

ARGUMENTS ON THE ORIGIN OF THE VERTEBRATE LIVER AND THE AMPHIOXUS HEPATIC DIVERTICULUM: A HYPOTHESIS ON EVOLUTIONARY NOVELTIES*

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Morphologic changes in evolution imply that all organs must descend with modifications from homologous organs of a common ancestor (Homology Principle). However, unlike other visceral organs, e.g., heart, the anatomical features of the liver/portal system have been highly conserved in vertebrate lineage. Already in the basal vertebrates (Cyclostomata), the visceral post-capillary blood is collected into the portal vein and directed to the liver, where it breaks into the capillaries again, forming venous rete mirabilia, the hallmark feature of the vertebrate liver. The anatomical stability of this complex arrangement in all vertebrates pleads for the search of the homologous precursor. Amphioxus possesses the midgut diverticulum, whose vascularization, developmental and topological characteristics are similar to those of the vertebrate liver/portal system. Experts have long suggested Amphioxus diverticulum as the homologous precursor of the vertebrate liver. The recent discovery of vertebrate liver-specific proteins in the Amphioxus diverticulum supports this hypothesis. However, the Homology Principle obligates us to ask the important question: What is the phylogenetic precursor of the complex Amphioxus diverticulum? There is no relevant evidence from putatively preceding forms (existing or fossil). However, recently discovered facts on Amphioxus' diverticulum development (A.O. Kovalevsky and L.Z. Holland) and function, combined with yolk sac vascularization in Cyclostomata and fishes by venous visceral blood, reinforce the hypothesis that Amphioxus' diverticulum evolved from a yolk sac of an advanced chordate ancestor, as suggested by A.S. Romer in his analysis on the transition from "visceral" to "somatic" animals in the evolution of chordates. Earlier, Charles Darwin, based on A.O. Kovalevsky's observations, supported similar phylogeny of chordates.

Keywords: evolution; homology; chordate; vertebrate; liver; Amphioxus; phylogenetic precursor, Romer; Darwin.

АРГУМЕНТЫ О ПРОИСХОЖДЕНИИ ПЕЧЕНИ ПОЗВОНОЧНЫХ И ПЕЧЁНОЧНОГО ВЫРОСТА ЛАНЦЕТНИКА: ГИПОТЕЗА О ПРИРОДЕ ЭВОЛЮЦИОННЫХ НОВООБРАЗОВАНИЙ

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Морфологические изменения в эволюции предполагают, что органы животных трансформируются из гомологичных органов общего предшественника, с большими или меньшими модификациями (принцип Гомологии). В отличие от других внутренних органов (например, сердца и легких), система печени и воротной вены демонстрирует минимальные отличия между филогенетически очерченными группами позвоночных. Уже у круглоротых, наиболее "примитивных" позвоночных,

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висцеральная посткапиллярная кровь собирается в непарную воротную вену, которая впадает в печень и опять распадается на капилляры. Печеночные капилляры (синусоиды) вновь собираются в непарную полую вену, формируя таким образом капиллярную сеть между двумя венами – *rete mirabilia*, что является уникальной характеристикой печени всех позвоночных, включая млекопитающих. Неизбежно возникает вопрос: Что что является гомологичным филогенетическим предшественником столь сложной системы как воротная вена и печень, которая уже присутствует как сформированный орган у круглоротых? По мнению экспертов, гомологичным филогенетическим предшественником системы портальной вены/печени позвоночных является печёночный вырост Ланцетника (бесчерепные), эмбриональное развитие, анатомическая позиция и васкуляризация которого сходны с теми же характеристиками печени позвоночных. Однако, принцип Гомологии обязывает задать и следующий вопрос: Что может быть филогенетическим предшественником печёночного выроста Ланцетника, учитывая его сложную архитектуру и уникальную васкуляризации? Предполагаемые предшественники (живущие и ископаемые) не дают информации. Тем не менее, наблюдения А.О. Ковалевского и L.Z. Holland в эмбриологии Ланцетника, в сочетании с васкуляризацией желточного мешка у круглоротых и рыб висцеральной венозной кровью, подтверждают гипотезу о происхождении печёночного выроста Ланцетника из желточного мешка хордового предшественника, предложенную Альфредом Ромером в работах о переходе от «висцерального» к «соматическому» типу в эволюции хордовых. Ранее, Чарльз Дарвин, на основании наблюдений А.О. Ковалевского, предположил сходный путь эволюции хордовых наиболее вероятным.

Ключевые слова: эволюция; гомология; хордовые; позвоночные; печень; Ланцетник; филогенетический предшественник; Ромер; Дарвин.

Background: morphologic changes in evolution in light of the Homology Concept

In vertebrates, visceral organs show a variety of morphological transitional modifications between animal groups that carry features of major forms in vertebrate phylogeny, i.e., cyclostomata → fishes → amphibians → reptiles → mammals (this grouping is called the “accepted phylogenetic sequence”). The heart, for example, shows a transition from three consecutive chambers in cyclostomata (Augustinsson et al., 1956), to four consecutive chambers of chondrichthyans and bony fishes (Kardong, 2012), and to a double circulation in lungfishes (Icardo et al., 2015). Then it transitions from amphibians’ left and right atrial chambers (Jaffee, 1963; Kardong, 2012) to reptiles’ three-chambered hearts with two atria and one common ventricle (with the exception of crocodiles’ four chamber heart) (Kardong, 2012; O’Malley, 2005), and then to mammals’ hearts with four chambers and parallel double circulation circuits (Jensen et al., 2013; Kardong, 2012).

However, unlike other visceral organs, e.g., heart, the evolution of a portal/liver system has been highly conserved in the vertebrate lineage (Hildebrand and Goslow, 2004). The anatomical stability of this complex arrangement (elaborated portal/liver system with unique vascularization) and the absence of a homologous precursor creates a “transitional gap” in Darwinian interpretation. In Chapter VI “Difficulties on Theory” of the Origin of Species, Charles Darwin writes:

“If it could be demonstrated that any complex organ existed, which could not possibly have been formed by numerous, successive, slight modifications, my theory would absolutely break down. But I can find out no such case. No doubt many organs exist of which we do not know the transitional grades, more especially if we look to much-isolated species, round which, according to my theory, there has been much extinction. Or again, if we look to an organ common to all the members of a large class, for in this latter case the organ must have been first formed at an extremely remote period, since which all the many members of the class have been developed; and in order to discover the early transitional grades through which the organ has passed, we should have to look to very ancient ancestral forms, long since become extinct.

We should be extremely cautious in concluding that an organ could not have been formed by transitional gradations of some kind.”(Darwin, 1859).

Nevertheless, in animal groups representing the accepted phylogenetic sequence (cyclostomata → fishes → amphibians → reptiles → mammals), the only variations in the portal/liver system are those in Hagfishes and some Teleosts, in which the portal vein receives blood from the viscera and a caudal part of the body (Hildebrand, 1974; Johansen, 1960). In contrast, in amphibians and reptiles, the portal system also receives blood from the ventral abdominal wall (Hildebrand and Goslow, 2004). Therefore, already in the basal vertebrate Cyclostomata the visceral post-capillary venous blood is collected into

a single portal vein and directed to the already-formed liver, where it breaks into a capillary net again, forming hepatic sinusoids and communications with liver parenchyma typical for advanced vertebrates (Elias, 1955; Elias and Bengelsdorf, 1952).

Liver architecture shares the same fundamental plan in all vertebrates, from low to the highest subclasses. It is a continuous mass of cells tunneled by a labyrinth of lacunae, in which the network of sinusoids is suspended. This structure is termed a muralium ('wallwork', system of walls) (Elias and Bengelsdorf, 1952). The walls are predominantly two cells (hepatocytes) thick in lower vertebrates and one cell thick in a few mammals (Elias, 1955; Elias and Bengelsdorf, 1952). The only difference in liver design in vertebrates is that in the adult lamprey (subclass Cyclostomata), the liver contains no bile ducts. These elements are lost when the larval lamprey undergoes metamorphosis to become an adult (Peek et al., 1979; Sidon and Youson, 1983; Youson and Sidon, 1978).

The portal/liver systems in all vertebrates, including lampreys and hagfishes, show identical developmental, topological and morphological characteristics (Elias, 1955; Elias and Bengelsdorf, 1952; Zorn and Wells, 2009). Thus, this conservation suggests that even the ancestor of the most basal vertebrates – Cyclostomata – acquired a liver. This elaborate endodermal-derived organ with a unique vascularization pattern, i.e., a portal–liver system, is a hallmark feature of all vertebrates, including placental mammals.

However, morphologic changes in evolution imply that any organ of living animals must descend with modifications from a homologous organ present in their common ancestor (Darwin, 1859; Gee, 2001; Romer and Parsons, 1986b; Walker Jr and Liem, 1994; Wolpert, 2000).

An appeal to homology in biology writings is commonly complemented by specification of what particular "kind of homology" is discussed. The terms "homology" and "homologous" here and further are used only in a sense of a historical concept of homology (Brigandt and Griffiths, 2007; Minelli and Fusco, 2013): "Homology, as classically defined, refers to a historical continuity in which morphological features in related species are similar in pattern or form because they evolved from a corresponding structure in a common ancestor." (Shubin et al., 2009). While citing the above statement, I believe that the application of the Homology Concept in conjunction with "descent with modifications" does not give room for any other interpretations than in classical Darwinian logic. As Minelli and Fusco write "This is the reason why, when Darwin (1859) used homology to support his theory of descent with modification, he did not beg the question (Minelli and Fusco, 2013).

