The origin of vertebrates: a hypothesis based on kidney development

HANS DITRICH*

University of Vienna, Institute for Zoology, Department of Theoretical Zoology, Althanstrasse 14, A-1090 Vienna, Austria

The habitat of the earliest vertebrates (craniates) is still being debated. Marine as well as freshwater habitats and anadromous behaviour have been proposed. In contrast, an estuarine origin of vertebrates is suggested here, based on ontogenetic, comparative anatomical and functional data. This approach should resolve inconsistencies between the probable existence of glomeruli in the vertebrate ancestors and the marine habitat of all related extant groups (e.g. urochordates and cephalochordates). The kidney, as the main osmoregulatory organ, must have been developed according to the environmental prerequisites even in stem vertebrates. In the absence of fossil evidence only deductions from contemporary animals are possible. These data indicate that ancestral stem vertebrates probably had well-developed glomeruli, and were capable of at least some ion-exchange between urine and the body. However, they were probably unable to cope with a strong osmotic gradient with respect to their environment. The conclusion is that these animals were osmoconformers at around 300–350 mOsm and therefore were restricted to brackish water. © 2007 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2007, **150**, 435–441.

ADDITIONAL KEYWORDS: Cambrian – craniates – evolution – excretion – habitat – osmoregulation.

The development of ancestral stem vertebrates (i.e. ancestors common to hagfish, lampreys and gnathostomes, hereafter referred to as 'protovertebrates') probably started in the Cambrian period, about 550–530 million years (Myr) ago (e.g. Kumar & Hedges, 1998). Inherently, no palaeontological evidence exists for the initial forms, as they probably lacked fossilizable elements. Thus, even later fossils provide only limited information on the ecological niche in which the most ancestral forms developed.

The renal system would be ideally suited to provide clues on the habitat of the most primitive vertebrates (craniates). However, only indirect evidence from the excretory functions of extant primitive vertebrates can be considered because no fossilized kidney remains are known.

Two theories, based on the anatomy and ontogeny of recent species, were postulated: Romer (1949) and others deduced a marine origin, as in closely related groups (e.g. Cephalochordata, Urochordata, etc.), which are marine (cf. Denison, 1956). Smith (1932)

and followers, however, concluded a limnic origin, mainly based on anatomical and ontogenetic features of the primitive kidney.

Griffith (1987) attempted to harmonize these two theories by assuming an anadromous life cycle of the 'protovertebrate', i.e. spawning in freshwater and later migration into the sea. Anadromous behaviour, however, is comparatively rare and most probably represents a derived condition, raising doubts about attributing such a complicated life cycle to a primitive form.

Instead an estuarine origin of the vertebrates is proposed here, based on comparative anatomical and functional data. This approach would eliminate certain inconsistencies in the existing theories and – in the absence of 'hard facts' – help to resolve the conflicting hypotheses on the emergence of the vertebrates.

ONTOGENETIC BACKGROUND

The earliest functional excretory structure in most lower vertebrates is the pronephros. It develops from the intermediary mesoderm (mesomere) in several (3–

*E-mail: hans.ditrich@univie.ac.at

12) of the anterior postotic segments. This mesoderm forms a series of vesicles from the somatopleura (nephrotomes) that grow ciliated openings (nephrostomes) into the coelom, and elongate distally and caudally. The individual canals then fuse with their ipsilateral neighbours, thus forming the primary urinary (Wolffian) duct; this continues to grow caudally and finally opens near the ventro-medial end of the body cavity (Fig. 1A).

In most lower vertebrate species at least one pair of capillary tufts, supplied from the dorsal aorta and evaginating into the coelom, is developed. These primary glomeruli, however, may be a product of the fusion of initially segmentally arranged vascular networks that fuse later in development (Hickman & Trump, 1969; Kluge & Fischer, 1990).

This generalized pattern is typically present in all non-amniote vertebrates, although it is not always fully developed and is usually rather rudimentary in amniotes (see e.g. van den Broek, van Oordt & Hirsch, 1938; Torrey & Feduccia, 1979; Vize et al., 1997). Still, the pronephros is always the site of development of the primary urinary duct (Wolffian duct).

