reprinted with permission of John Wiley & Sons, Inc. from *Journal of Morphology*, **272**, Gonzales *et al.*, Gross and fine anatomy of the respiratory vasculature of the mudskipper, *Periophthalmodon schlosseri* (Gobiidae: Oxudercinae), 629–640, Fig. 5, © 2011, Wiley-Liss, Inc., a Wiley Company.

Periophthalmodon schlosseri shows a specialized microvascular arrangement of the secondary lamellae. The vascular pathway of the secondary lamellae con-

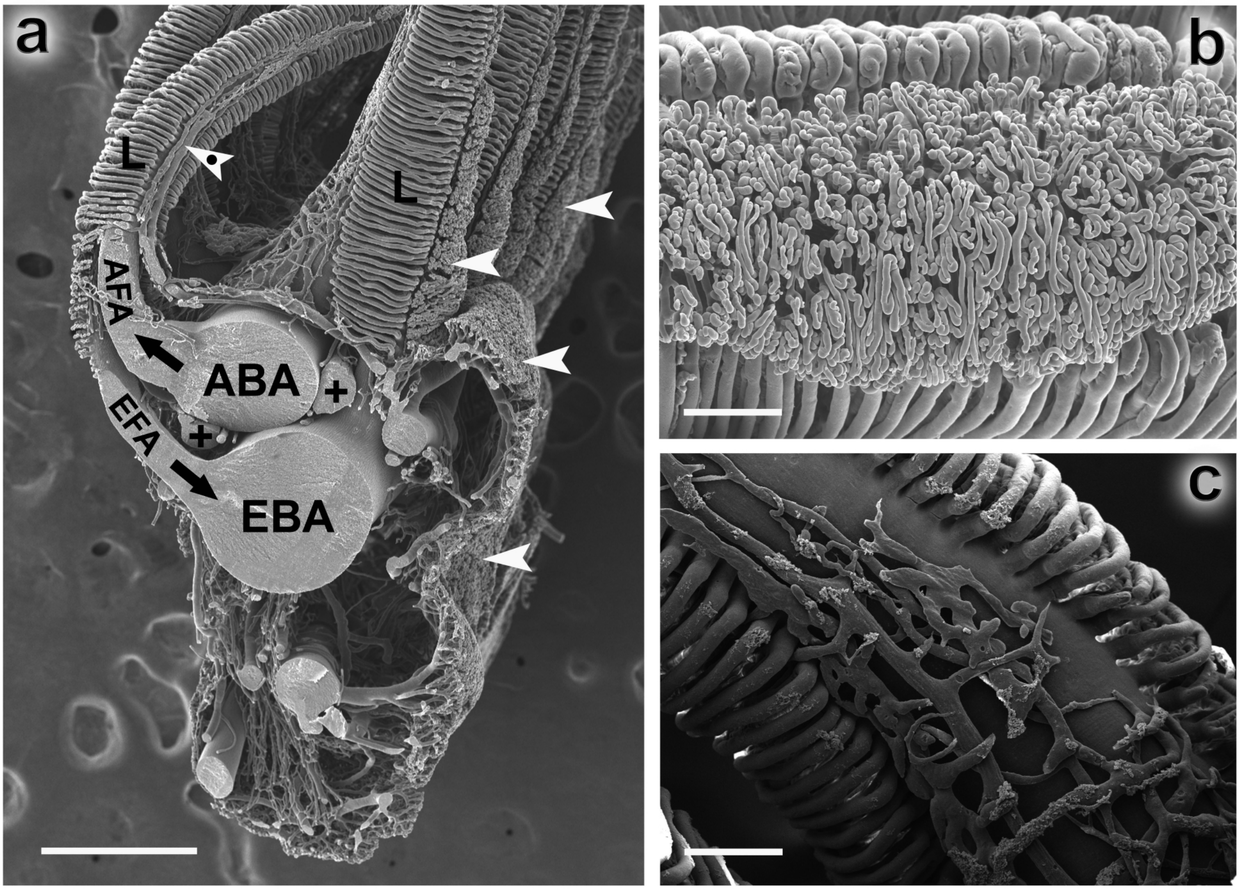

Fig. 5. Cross-section through a corrosion cast of the right first branchial arch in *Periophthalmodon schlosseri* (a). Surface views of the leading (b) and trailing (c) edges of a filament are shown with a higher magnification. The branchial arch and leading edge of the filaments are supplied by a dense network of capillary vessels (white arrowheads in a), while no such capillary network was observed on the trailing edge (white arrowhead with black dot inside in a). Plus signs are the branchial veins. Black arrows in (a) indicate the direction of blood flow. Bars 500 μm (a), 100 μm (b, c). ABA afferent branchial artery; AFA afferent filamental artery; EBA efferent branchial artery; EFA efferent filamental artery; L lamella. Reprinted with permission of John Wiley & Sons, Inc. from *Journal of Morphology*, **272**, Gonzales *et al*., Gross and fine anatomy of the respiratory vasculature of the mudskipper, *Periophthalmodon schlosseri* (Gobiidae: Oxudercinae), 629–640, Fig. 2, © 2011, Wiley-Liss, Inc., a Wiley Company.

sists of 5–8 parallel blood vessels (**Fig. 7c**), rather than forming lamellar sinusoids (narrow vascular spaces delineated by pillar cells), as seen in most teleosts (see Olson 2002; Evans *et al*. 2005). The internal diameter of the lamellar vessels of *P. schlosseri* (14.1 ± 2.5 μm) is larger than the size of the red blood cells (11.1 ± 8.8 μm) and would therefore impose less vascular resistance to blood than typical lamellar sinusoids. In aquatic fishes with typical secondary lamellae, the erythrocytes are squeezed, deformed and meander during their transit through the lamellar sinusoids, possibly to enhance oxygen uptake (Nilsson *et al*. 1995). During transit through the gills, 20 to 40% of the ventral aortic pressure is lost in typical water-breathing fishes (Olson and Farrell 2006). The channelization of the lamellar vessels in *P. schlosseri*, together with interlamellar fusion and thickening of the lamellar epithelia, would also help maintain sufficient blood flow to perfuse the aerial respiratory capillaries and the systemic beds, both located downstream of the lamellae, when the fish breathes air (Gonzales *et al*. 2011). Similar channelization of lamellar vasculature has also been reported in a few freshwater air-breathing fishes (e.g., *Amia calva*, Olson 1981; *Anabas testudineus*, Olson *et al*. 1986; *Lepisosteus oculatus*, Smatresk and Cameron 1982). It is currently unknown whether other mudskippers have similar vascular specialization in their lamellae.

The mudskipper skin is in general richly vascularized, although capillary density in the outermost layer of the epidermis varies between species and also among body parts within a species. Skin capillaries are distributed in higher densities and with shorter diffusion distance over more exposed parts of the body than in

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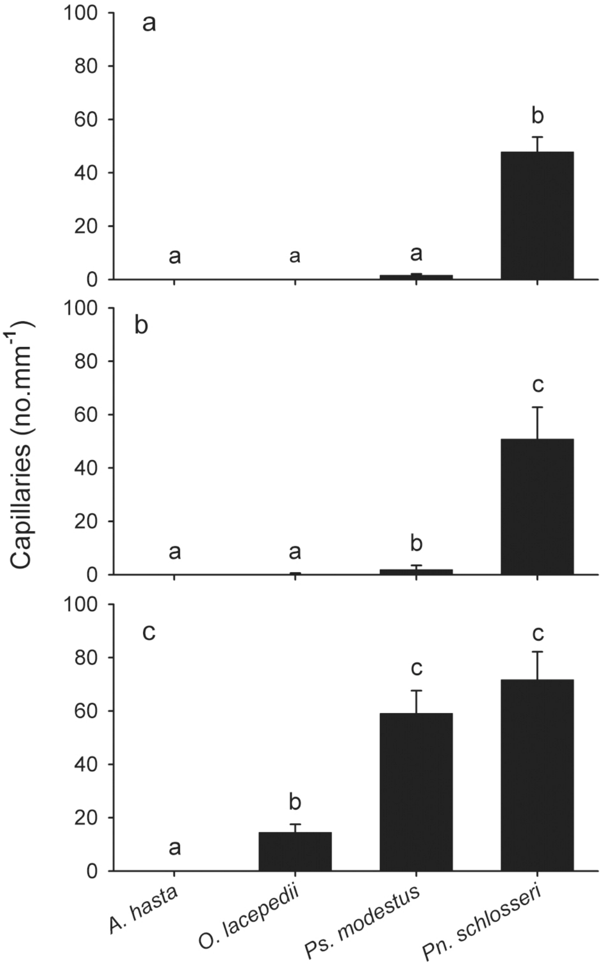

Fig. 6. Comparison of capillary densities within the 0–10 μm diffusion distance range in the tongue (a), palate (b) and operculum (c) among four species of gobies. *Acanthogobius hasta* is a non-air breather, *Odontamblyopus lacepedii* is a facultative air breather and *Periophthalmus modestus* and *Periophthalmodon schlosseri* are amphibious mudskippers. Values are mean + s.d. for 3 individuals. Different letters above the bars in each panel indicate significant differences between species in capillary density in respective epithelia. Reprinted with permission of John Wiley & Sons, Inc. from *Journal of Morphology*, **272**, Gonzales *et al*., Gross and fine anatomy of the respiratory vasculature of the mudskipper, *Periophthalmodon schlosseri* (Gobiidae: Oxudercinae), 629–640, Fig. 9, © 2011, Wiley-Liss, Inc., a Wiley Company.