Darwin writes: "... in order to discover the early transitional grades through which the organ has passed, we should have to look to very ancient ancestral forms...". The above notion

was applied to elucidate phylogenetic transitions in vertebrate hearts (Augustinsson et al., 1956; Icardo et al., 2015; Jaffee, 1963; Jensen et al., 2013; Kardong, 2012; O'Malley, 2005), and different hypotheses on the pre-vertebrate – vertebrate phylogenetic transition were outlined to suggest a homologous precursor of descendant forms (Fishman and Chien, 1997; Simões-Costa et al., 2005). Hence, the same inevitable question should be asked in regard to the vertebrate portal/liver system: What is the homologous phylogenetic precursor of the Cyclostomata portal/liver system, which already appears in this group of basal vertebrates as an elaborate organ with a unique vascularization pattern? This question must be asked for the sake of homology and because alternatively we would be forced to embrace the old notion that organs in evolution "... may be developed suddenly instead of gradually." (Mivart, 1871) and repudiate the Homology Principle together with Darwin theory.

Morphological arguments in favour of the origin of the vertebrate liver from the *Amphioxus* midgut diverticulum

Indeed, this quest always has long been raised, whether in a straightforward manner or indirectly. As the answer, all prominent experts grant the status of a phylogenetic homologous organ of *Amphioxus* (Cephalochordate)—the midgut diverticulum (Alexander, 1981; Barrington, 1979; Beklemishev, 1969; De Beer, 1928; Kardong, 2002; Lankester, 1889; Leake, 1975; Rähr, 1979a; Ulmer et al., 1962; Waterman, 1971; Willey, 1894; Young, 1962).

Such an appeal to a very distant, but presumably ancestral, group in a search of homology, when a morphologic characteristic in the group of interest shows little variation, was suggested by Darwin in the *Origin of Species* (Chapter 6. Difficulties Of The Theory. Sixth edition):

"In searching for the gradations through which an organ in any species has been perfected, we ought to look exclusively to its lineal progenitors; but this is scarcely ever possible, and we are forced to look to other species and genera of the same group, that is to the collateral descendants from the same parent-form, in order to see what gradations are possible, and for the chance of some gradations having been transmitted in an unaltered or little altered condition. But the state of the same organ in distinct classes may incidentally throw light on the steps by which it has been perfected." (Darwin, 1861).

All cephalochordates possess a sizable organ called a midgut diverticulum (Leake, 1975; Young, 1962); other terms are also common, e.g., hepatic or digestive caecum (Bhattacharya et al., 2008) or hepatic diverticulum (Ulmer et al., 1962), which includes a part of the intestine forming a sac and protruding from a midgut in the cranial-ventral-right direction (Fig. 1).

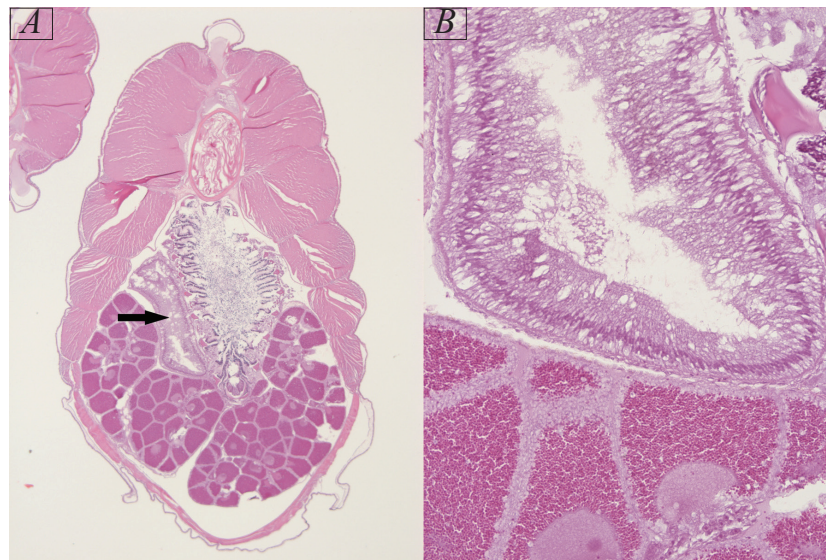


Fig. 1. *Amphioxus Branchiostoma floridae* (frontal view, female), H&E-stained paraffin section, front view:

A – Cross-section at the middle of the pharynx, arrow indicates the hepatic diverticulum, $\times 50$. B – The same area of the hepatic diverticulum and gonad, enlarged, $\times 400$.

The exceptional feature of this organ is that it is vascularized not by an arterial vessel but by a peculiar intestinal vein. In *Amphioxus*, venous blood from the post-capillary network of the caudal intestine is collected into an unpaired subintestinal vein, which breaks into a capillary network again and brings blood to the diverticulum (which appears as an intestinal sac, significantly extending cranially, laterally on the right from the midgut). Then the diverticulum's capillaries are again collected into a single vein – *vena Cardinales posterior* (analog of *vena Cava* in vertebrates) (Beklemishev, 1969; Ulmer et al., 1962).

Although this fact is not related to the aim of this analysis, it is worth mentioning that the appearance of the diverticulum in *Amphioxus* on the right side represents phylogenetically the earliest and most significant visceral asymmetry that occurs in chordate animals that otherwise maintain bilateral symmetry through adult life.

The vascularization of the *Amphioxus* midgut diverticulum was described in great detail by Hans Rähr (Rähr, 1979b). The important vascularization pattern of the *Amphioxus* midgut diverticulum and caudal intestine (hind-gut) could be demonstrated by a simplified schematic (Fig. 2):

Such a vascular pattern, i.e., intestinal venous blood again forming a capillary net between two veins (*reta mirabilia*) and supplying a derivative of intestine (i.e., portal/liver system), is a characteristic of both Cephalochordates and vertebrates. Although *Amphioxus* does not possess a liver as a solid gland, all prominent experts (a long time ago and now) share the opinion that this unique *Amphioxus* intestinal vein/diverticu-

lum arrangement is a homologous precursor to the portal vein/liver system in vertebrates (Alexander, 1981; Barrington, 1979; Beklemishev, 1969; De Beer, 1928; Kardong, 2002; Lankester, 1889; Leake, 1975; Rähr, 1979a; Shore, 1891; Ulmer et al., 1962; Waterman, 1971; Willey, 1894; Young, 1962).

Thomas Shore in 'Notes on the Origin of the Liver' writes :

"...we must not lose sight of the fact that this animal (*Amphioxus* – *VMS*) has now been shown to possess a "portal" system of veins quite comparable to that of higher vertebrates, and also of the fact that in no invertebrate has anything like a "portal" system of blood-vessels been shown to exist" (Shore, 1891).

Charles Weichert in 'Elements of Chordate Anatomy' directly associates the "portal" system of *Amphioxus* to the acquisition of a liver by vertebrates:

«Although no true liver is found in *amphioxus*, the presence of such a structure in higher chordates is foreshadowed in *Amphioxus* by a hollow, forward-projecting, ventral *hepatic caecum* which comes off the intestine just posterior to the branchial region. The lining of this pouch is ciliated, and it may have some digestive function. A system of veins coming from the intestine breaks up into capillaries on the hepatic caecum, thus presaging the appearance of the hepatic portal vein of higher form.» (Weichert, 1953).

However, there is an argument that the vascularization of a derivative of intestine by intestinal venous blood (portal circulation) does not alone constitute sufficient evidence to explain the differentiation of the midgut diverticulum of *Cephalochordata* into liver, as has been argued previously (Barrington,

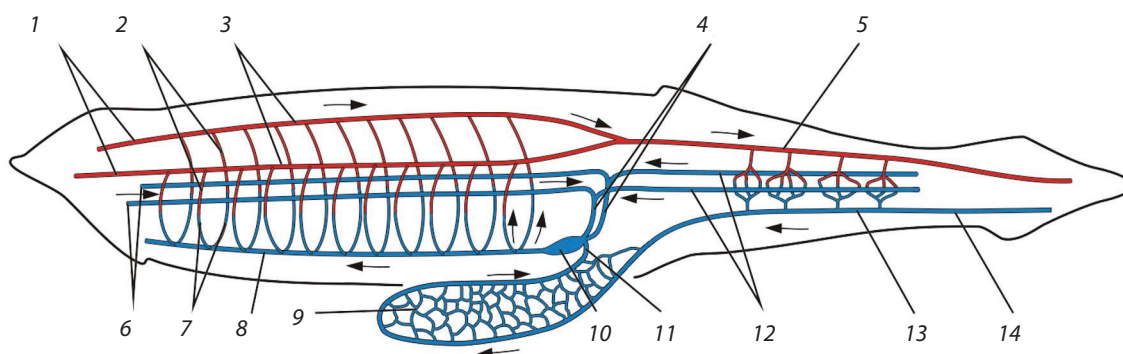


Fig. 2. Diagram of the circulatory system of Amphioxus.

13 – subintestine vein; 9 – capillary network surrounding the diverticulum; 11 – *vena Cardinales posterior*. From: Wikipedia (Wikipedia, 2017).

1937). In this case, finding other facts that support same hypothesis could favour the so called ‘Best Explanation’.

Although, at first glance, the above task appears unsophisticated, it constitutes a valid and important scientific tool, as stated in the most famous biology writing:

“...it is a method used in judging of the common events of life, and has often been used by the greatest natural philosophers.” (Darwin, *On the Origin of Species*, 1872, p. 545 (Darwin, 1861)).