Later, when the more posterior segments develop the opisthonephros from the (unsegmented) nephrogenic ridge, these newly formed tubules connect to the primary urinary duct and gradually replace the excretory function of the pronephros. The latter then often degenerates. In several lower vertebrates, however, the pronephros remains functional even in the adult (e.g. myxinoids, Chondrostei, and a few teleosts) or transforms into a reticulo-lymphoid organ (most teleosts).
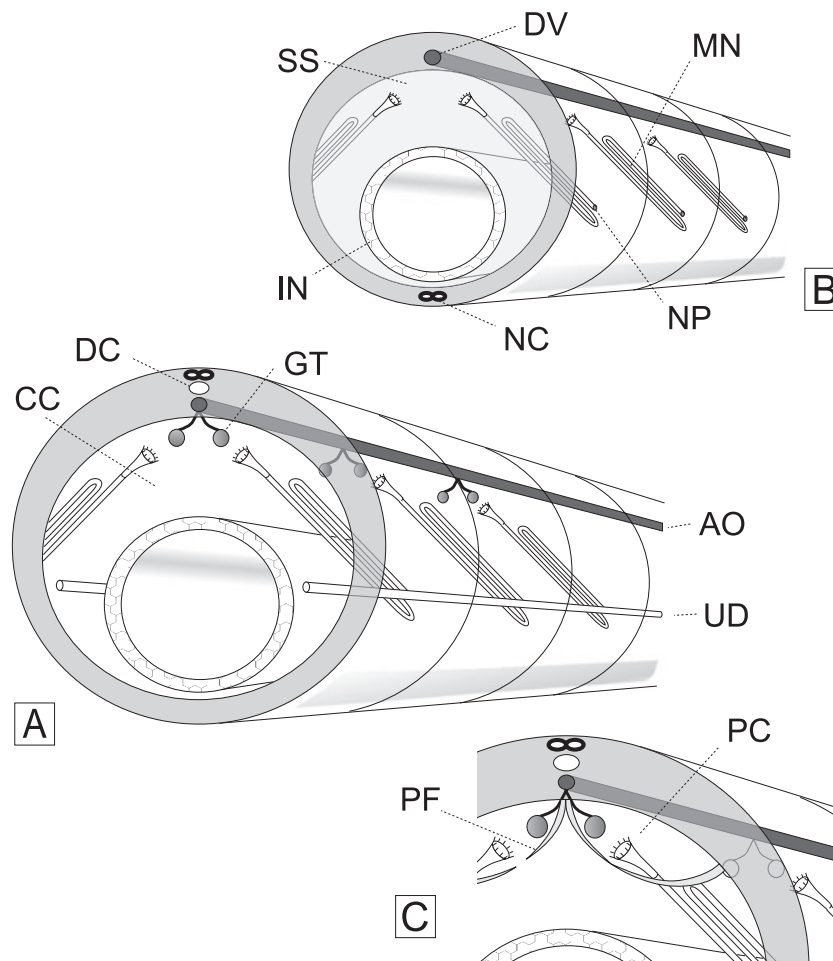

Figure 1. Schematic drawing of the excretory apparatus in the 'protovertebrate' (A), in higher annelids (B), and in an advanced developmental stage in 'protovertebrates' (C, modified detail from A). Note that the peritoneal fold in (C) is still open towards the coelom on the left side, whereas it is already closed in a later stage (right side), thus forming a separate nephric chamber. Both conditions can be found in the pronephros of extant lower vertebrates. Abbreviations: AO, dorsal aorta; CC, coelomic cavity; DC, dorsal chorda; DV, dorsal vessel; GT, glomerular tuft; IN, intestine; MN, metanephridium; NC, neural chord; NP, nephroporus; PC, pronephric chamber; PF, peritoneal fold; SS, segmental septum; UD, urinary duct.

Crude recapitulation of the phylogenetic history during embryological development (Haeckel's law) can be accepted as a clue to the evolutionary path, although with some caveats. Accordingly, it can be assumed that the kidney of the ancestral stem vertebrate resembled, to some extent, the ontogenetically most primitive, i.e. differentiated, pronephroi.