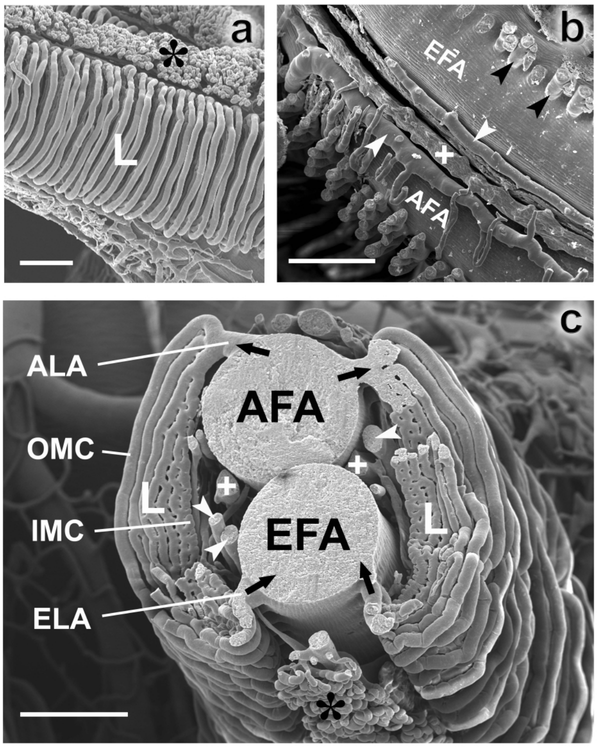

Fig. 7. A corrosion cast of *Periophthalmodon schlosseri* to show a filament of the left second branchial arch showing the lamellae covering one side of the filament (a) and the interlamellar circuit when lamellae were removed (b). A cross-section through a filament shows the vascular details of the arterio-arterial and interlamellar circuits (c). Plus signs in (b) and (c) are filamental veins; white arrowheads in (b) and (c) are interlamellar vessels of unknown origin; black arrowheads in (b) are the efferent lamellar arterioles; asterisks in (a) and (c) are capillaries in the leading edge of the filament. Black arrows in (c) indicate the direction of blood flow. Bars 100 μm (a–c). AFA afferent filamental artery; ALA afferent lamellar arteriole; EFA efferent filamental artery; ELA efferent lamellar arteriole; IMC inner marginal channel; L lamella; OMC outer marginal channel. Reprinted with permission of John Wiley & Sons, Inc. from *Journal of Morphology*, **272**, Gonzales *et al*., Gross and fine anatomy of the respiratory vasculature of the mudskipper, *Periophthalmodon schlosseri* (Gobiidae: Oxudercinae), 629–640, Fig. 3, © 2011, Wiley-Liss, Inc., a Wiley Company.

ventral surfaces, which are in contact with the mud when the mudskippers emerge (Park *et al*. 2006; Zhang *et al*. 2000, 2003). In *Boleophthalmus* and to a lesser extent in *Scartelaos*, the entire body surface, except the ventral surface, is studded with characteristic pappillary protrusions called “dermal bulges”. An artery runs through the dermis into each dermal bulge at its center and sends off radially arranged capillaries. Effluent blood from the dermal bulge is drained by a vein running parallel to the artery (Schöttle 1931; Zhang *et al*. 2000; Park *et al*. 2003). *Periophthalmodon* or *Periophthalmus* lacks a dermal bulge over their skin.

Respiratory capillaries of the bucco-opercular cavity and the skin are believed to originate from the systemic arteries such that systemic beds and aerial respiratory beds are connected in parallel (Schöttle 1931). In this design, the aerial respiratory capillaries are supplied with arterial blood having the same Po_2 and oxygen content as systemic capillaries would receive, which may be considered as physiologically ineffective. Oxygenated blood from the respiratory epithelia is drained by systemic veins and therefore, likely mixed

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with deoxygenated systemic venous blood before returning to the heart.

3-3. Swamp eels

The main aerial respiratory surface resides in the buccopharyngeal and opercular epithelia in *Monopterus* swamp eels (Munshi *et al.* 1989, 1990). Several papers have stated that *Synbranchus marmoratus* uses the gills for aerial respiration (Carter and Beadle 1931; Johansen 1966; Graham and Baird 1984) but this has never been confirmed by blood gas analysis. As is generally the case in other air-breathing fishes, the gills become atrophied with increasing reliance on air breathing. Thus, exclusively water-breathing *Macrotrema caligans* has four pairs of holobranchs resembling those of purely aquatic teleosts (Liem 1987; Rosen and Greenwood 1976), as does facultative air-breathing *Synbranchus* (Rosen and Greenwood 1976), whilst the filaments are arranged alternately along the length of a gill arch in *Synbranchus* (Liem 1987). Species of *Monopterus* show different degrees of reductions in gill dimension; the most advanced stage of gill specialization is seen in *Monopterus boueti*, in which "the gill filament becomes a small, finger-like appendage devoid of secondary filaments (=lamellae, Liem 1987)". Rosen and Greenwood (1976) also stated "*Monopterus boueti* has no free gill filaments on any arch; the 1st to 3rd arches each carrying a narrow flange of tissue, the flange on the 1st arch being the smallest". Munshi *et al.* (1990) showed that *Monopterus cuchia* has filaments or filamental-like structures only on the 2nd and 3rd gill arches, but Liem's diagram indicates that the gill filaments also occur on the 1st gill arch (**Fig. 8**).

Cardiovascular anatomy has been described in detail only for *Monopterus ablus* (Wu and Liu 1943; Liem 1961, **Fig. 8**) and *Monopterus cuchia* (Munshi *et al.* 1990). Rosen and Greenwood (1976) presented simplified diagrams of the central vascular system of *Synbranchus marmoratus*, *Synbranchus madeirae*, *Ophisternon afrum*, *Monopterus albus*, *Monopterus cuchia* and *Monopterus boueti* (see Subsection 6-1 Anatomical issues). A consistent deviation of the synbranchid vasculatures from the typical teleost design is the occurrence of the hyoidean artery branching off from the elongate ventral aorta to supply the respiratory vasculature within the mouth. Thus, the respiratory capillaries of this region are perfused directly with ventral aortic blood (similar to the descriptions for *Boleophthalmus viridis*, *Boleophthalmus boddarti* and *Pseudapocryptes elongatus*, see Subsection 3-2 Mudskippers). Even more notable is the presence of large thoroughfare (shunt) vessels in the 4th gill arch in all *Monopterus* species, which form direct conduits between the ventral and dorsal aortae. To my knowledge, a direct vascular connection between the two aortae has been described only for these *Monopterus* species among teleosts and possibly, for *Electrophorus electricus* (Carter 1935, but see also Subsection 3-5 Lungfishes). Oxygenated blood from the aerial respiratory surface is drained by the anterior

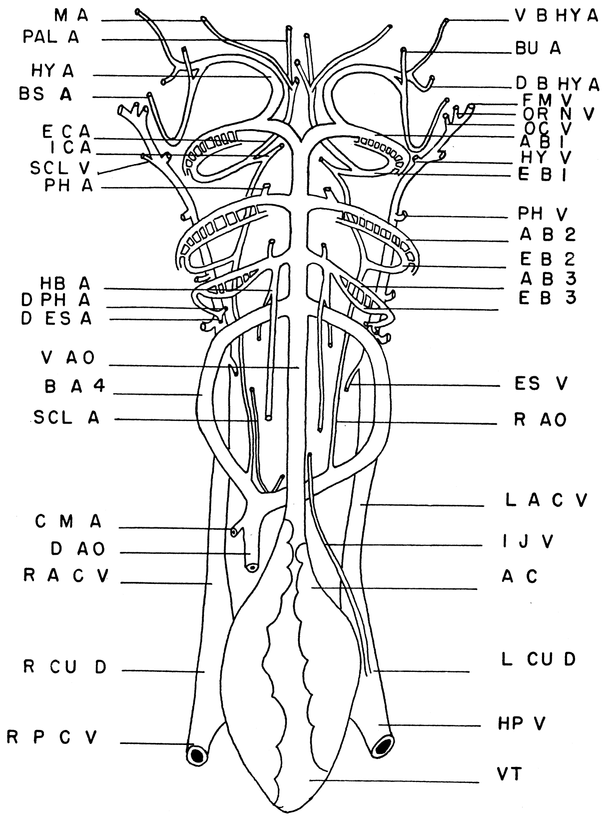

Fig. 8. The branchial and cranial blood vessels of *Fluta alba* (=*Monopterus albus*, Rosen and Greenwood 1976). Ventral view. AB1-3 1st to 3rd afferent branchial arteries; AC auriculae cordis; BA4 4th branchial artery; BS A branchiostegal artery, BU A buccal artery; CMA coeliacomesenteric artery; D AO dorsal aorta; D B HY A dorsal branch of hyoidean artery; D ES A dorsal esophageal artery; D PH A dorsal pharyngeal artery; EB1-3 1st to 3rd efferent branchial arteries; E C A external carotid artery; ES V esophageal vein; F M V facialis-mandibularis vein; HB A hypobranchial artery; HP V hepatic vein; HY A hyoidean artery; HY V hyoidean vein; I C A internal carotid artery; I J V inferior jugular vein; L A C V left anterior cardinal vein; L CU D left Cuvierian duct; MA mandibular artery; OC V occipital vein; OR N V orbitonasal vein; PAL V palatine vein; PH A pharyngeal artery; PH V pharyngeal vein; RAC V right anterior cardinal vein; R AO radix aorta; R CU D right Cuvierian duct; RPCV right posterior cardinal vein; SCL A subclavian artery; SCL V subclavian vein; V AO ventral aorta; V BH A ventral branch of hyoidean artery; VT ventricle. Reprinted with permission of John Wiley & Sons, Inc. from *Journal of Morphology*, **108**, Liem, Tetrapod parallelisms and other features in the functional morphology of the blood vascular system of *Fluta alba* Zuiew (pisces: Teleostei), 131–143, Fig. 3, © 2005, Wiley-Liss, Inc., a Wiley Company.

cardinal vein and the inferior jugular vein as in *Odontamblyopus*, *Periophthalmodon* and *Channa*. Munshi *et al*. (1990) demonstrated for *Monopterus cuchia* that afferent and efferent filamental arteries are connected by single channel capillaries or several parallel capillaries supplied by the lamellar arterioles.

The cardiac anatomy of *Monopterus albus* was described by Liem (1961) and Lai *et al*. (1998) and that of *Monopterus cuchia* by Munshi and Mishra (1974). The heart of all *Monopterus* species is situated far posterior to the usual position of teleost hearts at the level of the pectoral girdle and therefore has an unusually long ventral aorta. Liem (1961) described that the fish has three sino-atrial valves in *Monopterus albus* but Lai *et al*. (1998) denied it for the same species. Sino-atrial valves are absent from *Monopterus cuchia* (Munshi and Mishra 1974). Liem (1961) stated “A rather distinct dorsal median groove seems to divide the sinus venosus partly into two halves”, which was later confirmed by Munshi and Mishra (1974). The atrium of *Monopterus* has abnormal morphologies located dorsal to and surrounding the bulbus arteriosus. It is unknown whether these peculiarities have any functional significance.

3-4. Snakeheads

The air-breathing organ of snakeheads forms a pair of diverticula, often called suprabranchial chambers, located in the dorsal side of the buccopharyngeal cavity (**Fig. 9**, Ishimatsu 1982; Liem 1984). The organ is protected dorsally by the heavy, bony plates of the skull and posterolaterally communicates with the opercular cavities. Into the lumen of the suprabranchial chambers, three pairs of cartilaginous processes project, anteroposteriorly from the parasphenoid, hyomandibular and epibranchial of the 1st gill arch (**Fig. 9a**). Among them, the hyomandibular process is the largest and almost completely traverses the lumen of the organs (**Fig. 9b**). The respiratory surface of the organ is studded with small papilla-like projections, which increase in number and complexity with fish growth. In addition, the aerial respiratory surface extends to the roof of the buccal cavity anterior to the suprabranchial chambers and on the surface of the tongue (Ishimatsu and Itazawa 1983a). The gill filaments are shorter compared with those of the strictly water-breathing fishes (**Fig. 10**). The 1st to 3rd gill arches are larger and composed of complete holobranchs, while the 4th arch is much smaller and the inner hemibranch of the 4th arch is rudimentary in *Channa argus*, *Channa maculata* (Ishimatsu *et al*. 1979), and *Channa marulius* (Olson *et al*. 1994). In comparison, the 4th gill arch of facultative air-breathing *Channa punctata* is anatomically similar to the other three arches, although smaller in size (Olson *et al*. 1994).