Needless to say, the entire Darwin argument was based on the same abductive reasoning. This type of reasoning was termed by G.H. Harman as “Inference to the Best Explanation” (Harman, 1965) and is widely used in scientific analyses (Lipton, 2003; Thagard, 1978).

Additional facts supporting the origin of the vertebrate liver from the Amphioxus midgut diverticulum

I. Amphioxus diverticulum expresses vertebrate liver-specific proteins

One class of supporting facts consists of the number of vertebrate liver-specific markers that are expressed in the Amphioxus hepatic diverticulum, e.g., glutathione-S-transferase, plasminogen-like protein, antithrombin, and cytochrome P450 (Bhattacharya et al., 2008; Fan et al., 2007b; Liang and Zhang, 2006; Liang et al., 2006; Mizuta and Kubokawa, 2007). This expression supports the homology hypothesis above. Another piece of support comes from the fact that Amphioxus’ diverticulum is the sole tissue producing vitellogenin in Amphioxus (Han et al., 2006; Wang et al., 2015), thereby “reinforcing the homology of digestive diverticulum to vertebrate liver” (Fan et al., 2007a; Li and Zhang, 2010; Liang and Zhang, 2006; Liang et al., 2006; Liem et al., 2001). (Note: vitellogenin is never synthesized by oocytes themselves; in vertebrates, this synthesis

occurs mainly in the liver, and then vitellogenin is concentrated in oocytes (Jorgensen, 2008).

Additional facts supporting the origin of the vertebrate liver from the Amphioxus midgut diverticulum

II. Co-evolution of insulin-producing tissue and liver in the chordate/vertebrate lineage

Another group of facts that could be explained by the same hypothesis are facts on co-evolution of insulin-producing tissues and liver in the chordate – vertebrate lineage. The transition of the *Cephalochordata* midgut diverticulum differentiation into the liver in the chordate – vertebrate lineage could be inferred from comparative morphology and phylogeny of insulin-producing tissue, the Islets of Langerhans. It is well-documented that certain components of portal blood – insulin and augments of liver regeneration – exert morphogenic properties for hepatocyte differentiation (Junge and Creutzfeldt, 1981; Parzefall et al., 1996; Starzl et al., 1976a; Starzl et al., 1976b) (Nalesnik et al., 2017; Starzl et al., 1975). It was also shown that insulin receptor substrate-2 is crucial for liver development and hepatocyte survival (Giddings and Carnaghi, 1992; Khamzina et al., 2003).

In invertebrates, insulin is mainly produced by neural cells (e.g., (Birse et al., 2011), (for review see (Conlon et al., 1988; Heller, 2010)). However, in Amphioxus, the cells expressing insulin-like growth factor are mainly scattered epithelial cells of hepatic diverticulum and caudal intestine (hind-gut) (Guo et al., 2009; Reinecke et al., 1993) that are not yet organized into an islet organ (Heller, 2010). Cyclostomes are the first Chordates that develop compact insulin-producing tissues (Islet of Langerhans) in conjunction with portal circulation (Epple and Brinn, 1975; Schirner, 1959; Yegorov et al., 2014), and they simultaneously acquire the liver.

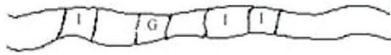



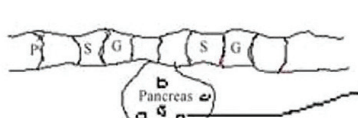

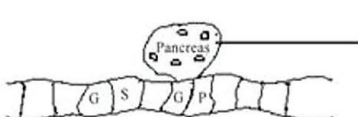



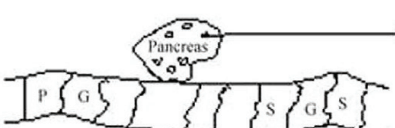

Phylogeny	Gut	Islet Organ	Notes
Invertebrates (Flies/silk worm)		None	Insulin and glucagon like peptides in the gut
Protochordates (amphioxus, tunicates)		None	First appearance of PP and SS like cells
Cyclostomes (hagfish, lamprey)			First islet like organ with two cell types and lumen
Cartilagenous fish (shark, ray) Bony fish (teleost, lungfish)			First real islet organ with 3-4 unique cell types
Amphibians (salamander, frog) Reptiles (turtle, snake)			Islets with all four principal hormones. Scattered endocrine cell
Birds (chicken, ducks)			Multilobed pancreas in some birds and many glucagon cells in islets. Ghrelin is found in some species
Mammals			Islets with five endocrine cell in some species

Fig. 3. Evolution of the Islet organ from invertebrates to mammals. Considerable species variation occurs in all classes, but the scheme is meant to be semi-representative. Family member cell types that remain in the gut are represented by single letters. I = insulin, G = glucagon peptides, SS = somatostatin peptides, P = PP family peptides. The cyclostomes are the first species in which islet-like clusters have migrated out of the gut tube into a separate cluster (islet) surrounding the common bile duct. It is with the cartilaginous and bony fish that the first real pancreas is formed with islets containing three and sometimes four hormones. These islets can lie within large islets (Brockmann bodies) or multiple islets within an exocrine pancreas. Reptiles and Amphibia are the first species with islets containing all four of the major hormones. Some species of Aves have multilobed pancreata, and the islets tend to contain a lot of glucagon cells. This is the first appearance of ghrelin cells in some species. Mammals have a diverse range of structures but are generally round and contain four or five islet hormones. Insulin (*red*), Glucagon (*green*), Somatostatin (*blue*), Pancreatic Polypeptide (*yellow*), Ghrelin (*purple*). BD = bile duct. From: Heller RS, The comparative anatomy of islets. (Heller, 2010), reproduced with permission of the Publisher.

The best comparative and phylogenetic model on the Islet of Langerhans is given by R. Scott Heller in his chapter "The Comparative Anatomy of Islets" (Heller, 2010), as illustrated in Fig. 3:

Therefore, it is conceivable that during Chordate evolution between Early (Benton, 2005; Mallatt and Holland, 2013) – Middle Cambrian (Morris and Whittington, 1979) and upper Cambrian – lower Ordovician (Chaline, 1990; Dineley, 1964), venous blood drained from intestine began to carry hor-

mones of the pancreatic family to the midgut diverticulum of an Amphioxus-like animal via pre-existing portal circulation. A transition of the brain-gut axis in regards to the pancreatic family hormones from neural to intestinal epithelial cells is well documented in the evolution of protochordates and chordates (Conlon et al., 1988; Emdin and Falkmer, 1977; Falkmer, 1979; Falkmer et al., 1985; Falkmer et al., 1976; Falkmer et al., 1978; Guo et al., 2009; Reinecke et al., 1993). From all these facts, we can suggest that 1) the transition of pancreatic family hormone

expression from neural cells to intestinal epithelial cells and 2) portal circulation by bringing hormones of the pancreatic family back to the epithelial cells of Amphioxus diverticulum, promoted differentiation of the midgut diverticulum of *Cephalochordata* into the liver of vertebrates, followed by selection.

From the Inference to the Best Explanation (Harman, 1965) (Lipton, 2003; Thagard, 1978), the hypothesis of vertebrate liver phylogeny from the Amphioxus diverticulum is the most parsimonious model that complies with the Homology Principle and unites facts on co-evolution of the Islets of Langerhans (Conlon et al., 1988; Emdin and Falkmer, 1977; Falkmer, 1979; Falkmer et al., 1985; Falkmer et al., 1976; Falkmer et al., 1978; Guo et al., 2009; Heller, 2010; Reinecke et al., 1993.) and liver (Alexander, 1981; Barrington, 1979; Beklemishev, 1969; Bhattacharya et al., 2008; De Beer, 1928; Fan et al., 2007b; Kardong, 2002; Lankester, 1889; Leake, 1975; Liang and Zhang, 2006; Liang et al., 2006; Liem et al., 2001; Mizuta and Kubokawa, 2007; Rähr, 1979a; Ulmer et al., 1962; Waterman, 1971; Willey, 1894; Xu, 2015; Young, 1962) in the chordate/vertebrate lineage.

Therefore, the hypothesis that Cephalochordate's hepatic diverticulum is a homologous phylogenetic precursor of vertebrate liver sounds very substantial and is shared by most experts (Alexander, 1981; Barrington, 1979; Beklemishev, 1969; Bhattacharya et al., 2008; De Beer, 1928; Fan et al., 2007b; Kardong, 2002; Lankester, 1889; Leake, 1975; Liang and Zhang, 2006; Liang et al., 2006; Liem et al., 2001; Mizuta and Kubokawa, 2007; Rähr, 1979a; Ulmer et al., 1962; Waterman, 1971; Willey, 1894; Xu, 2015; Young, 1962). However, the Homology Concept equally obligates us to ask the following important question: What could be a phylogenetic precursor of the Amphioxus hepatic diverticulum, considering its elaborate architecture and unique vascularization pattern?

Facts and arguments in favor of the origin of the Amphioxus diverticulum from a yolk sac of a preceding advance chordate – a hypothesis

Again, if we accept the Homology Concept, this question also must be asked because alternatively, we would be forced to embrace the old notion that organs in evolution "... may be developed suddenly instead of gradually." (Mivart, 1871) and repudiate the Homology Principle. Although, to the best of my knowledge, this question never appeared in publications, I believe that such an enquiry should be a natural quest for anyone studying the topic. I speculate that the reason why this question is traditionally omitted is that the putatively preceding forms (living animals or fossils) apparently do not provide any relevant evidence for the answer.