Segmentally arranged pairs of ciliated funnels that drain to the outside in the next segment via a tortuous duct (metanephridiae) are present in higher annelids (Fig. 1B). Hypothetically, analogous segmental coelomatic ducts might have joined laterally, thus forming a pair of urinary ducts that open near the anus in 'protovertebrates'.

Glomeruli might have formed as aortal sprouts that developed into vascular tufts, apparently to enhance the diffusion of substances from the blood into the coelomic fluid. Depending on physiological parameters – mainly size and metabolic activity of the organism – pronephric chambers might have differentiated from the general coelom; this would have further enhanced the efficiency of the transport of substances from the blood to the exterior (Fig. 1C). A similar pattern, although strongly modified, is found in many lower vertebrates.

The development of dedicated vascular structures for enhanced diffusion from the blood (i.e. glomeruli) is interpreted here as being quite unrelated to water secretion. Moreover, a major role of the glomeruli in the excretion of nitrogenous metabolic waste products seems unlikely, as ammonia – the main excretory product of aquatic lower vertebrates – diffuses freely through all thin epithelia. Instead, one can assume that the ionic regulatory processes by the renal tubules provide a functional requirement for the development of enhanced diffusion from the blood into the coelomic fluid. Although such structures might not be essential for very small, freely floating, thin-walled embryos (or 'protovertebrates'), ionic regulation is no doubt stimulated by larger body size, the development of a calcified, internal or external skeleton (e.g. Pteraspidomorphi), and higher metabolic, especially neural, activity.

PHYLOGENETIC BACKGROUND

Hagfish probably retained many of the generalized characters that can be assumed to have been present in the 'protovertebrates' (e.g. Kuratani, Kuraku & Murakami, 2002). They have, among many other peculiarities, a functional (although modified) pronephros; unlike all other nonmammalian vertebrates, they lack a renal portal venous system (van den Broek et al., 1938; Marinelli & Strenger, 1956).

The oldest fossil hagfish, however, are found in the late Carboniferous, over 300 Myr ago (Bardack, 1991; Janvier, 1996). Thus about 230 Myr had probably already passed since the emergence of the 'protovertebrates'. Accordingly, myxinoids cannot be expected to represent a 'basic type'. Instead, together with the line of development of the gnathostomes, their excretory system provides clues on how the 'protovertebrate' kidney might have been organized.

In hagfish, as well as in lampreys and in all gnathostomes, the anterior portion of the kidney, the pronephros, initially shows a segmental arrangement. Nephrostomes and comparatively large glomeruli are present in the hagfish pronephros (Table 1). They remain in the adult as a glomus (fused glomeruli) bulging into the pericardial space with numerous pronephric tubules (most of them formed by splitting of the initial generation); their nephrostomes open into the pericardial coelom (e.g. van den Broek et al., 1938; Marinelli & Strenger, 1956).

Although being freshwater forms, the ammocoetes larvae of lampreys show a similar pronephric construction. Their nephrostomes open into a common coelomic cavity near the (fused) glomus (e.g. Kluge & Fischer, 1990).

In chondrichthyans, the pronephros forms as a series of segmental pronephric vesicles that develop into tubules with nephrostomes. However, neither functional glomeruli nor nephric chambers are formed, and the whole complex is later either incorporated in the female genital system or obliterated in males.

In several osteichthyans that retained presumably ancestral features, such as sturgeons and paddlefish (Chondrostei) or the brachyopterygian *Polypterus*, the pronephros participates in excretory activity, although often in modified form. In most bony fish, however, the pronephros develops into a lymphoreticular and/or haematopoetic organ – the head kidney (see References in Ditrich, 2005).

Note that all aglomerular teleosts (e.g. Syngnathidae, Batrachidae) represent relatively derived, distantly related groups that cannot be regarded as ancestral types.

The conclusion is that the hypothetical 'stem-kidney' was developed from a series of nephric tubules opening into the coelom (or into a nephric chamber in a more evolved state), each with a ciliated nephrostome, vis-à-vis to a glomerulus. These tubules would laterally join a common duct that runs caudally to open near the anus (cf. Fig. 1A). This model is strongly modified in all extant vertebrates. However, in males that have a urogenital connection, the nephrons that later participate in seminal transport outline this pattern during differentiation.