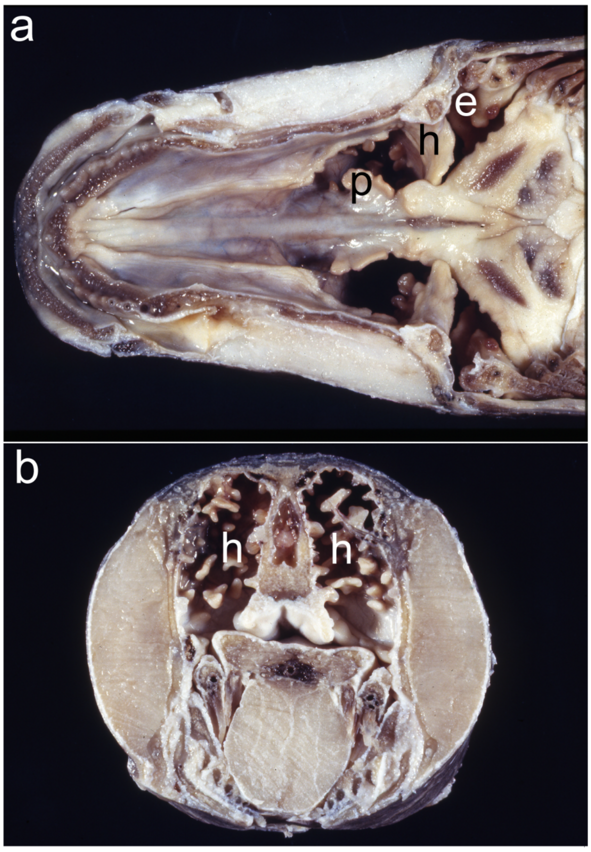

Fig. 9. Horizontal (palatine view, a) and cross (frontal view, b) sections of the head of *Channa argus* to show the suprabranchial chambers. Note that cartilaginous processes from the parasphenoid (p), hyomandibular (h) and the epibranchial of the 1st gill arch (e) projecting into the chambers (a). The inner margin of the hyomandibular processes (h) abuts to the inner wall of the chambers and thereby divides the cavity when the operculum is adducted (b). The capillary network extends to the palatine region anterior to the chambers and the surface of the tongue. Original photographs.

The central cardiovascular system of *Channa* species shows several unique deviations from typical teleost design (**Fig. 11a**). Most notable is the presence of two (anterior and posterior) ventral aortae (**Figs. 11a, b**, Ishimatsu *et al*. 1979; Ishimatsu and Itazawa 1983a; Munshi *et al*. 1994). Equally remarkable is the almost complete separation of the respiratory and systemic circuits on the arterial side. Thus, the vascular route originating from the anterior ventral aorta takes on the majority of the respiratory role, whereas the posterior vascular pathway is specialized almost as a conduit between the ventral and dorsal aortae in snakeheads. The anterior ventral aorta gives off the afferent branchial arteries of the 1st and 2nd arches, which exclusively perfuse the capillary bed of the air-breathing organ but have no vascular connection with the systemic arteries. On the other hand, the posterior ventral

Fig. 10. Lateral views of the corrosion casts of the left gill arches of *Channa argus*. From top to bottom, the 1st to 4th gill arches. Note the much smaller size of the 4th gill arch. Original photograph.

aorta sends blood to the 3rd and 4th afferent branchial arteries, which after passing through gill arch vasculatures, supply the entire systemic arterial system. The description by Munshi *et al.* (1994) on the postero-ventral and antero-dorsal origins of the posterior and anterior ventral aortae, respectively from the bulbus arteriosus, seems to be due to misinterpretation of their casts (Ishimatsu, personal observation). Earlier descriptions also support the dorsal origin of the ventral aorta to the 3rd and 4th gill arches (Das and Saxena 1956; Marathe and Kulkarni 1957). Other morphological specializations of the *Channa* heart include absence of the sino-atrial valves, the highly trabeculate nature of the ventricle and muscular ridges running longitudinally on the wall of the bulbus arteriosus (**Fig. 11b**, Ishimatsu and Itazawa 1983a).

Respiratory vasculature of the air-breathing organ originates from the 1st and 2nd efferent branchial arteries; the 1st one irrigates the antero-dorsal and lateral surfaces of the organ, while the 2nd one supplies the postero-ventral surfaces (Ishimatsu *et al.* 1979). Each terminal respiratory capillary forms a coiled spiral with several loops parallel to the respiratory surface (Olson *et al.* 1994). Blood oxygenated in the air-breathing organ is drained by tributaries of the anterior cardinal vein (**Fig. 11a**), which also drains systemic vascular beds of the head. The inferior jugular vein drains the respiratory surface on the tongue (Ishimatsu and Itazawa 1983a; Munshi *et al.* 1994).

At microscopic levels, the lamellar vasculature of the 1st to 3rd gill arches is modified into parallel channels, as in *Periophthalmodon schlosseri* (see **Fig. 7**). The filaments in the 4th gill arch of obligate air-breathing snakeheads (*Channa argus*, *Channa maculata* and *Channa marulius*) are atrophied to the extent that the inner hemibranch bears only very short filaments with nearly no secondary lamellae and has direct vascular connections (shunts) between the afferent and efferent branchial arteries (Ishimatsu *et al.* 1979; Olson *et al.* 1994). The 4th-arch gill vasculature of facultative air-breathing *C. punctata* was reported to lack shunt vessels (Munshi *et al.* 1994).

3-5. Lungfishes

Lungfishes are distinct from most other living air-breathing fishes in that they possess "true" lungs (Graham 1997; Perry *et al.* 2001). The lungs of all lungfishes are positioned dorsal to the alimentary canal as a single (in *Neoceratodus*) or paired (in *Protopterus* and *Lepidosiren*) sac (Goodrich 1958; Maina 1987; Graham 1997). The pneumatic duct opens in the ventral wall of the esophagus and runs dorsally round the right side of the esophagus. Internally, the lungs are subdivided into numerous compartments or alveoli by progressively dividing septa and trabeculae (Grigg 1965; Hughes and Weibel 1976).

Neoceratodus has four pairs of holobranchs and a pseudobranch on each side of the head, enclosed by an operculum. Each holobranch bears well-developed gill filaments, which carry densely spaced secondary lamellae on either side (Gannon *et al.* 1983). The gill microvasculature of *Neoceratodus* is reminiscent of that reported for elasmobranches; each afferent filamental artery delivers blood to a flattened plexus of interconnecting vessels, the corpus cavernosum, from which the afferent lamellar arterioles feed the lamellar sinusoids of the well-developed secondary lamellae (Gannon *et al.* 1983). The efferent branchial arteries of all four gill arches of *N. forsteri* unite to form the dorsal aorta and other major arteries of the head. In addition, the pulmonary artery originates from the 4th efferent branchial artery (i.e., 6th aortic arch) of either side (Goodrich 1958). Parallel to the curved course of the pneumatic duct described above, the left pulmonary artery from the 4th left efferent branchial artery loops around the esophagus before it reaches the ventral wall of the lung. The 4th right pulmonary artery branches off from the right efferent branchial artery to perfuse the dorsal wall of the lung. The two pulmonary veins drain the lung, join to form the common pulmonary vein running down on the right side of the esophagus and empty into the left side of the atrium (Grigg 1965; Gannon *et al.* 1983).

The gills of *Protopterus* are remarkably specialized

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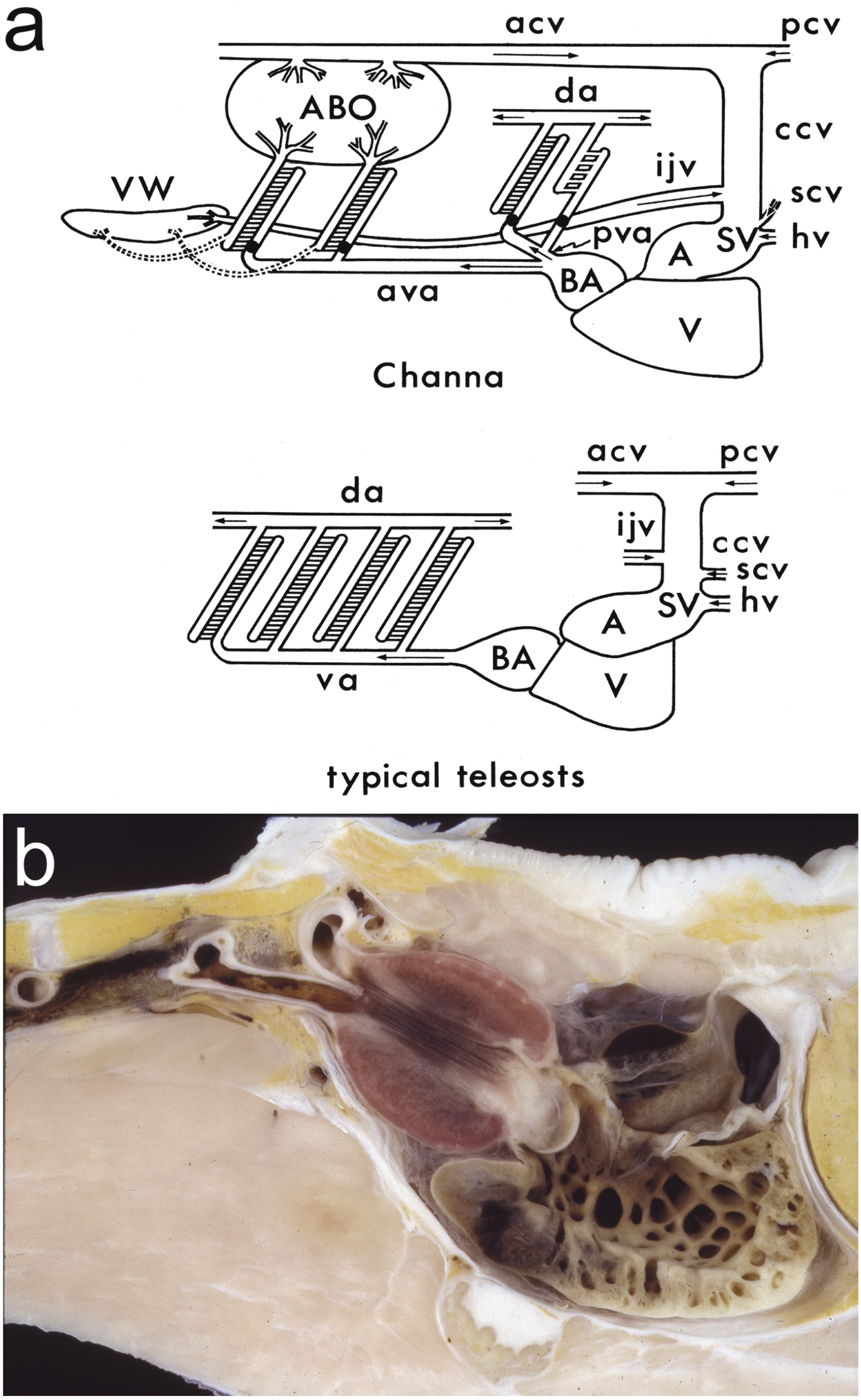

Fig. 11. (see next page for caption).