Recently, I came across some information that may shed light on the origin of the Amphioxus hepatic diverticulum.

I have received access to the original magistrate thesis of Alexander Kovalevsky (Ковалевский, 1865) on the development of *Amphioxus Lanceolatus*, published in Russian in 1865. A shortened version of the thesis was re-published as a research article in German in 1867 (Kovalevsky, 1867) and much later in Russian as part of 'The Selected Manuscripts of Kovalevsky' (Ковалевский, 1951). By reading, side to side, the earliest (Ковалевский, 1865) and later editions (both German and Russian) (Kovalevsky, 1867; Ковалевский, 1951), I found that the later editions were published without one crucial fragment, which reads:

"Developing diverticulum stretches from the gut. Some considered Amphioxus' diverticulum as the organ homologous to liver. Indeed, all cells of the diverticulum are filled with a yellow-green substance; interestingly, even before formation of the diverticulum, its function was performed by a straight part of the gut; the color of intestinal wall in this location is completely green." (Ковалевский, 1865) (page 31), (*VMS translation*).

Available publications on Amphioxus development do not provide additional information about the hepatic diverticulum (Conklin, 1932; Holland and Onai, 2012). Since I have only adult specimens of Amphioxus, I asked a prolific expert on the Lancelet to share personal observations on Amphioxus development, in particular on the development of the hepatic diverticulum. The expert replied:

"The diverticulum forms at the very end of metamorphosis as an outgrowth of the gut. The more well fed the animals, the larger the diverticulum. Food moves into the diverticulum, which seems to store the food. Before the diverticulum forms, if the animals do not have food for a period of hours, the gut empties, they stop eating and never start again. After the diverticulum forms, if the animals do not have food for a day, the main gut empties, but the diverticulum remains full of food and if food is provided the animals will eat it and do fine." (L.Z. Holland, personal communication) (Holland, 2014b).

Notes on early midgut development by Kovalevsky and the above observations from Dr. Holland stating that the diverticulum functions in food storage motivated me to examine the hypothesis that a homologous phylogenetic precursor of the Amphioxus' diverticulum could be a yolk sac of a preceding animal. The reasons for this unconventional proposition are very simple: If we believe that an organ of a living animal must descend from a homologous organ of an ancestor animal, we have to hypothesize a model with such a homologous precursor and first test this model for its internal consistency. Under the above theoretical constraints, there is no structure other than a yolk sac that could be suggested. Another reason (also simple) is the anatomical similarity between Amphioxus' diverticulum and yolk sac: both appear as extensions of intestine.

The possible connection between embryonic nutrition and the yolk sac ("large yolked ova") was suggested earlier by Thomas Shore:

"...that this organ primarily arose as an embryonic «organ of nutrition,» evolved *pari passu* with the evolution of those vertebrates which produced large yolked ova." (Shore, 1891).

At this point, I can foresee a loud objection: Amphioxus has a microlecithal or alecithal oocyte, which is traditionally considered as "primary alecithal" (in contrast to "secondary alecithal" oocytes of marsupials and placental mammals) and no yolk sac by default. Therefore, where did "a yolk sac of a preceding animal" come from?

Yolk sac consists a yolk in minimal/moderate to significant amounts, and the contained tissues – endodermal, mesenchymal, and ectodermal. These tissues are formed in all animals with bilateral symmetry. If we agree that these tissues are always present, let us test this model for its internal consistency; "yolk issue" will be elaborated later.

The first step in evaluating the hypothesis is to determine whether there is a possible anatomical similarity between the Amphioxus diverticulum and the yolk sac.

To visualise this comparison, I invite my readers to perform an imaginary transposition of the Amphioxus diverticulum. Imagine that a midgut diverticulum, surrounded by skin with feeding and draining vessels, is being stretched and protruded down from the Lancelet ventral site, together with skin and vasculature. Anatomically, this repositioned midgut diverticulum, with its unique vascular pattern, would be homologous to a yolk sac (Fig. 4).

The hypothesis: *Argumenta Pro et Contra*

The main benefit of this hypothesis is that it suggests a real organ – a yolk sac – as a precursor of the Amphioxus' diverticulum. Although Amphioxus has a microlecithal oocyte with no yolk sac, in theory, the presence of a yolk sac in a preceding form is plausible. Another advantage of this suggestion is that there is no other organ/structure that could be morphologically suggested as a homologous phylogenetic precursor of the Amphioxus' diverticulum.

Facts from studies of early development of the digestive system in cyclostomes and fishes provide additional support for this hypothesis.

In a morphological study on the early development of the lamprey digestive and intestinal blood systems (15 days, about 5 mm long), E.W. Baxter (1957) writes:

"In these larvae the blood can be seen traversing the lateral walls of the gut near the anterior end of the yolk mass and by this route a steady trickle of blood reaches the now mid-ventral sub-intestinal vein. In this vessel the blood passes forwards to the liver, which has now reached the stage of a hollow sac,

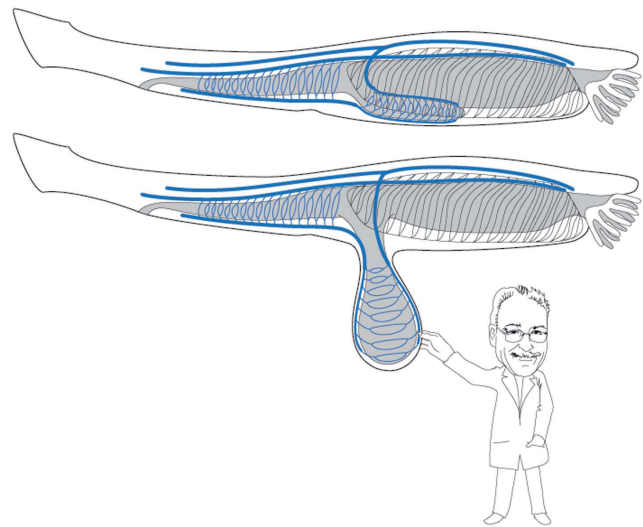


Fig. 4. Imaginary transposition of a midgut diverticulum, surrounded by skin with feeding and draining vessels, such that it became stretched and protruded down from Lancelet. Morphologically, this midgut diverticulum, with its unique vascular architecture, is homologous to a yolk sac (highly schematic).

and from anastomosing vessels in its walls the hepatic blood is returned to the heart." (Baxter, 1957).

Please note that in lamprey larva, the only vessel feeding the yolk sac is an unpaired subintestinal vein. The fact that the lamprey has two hollow sacs (liver and yolk sac) with similar vascularization patterns is puzzling, but its deliberation is beyond the scope of this communication. It can only be speculated that the yolk sac was duplicated (i.e., gene duplication) in an ancestor, with one copy taking on the new function as the digestive/secretory organ, while the other maintained its role in food storage.

The crucial fact is that the lamprey's yolk sac has a vascularization pattern similar to that of the Amphioxus' diverticulum.

Another relevant note was written by the famous evolutionary scholar Harland W. Mossman in a manuscript published in the *Biological Reviews of the Cambridge Philosophical Society*:

"...the blood supply of the yolk sac of teleost fishes comes from somatic veins, such as the caudal and cardinals, instead of from vitelline arteries branching off from the aorta as in amniotes." (Mossman, 1948).

Although later studies showed that arterial supply to the yolk sac also exists in teleost fishes, e.g., (Isogai and Horiguchi, 1997), the early participation of the subintestinal and the posterior cardinal veins in yolk sac vascularization (Isogai and Horiguchi, 1997; Mossman, 1948) favors homology between the yolk sac and Amphioxus' hepatic diverticulum.

A study on anatomical interactions between the yolk sac and intestine during early fish development was conducted by O.I. Schmalhausen (1991). In descriptions on prelarval development of Russian Sturgeon (Schmalhausen, 1991), which belongs to a phyletically ancient fish group (Foreman et al., 2013), Olga I. Schmalhausen writes:

“At the stage of hatching, the digestive system consists of the alimentary canal and rudiments of the digestive glands, liver, and dorsal pancreas. The alimentary canal is divided into two parts, a widened anterior (yolk sac) part and a narrow posterior part.” (Schmalhausen, 1991).

Although this description of a Sturgeon yolk sac is short, it shows the same anatomical relation of the yolk sac to intestine, as it appears in *Amphioxus* between the hepatic diverticulum and the caudal intestine.

Again, this analysis is a theoretical exercise aiming to suggest a homologous phylogenetic precursor of the *Amphioxus*' hepatic diverticulum. To facilitate this goal, arguments are borrowed from a range of different studies and representatives or are hypothetical suggestions based on logic. Since we do not have facts suggesting a putative precursor of *Amphioxus*' diverticulum, aligning the above arguments from different fields/subjects may help to test the hypothesis for its internal coherence.

The only condition that is firmly embedded in the hypothesis is that the hypothesized phylogenetic forerunner of *Amphioxus* must be an advanced chordate with a yolk sac. Within this assumption, the main objection is the deviation from the usual phylogenetic trend in ovum size: from smaller – microlecithal oocyte (no yolk sac) to bigger – mesolecithal and telolecithal oocytes (presence of yolk sac). (Note: changing the size of the ovum due to viviparity occurring in different vertebrate classes is beyond the scope of this analysis.) However, the opposite trend is also plausible under variations of nutrient availability: The transition of the feeding pattern in larval forms from lecithotrophy to planktotrophy, or to that of facultative feeding and other intermediate forms, is known and theoretically possible in both directions (Allen and Pernet, 2007; Allen et al., 2006), which could affect gut morphogenesis (Pernet and McHugh, 2010).