PHYSIOLOGICAL BACKGROUND

The function of the excretory system is crucial in considering the possible environment of the 'protoverte-

Table 1. Comparison of ecological, physiological, and anatomical parameters in lower vertebrates. Data compiled from Hickman & Trump (1969), Griffith & Pang (1979), and Bone & Marshall (1985)

Group	Biotope	Osmolarity (approx. mOsm)	Glomeruli (adult)	Pronephros
Myxionids	marine	1000	very large	excretory
Petromyzonts	freshwater & anadromous	200/300	large	reduced
Chondrichthyes	marine*/freshwater	1100/500	large	reduced
Actinistians	marine*	900**	large	lacking (?)
Chondrosteans	freshwater & anadromous	300	large	excretory
Holosteans	freshwater	330	large	reticulary
Teleosts	marine	350	very small/lacking	excretory/reticulary/reduced
Teleosts	freshwater	300	medium	reticulary/reduced

*High internal urea concentration and NaCl-secreting rectal gland; **might be restricted to submarine freshwater upwellings (Griffith & Pang, 1979).

brate'. Several main functions can be attributed to all vertebrate excretory systems:

1. excretion of nitrogenous waste products;
2. maintaining homeostasis with regard to ions (i.e. salt balance);
3. regaining valuable substances (glucose, salts, amino acids, etc.);
4. maintaining a physiological osmotic value (i.e. water balance).

Nitrogenous waste excretion can be disregarded with respect to the hypothetical 'protovertebrate' habitat. Both freshwater and marine vertebrates, as well as most aquatic invertebrates, synthesize ammonia as their main excretory product. Ammonia is readily water soluble and freely diffuses through all thin epithelia (most notably the gills). Thus, the 'protovertebrate' would not require a kidney at all for nitrogen excretion, provided that body size was small and no diffusion-restricting structures (armor, shell) had been present.

Ion turnover and reclaiming substances from the primary urine cannot be viewed as isolated processes. Monovalent ions (Na^+, Cl^-) are mainly handled by the gills in most extant aquatic vertebrates. Divalent ions, as well as several other substances, are mainly exchanged by active and thus ATP-consuming processes of the renal tubular epithelium. Ca^{2+} and Mg^{2+} ion regulation/recovery are especially essential for developing internal and external skeletal structures, and efficient neuronal function, respectively. The organic substances that are still of value for the organism (e.g. glucose, amino acids, lipids, peptides, etc.) are similarly regained by epithelial transport, and are also regained passively following concentration gradients. It is proposed here that these tubular exchange processes were the main function of the 'protovertebrate' kidney. These epithelial transport processes

require a certain volume of primary urine, provided by glomerular filtering and/or peritoneal funnels, as a substrate. Short-time regulatory capacity must also be assumed, according to the fluctuating physiological needs.

Osmotic balance is a special challenge for the ion exchange mechanisms. The energy consumption of isotonic ion exchange is probably rather low. However, pumping against a strong osmotic difference – whether in fresh water or in sea water – requires efficient and elaborate mechanisms and the necessary energetic capacity. Although the latter is merely 'costly' with respect to foraging, the former postulates a certain evolutionary progression and may therefore be regarded as a derived condition.

Among extant lower vertebrates, most osteichthyans, whether marine based or in fresh water, have an osmolarity of about 300–350 mOsm (see Table 1). Marine chondrichthyan osmolaritiy is slightly above that of sea water as a result of their conspicuous urea retention system. The few freshwater inhabitants have osmolarities around 500 mOsm (e.g. Bone & Marshall, 1985). Interestingly, the coelacanth *Latimeria chalumnae* (Smith, 1939) developed a strategy similar to that of chondrichthyans for raising the body osmolarity near to the environmental level (Griffith & Pang, 1979). This is likely to be a homoplastic physiological property, although a (rather arduous) mechanism of elevating the internal osmolarity apparently evolved at least twice (cf. Arnason, Gullberg & Janke, 2001).

Petromyzonts are anadromous or freshwater living and maintain osmotic values around 300 mOsm, similar to bony fish.