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(**Fig. 12**). The 1st arch bears a hemibranch, which Laurent *et al.* (1978) considered to be the pseudobranch. The 2nd and 3rd arches almost completely lack the gill lamellae and instead, form direct pathways between the heart and the dorsal aorta (Laurent *et al.* 1978). The posterior three pairs of arches (the 4th to 6th arches) bear the gills. The gill respiratory microvasculature of *Protopterus* is composed of a loose network of interconnecting vessels, which is radically different from the lamellar sinusoids of *Neoceratodus* and most teleosts. The primary lamellae of *Protopterus aethiopicus* have afferent-efferent shunts, which dilate during aestivation (Laurent *et al.* 1978). The efferent branchial arteries of these posterior gill arches unite and send off two major vessels, i.e., the pulmonary artery to perfuse the lungs and the ductus arteriosus leading to the dorsal aorta (Szidon *et al.* 1969; Laurent *et al.* 1978).

Information on the gills of *Lepidosiren* is more limited and confusing. Wright (1974) reported that "In *Lepidosiren*, there are six branchial arches which are short and rodlike. Gills are found on the last three branchial arches only. These are small and each consists of 8–15 lobe-like filaments of varying size, some with irregular, knob-like secondary and often tertiary branches". In contrast, de Moraes *et al.* (2005) demonstrated that gills are present on five gill arches; "irregularly arranged, short, papillar gill lamellae are supported by the five gill arches: hyoid and branchial arches 1, 2, 3, and 4". Regarding the central vasculature of *Lepidosiren*, Robertson (1913) described that "four afferent vessels arise in two sets of two in close proximity on either side, from the very short ventral aorta at the anterior end of the bulbus cordis". According to Robertson (1913), these are the aortic arches 3, 4, 5, and 6. She also described afferent and efferent vessels of the hyoidean hemibranch originating from the 3rd aortic arch. I am not aware of any recent publication on the vascular anatomy of *Lepidosiren*.

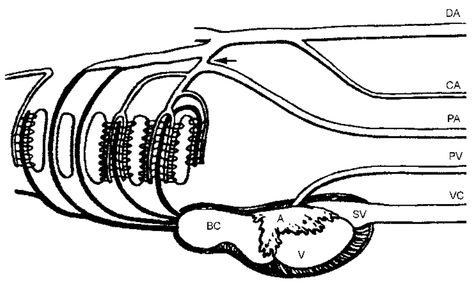

Fig. 12. Schematic drawing of the central circulation in *Protopterus aethiopicus*. Modified from Johansen *et al.* 1968a on the basis of the diagram given by Laurent *et al.* (1978) and the photograph in Szidon *et al.* 1969. A atrium; BC bulbus cordis; CA coeliac artery; DA dorsal aorta; PA pulmonary artery; PV pulmonary vein; SV sinus venosus; V ventricle; VC vena cava. Arrow indicates ductus arteriosus. Springer and the original publisher (*Zeitschrift für Vergleichende Physiologie*, **59**, 1968, 157–186, Cardiovascular dynamics in the lungfishes, Johansen, Fig. 1) is given to the publication in which the material was originally published, by adding: "With kind permission from Springer Science and Business Media."

The atrium and ventricle of all lungfishes are incompletely divided into right and left sides by median septa (**Fig. 13**). Systemic venous blood from the central veins (the vena cava, the right and left ductus Cuvieri and the hepatic veins) is drained into the sinus venosus and conveyed to the right side of the atrium and ventricle (Burggren and Johansen 1986). Pulmonary venous blood is separately drained by the pulmonary vein, which fuses with the pulmonary fold within the sinus venosus, and returns to the left side of the atrium (Bugge 1960; Klitgaard 1978; Icardo *et al.* 2005a). *Protopterus* and *Lepidosiren* show a somewhat higher degree of the specialization of the cardiac anatomy than *Neoceratodus*, in line with the higher dependence on aerial gas exchange in the former two genera (Burggren and Johansen 1986). The bulbus cordis of *Protopterus* and *Lepidosiren* (or conus arteriosus, see Icardo *et al.* 2005b) takes a characteristic S-shaped bend and contains a spiral fold and an opposing shorter fold, which nearly completely divides the lumen into two channels. Most anteriorly, a horizontal septum divides the

Fig. 11. Comparison of the central circulatory system of *Channa* and of typical teleosts (a) and the photograph of the heart of *Channa argus* after a sagittal section was cut on the median plane (b). Two ventral aortae arise from the bulbus arteriosus. The anterior aorta extends anteriorly but is partly cut between the 1st and 2nd afferent branchial arteries in b. The posterior aorta runs more dorsally and soon divides into the 3rd and 4th afferent branchial arteries. Note also the highly trabeculate ventricular lumen, muscular ridges running on the inner wall of the bulbus and the absence of the sino-atrial valve. The ventral vascular wall of the buccal cavity is also highly vascular, supplied with blood from the efferent side of the 1st and 2nd gill arches (dotted vessels in a). *Arrows* in a indicate the direction of blood flow. *A* atrium; *ABO* air-breathing organ; *acv* anterior cardinal vein; *ava* anterior ventral aorta; *BA* bulbus arteriosus; *ccv* common cardinal vein (=ductus Cuvieri); *da* dorsal aorta; *hv* hepatic vein; ijv inferior jugular vein; *pcv* posterior cardinal vein; *pva* posterior ventral aorta; *scv* subclavian vein; *SV* sinus venosus; V ventricle; *va* ventral aorta; *VW* vascular wall of the buccal cavity. With kind permission from Springer Science + Business Media: <*Journal of Comparative Physiology B*, Difference in blood oxygen levels in the outflow vessels of the heart of an air-breathing fish, *Channa argus*: Do separate blood streams exist in a teleostean heart?, **149**, 1983, 435–440, Ishimatsu and Itazawa, Figs. 1 and 2>.

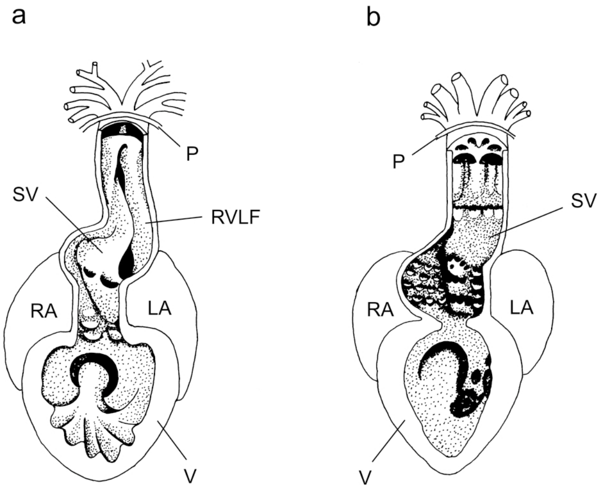

Fig. 13. The hearts of *Protopterus* (a) and *Neoceratodus* (b). The ventricle and bulbus cordis are cut to show the internal structure (ventral view). LA, left part of the atrium; P, pericardium; RA, right part of the atrium; RVLF, right ventrolateral folds; SV, spiral valve; V, ventricle. Reprinted and modified with permission of John Wiley & Sons, Inc. from *Journal of Morphology*, **190**, Burggren and Johansen, Circulation and respiration in lungfishes (dipnoi), 217–236, Fig. 3, © 1986, Wiley-Liss, Inc., a Wiley Company.

bulbar lumen to the ventral and dorsal channels; the ventral channel gives rise to the vessels to the anterior arches, while the dorsal channel sends vessels off to the posterior arches (**Fig. 13a**, Robertson 1913; Bugge 1960; Icardo *et al*. 2005b). The bulbus cordis of *Neoceratodus* also takes a similar S-shaped course and contains a spiral fold in its proximal portion. However, it has four rows of longitudinal valves in the distal portion and lacks the septum in its most cranial end. The disposition of the vessels emanating from the bulbus is similar to that in the other two genera, i.e., the anterior arch arteries come out ventrally and the posterior arch arteries dorsally (**Fig. 13b**, Klitgaard 1978).

4. Physiology of the cardiorespiratory system in aquatic air-breathing fishes

4-1. Eel gobies

Relatively little is known about the cardiorespiratory physiology of eel gobies, except the air-breathing threshold at which *Odontamblyopus lacepedii* switches from aquatic to bimodal respiration, total gas volume within the air-breathing organ and the tidal volume relative to the total gas volume (Gonzales *et al*. 2006). *Odontamblyopus lacepedii* is a facultative air-breather, which satisfies its oxygen requirement entirely through aquatic gas exchange in normoxic water, and starts air breathing only when water Po_2 is decreased to around 20 torr (Gonzales *et al*. 2006). The volume of its air-breathing organ is about 5% of its body volume and the entire volume of the gas is renewed in each breathing cycle, as evidenced by near matching of the total gas volume and the tidal volume. The duration of breath holding ranges from a few seconds up to about 30 min.

4-2. Mudskippers

Mudskippers are distinct from most aquatic air-breathing fishes in their respiratory capability on land. First, mudskippers are capable of maintaining oxygen uptake at a similar or even higher rate in air than in water (Graham 1997; Ishimatsu and Gonzales 2011). Second, mudskippers are able to eliminate CO_2 at a normal rate while out of water, and thereby maintain blood pH. Third, mudskippers do not need water for aerial ventilation of their air-breathing organ. However, other than comparisons of aerial and aquatic respiratory rates, not much is known about the respiratory physiology of mudskippers. Aguilar *et al*. (2000) determined the aerial tidal volume of *Periophthalmodon schlosseri* and found that it amounts to 54% of the total volume of the air-breathing organ (buccopharyngeal and opercular cavities). Similar to aerial ventilatory responses to air and water Po_2/Pco_2 shown for *Channa argus* (see below), aquatic hypoxia had little, if any, effect on aerial ventilation, while aerial hypoxia greatly stimulated it. Unlike the findings for *Channa argus*, however, both ventilatory frequency and tidal volume increased under hypoxia. Thus, *Periophthalmodon schlosseri* is able to improve gas exchange efficiency by renewing a larger percentage of the gas in the air-breathing organ in response to limited O_2 availability. The respiratory system of *Periophthalmodon schlosseri* is likely better adapted to an aerial rather than aquatic environment. This has been supported by the fact that the fish can repay oxygen debts after exhaustive exercise in air but not in water (Takeda *et al*. 1999). When the fish was placed in air after exhaustive exercise, oxygen uptake rate immediately increased 2.5 times, whereas no such increase occurred when the fish was confined in water after exercise.