Therefore, I hypothesize that *Amphioxus* evolved from an advanced motile chordate ancestor with a yolk sac, and during this transition (or cephalochordate phylogeny itself), the yolk sac ceased to function in food storage, became internalized, and acquired functions of a digestive organ. It is worth noting that the internalization/somatization of the yolk sac is a normal morphogenesis process in the development of many living fishes (Mossman, 1987; Schmalhausen, 1991). Obviously, the chordate lineage acquired a liver after the portal system had been acquired.

Since we agree that formation of the liver followed the acquisition of a portal system in the phylogeny of chordates, a well-documented transition of the brain-gut axis in regards to the pancreatic family hormones from neural to intestinal epithelial cells in the evolution of protochordates and chordates (Conlon et al., 1988; Emdin and Falkmer, 1977; Falkmer, 1979; Falkmer et al., 1985; Falkmer et al., 1976; Falkmer et al., 1978; Guo et al., 2009; Reinecke et al., 1993) (Epple and Brinn, 1975; Schirner, 1959; Yegorov et al., 2014) (Heller, 2010) could serve as the causal explanation of co-evolution of insulin-producing tissue and liver.

My hypothesis is congruent with the model of chordate evolution, advanced by Alfred Sherwood Romer, who is a renowned for his contributions to the study of vertebrate evolution. In his work, Professor Romer advocated the hypothesis that chordate phylogenesis began with primitive sessile (attached) “visceral” “arm-feeding” animals, which evolved into sessile gill filter-feeding animals. Romer's hypothesis suggests further evolution with selection of ancestral tunicates, whose free-swimming larva evolved into a motile, advanced chordate. Romer suggested that the motile, advanced chordate is an ancestor of both a basal vertebrate and *Amphioxus* (Romer and Parsons, 1986a; Romer, 1967; Romer, 1972, 1959), (Fig. 4):

Obviously, the above model contradicts the recently proposed rearrangements of phyletic relations in the phylum Chordata, based on an analysis of molecular data (Delsuc et al., 2006; Delsuc et al., 2008). Traditional (Holland et al., 2004; Romer, 1967; Schaeffer, 1987; Stach, 2008; Stach, 2014; Stokes and Holland, 1998; Wada and Satoh, 1994) or ‘standard’ (Gee, 2001) perception of phylogenetic relations between Chordate subphyla (based on both morphologic and molecular data) suggested Cephalochordata as the most closely preceding subphylum to Vertebrata. On the contrary, recent phylogenetic analysis of a large set of molecular data suggested Tunicata as a sister taxon to Vertebrata (Delsuc et al., 2006; Delsuc et al., 2008). (It should be mentioned that one classical morphologic analysis on phylogeny of low chordates, while outlining all possible relations between Chordate subphyla, still favors a view of Tunicata as a sister taxon to Vertebrata (O.M. Ivanova-Kazas, 1995, p. 14) (Ivanova-Kazas, 1995)).

In regard to the Homology concept, there is a concern that the recent trend in phylogenetic reconstructions disregards morphologic evidence (Assis, 2009; Giribet, 2015; Jenner, 2004; Wanninger, 2015; Wiens, 2004; Wipfler et al., 2016). I share these concerns (Assis, 2009; Giribet, 2015; Jenner, 2004; Wanninger, 2015; Wiens, 2004; Wipfler et al., 2016), and would like to highlight particular differences and similarities in the morphology of vascular systems among Chordate subphyla.

Among chordates, the representatives of the Tunicata phylum showed the greatest diversity in body plan (Holland,

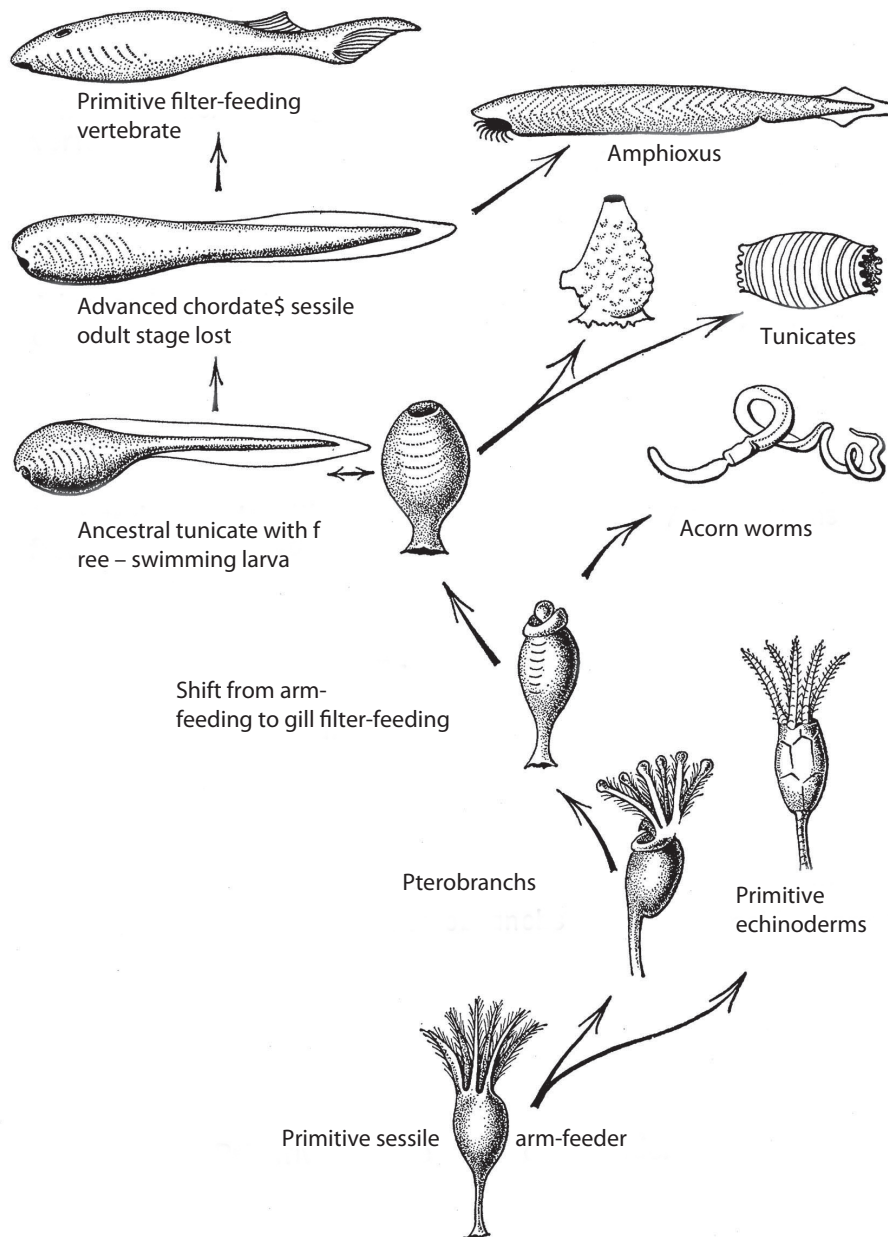


Fig. 5. Romer's diagram on the probable course of chordate evolution. "From a primitive sessile arm-feeder to the tunicates, there evolved successive improvements in the «visceral» animals. With the free-swimming larva of the tunicate type, there began a second evolutionary series, with emphasis on the dominant «somatic» animal." From: Alfred Sherwood Romer, *The vertebrate as a dual animal – somatic and visceral*, 1972 (Romer, 1972). Reproduced with permission of University of Chicago Press.

2014a). However, in spite of such diversity, all tunicates, without exception, possess a unique feature that separates them from both Cephalochordata and Vertebrata – a unique reverse pattern of blood circulation (Bone et al., 1997; Goddard, 1973; Konrad, 2016). O. F. Kampmeier writes:

"The circulation of blood in tunicates presents a phenomenon that is without parallel in the animal kingdom. The heart

reverses its pulsations periodically; in other words, the waves of contraction pass along it from end to end first in one direction for a certain number of beats (from 60 to 100) and then, after a slight pause, in opposite direction (Kampmeier, 1969, p.163)" (Kampmeier, 1969).

Obviously, this unique trait – the reverse pattern of blood circulation – must evolve in ancestral tunicates prior to their

diversification. Another indirect piece of evidence that this pattern was acquired early in tunicate phylogeny is the fact that the tunicate heart has a reverse direction in the earliest studied animals (3 days after attachment) (Konrad, 2017). As far as we know, no Vertebrata shares this trait, which creates a morphology gap in phyletic relations between Tunicata and Vertebrata. On the other hand, Cephalochordata and Vertebrata share the same vascular system design, including such unique feature as the portal/liver vascular pattern, which makes a morphology bridge for phyletic relations between Cephalochordata and Vertebrata. Such strong homology and dissimilarity argue in favor of the traditional schema on phylogenetic relations between Chordate subphyla, in which Cephalochordata is suggested as the closest subphylum to Vertebrata (Gee, 2001; Holland et al., 2004; Romer, 1967; Schaeffer, 1987; Stach, 2008; Stach, 2014; Stokes and Holland, 1998; Wada and Satoh, 1994).