Myxinoids are exclusively marine based and are the only recent craniates group that is isotonic to sea water. Their capacity of responding to fluctuations in environmental osmolarity is very restricted (cf. Dantzler,

1988). Accordingly, myxinoids must either have lost their osmoregulatory potential at some point in their evolution or they have always lacked this attribute. Given the rather basic construction of the myxinoid kidney, the latter interpretation is more probable.

SYNOPSIS

Considering the constructional premises, the following hypothetical anatomy of the 'protovertebrate' kidney can be proposed (with liberal use of Occam's razor).

The excretory system is formed by a series of paired, segmental nephrons that begin with a nephrostome opening into the coelomic cavity. A pair of glomeruli per segment, supplied by branches from the aorta, projects into the coelomic cavity close to these nephrostomes. At a later stage of development, the glomerulus/nephrostome area becomes separated from the rest of the coelomic cavity by an epithelial fold. The nephrons connect to a duct that is formed by caudal growth of the most anterior nephric tubules. These paired urinary ducts open near the anal region.

The coelomic fluid that is passed into the tubules by the ciliary action of the nephrostomes is modified by epithelial transport processes. The latter recover – to the greatest extent possible – those substances that are still useful for metabolism by isotonic exchange mechanisms. These cellular transport processes involve pinocytosis, exocytosis, and membrane pumps, and are thus ATP dependent. However, neither a urea retention mechanism, for increasing the osmolarity of the body to the level of sea water, nor specialized sodium (and chloride) excreting mechanisms (ionocytes: chloride cells, salt glands, etc.) are present. 'Protovertebrates' therefore could not remove the salt that inevitably would enter the body through all thin epithelia (mainly gut and gills) in a hyperosmotic medium. Nor could they recover the ions that would diffuse out in fresh water. Under these premises, 'protovertebrates' must have been isotonic, but not isoionic, with their surroundings.

Given these physiological constraints, 'protovertebrates' would be restricted to environments with a salt concentration similar to their body fluids. Assuming that the internal osmolarity of the body was around 350 mOsm, as is the case in most living vertebrates (except for chondrichthyans and *Latimeria*), the salt concentration of the habitat must have been in that range. Consequently the possible habitats include estuaries, or more or less isolated seas with a salt content of about a third of today's oceans. Although such a biotope probably has the advantage of reduced competition and predation pressure, osmotic fluctuations would pose an inherent threat to the 'protovertebrates'.

Clearly the concept presented above has its limits. It cannot be verified by direct observation or experimental set-ups. It is, however, consistent with the available data and avoids two main shortcomings in the previous theories of vertebrate origin.

1. The presence of well-developed glomeruli does not per se indicate a limnic origin. In an isotonic environment, even large glomeruli would not result in water loss. Instead, the development of glomeruli would be a consequence of an increase in body size and/or metabolic rate, compared with aglomerular ancestors, which resulted in the need for a more efficient diffusion of substances into the coelomic fluid.
2. Marine habitats (based on present day salinity) are encountered with a variety of highly evolved excretory mechanisms by extant vertebrates. It is neither likely that a primitive ancestral organism would initially have developed such complex features, nor likely that such intricate mechanisms evolved to replace an already existing, functional osmoregulatory system that was apparently sufficient to live in marine niches.

It may be further deduced from this hypothesis that the later development of the osmoregulatory system evolved in (at least) two different directions (Fig. 2).

Ionocytes in the gills and a corresponding 'short cut' for the ingested salt in sea water (via the oesophagus and branchial arteries) have evolved in bony fish, maintaining their internal osmolarity at around 350 mOsm. In chondrichthyans (and also in actinistians) the internal osmolarity increased above the surroundings by the development of au urea retention mechanism and an additional means of salt excretion – the anal gland. Only myxinoids retained the presumably basic, osmoconform system.
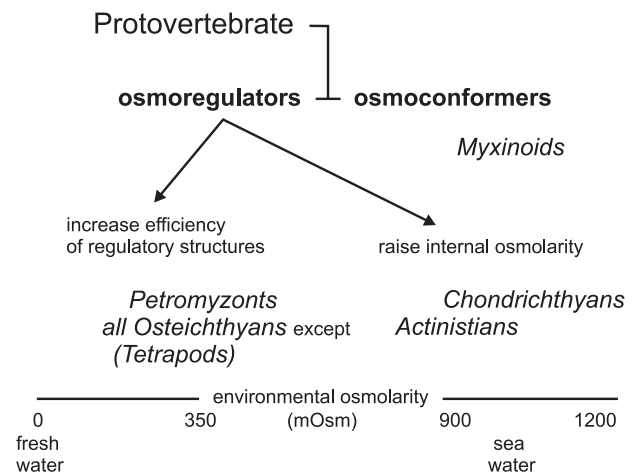