Compared with the amount of data available on the gas exchange physiology of mudskippers, far less is known about the blood gas levels and cardiovascular physiology of these fishes. This lack of data stems mainly from the difficulty of working with mudskippers, which usually weigh from only a few grams to 50 g. Electrocardiograms have been recorded for only a few species of mudskipper (Garey 1962; Gordon *et al*. 1969). An exception is *Periophthalmodon schlosseri* (larger specimens weighing over 200 g). We have shown that *Periophthalmodon schlosseri* maintained normal heart rate and blood gas levels when they were placed on land (**Fig. 14**, Ishimatsu *et al*. 1999). Arterial Po_2 was unaffected, Pco_2 slightly decreased and pH became higher by air exposure. Neither arterial blood pressure nor heart rate significantly changed during the air exposure or recovery. In contrast, forced submersion may elicit significant responses to the cardiorespiratory parameters of mudskippers. Garey (1962) was the first to demonstrate a lowering of heart rate upon submersion (water oxygen level not stated) in a mudskipper (*Periophthalmus australis* = *Periophthalmodon freycineti*, Murdy 1989). We also have shown in *Periophthalmodon schlosseri* that heart rate rapidly decreased in response to forced submersion in normoxic seawater but rose to a higher than pre-submersion level when the fish took the first air breath upon re-emersion (**Fig. 15**). Blood pressure was depressed during submersion and did not show any overshoot upon re-emersion (Ishimatsu *et al.*, 1999). Kok *et al.* (1998) reported that a similar decrease in heart rate occurred during submersion in *Periophthalmodon schlosseri* but not in *Boleophthalmus boddarti*. Martin and Bridges (1999) reported relative changes in heart rate, cardiac output and stroke amplitude of *Periophthalmus argentilineatus* under aquatic hypoxia, hyperoxia and emersion.

4-3. Swamp eels

Swamp eels are relatively well studied for their cardiorespiratory functions because of the peculiar arrangement of the central vascular connections (see Subsection 3-3 Swamp eels). In addition, the relatively large size of these fishes (up to the body length of 1.5 m, Graham 1997) facilitates the application of modern physiological techniques for blood flow and pressure measurements, and blood sampling (Graham *et al.* 1995; Lai *et al.* 1998; Skals *et al.* 2006; Iversen *et al.* 2011). The facultative air-breathing *Synbranchus marmoratus* relies exclusively on water breathing in normoxic water and switches to air breathing when water Po_2 declines to 40–50 torr (Graham and Baird 1984; Skals *et al.* 2006). In contrast, the obligate air-breathing *Monopterus cuchia* satisfies about 65% of its O_2 requirements by aerial gas exchange even in normoxic water with free access to air (Lomholt and Johansen 1976). In response to breathing air in hypoxic water, cardiac output increased by 70% as a result of a *ca*. 35% increase in stroke volume and heart rate in *S. marmoratus* (Skals *et al.* 2006). Mean central filling pressure also increased in response to air breathing, which likely resulted in a rise in stroke volume in spite of an increased heart rate. These authors presumed that the observed increases in venous tone were probably due to a constriction of the small veins and venules by an increased sympathetic tone. A doubling of the heart rate was reported also for *M. cuchia* during air breathing (Lomholt and Johansen 1976).

Blood flow pattern during air breathing and breath holding was estimated for *Monopterus cuchia* by

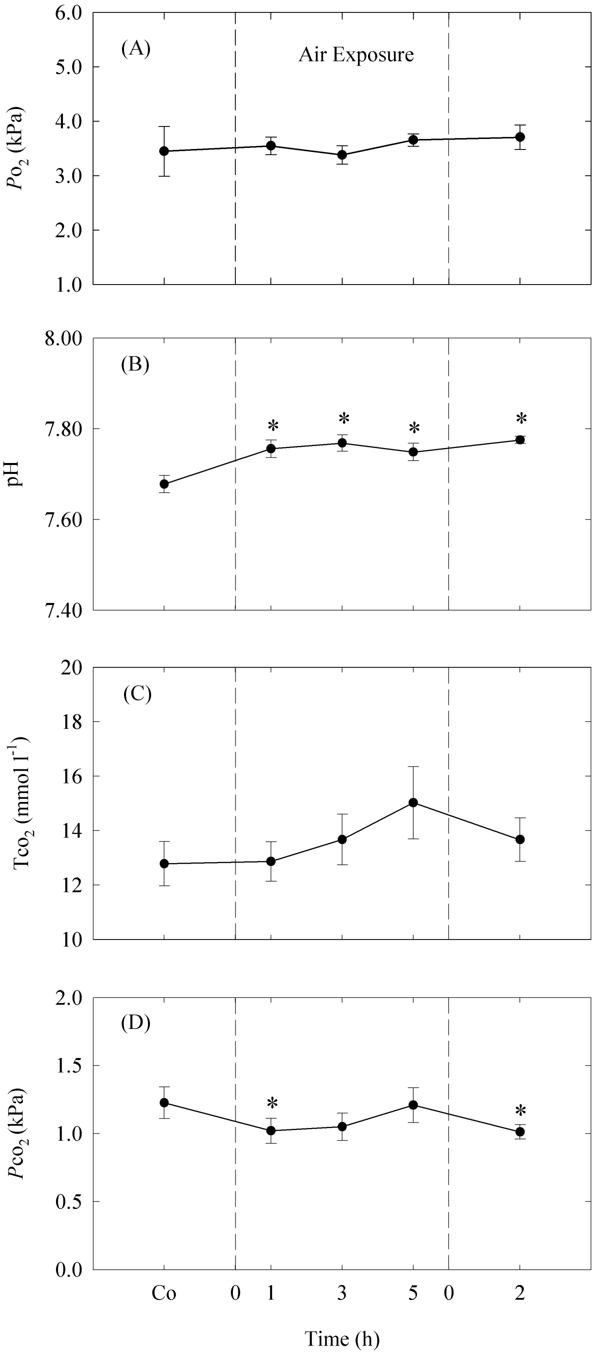

Fig. 14. Changes in arterial Po_2 (A), pH (B), total CO_2 (TCO_2, C) and Pco_2 (D) of *Periophthalmodon schlosseri* under control (Co) conditions and during 6 h of air exposure and 2 h of recovery. Before and after the air exposure, the fish were partially immersed in normoxic 50% sea water with free access to air. Values are means ± S.E.M. ($N = 7$). Asterisks indicate significant differences from the corresponding control value ($P < 0.05$). Reprinted with permission from the *Journal of Experimental Biology*, **202**, Ishimatsu *et al.*, Arterial blood gas levels and cardiovascular function during varying environmental conditions in a mudskipper, *periophthalmodon schlosseri*, 1753–1762, Fig. 1, © 1999, The Company of Biologists Ltd.

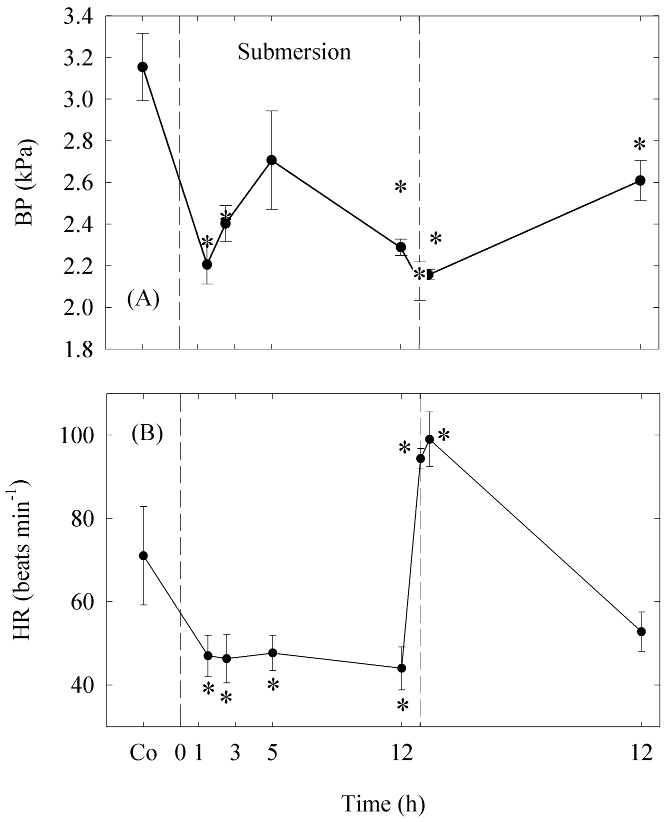

Fig. 15. Changes in blood pressure (A) and heart rate (B) of *Periophthalmodon schlosseri* under control (Co) conditions and during 12 h of forced submersion in normoxic water without access to air and 12 h of recovery. Before and after the submersion period, the fish were partially immersed in normoxic 50% sea water with free access to air. Values are means ± S.E.M. ($N = 6$). Asterisks indicate significant differences from the corresponding control value ($P < 0.05$). Reprinted and modified with permission from the *Journal of Experimental Biology*, **202**, Ishimatsu *et al.*, Arterial blood gas levels and cardiovascular function during varying environmental conditions in a mudskipper, *periophthalmodon schlosseri*, 1753–1762, Fig. 6, © 1999, The Company of Biologists Ltd.

Johansen (1982) from the data of Lomholt and Johansen (1976). The analysis was based upon the measured values of Po_2 and pH of arterial and venous blood, O_2 capacity and O_2 dissociation curve of the blood, and oxygen uptake rates, assuming complete equilibration of blood and gas across the capillary in the air-breathing organ. Even though some assumptions were not fully explained in the text, the analysis demonstrated that during air breathing when the air-breathing organ was filled with air of relatively high Po_2, a large fraction of cardiac output was directed to the air-breathing circuit, whilst during breath holding when the air was expelled from the organ, by far the greater portion of cardiac output perfused the systemic vascular bed (**Fig. 16**). This led Johansen (1982) to the idea of temporal, not spatial, separation of systemic and air-breathing circuits in these fishes. Johansen (1982) assumed total mixing of systemic venous blood and O_2-rich blood from the air-breathing organ on the basis of the anatomy of the central circulatory system.