The alternative model suggests the complete loss of the general vascular design, including the portal/liver pattern and one-directional blood flow, during the transition from pre-Cephalochordata to pre-Tunicata and the acquisition of the reverse pattern of blood circulation in phylogeny of Tunicata. Positioning of Tunicata as a sister taxon to Vertebrata also inevitably suggests loss and acquisition of the same traits but in the opposite sequence: loss of the reverse blood circulation, acquisition of one-directional circulation, and re-acquisition of the general one directional vascular design, including the portal/liver vascular pattern, which makes such modeling less parsimonious. Of course, it could be disputed that Cephalochordata represent a relatively recent offshoot of ascidians stem (Medawar, 1951), but Amphioxus' hepatic diverticulum argues against this idea. Additionally, common sense favors tunicate divergence before the appearance of the motile advanced chordate (suggested precursor of both vertebrates and Amphioxus) because this model requires the fewest evolutionary events (Steel and Penny, 2000) and, therefore, is parsimonious.

Concluding remarks

My hypothesis only adds a yolk sac to the advanced chordate of the Romer model. The Amphioxus phylogeny from an advanced chordate was initially suggested by A.S. Romer in his hypothesis on the transition from "visceral" to "somatic" animals in evolution of the chordate (Romer and Parsons, 1986a; Romer, 1967; Romer, 1972, 1959). (Note: I do not think that the term "Garstang-Berrill-Romer hypothesis" (Holland, 2011; Lacalli, 2005) is correct, because Romer's model suggested origin of Amphioxus from advanced motile chordate and not from a tadpole-like protochordates, as Lacalli depicted (Lacalli, 2005). In my model, the yolk sac of the advanced chordate predecessor is suggested to be the homologous precursor of the Amphioxus hepatic diverticulum.

This analysis is based on the idea that all organs of living animals must descend, with modifications great or small, from homologous organs of a common ancestor. My inquiry into origin of the vertebrate liver and the Amphioxus hepatic diverticulum was thought about and revised for twenty five years, but it only recently gained traction due to the discovery of the Alexander Kovalevsky and Linda Holland observations. Therefore, I suggest that within the Homology concept and according to the Inference to the Best Explanation principle (Harman, 1965) (Lipton, 2003; Thagard, 1978), the only organ that could be hypothesized as the homologous precursor for Amphioxus' diverticulum is the yolk sac of a preceding advanced motile chordate ancestor. I also hypothesize that during the transition from the presumably advanced chordate to Amphioxus (or during cephalochordate phylogeny itself), the yolk sac ceased to function in food storage, became internalized, and acquired functions of a digestive organ.

I also suggest that within the Homology concept and in congruence with morphologic evidences, the traditional (Gee, 2001; Holland et al., 2004; Romer, 1967; Schaeffer, 1987; Stach, 2008; Stach, 2014; Stokes and Holland, 1998; Wada and Satoh, 1994) perception of phylogenetic relations between Chordate subphyla is a parsimonious model.

Similarly, in light of the Homology concept, the only organ that could be hypothesized as the homologous precursor for Amphioxus' diverticulum is the yolk sac of a preceding advanced motile chordate ancestor. I also hypothesize that during the transition from the presumably advanced chordate to Amphioxus (or during cephalochordate phylogeny itself), the yolk sac ceased to function in food storage, became internalized, and acquired functions of a digestive organ, establishing the homologous phylogenetic precursor of vertebrate liver.

I also believe that the consistency of my hypothesis with the accepted model of Islets of Langerhans phylogeny (Heller, 2010) (Epple and Brinn, 1975; Guo et al., 2009; Reinecke et al., 1993; Schirner, 1959; Yegorov et al., 2014), as well as congruence with the well-thought out model on the probable course of chordate evolution outlined by the profound evolutionary scholar Alfred Sherwood Romer, is supportive. In this avenue, I find the following citation as endorsing the Romer model and my small contributions to it:

"Some observations lately made by M. Kowalevsky,²² since confirmed by Prof. Kuppfer, will form a discovery of extraordinary interest, if still further extended, as I hear from M. Kowalevsky in Naples he has now effected. The discovery is that the larvae of Ascidians are related to the Vertebrata, in their manner of development, in the relative position of the nervous system, and in possessing a structure closely like the *chorda dorsalis* of vertebrate animals. It thus appears, if we may rely on embry-

ology, which has always proved the safest guide in classification, that we have at last gained a clue to the source whence the Vertebrata have been derived. We should thus be justified in believing that at an extremely remote period a group of animals existed, resembling in many respects the larvæ of our present Ascidiæ, which diverged into two great branches – the one retrograding in development and producing the present class of Ascidiæ, the other rising to the crown and summit of the animal kingdom by giving birth to the Vertebrata.”

Charles Darwin. *The Descent of Man and Selection in Relation to Sex* (pp. 205-206) (Darwin, 1871).

My analysis, based on the Homology Principle, is aligned with traditional perception of phylogenetic relations between Chordate subphyla (Gee, 2001; Holland et al., 2004; Romer, 1967; Schaeffer, 1987; Stach, 2008; Stach, 2014; Stokes and Holland, 1998; Wada and Satoh, 1994), which supports cephalochordates as the closest living relatives of vertebrates. In contrast, new results from a phylogenetic analysis of a large set of molecular data suggested a dramatic rearrangement in relationships between Chordate subphyla (Delsuc et al., 2006; Delsuc et al., 2008). Employing the same Homology principle

on the molecular level, Delsuc and co-authors provided compelling evidence that tunicates, and not cephalochordates, represent the closest living relatives of vertebrates. How can we reconcile the above facts?

I believe that we shall overcome the contradiction (for now) by employing the famous notion “Homology cannot be proven; it is always inferred.” (Mayr, 2001). As Alessandro Minelli put it: “This circumstance shows how much our trees are still dependent on a body of evidence that is obviously growing, and thus changing, rapidly, so that we must be cautious before accepting this or that grouping as definitive.”

Alessandro Minelli, *Perspective in Animal Phylogeny and Evolution*. (Minelli, 2009)

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References

- Alexander, R.M. 1981. The chordates.
- Allen, J. D., and B. Pernet. 2007. Intermediate modes of larval development: bridging the gap between planktotrophy and lecithotrophy. *Evolution & development* 9: 643-653.
- Allen, J. D., C. Zakas, and R. D. Podolsky. 2006. Effects of egg size reduction and larval feeding on juvenile quality for a species with facultative-feeding development. *Journal of Experimental Marine Biology and Ecology* 331: 186-197.
- Assis, L. 2009. Coherence, correspondence, and the renaissance of morphology in phylogenetic systematics. *Cladistics* 25: 528-544.
- Augustinsson, K.-B., R. Fänge, A. Johnels, and E. Östlund. 1956. Histological, physiological and biochemical studies on the heart of two cyclostomes, hagfish (*Myxine*) and lamprey (*Lampetra*). *The Journal of physiology* 131:257-276.
- Barrington, E. J. W. 1937. The digestive system of *Amphioxus* (*Branchiostoma*) *lanceolatus*. *Philosophical Transactions London* 228: pp. 269-312.
- Barrington, E. J. W. 1979. *Essential features of lower types*. University of Chicago Press, Chicago & London.
- Baxter, E. W. 1957. The development of the intestinal blood-system of lampreys (*Petromyzonidae*) with an account of the origin of vascular couples. *Proc Zool Soc London* 129: 371-396.
- Beklemishev, V. N. 1969. *Principles of comparative anatomy of invertebrates*. 1. Promorphology. 2. Organology. Oliver & Boyd.
- Benton, M.J. 2005. *Vertebrate palaeontology*. Third edition. Blackwell Publishing.
- Bhattacharya, H., S. Zhang, and Q. Xiao. 2008. Comparison of histopathological alterations due to sublethal CCl4 on Rosy Barb (*Puntius conchonius*) and *Amphioxus* (*Branchiostoma belcheri*) with implications of liver ontogeny. *Toxicology mechanisms and methods* 18: 627-633.
- Birse, R. T., J. A. E. Soderberg, J. L. Luo, A. M. E. Winther, and D. R. Nassel. 2011. Regulation of insulin-producing cells in the adult *Drosophila* brain via the tachykinin peptide receptor DTKR. *J. Exp. Biol.* 214: 4201-4208.
- Bone, Q., J. Braconnot, and C. Carre. 1997. On the heart and circulation in doliolods (Tunicata: Thaliacea). *SCIENTIA MARINA* 61: 189-194.
- Brigandt, I., and P. E. Griffiths. 2007. The importance of homology for biology and philosophy. *Biology & Philosophy* 22: 633-641.
- Chaline, J. 1990. *Paleontology of vertebrates*. Springer-Verlag, Berlin, Heidelberg etc.
- Conklin, E. G. 1932. The embryology of *Amphioxus*. *Journal of Morphology Philadelphia* 54: pp. 69-118.
- Conlon, J., M. Reinecke, M. Thorndyke, and S. Falkmer. 1988. Insulin and other islet hormones (somatostatin, glucagon and PP) in the neuroendocrine system of some lower vertebrates and that of invertebrates—a minireview. *Hormone and Metabolic Research* 20: 406-410.
- Darwin, C. 1859. *On the Origin of Species By Means of Natural Selection, or The Preservation of Favoured Races in The Struggle for Life*. First ed. John Murray, London.
- Darwin, C. 1861. *On the Origin of Species By Means of Natural Selection, or The Preservation of Favoured Races in The Struggle for Life*. 6th ed. John Murray, London.
- Darwin, C. R. 1871. *The descent of man and selection in relation to sex: Vol. I*. J. Murray.
- De Beer, G. R. 1928. *Vertebrate zoology An introduction to the comparative anatomy, embryology, and evolution of chordate animals*. With an introduction by JULIAN S. HUXLEY. Macmillan Co., New York.
- Delsuc, F., H. Brinkmann, D. Chourrout, and H. Philippe. 2006. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature*. 439: 965-968.