Figure 2. Diagram of potential pathways of osmoregulatory strategies related to the osmolarity in aquatic environments.

Each of the above pathways, however, also has inherent drawbacks: Osmoconformers experience variations of the environmental salt content as direct alterations in the composition of their body fluid – a problem that is known to strongly affect myxinoids.

The excretion of surplus salts in sea water, or vice versa the removal of instreaming water and salt uptake in freshwater, not only presupposes the development of the pertinent mechanisms, but also implies high energetic demands that must be met by foraging and respiration. Raising the body osmolarity by accumulation of urea and other organic osmolytes – as elasmobranchs do – requires the development of urea retention mechanisms and biochemical strategies to protect the body (e.g. certain proteins) against such high urea concentrations.

Any attempt to bring the developmental achievements postulated in this paper in accord with the geological timescale, or with fossil evidence, remains hypothetical. The evolution of an excretory system that molds the intricate osmoregulatory structures in later vertebrates might have originated in an organism similar to conodonts (see e.g. Sweet & Donoghue, 2001). However, traces of the internal structure of these animals are scarce and any statements on their excretory system remain speculative. The Chengjiang Myllokunmingiids, *Myllokunmingia* and *Haikouichthys* (Chen, Huang & Li, 1999; Shu *et al.*, 1999, 2002; see also Zhang & Hou, 2004) already show many vertebrate features. Still, no details of their excretory system have been identified. These species were probably marine dwellers, but larger body sizes and large mineralized skeletal structures seem to have evolved much later. The Pteraspidomorphi have a distinct fish-like appearance. They probably had at least some of the ion exchange and regulatory features that are depicted in this paper. However, fossils of the possible earliest forms from this group (*Anatolepis*) are fragmentary. Undisputed remains of pteraspidomorphs (Arandaspida), however, have not been found earlier than about 480 Myr ago. Accordingly, the 'protovertebrate' postulated in this paper might be placed in the historical vicinity of Yunnanozoan-/Myllokunmingiid-like forms and early pteraspidomorphs. In the established timescale (Shergold & Cooper, 2005), the Chengjiang fossils (early Cambrian) and the Ordovician arandaspids are approximately 50 million years apart. Note also that the Haikou–Chengjiang fossils represent an exceptionally rich and well-preserved site that might or might not be representative for other coastal aquatic communities. The postulated estuarine environment of the 'protovertebrate' was no doubt geographically rather restricted, and might therefore not have been preserved or discovered so far. Accordingly the hypothetical 'protovertebrate' cannot be precisely designated, either in time or in location.

Theories on the development of vertebrates can have various starting points: palaeontological evidence, comparative anatomy, and, more recently, genetic analyses. However, a functional basis of considerations on vertebrate ancestry is certainly helpful to develop a realistic scenario. Even though it may sound trivial, the ancestral vertebrate was certainly fully competent to survive in its environment, reproduce, compete with others, and avoid predation. Thus no functionally 'incipient' or 'rudimentary' organ systems can have been present. Finally, a rather limited ecological niche-like estuary would probably provide a better 'adapting space' for new developmental achievements than an environment with already rich, well-established species interdependencies, such as the ocean or limnic habitats would.

ACKNOWLEDGEMENT

REFERENCES

Arnason U, Gullberg A, Janke A. 2001. Molecular phylogenetics of gnathostomous (jawed) fishes: old bones and new cartilage. *Zoologica Scripta* **30:** 249–255.

Bardack D. 1991. First fossil hagfish (Myxinoidea): a record from the Pennsylvanian of Illinois. *Science* **254:** 701–703.

