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 O₂-rich effluent blood of the air-breathing organ from O₂-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.

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; Niedzwiedzki 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-
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 O₂-poor systemic venous blood to the pulmonary arteries with a relatively low pressure, whilst the left chambers eject O₂-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 phylogeny of the cardiorespiratory system of 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.

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.

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 sympatric mudskippers (Beleophthalmus pectinirrostris and Periophthalmus modestus), O.
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 \( P_{o2} \) 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; Kutscher 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 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 caudal fin. An other 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 (Stieu 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 Lapiote 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...
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 (Odua 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. *Protoperus 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 threat of over-exploitation (Mlewa et al. 2011).

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 filamentary arteries. The afferent filamentary 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, *O₂*-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 fishes, such as blennies (Graham 1976). The suprabranchial chamber, which was reported for *Periophthalmodon schlosseri* by Yadav et al. (1990) and for another oxudercine *Pseudopocryptes lanceolatus* (= *Pseudopocryptes 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 *Periophthalmodon* species (Tamura and Moriyama 1976; Graham 1997; Graham et al. 2007). Gill filaments of *Periophthalmodon* and *Periophthalmodon* are short, sparsely spaced and twisted (*Periophthalmodon*). The gills of *Boleophthalmus* are 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 viridus* (this scientific name is no longer valid, possibly *Scartelaos histophorus*) extends further anteriorly after giving off the first afferent branchial arteries and...
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 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 bodarti*, 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.

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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 lacepedi*, and two species of mudskippers (*Periophthalmodon 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*, *Periophthalmodon chrysospilios*, *P. modestus*, *Pseudapocryptes elongatus*, and *Scartelaos histophorus*.

*Periophthalmodon schlosseri* shows a specialized microvascular arrangement of the secondary lamellae. The vascular pathway of the secondary lamellae con-
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...
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 papillary 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 Periophthalmodon 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 PO2 and oxygen content as systemic capillaries would receive, which may be considered as physiologically inefficient. Oxygenated blood from the respiratory epithelia is drained by systemic veins and therefore, likely mixed
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 1978) but this has never been confirmed by blood gas analysis. As generally the case in other air-breathing fishes, the gills become atrophied with increasing reliance on air breathing. Thus, exclusively water-breathing Macrotema 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 madeirensis, Ophisternon afrom, 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 hyoidian 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 viridescens, Boleophthalmus boddarti and Pseudocypreps 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
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
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 Propterus 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 Propterus are remarkably specialized...
Fig. 11. (see next page for caption).
(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 Prototerus 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 Prototerus 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.

The atrium and ventricle of all lungfishes is incompletely divided into right and left sides by median septa (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). Prototerus 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 Prototerus 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...
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 lacepedius 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 lacepedius 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 PO₂ 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 CO₂ 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 cardiorespiratory 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 pouches). Similar to aerial ventilatory responses to air and water PO₂/Paco₂ 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 O₂ 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 PO₂ was unaffected. Pco₂ slightly decreased and pH became higher by air exposure. Neither arterial

Fig. 13. The hearts of Proteoptyerus (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, 198, Burggren and Johansen, Circulation and respiration in lungfishes (dipnoi), 217–236, Fig. 3, © 1986, Wiley-Liss, Inc., a Wiley Company.

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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 argenteilineatus* 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 *P* _O_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 _P*_O_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.
Johansen (1982) from the data of Lomholt and Johansen (1976). The analysis was based upon the measured values of Po2 and pH of arterial and venous blood, O2 capacity and O2 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 Po2, 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 O2-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 Po2 ~ 40 torr) water, respectively (Itazawa and Ishimatsu 1981). In contrast, aerial gas exchange makes only a minor (15%) contribution to total CO2 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 expels 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 Po2 in-
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 Po2 (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 O2-rich blood from the air-breathing organ and O2-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 Po2 and O2 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 Po2 and O2 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.

### 4-5. Lungfishes

Protopterus and *Lepidosiren* are obligate air-breathing fishes. These lungfishes satisfy nearly 90% of their O2 requirement through aerial respiration even in normoxic water (Protopterus, Lenfant and Johansen 1968; McMahon 1970; Lepidosiren, Sawaya 1946), although the relative importance of aerial O2 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 CO2 elimination such that 70% of CO2 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 O2 uptake and 76% of CO2 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 CO2 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 CO2 elimination increased from 13 to 74% (as estimated from their Fig. 3). *Neoceratodus* satisfied almost all of its O2 demand through aquatic gas exchange when the fish rested in normoxic water, but commenced air breathing in response to decreasing water P.O2 (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.
Johansen 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; Iverson 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 PO2 but not O2 content or saturation and therefore, probably overestimated the fraction mainly because of low systemic venous PO2. 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 PO2 (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 x 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.

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 oc-
Double circulation 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 separate afferent conduits to the air-breathing organ of Graham 1997). The case of air-breathing fishes known (374 species at the time of Graham 1997) 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 O₂-rich and O₂-poor blood seen in lungfishes and C. argus is likely to be of physiological significance, although the PₐO₂ difference may be quite small. The difference in the systemic and pulmonary arterial blood PₐO₂ in lungfishes ranges from 7 to 16 torr (Johansen et al. 1968a) but because the blood PₐO₂ levels are at the steep portion of their oxygen dissociation curve (Lenfant and Johansen 1968), the difference in O₂ saturation level could be up to 30%. Similarly, the PₐO₂ difference in the anterior and posterior ventral aortic blood in Channa argus ranges from 12 to 22 torr but O₂ 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 Polypterus and Channa show differential development of gill tissues between the arches; those gill arches carrying O₂-rich blood have become either gill-less (Proopterus, 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. They 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 (Bittun et al. 2004). Thus, the secondary lamellae protrude from the filamentous tissues as in ordinary fishes in small individuals but are embedded in filamentous 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 re-structuring 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. Gulpfeeding 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 ratio 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.

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


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 madeirensis, C Ophisternon afrum, D Monopterus albus, E Monopterus cuchia, F Monopterus boneti. 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 the American Museum of Natural History, © 1976, courtesy The American Museum of Natural History.

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 Ophisternon possess gills on all four arches but species of Monopterus are radically different from them in gill vascular anatomy.

6. 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 anabatoids 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 circula-

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 O2 uptake through the gills (1), Tazawa and Johansen (1987) arrived at equations for shunt fractions (per-
cent 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 O2 uptake. Even if one assumes the complete equilibration 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 (two of the ventral aortae and 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-Po2 sen-
sors 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 vas-
sular 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; Boutillier 1990; Hicks et al. 1996; Ishimatsu et al. 1996; Wang et al. 1997, 1999). Partial mixing of blood likely occurs within the verteicle and the conus arteriosus in am-
phibians (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-ven-
tricular septa divide the respective lumens completely and therefore, volume outputs into the two sides of the bulbus cords are likely determined by the vascular resistance ratios of the pulmonary and systemic cir-
cuits, 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 cords in relation to the position of the inter-ventricular septum is also important. Sizodon et al. (1969) demonstrated in Propterus 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 impor-
tant 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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