4-4. Snakeheads

Channa argus takes up 60% and 85% of its total oxygen requirement by air breathing when breathing bimodally in normoxic and hypoxic (water Po_2 ~ 40 torr) water, respectively (Itazawa and Ishimatsu 1981). In contrast, aerial gas exchange makes only a minor (15%) contribution to total CO_2 elimination, as in most aquatic air-breathing fishes (Graham 1997). The breathing pattern always consists in a rapid breathing movement interspersed with variable periods of breath holding. Upon surfacing, the fish expires the gas inside the air-breathing organ before inspiring fresh air. Aerial ventilation of the air-breathing organ is effective only in water since the gas is expelled by a reversed flow of water from the gill openings, which will completely replace the gas in the organ and thereby attain a complete renewal of the gas upon inspiration (Ishimatsu and Itazawa 1981; Liem 1984). When the fish is experimentally placed out of water, the blood Pco_2 in-

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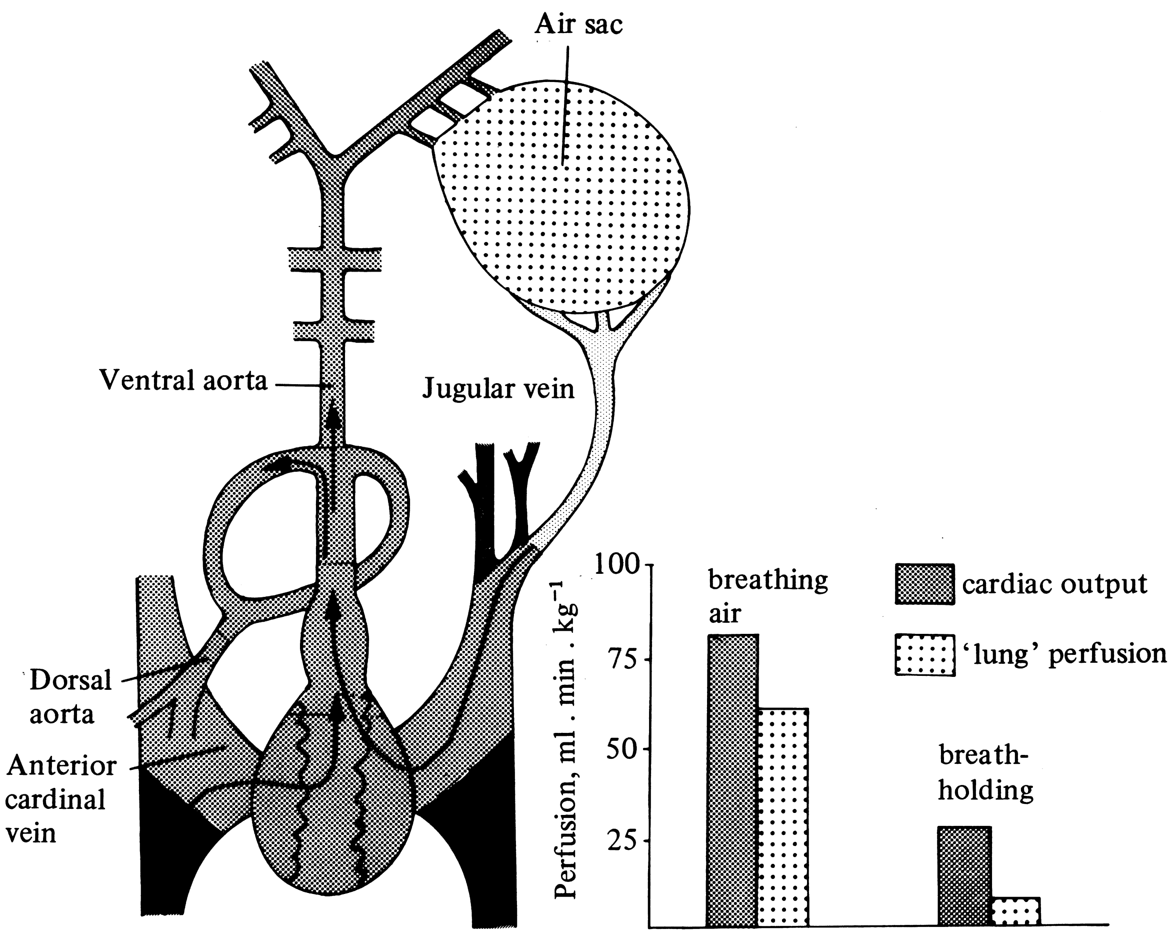

Fig. 16. Left: Schematic drawing of the heart and its major in- and outflow vessels in *Amphipnous cuchia* (=*Monopterus cuchia*, Rosen and Greenwood 1976). Note the paired shunt vessels (=BA4 of **Fig. 7**) from the ventral aorta forming the dorsal aorta. The effluent blood from the air-breathing organ converges with systemic venous blood in the jugular veins. Right: Air-breathing increases cardiac output and the fraction of the cardiac output perfusing the air-breathing organ. During breath holding, a major fraction of the cardiac output is shunted from the ventral to the dorsal aorta. Reprinted with permission from *A Companion to Animal Physiology*, Taylor, Johansen, Bolis (eds.), Blood, circulation, and the rise of air breathing: Passes and bypasses, Johansen, 91–105, Fig. 7.2, © 1982, Cambridge University Press.

creases significantly and the fish suffers from uncompensated respiratory acidosis (Ishimatsu and Itazawa 1983b) in spite of greatly increased ventilatory frequency (Ishimatsu and Itazawa 1981). During bimodal respiration, aerial ventilation is strongly enhanced by aerial hypoxia but not by lowered aquatic Po_2 (Glass *et al.* 1986). The enhanced aerial ventilation is largely due to increased ventilatory frequency, with little changes in tidal volume. Temperature affects the aerial ventilatory responses to hypoxia such that much higher responses were evoked at 25°C than at 15°C. Aerial hypercapnia had little effect on aerial ventilation of *Channa argus*.

The unusual cardiovascular function of snakeheads is that the fish has the capacity of functional separation of O_2-rich blood from the air-breathing organ and O_2-poor systemic venous blood with no morphological septation within the heart (Ishimatsu and Itazawa 1983a, **Fig. 17**). Determinations of blood oxygen levels from chronically cannulated free-swimming fish revealed that both Po_2 and O_2 content are significantly higher for the blood sampled from the 3rd and 4th afferent branchial arteries originating the posterior ventral aorta than the blood sampled from the 1st and 2nd afferent branchial arteries derived from the anterior ventral aorta. The Po_2 and O_2 content differences between the two ventral aortic blood was 12–22 torr and 0.6–1.4 mmol l^{-1}. Thus, there must be at least partially separated blood streams within the heart of *Channa argus*, which is presumably brought about by the presence of laminar blood streams since the heart of *C. argus* lacks any anatomical septation. The morphological specializations of the heart (see Subsection 3-4 Snakeheads) likely help prevent total mixing of the two types of blood and also channeling the outflow from the heart.

Flow dynamics in the central circulatory system of *Channa argus* was examined by angiocardiography (Andresen *et al.* 1987). The results demonstrated nearly complete ventricular emptying at the end of systole and high elasticity of the bulbus arteriosus, which almost completely absorbed ventricular output shortly after the completion of ventricular systole. A preference for a greater volume of blood from the air-breathing organ through the heart to the posterior ventral aorta was suggested by the larger diameter of the X-ray image of and a higher density of contrast medium within the posterior ventral aorta, even though this needs more rigorous investigation.

Regarding possible regulatory mechanisms for blood-flow partitioning between the air-breathing organ and systemic circulations in *C. argus*, a preliminary data by Ishimatsu *et al.* (1986) on perfused head preparations illustrated that both acetylcholine and adrenaline added to the perfusion fluid increased the resistance of the vascular bed downstream of the anterior ventral aorta, and redistributed perfusion flows away from the air-breathing circuit (as determined in the anterior venous flow) to the general systemic circuit (as determined in the dorsal aortic flow). Stimulation of a vagal branch innervating the air-breathing organ mimicked the effects of acetylcholine or adrenaline. This innervation is probably non-adrenergic, since no adrenergic nerve fibers could be demonstrated in the vasculature of the air-breathing organ with Falck-Hillarp histochemistry.

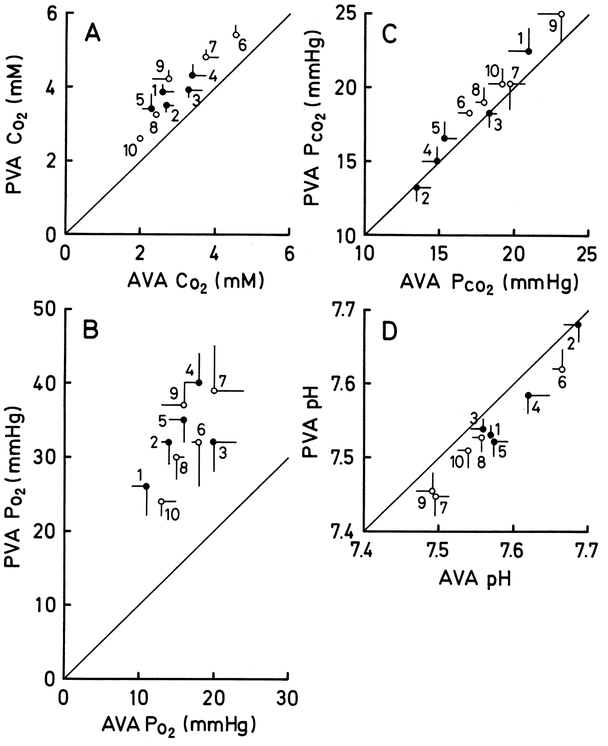

Fig. 17. Comparison of oxygen content (Co_2), Po_2, Pco_2 and pH of the blood taken from the anterior and posterior ventral aortic circulations (AVA and PVA) of freely swimming, chronically cannulated *Channa* at 25°C. Ordinal values are blood gas parameters from afferent branchial artery (ABA) 3 and 4 and those from ABA 1 and 2 are plotted along the abscissa. ABA 1 and 2 branch off from the anterior ventral aorta (AVA) and ABA 3 and 4 from the posterior ventral aorta (PVA). Mean ± SD of six measurements made on each fish are given. *Dots* indicate fish cannulated at ABA 2 and 4, *circles* indicate fish cannulated at ABA 1 and 3. *Figures* next to the points are experimental fish numbers. With kind permission from Springer Science + Business Media: <*Journal of Comparative Physiology B*, Difference in blood oxygen levels in the outflow vessels of the heart of an air-breathing fish, *Channa argus*: Do separate blood streams exist in a teleostean heart?, **149**, 1983, 435–440, Ishimatsu and Itazawa, Fig. 3>.

4-5. Lungfishes

Protopterus and *Lepidosiren* are obligate air-breathing fishes. These lungfishes satisfy nearly 90% of their O_2 requirement through aerial respiration even in normoxic water (*Protopterus*, Lenfant and Johansen 1968; McMahon 1970; *Lepidosiren*, Sawaya 1946), although the relative importance of aerial O_2 uptake is size-dependent. Thus, Johansen *et al.* (1976) reported that *Protopterus* with body weight of 4 g took up 70% of total oxygen demand aquatically but the value diminished to 10–15% in fish larger than 50 g. In comparison, aquatic respiration is generally more important for CO_2 elimination such that 70% of CO_2 elimination is through aquatic breathing even in adult *Protopterus* (Lenfant and Johansen 1968). Somewhat in conflict with those earlier findings, Perry *et al.* (2005) claimed that the lung was responsible for 91% of O_2 uptake and 76% of CO_2 elimination in *P. dolloi*. However, this might be due to very high pulmonary ventilation frequencies of their animals (6–48 breaths min^{-1} at 25°C), which would enhance aerial CO_2 excretion. Working on *Lepidosiren*, Amin-Naves *et al.* (2004) tested the effects of acute temperature changes on respiratory partitioning of aerial and aquatic gas exchange. Pulmonary ventilation increased from 0.5 to 8.1 ml BTPS (body temperature and pressure, saturated with water vapor) kg^{-1} min^{-1} when temperature was raised from 15 to 35°C, exclusively due to increased frequency of air ventilation. Concomitantly, the relative contribution of aerial respiration to CO_2 elimination increased from 13 to 74% (as estimated from their Fig. 3). *Neoceratodus* satisfied almost all of its O_2 demand through aquatic gas exchange when the fish rested in normoxic water, but commenced air breathing in response to decreasing water Po_2 (Kind *et al.* 2002) or during exercise (Grigg 1965). Control of ventilation in lungfishes has been reported for *Neoceratodus* by Johansen *et al.* (1967) and Fritsche *et al.* (1993), for *Lepidosiren* by Sanchez and Glass

(2001), Sanchez *et al.* (2001) and Amin-Naves *et al.* (2007) and for *Protopterus* by Johansen and Lenfant (1968) and Perry *et al.* (2008, see also earlier studies cited in these papers). Lungfishes breathe air through the mouth as in all other air-breathing fishes (McMahon 1969).