23. Delsuc, F., G. Tsagkogeorga, N. Lartillot, and H. Philippe. 2008. Additional molecular support for the new chordate phylogeny. *Genesis*. 46: 592-604.
24. Dineley, D. L. 1964. Armor-plated and jawless Devonian fish. Fossil record is clue to pteraspid habitat. *Natur Hist* 73: 48-53.
25. Elias, H. 1955. Liver morphology. *Biological Reviews* 30: 263-310.
26. Elias, H., and H. Bengelsdorf. 1952. The structure of the liver of vertebrates. *Acta anatomica* 14: 297-337.
27. Emdin, S. O., and S. Falkmer. 1977. Phylogeny of insulin. Some evolutionary aspects of insulin production with particular regard to the biosynthesis of insulin in *Myxine glutinosa*. *Acta Paediatr Scand Suppl*: 15-25.
28. Epple, A., and J. E. Brinn, Jr. 1975. Islet histophysiology: evolutionary correlations. *General and comparative endocrinology* 27: 320-349.
29. Falkmer, S. 1979. Immunocytochemical studies of the evolution of islet hormones. *Journal of Histochemistry & Cytochemistry* 27: 1281-1282.
30. Falkmer, S. et al. 1985. Phylogenetical aspects on islet hormone families: a minireview with particular reference to insulin as a growth factor and to the phylogeny of PYY and NPY immunoreactive cells and nerves in the endocrine and exocrine pancreas. *Peptides* 6 Suppl 3: 315-320.
31. Falkmer, S. et al. 1976. Tumor pathology of the hagfish, *Myxine glutinosa*, and the river lamprey, *Lampetra fluviatilis*. A light-microscopical study with particular reference to the occurrence of primary liver carcinoma, islet-cell tumors, and epidermoid cysts of the skin. *Prog. Exp. Tumor. Res.* 20: 217-250.
32. Falkmer, S., S. Marklund, P. E. Mattsson, and C. Rappe. 1978. Hepatomas and other neoplasms in the atlantic hagfish (*Myxine glutinosa*): a histopathologic and chemical study. *Annals of the New York Academy of Sciences* 298: 342-355.
33. Fan, C. et al. 2007a. Identification and expression of a novel class of glutathione-S-transferase from amphioxus *Branchiostoma belcheri* with implications to the origin of vertebrate liver. *The international journal of biochemistry & cell biology* 39: 450-461.
34. Fan, C. et al. 2007b. Identification and expression of a novel class of glutathione-S-transferase from amphioxus *Branchiostoma belcheri* with implications to the origin of vertebrate liver. *The international journal of biochemistry & cell biology* 39: 450-461.
35. Fishman, M. C., and K. R. Chien. 1997. Fashioning the vertebrate heart: earliest embryonic decisions. *Development* 124: 2099-2117.
36. Foreman, R. E., A. Gorbman, J. Dodd, and R. Olsson. 2013. *Evolutionary biology of primitive fishes*. Springer Science & Business Media.
37. Gee, H. 2001. Deuterostome phylogeny: the context for the origin and evolution of the vertebrates.
38. Giddings, S. J., and L. R. Carnaghi. 1992. Insulin receptor gene expression during development: developmental regulation of insulin receptor mRNA abundance in embryonic rat liver and yolk sac, developmental regulation of insulin receptor gene splicing, and comparison to abundance of insulin-like growth factor 1 receptor mRNA. *Mol. Endocrinol.* 6: 1665-1672.
39. Giribet, G. 2015. Morphology should not be forgotten in the era of genomics—a phylogenetic perspective. *Zoologischer Anzeiger-A Journal of Comparative Zoology*. 256: 96-103.
40. Goddard, C. 1973. Vascular physiology of the ascidian *Pyura praeputialis*. *Journal of Zoology* 170: 271-298.
41. Guo, B., S. Zhang, S. Wang, and Y. Liang. 2009. Expression, mitogenic activity and regulation by growth hormone of growth hormone/insulin-like growth factor in *Branchiostoma belcheri*. *Cell and tissue research*. 338: 67-77.
42. Han, L., S. Zhang, Y. Wang, and X. Sun. 2006. Immunohistochemical localization of vitellogenin in the hepatic diverticulum of the amphioxus *Branchiostoma belcheri tsingtauense*, with implications for the origin of the liver. *Invertebrate Biology*. 125: 172-176.
43. Harman, G. H. 1965. The inference to the best explanation. *The Philosophical Review*. 74: 88-95.
44. Heller, R. S. 2010. The comparative anatomy of islets. In: S. Islam (ed.) *The Islets of Langerhans* No. 654. p 21-37. Springer, London.
45. Hildebrand, M. 1974. *Analysis of vertebrate structure*. John Wiley & Sons, New York, London, etc.
46. Hildebrand, M., and G. E. Goslow. 2004. *Analysis of vertebrate structure*: 5th edition.
47. Holland, L. Z. 2014a. Genomics, evolution and development of amphioxus and tunicates: The goldilocks principle. *Journal of experimental zoology. Part B, Molecular and developmental evolution*.
48. Holland, L. Z. 2014b. Personal communication.
49. Holland, L.Z., V. Laudet, and M. Schubert. 2004. The chordate amphioxus: an emerging model organism for developmental biology. *Cellular and molecular life sciences : CMLS* 61: 2290-2308.
50. Holland, L.Z., and T. Onai. 2012. Early development of cephalochordates (amphioxus). *Wiley interdisciplinary reviews. Developmental biology* 1: 167-183.
51. Holland, N. D. 2011. Walter Garstang: a retrospective. *Theory in Biosciences* 130: 247-258.
52. Icardo, J. M., B. Tota, and Y. K. Ip. 2015. *Anatomy of the Heart and Circulation in Lungfishes. Phylogeny, Anatomy and Physiology of Ancient Fishes*: 133.
53. Isogai, S., and M. Horiguchi. 1997. The earliest stages in the development of the circulatory system of the rainbow trout *Oncorhynchus mykiss*. *Journal of morphology* 233: 215-236.
54. Ivanova-Kazas, O. 1995. An essay on the phylogeny of lower chordates. *Trudy Sankt-Peterburgskogo Obshchestva Estestvoispytatelei* 84: 1-158.
55. Jaffee, O. C. 1963. Bloodstreams and the Formation of the Interatrial Septum in the Anuran Heart. *The Anatomical record* 147: 355-357.
56. Jenner, R. A. 2004. Accepting partnership by submission? Morphological phylogenetics in a molecular millennium. *Systematic biology* 53: 333-342.
57. Jensen, B., T. Wang, V. M. Christoffels, and A. F. Moorman. 2013. Evolution and development of the building plan of the vertebrate heart. *Biochim Biophys Acta* 1833: 783-794.
58. Johansen, K. 1960. Circulation in the hagfish, *Myxine glutinosa* L. *Biol Bull* 118: 289-295.
59. Jorgensen, P. 2008. Yolk. *Current biology : CB* 18: R103-104.
60. Junge, U., and W. Creutzfeldt. 1981. Trophic effect of portal blood in maintenance cultures of adult rat hepatocytes. *Digestion* 22: 216-224.
61. Kampmeier, O. F. 1969. *Evolution and comparative morphology of the lymphatic system*. Thomas.
62. Kardong, K. V. 2002. *Vertebrates: comparative anatomy, function, evolution*. 3rd edition.
63. Kardong, K. V. 2012. *Vertebrates: comparative anatomy, function, evolution*. Sixth ed.
64. Khamzina, L., P. A. Gruppuso, and J. R. Wands. 2003. Insulin signaling through insulin receptor substrate 1 and 2 in normal liver development. *Gastroenterology* 125: 572-585.
65. Konrad, M. 2017. Personal communication: Tunicate heart has reverse direction in the earliest animals (3 days after attachment).
66. Konrad, M. W. 2016. Blood circulation in the ascidian tunicate *Corella inflata* (Corellidae). *PeerJ* 4: e2771.