Bone Q, Marshall NB. 1985. Osmoregulation und Ionenbalance. *Biologie der Fische*. Stuttgart: Fischer, 96–117.

van den Broek AJP, van Oordt GJ, Hirsch GC. 1938. Urogenitalsystem. In: Bolk L, Göppert E, Kallius E, Lubosch W, eds. *Handbuch der vergleichenden Anatomie der Wirbeltiere* Vol. 5. Berlin: Urban und Schwarzenberg, 683–854.

Chen JY, Huang DY, Li CW. 1999. An early Cambrian craniate-like chordate. *Nature* **402:** 518–522.

Dantzler WH. 1988. *Comparative Physiology of the Kidney*. Berlin: Springer.

Denison RH. 1956. A review of the habitat of the earliest vertebrates. *Fieldiana Geology Memoirs* **11:** 359–457.

Ditrich H. 2005. Renal structure and function in Vertebrates. In: Dutta HM, Kline DW, eds. *Biological Systems in Vertebrates* Vol. 2. Enfield: Science Publishers.

Griffith RW. 1987. Freshwater or marine origin of vertebrates? *Comparative Biochemistry and Physiology* **87:** 523–531.

Griffith RW, Pang. RK. 1979. Mechanisms of osmoregulation in the coelacanth, Latimeria chalumnæ: evolutionary implications. The biology and physiology of the living Coelacanth. *Occasional Papers of the California Academy of Sciences* **134:** 79–93.

Hickman CP, Trump BF. 1969. The kidney. In: Hoar WS, Randall DJ, eds. *Fish Physiology 1. Excretion, Ionic Regulation and Metabolism*. New York: Academic Press, 91–239.

Janvier P. 1996. *Early Vertebrates*. Oxford Monographs in Geology and Geophysics 33. Oxford: Oxford University Press.

Kluge B, Fischer A. 1990. The pronephros of the early ammocoete larva of lampreys (Cyclostoma, Petromyzontes): Fine structure of the external glomus. *Cell and Tissue Research* **260:** 249–259.

Kumar S, Hedges SB. 1998. A molecular timescale for vertebrate evolution. *Nature* **392:** 917–920.

Kuratani S, Kuraku S, Murakami Y. 2002. Lamprey as an evo-devo model: lessons from comparative embryology and molecular phylogenetics. *Genesis* **34:** 175–183.

Marinelli W, Strenger A. 1956. Vergleichende Anatomie und Morphologie der Wirbeltiere. *Myxine glutinosa*. Vienna: Deuticke, 81–172.

Romer AS. 1949. *The Vertebrate Body.* Philadelphia: Saunders.

Shergold JH, Cooper RA. 2005. The Cambrian period. In: Gradstein FM, ed. *A Geologic Timescale 2004*. Cambridge: Cambridge University Press, 147–164.

Shu DG, Luo HL, Zhang XL, Hu SX, Chen L, Han J, Zhu M, Li Y, Chen LL. 1999. Lower Cambrian vertebrates from South China. *Nature* **402:** 42–46.

Shu DG, Morris SC, Han J, Zhang ZF, Yasui K, Janvier P, Chen L, Zhang XL, Liu JN, Li Y, Liu HQ. 2002. Head and backbone of the early Cambrian vertebrate *Haikouichthys*. *Nature* **421:** 526–529.

Smith H. 1932. Water regulation and its evolution in the fishes. *Quarterly Reviews in Biology* **7:** 1–26.

Smith JLB. 1939. A living fossil. *Cape Naturalist* **6:** 187–194.

Sweet WC, Donoghue PCJ. 2001. Conodonts: past present future. *Journal of Paleontology* **75:** 1174–1184.

Torrey TW, Feduccia A. 1979. The urogenital system. *Morphogenesis in Vertebrates*. New York: Wiley, 365–401.

Vize PD, Seufert DW, Caroll TJ, Wallingford JB. 1997. Model systems for the study of kidney development: use of the pronephros in the analysis of organ induction and patterning. *Developmental Biology* **188:** 189–204.

Zhang XG, Hou XG. 2004. Evidence for a single median finfold and tail in the lower Cambrian vertebrate *Haikouichthys ercaicunensis*. *Journal of Evolutionary Biology* **17:** 1162–1166.