The investigation into the cardiovascular dynamics of lungfishes was pioneered by Kjell Johansen and his colleagues. Heart rate and cardiac output of *Protopterus aethiopicus* increased immediately after each air breath and subsequently subsided (Johansen *et al.* 1968a), as has been shown for swamp eels (Skals *et al.* 2006; Iversen *et al.* 2011) and electric eel (Johansen *et al.* 1968b). A radiological study of *P. aethiopicus* by Johansen and Hol (1968) revealed that injections of a contrast medium in the pulmonary vein preferentially filled the left cardiac chambers to be selectively dispatched to the anterior gill-less arches, which give rise to the major systemic arteries. In contrast, injections of the medium in the vena cava was conveyed into the sinus venosus and then to the right, posterior part of the atrium. Johansen *et al.* (1968a) estimated distribution of the systemic and pulmonary venous blood into anterior and posterior arches of *Protopterus aethiopicus*. Their calculations estimated that over 90% of blood flow through the anterior arches was derived from the pulmonary venous blood immediately after air breathing and then the fraction decreased down to 65% in four minutes (**Fig. 18**). However, these calculations used blood Po_2 but not O_2 content or saturation and therefore, probably overestimated the fraction mainly because of low systemic venous Po_2. In addition, their experimental animals may not have recovered sufficiently from rather invasive surgical procedures (implantation of five catheters and four electromagnetic flow probes), guessed from the very high frequency of air-breathing (once in several minutes) reported in these studies, as compared with the much lower air-breathing frequency (*ca.* 5 breaths h^{-1}) reported for intact *Lepidosiren* by Sanchez and Glass (2001), which is allegedly more dependent on air breathing than *Protopterus* (Johansen *et al.* 1968a). Incomplete recovery may also have resulted in a relatively low arterial Po_2 (28–38 torr) reported for *Lepidosiren* by Johansen *et al.* (1968a), as compared with the values (80–90 torr) reported for the same species subjected to less invasive surgery (a dorsal aortic catheter alone) by da Silva *et al.* (2008) and Bassi *et al.* (2010). Bassi *et al.* (2010) estimated %R-L shunt (=Qsh/Qtot × 100, where Qsh is systemic venous blood flow recirculated into the systemic circuit and Qtot is the total systemic blood flow) of *Lepidosiren* to be 19%, based on the equation given by Piiper (1993). This means that systemic blood is composed of 81% of pulmonary venous blood and 19% of systemic venous blood, which agrees with the estimate given by Johansen *et al.* (1968a) for *Protopterus* in the middle of a breath-holding period. Nearly nothing is known about blood separation in *Neoceratodus*; blood gas analysis by Johansen *et al.* (1968a) indicated that 84% of blood flow through the anterior arch originated from the pulmonary venous blood in hypoxic water, but the value declined to 33% in normoxic water.

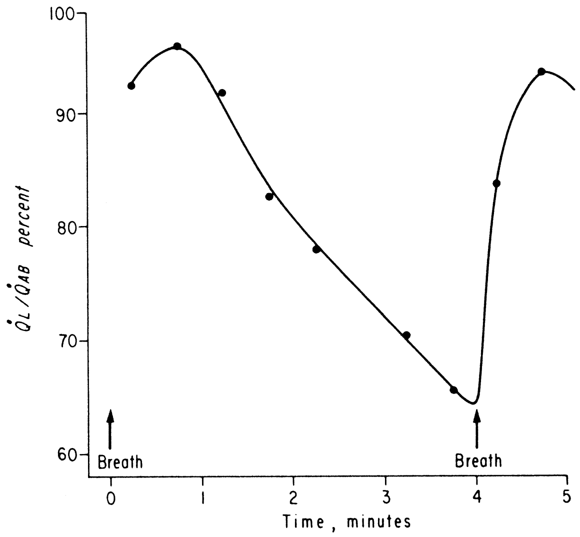

Fig. 18. Time course of the proportion of pulmonary flow (Q_L) to total flow perfusing the anterior gill-less branchial arteries (Q_{AB}) during an interval between air breaths in *Protopterus aethiopicus*. Springer and the original publisher (*Zeitschrift für Vergleichende Physiologie*, **59**, 1968, 157–186, Cardiovascular dynamics in the lungfishes, Johansen, Fig. 23) is given to the publication in which the material was originally published, by adding: "With kind permission from Springer Science and Business Media."

5. Conclusion

In his influential review on the evolution of double circulation in vertebrates, Foxon (1955) listed common features shared by vertebrates with double circulation. These are (1) separate return of oxygenated and deoxygenated blood into the heart, (2) at least a considerable amount of anatomical guiding of the two streams in the heart, if not complete separation and (3) separate vessels by which the oxygenated and deoxygenated bloods leave the heart. **Table 1** compiles the available information on these three conditions for air-breathing fishes. What emerges from this table is (1) the highly derived anatomy of lungfish hearts is not shared with any actinopterygian fishes, (2) the scattered occurrence of each condition among actinopterygian fishes, which appears to have no correlation with the taxonomic position of the species, and (3) separate outflow vessels from the heart occurs more frequently in these fishes.

Separate vessels to drain the air-breathing organ occur in lungfishes and several 'primitive' air-breathing fishes but it is only in lungfishes that these vessels directly open into the heart. This condition prevails in all three genera of lungfish (note that the statement of pulmonary return only into the sinus venosus for *Neoceratodus* given by Graham 1997 is incorrect, see Klitgaard 1978). *Amia*, *Gymnarchus* and *Polypterus* have separate air-bladder veins but these do not reach the heart. Cardiac septum is almost nonexistent in air-breathing fishes except lungfishes. A central septum was reported for the sinus venosus of *Monopterus* but no detailed account was given (Liem 1961; Munshi and Mishra 1974). Unusual division of the atrium into two halves in *Gymnarchus* was reported in a paper published more than 100 years ago (Assheton 1907) but no morphological study has subsequently been published to confirm this. Apart from lungfishes, separate outflow vessels from the heart to the air-breathing organ circuits have been found in *Polypterus*, *Gymnarchus*, two closely related catfishes, *Clarias* and *Heteropneustes* in the superfamily Siluroidea, *Anabas* and *Channa*. According to Allis (1922), the truncus arteriosus of *Polypterus* gives off, immediately after issuing from the pericardial chamber, a large vessel on either side, which splits into the 2nd afferent branchial artery and the united trunk of the 3rd and 4th afferent branchial arteries. The latter sends off the pulmonary artery. The bulbus arteriosus of *Gymnarchus* sends off four and that of *Heteropneustes* and *Clarias* emits three separate vessels. Thus, the occurrence of more than a single ventral aorta is more frequently seen among air-breathing fishes than the two other conditions, even though it is still quite rare considering the large number of air-breathing fishes known (374 species at the time of Graham 1997). The case of *Channa* indicates that separate afferent conduits to the air-breathing organ are the most crucial of the three conditions proposed by Foxon (1955) to achieve partial if not complete double circulation.

Partial separation of the O_2-rich and O_2-poor blood seen in lungfishes and *C. argus* is likely to be of physiological significance, although the Po_2 difference may be quite small. The difference in the systemic and pulmonary arterial blood Po_2 in lungfishes ranges from 7 to 16 torr (Johansen *et al.* 1968a) but because the blood Po_2 levels are at the steep portion of their oxygen dissociation curve (Lenfant and Johansen 1968), the difference in O_2 saturation level could be up to 30%. Similarly, the Po_2 difference in the anterior and posterior ventral aortic blood in *Channa argus* ranges from 12 to 22 torr but O_2 content in the posterior ventral aortic blood is on the average 34% higher than that in the anterior ventral aortic blood (range 18% to 52%, Ishimatsu and Itazawa 1983a).

It is worth noting that both *Protopterus* and *Channa* show differential development of gill tissues between the arches; those gill arches carrying O_2-rich blood have become either gill-less (*Protopterus*, **Fig. 12**) or reduced in size, with shunt vessels connecting the afferent and efferent arteries (*Channa*, **Fig. 10**). *Neoceratodus* does not show such differentiation in gill anatomy, probably reflecting the facultative air-breathing habit of the fish. The vascular anatomy in *Lepidosiren* is obscure in this regard (see Subsection 3-5 Lungfishes). Reductions in gill surface area in air-breathing fishes have been interpreted as an adaptation to reduce oxygen loss to hypoxic water for aquatic air-breathing fishes (Graham 1997). Gill tissues are proven to be highly plastic. Gills can change surface area by filling or exposing interlamellar space with cell mass in response to ambient conditions such as hypoxia and temperature, probably to balance physiological needs for gas exchange and unavoidable passive loss or gain of water and ions across the gill surface (Nilsson 2007). The gills of obligate air-breathing *Arapaima gigas* change anatomy with growth (Brauner *et al.* 2004). Thus, the secondary lamellae protrude from the filamental tissues as in ordinary fishes in small individuals but are embedded in filamental tissues in larger fish, which probably rely upon air breathing more heavily than smaller fish (Johansen *et al.* 1976). Further, Bond (1960) demonstrated a reverse relationship between gill area and ambient oxygen levels for salamanders. It then is tempting to speculate that those gill arches receiving more oxygenated blood slow their development rates or stop development at some stage and maintain a pedomorphic state. Then, those fishes showing differential development among arches might have the capacity of separate blood streaming through the heart and then the gill arches.

The above reasoning leads to the idea that double circulation in vertebrates initially evolved as a means of reducing oxygen loss to hypoxic surrounding water, in which air breathing probably evolved (Graham 1997; Clack 2002, 2007; Clement and Long 2010). Physiological measurements must be made to clarify whether double circulation occurs or not in modern air-breathing fishes. The reasons why modern amphibious fishes can invade land to some extent without restructuring their cardiovascular system (and therefore possibly without the capacity of double circulation) might be because of the location of the gas exchange surface within the mouth and their small size. Gulping air inside the mouth and not ventilating the gills during breath holding, mudskippers are probably not subjected to the risk of oxygen loss through the gills. In addition, the small size of mudskippers should facilitate oxygen uptake through the skin, due to the large surface-to-volume rate of the body. The Devonian sarcopterygians are generally much larger. For instance, *Panderichthys* had a total body length of over a meter (Clack 2002), the lower jaws alone of *Tiktaalik* range from 170 to 310 mm (Daeschler *et al.* 2006), the skulls of *Ichthyostega* and *Acanthostega* can be 250 and 200

Table 1. Comparison of central cardiovascular anatomy in air-breathing fishes relating to potential separation of oxygenated blood from the air-breathing organs and deoxygenated blood from the body.