67. Kovalevsky, A. O. 1867. Entwicklungsgeschichte des Amphioxus lanceolatus. Mem. de l'Acad. d. Sci de St. Petersb. VII, ser., XI: 1-17.
68. Lacalli, T. C. 2005. Protochordate body plan and the evolutionary role of larvae: old controversies resolved? Canadian Journal of Zoology 83: 216-224.
69. Lankester, E. R. 1889. Contributions to the Knowledge of Amphioxus lanceolatus, Yarrell. Quarterly J. Micr. Soc xxxix: pp. 365-408.
70. Leake, L. D. 1975. Comparative histology. An introduction to the microscopic structure of animals.
71. Li, H., and S. Zhang. 2010. Hepatic caecum of amphioxus and origin of vertebrate liver. Yi chuan= Hereditas/Zhongguo yi chuan xue hui bian ji 32: 437-442.
72. Liang, Y., and S. Zhang. 2006. Demonstration of plasminogen-like protein in amphioxus with implications for the origin of vertebrate liver. Acta Zoologica 87: 141-145.
73. Liang, Y., S. Zhang, L. Lun, and L. Han. 2006. Presence and localization of antithrombin and its regulation after acute lipopolysaccharide exposure in amphioxus, with implications for the origin of vertebrate liver. Cell and tissue research 323: 537-541.
74. Liem, K. F., W. F. Walker, W. E. Bemis, and L. Grande. 2001. Functional anatomy of the vertebrates: an evolutionary perspective. Harcourt College Publishers Philadelphia.
75. Lipton, P. 2003. Inference to the best explanation. Routledge.
76. Mallatt, J., and N. Holland. 2013. Pikaia gracilens Walcott: stem chordate, or already specialized in the Cambrian? Journal of experimental zoology. Part B, Molecular and developmental evolution 320: 247-271.
77. Mayr, E. 2001. What evolution is. Phonex, Great Britain.
78. Medawar, P. B. 1951. Asymmetry of larval Amphioxus. Nature. 167: 852-853.
79. Minelli, A. 2009. Perspectives in animal phylogeny and evolution. Oxford University Press.
80. Minelli, A., and G. Fusco. 2013. Homology The Philosophy of Biology. p 289-322. Springer.
81. Mivart, S. G. 1871. On the Genesis of Species.
82. Mizuta, T., and K. Kubokawa. 2007. Presence of sex steroids and cytochrome P450 genes in amphioxus. Endocrinology 148: 3554-3565.
83. Morris, S. C., and H. B. Whittington. 1979. The animals of the Burgess Shale. Scientific American 241: 122-135.
84. Mossman, H. W. 1948. Circulatory cycles in the vertebrates. Biol Rev Cambridge Phil Soc 23: 237-255.
85. Mossman, H. W. 1987. Vertebrate fetal membranes. The Macmillan Press Ltd, Basingstoke & London.
86. Nalesnik, M. A., C. R. Gandhi, and T. E. Starzl. 2017. Augmenter of Liver Regeneration: A Fundamental Life Protein. Hepatology (Baltimore, Md).
87. O'Malley, B. 2005. Clinical anatomy and physiology of exotic species. WB Saunders Company.
88. Parzefall, W., E. Erber, E. Kainzbauer, and R. SchulteHermann. 1996. Effects of insulin, glucagon and triiodothyronine on DNA synthesis in rat hepatocyte primary cultures induced by liver tumour promoters and EGF. Toxicology in Vitro 10: 183-193.
89. Peek, W. D., E. W. Sidon, J. H. Youson, and M. M. Fisher. 1979. Fine structure of the liver in the larval lamprey, Petromyzon marinus L.; hepatocytes and sinusoids. The American journal of anatomy 156: 231-250.
90. Pernet, B., and D. McHugh. 2010. Evolutionary changes in the timing of gut morphogenesis in larvae of the marine annelid *Streblospio benedicti*. Evolution & development 12: 618-627.
91. Rähr, H. 1979a. Circulatory-system of Amphioxus (Branchiostoma lanceolatum (Pallas) - light-microscopic investigation based on intra-vascular injection technique. Acta Zoologica. 60: 1-18.
92. Rähr, H. 1979b. The circulatory system of Amphioxus (Branchiostomata lanceolatum (Pallas)). A light-microscopic investigation based on intravascular injection technique. Acta Zool (Stockh). 60: 1-18.
93. Reinecke, M., C. Maake, S. Falkmer, and V. R. Sara. 1993. The branching of insulin-like growth factor-i and insulin - an immunohistochemical analysis during phylogeny. Regulatory peptides. 48: 65-76.
94. Romer, A., and T. Parsons. 1986a. The vertebrate body. Philadelphia, Saunders College Publishing.
95. Romer, A. S. 1959. The vertebrate story.
96. Romer, A. S. 1967. Major steps in vertebrate evolution. Science 158: 1629-1637.
97. Romer, A. S. 1972. The vertebrate as a dual animal—somatic and visceral. In: T. M. Dobzhansky, et al. (ed.) Evolutionary biology. p. 121-156. Springer.
98. Romer, A. S., and T. S. Parsons. 1986b. The vertebrate body.
99. Schaeffer, B. 1987. Deuterostome Monophyly and Phylogeny. In: M. K. Hecht (ed.) Evolutionary Biology No. 21. p. 179-236. Springer.
100. Schirner, H. 1959. On the use of pseudoiso-cyanin for the study of the pancreas of the Cyclostomata and the production of insulin. Zeitschr Naturforsch 14 b: 690-691.
101. Schmalhausen, O. 1991. The Russian Sturgeon *Acipenser Güldenstädti* Part II. Later Prelarval Development Animal species for developmental studies. p. 67-88. Springer.
102. Shore, T. W. 1891. Notes on the Origin of the Liver. Journal of anatomy and physiology 25: 166-197.
103. Shubin, N., C. Tabin, and S. Carroll. 2009. Deep homology and the origins of evolutionary novelty. Nature. 457: 818-823.
104. Sidon, E., and J. Youson. 1983. Morphological changes in the liver of the sea lamprey, *Petromyzon marinus* L., during metamorphosis: I. Atresia of the bile ducts. Journal of morphology. 177: 109-124.
105. Simões-Costa, M. S. et al. 2005. The evolutionary origin of cardiac chambers. Developmental biology. 277: 1-15.
106. Stach, T. 2008. Chordate phylogeny and evolution: a not so simple three-taxon problem. Journal of Zoology. 276: 117-141.
107. Stach, T. 2014. Deuterostome phylogeny – a morphological perspective.
108. Starzl, T. E., I. Y. Lee, K. A. Porter, and C. W. Putnam. 1975. The influence of portal blood upon lipid metabolism in normal and diabetic dogs and baboons. Surgery, gynecology & obstetrics. 140: 381- 396.
109. Starzl, T. E., K. A. Porter, and C. W. Putnam. 1976a. Insulin, glucagon, and control of hepatic structure, function, and capacity for regeneration. Metabolism-Clinical and Experimental. 25: 1429-1434.
110. Starzl, T. E., K. A. Porter, K. Watanabe, and C. W. Putnam. 1976b. Effects of insulin, glucagon, and insulin/glucagon infusions on liver morphology and cell division after complete portacaval shunt in dogs. Lancet. I: 821-825.
111. Steel, M., and D. Penny. 2000. Parsimony, likelihood, and the role of models in molecular phylogenetics. Molecular biology and evolution. 17: 839-850.
112. Stokes, M. D., and N. D. Holland. 1998. The Lancelet. Am. Scientist. 86: 552-560.
113. Thagard, P. R. 1978. The best explanation: Criteria for theory choice. The journal of philosophy. 75: 76-92.

114. Ulmer, M. J., R. E. Haupt, and E. A. Hicks. 1962. Comparative chordate anatomy. A laboratory manual.
115. Wada, H., and N. Satoh. 1994. Details of the evolutionary history from invertebrates to vertebrates, as deduced from the sequences of 18S rDNA. *Proceedings of the National Academy of Sciences of the United States of America* 91: 1801-1804.
116. Walker Jr, W. F., and K. F. Liem. 1994. Functional anatomy of the vertebrates: an evolutionary perspective. Harcourt Brace College Orlando, FL.
117. Wang, Y., H. Wang, M. Li, Z. Gao, and S. Zhang. 2015. Identification, expression and regulation of amphioxus G6Pase gene with an emphasis on origin of liver. *General and comparative endocrinology*. 214: 9-16.
118. Wanninger, A. 2015. Morphology is dead—long live morphology! Integrating MorphoEvoDevo into molecular EvoDevo and phylogenomics. *Frontiers in Ecology and Evolution*. 3: 54.
119. Waterman, A. J. 1971. Chordate structure and function. The Macmillan Company, New York; Collier-Macmillan Limited, London.
120. Weichert, C. K. 1953. Elements of chordate anatomy. New York, Toronto & London: McGraw-Hill Book Co., Inc.
121. Wiens, J. 2004. The role of morphological data in phylogeny reconstruction. *Systematic biology*. 53: 653-661.
122. Wikipedia. 2017. Lancelet's circulatory system.
123. Willey, A. 1894. Amphioxus and the ancestry of the vertebrates.
124. Wipfler, B., H. Pohl, M. I. Yavorskaya, and R. G. Beutel. 2016. A review of methods for analysing insect structures – the role of morphology in the age of phylogenomics. *Current Opinion in Insect Science*. 18: 60-68.
125. Wolpert, L. 2000. What is evolutionary developmental biology? In: *Novartis Foundation symposium*. p. 1-14.
126. Xu, A. 2015. Amphioxus Immunity: Tracing the Origins of Human Immunity. Elsevier.
127. Yegorov, S., J. Bogerd, and S. V. Good. 2014. The relaxin family peptide receptors and their ligands: New developments and paradigms in the evolution from jawless fish to mammals. *General and comparative endocrinology*.
128. Young, J. Z. 1962. The life of vertebrates.
129. Youson, J. H., and E. W. Sidon. 1978. Lamprey biliary atresia: first model system for the human condition? *Experientia* 34: 1084-1086.
130. Zorn, A.M., and J.M. Wells. 2009. Vertebrate endoderm development and organ formation. *Annual review of cell and developmental biology*. 25: 221.
131. Ковалевский, А.О. 1865. История развития Amphioxus lanceolatus или Branchiostoma lumbricum. Диссертация для получения степени магистра зоологии. Dissertation for Master Degree in Zoology, Санкт-Петербургский Университет. Тип. Н. Тиблена и Комп. (Н. Неклюдова), СПб.
132. Ковалевский, А.О. 1951. История развития Amphioxus lanceolatus Избранные работы. р. 7-40. АН СССР, Ленинград.

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