Fish	O_2 difference in ABS and ABA	Separate origin of outflow vessels from the heart into systemic and ABO circuits	Cardiac septation	Separate efferent vessels from the air-breathing organ to the heart*
Lepisosteus	Unknown	No (1)	No (2)	No (1)
Amia	Unknown	No (3)	No (4)	No (into the right ductus Cuvieri) (4)
Arapaima	Unknown	No (5)	Unknown	No (5)
Odontamblyopus	Unknown	No (6)	No (7)	No (6)
Periophthalmodon	Unknown	No (8)	No (9)	No (8)
Polypterus	Unknown	Yes (10)	No (3)	No (into the hepatopulmonary vein) (11)
Gymnarchus	Unknown	Yes (12)	Partial in atrium (12)	No (into the right ductus Cuvieri) (12)
Monopterus	Unknown	No (13, 14)	Partial in sinus venosus (15)	No (14)
Heteropneustes	Unknown	Yes (16)	No (17)	No (16)
Clarias	Unknown	Yes (18)	Unknown	No (18)
Anabas	Unknown	Yes (19, 20)	No (21)	No (19, 20)
Channa	Yes (22)	Yes (22, 23)	No (22, 23)	No (22, 23)
Neoceratodus	Yes (24)	Yes (two circuits connected by DA) (13)	Partial in both atrium and ventricle (25)	Yes (into the right side of the atrium) (25)
Protopterus	Yes (24)	Yes (two circuits connected by DA) (26, 27)	Partial in both atrium and ventricle (28)	Yes (into the right side of the atrium) (29)
Lepidosiren	Yes (24)	Yes (two circuits connected by DA) (30)	Partial in both atrium and ventricle (30)	Yes (into the right side of the atrium) (30)

(1) Potter 1927, (2) Zaccone *et al.* 2011, (3) Parsons 1930, (4) Goodrich 1958, (5) Greenwood and Liem 1984, (6) Gonzales *et al.* 2008b, (7) personal observation, (8) Gonzales *et al.* 2011, (9) Schöttle 1931, (10) Allis 1922, (11) Abdel Magid 1967, (12) Assheton 1907, (13) Liem 1961, (14) Munshi *et al.* 1990, (15) Munshi and Mishra 1974, (16) Olson *et al.* 1990, (17) Thomas 1967, (18) Olson *et al.* 1995, (19) Olson *et al.* 1986, (20) Munshi *et al.* 1986, (21) Thomas 1976, (22) Ishimatsu and Itazawa 1983a, (23) Ishimatsu *et al.* 1979, (24) Johansen *et al.* 1968a, (25) Klitgaard 1978, (26) Bugge 1960, (27) Szidon *et al.* 1969, (28) Icardo *et al.* 2005b, (29) Icardo *et al.* 2005a, (30) Robertson 1913.

ABS, afferent blood to the systemic bed (= systemic arterial blood); ABA, afferent blood to air-breathing organs (ABO); DA, ductus arteriosus. * drainage pathway stated when a pulmonary vein or an air-bladder vein exists.

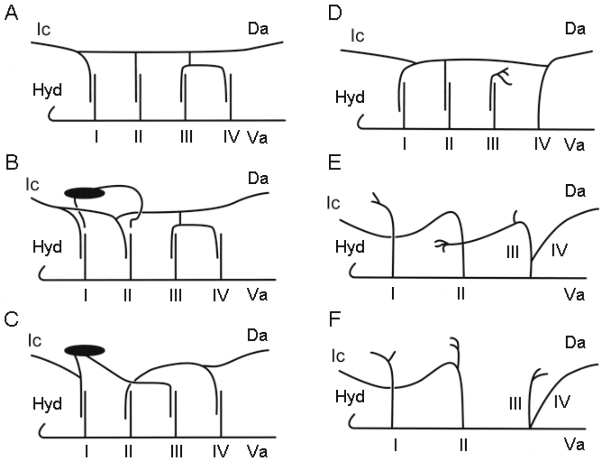

Fig. 19. Schematics of the central circulation in synbranchids. A *Synbranchus marmoratus*, B *Synbranchus madeirae*, C *Ophisternon afrum*, D *Monopterus albus*, E *Monopterus cuchia*, F *Monopterus boueti*. Da dorsal aorta; Hyd hyoidean artery; Ic internal carotid artery; Va ventral aorta. Roman numerals indicate the number of gill arches. Black ellipsoids in B and C represent the median sinus in the buccopharyngeal epithelium. Reprinted and modified with permission from *Am. Mus. Nat. Hist.*, **157**, Rosen and Greenwood, A fourth neotropical species of synbranchid eel and the phylogeny and systematics of synbranchiform fishes, 1–69, Figs. 51–56, © 1976, courtesy The American Museum of Natural History.

mm long, respectively (Clack 2002). These large animals might have needed to develop a more efficient cardiovascular system to sustain activities on land with a lesser contribution of cutaneous gas exchange.

6. Future research topics

The following lists several more important questions in the anatomical and physiological research of air-breathing fishes. It is most important to analyze blood gas levels of the different outflow vessels from the heart for those fish in which double circulation is a possibility.

6-1. Anatomical issues

Early descriptions of the cardiovascular anatomy of air-breathing fishes often lack sufficient accuracy for the interpretation of function and therefore, need to be re-examined with modern techniques such as corrosion vascular casting. The more important species for the study include *Lepidosiren*, *Electrophorus*, *Gymnarchus* and synbranchids.

The cardiovascular anatomy of *Lepidosiren* is obscure. Laurent (1984) illustrated the schematics of the arrangement of gill arches in different fish groups including *Lepidosiren* but did not cite original sources. According to Laurent (1984), the central vasculature of *Lepidosiren* is different from that of *Protopterus* in that the efferent arteries of the posterior arches do not bifurcate and that there is no afferent or efferent vessel on the posterior wall of the 6th gill cleft.

Electrophorus is the only teleost, together with *Monopterus*, that might have direct vascular connections between the ventral and dorsal aortae. The diagram in Carter (1935) illustrates that all four branchial arteries connect the ventral aorta directly to the dorsal aorta. *Gymnarchus* is the only fish that is said to have partial separation of the heart (Assheton 1907 as cited by Graham 1997). A description of the peculiar anatomy drawn by Graham (1997), i.e., partly divided ductus Cuvieri, direct drainage of venous blood from the air-breathing organ (gas bladder) into the left side of the atrium and almost separate origins of four afferent branchial arteries from the heart, requires careful investigation.

Figure 19 demonstrates diagrams of synbranchid central vessels reported by Rosen and Greenwood (1976). The diagrams depict only the course of major vessels. Nonetheless, it is apparent that gradual loss of gills and re-modeling of vascular connections occurred presumably with an increasing dependency on air breathing. The two species of *Synbranchus* and one of *Opisternon* possess gills on all four arches but species of *Monopterus* are radically different from them in gill vascular anatomy.

6-2. Physiological issues

If differential development of gill size/vasculature is a feature to indicate the presence of separate blood streams as hypothesized above, then fishes with different sizes of gill arches may be of higher priority for research. These include *Anabas* and other anabatoids and synbranchids. The climbing perch *Anabas testudineus* shows a central vascular anatomy almost identical with that in *Channa*. Gill vascular specialization is even further advanced in *A. testudineus* such that shunt vessels between the afferent and efferent gill vessels occur not only in the 4th but also in the 3rd gill arch. Other anabantoids likely share the central vascular pattern known for *A. testudineus*, including two ventral aortae and thereby meet the supposedly basic anatomical requirement for double circulation (Machowicz and Gray 1955; Burggren 1979). Synbranchids also show a variable degree of development between gill arches (**Fig. 19**) but the presence of an unusually long ventral aorta would promote blood mixing. Although *Polypterus*, *Amia* and *Gymnarchus* show different courses of pulmonary veins, their gills are more or less equally developed between arches. Still, these are important species in the consideration of cardiovascular evolution in fishes and therefore, should be tested for their capacity of double circulation. Farrell (2007) discussed the cardiovascular anatomy and physiology of polypterids, gars, *Amia* and lungfishes.

Full analysis of blood partitioning within the central vasculature of *Channa* is challenging. Assuming that total mixing of blood from the air-breathing organ and systemic beds in the common cardinal vein and no O_2 uptake through the gills (!), Tazawa and Johansen (1987) arrived at equations for shunt fractions (percent of common cardinal vein blood directed to the anterior ventral aorta and percent of hepatic venous blood directed to the posterior ventral aorta) within snakehead's heart. Their analysis still requires blood oxygen data from five different sites in addition to aerial O_2 uptake. Even if one assumes the complete equilibrium of the blood draining the air-breathing organ and the gas within the organ (as did Johansen 1982), the fish must be implanted with four catheters (the two ventral aortae or afferent branchial arteries from them, a systemic vein and the common cardinal vein). I have attempted such surgery but found it to be technically highly difficult. There is also a possibility that different types of blood within the anterior and common cardinal veins are not totally mixed but conveyed as separate streams. Implantable micro-Po_2 sensors might prove useful. Measurements of blood pressure on a freely swimming *C. argus* demonstrated nearly simultaneous upstroke of blood pressures in the two artae (Ishimatsu, unpublished). Resolving how blood streams can be separated within the central vascular system of *Channa* needs novel techniques of blood flow and pressure determination and tracers that are applicable to free-ranging animals.

The hemodynamic basis for the blood separation in lungfishes is almost totally unknown. In this regard, knowledge is more advanced for amphibians and reptiles (Johansen and Burggren 1985; Boutilier 1990; Hicks *et al.* 1996; Ishimatsu *et al.* 1996; Wang *et al.* 1997, 1999). Partial mixing of blood likely occurs within the ventricle and the conus arteriosus in amphibians (Wang *et al.* 1999), whereas the volume of blood residing in the cavum venosum is thought to be an important factor for blood mixing in reptiles. Within the lungfish heart, neither intra-atrial nor intra-ventricular septa divide the respective lumens completely and therefore, volume outputs into the two sides of the bulbus cordis are likely determined by the vascular resistance ratios of the pulmonary and systemic circuits, which are related to diastolic pressures in the two circuits (Hicks *et al.* 1996). In this consideration, the position of the orifice of the bulbus cordis in relation to the position of the inter-ventricular septum is also important. Szidon *et al.* (1969) demonstrated in *Protopterus aethiopicus* that the orifice is far to the left of the inter-ventricular septum. If this is the case, the difference in diastolic pressure may be an important determinant for blood distribution between the pulmonary and systemic circuits in this fish, as proposed for the turtle by Hicks *et al.* (1996). In contrast, Icardo *et al.* (2005b) stated that the orifice of the bulbus cordis is positioned only slightly to the right of the ventricular septum in *Protopterus dolloi*.

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