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Parallel avenues in the evolution of hearts and pumping organs

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Abstract. Research in animal models established that tinman, a key gene *in Drosophila* dorsal vessel development, is an orthologue of Nkx2-5, a key gene in vertebrate cardiac development. Similarities between the arthropod dorsal vessel and vertebrate hearts are interpreted in light of concepts such as homology or convergence. We discuss this controversy in the context of the evolution of animal circulatory pumps and propose the distinction between peristaltic and chambered pumps as a fundamental parameter for evolutionary comparisons between bilaterian pumps. Neither homology nor convergence is satisfactory to explain the origins of hearts and pumping organs. Instead, we propose that animal pumps derive from parallel improvements of an ancestral, peristaltic design represented by a layer of myocytes at the external walls of primitive vessels. This paradigm unifies disparate views, impacts our understanding of bilaterian evolution and may be helpful to interpret similarities between pumping organs of phylogenetically relevant species and emerging models.

Keywords. Heart, dorsal vessel, cardiac chambers, peristaltic vessel, vertebrate, *Drosophila*, evolution, development, homology, homoplasy, paralleli.

Prologue

Animal models are responsible for a significant share of our current understanding of biology. Knowledge obtained in experimental animals led to the development of concepts and technologies that have a major impact on how we comprehend, diagnose and treat human diseases. One of the most impressive affirmations of the importance of animal models was the discovery, in the space of only 5 years, that a vertebrate orthologue of a gene initially described in the dorsal vessel of the fruit fly *Drosophila melanogaste*r [1] is crucial for cardiac development in mammals [2, 3], as well as responsible for frequent forms of congenital heart disease in humans [4]. This remarkable tale of success has been a powerful advocate for the idea that the pumping organs of *Drosophila* and man display a similar structural organization and share a common evolutionary origin. However, recent advances in our understanding of the basic genetic circuits employed in animal development suggest that the homologies proposed between the pumping organs of *Drosophila* and men can also be understood as homologies at the level of gene regulatory pathways that make a myocyte, rather than at the level of the specific blueprints that organize these cells in a tridimensional pumping unit [5]. Here we discuss the evidence for and against homology of these organs in the specific context of heart evolution and review the origins of pumping organs in animals. Armed with this knowledge, we evaluate the pros and cons of different hypotheses concerning the homology between circulatory pumps of animals, addressing the issue of homology at specific levels of organization, rather than across all possible levels. A balanced discussion of the evolutionary origins of pumping organs reveals opportunities for unified views on this controversial subject and also offers support for an increased awareness of the roles of

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physical factors in the development of the cardiovascular system.

Introduction

Opening the black box of cardiac development

The study of cardiac morphogenesis is now a field where developmental biology integrates with molecular biology, genetics and cardiology. The rise of the developing heart as such a rich model is recent and can be traced back to several key studies. The initial impetus for the inauguration of molecular approaches in cardiac morphogenesis was the demonstration that the skeletal muscle cell phenotype could be induced in a fibroblast by transfection of the basic Helix-loop-Helix factor MyoD [6]. This landmark study galvanized investigators and prompted them to look for the putative basic Helix-loop-Helix factor that embodied the master gene of vertebrate cardiac development [7]. Quite unexpectedly, the breakthrough came instead from a different line of investigation. Working in the arthropod insect *Drosophila melanogaster*, Bodmer and colleagues [8] cloned and described the expression patterns of msh-2/tinman [9]. Tinman expression was shown to be an absolute requirement for the development of visceral muscles and of the dorsal vessel, the circulatory pump of *Drosophila* [1]. This latter role of tinman raised expectations that a vertebrate ortholog of tinman could fill the slot reserved for the cardiac master gene. The mouse tinman orthologue, Csx/Nkx2-5, was eventually cloned by Komuro and Izumo, [10] and Lints and colleagues [11], and the major roles of Nkx2-5 in vertebrate cardiac development were subsequently demonstrated by targeted recombination [2, 3]. These studies demonstrated a clear requirement for Nkx2-5 in cardiac morphogenesis. However, these studies showed that, in contrast to the null alleles of tinman, those of Nkx2-5 were still compatible with the formation of a primitive heart [2, 3]. These studies dashed hopes that vertebrate cardiac development could be understood on the basis of master regulatory genes (see [12]) and, instead, opened way for the modern view of cardiac development, which envisions cardiac specification as a complex process that requires integration of multiple stimulatory and inhibitory signals [13–16]. These landmark contributions established a fruitful research paradigm for the study of cardiac development [17, 18]. It was clear that the morphogenesis of the *Drosophila* dorsal vessel was a rich source of inspiration in the search for genetic pathways of vertebrate cardiac development [17]. Vertebrate cardiac biology thus owes much to the tiny *Drosophila* dorsal vessel.

Resemblances between development and genetic regulation of the *Drosophila* dorsal vessel and of vertebrate hearts have been extensively reviewed [17, 19–21]. It is generally accepted that similarities lie at the initial morphogenetic stages and at the developmental regulatory networks, but that adult morphologies are very different [17]. Some of the major similarities are, 1) the theme of paired precursors migrating towards the midline and fusing to form a tube [17]; 2) the existence of multiple ortholog genes playing critical roles in pump development and function (e.g. tinman/Nkx2-5; d-mef2/Mef2s, pannier/Gata, dpp/Bmps, mad/Smad1, medea/Smad4, wingless/Wnt, seven-up/Couptf-II) [8, 10, 11, 20–31]; 3) the early establishment of an anterior-posterior (AP) (cranial-caudal) pattern by genetic pathways that may converge on members of the Hox family (e.g. direct regulation of posterior fates by abd-A in *Drosophila* and by retinoic acid (RA) signaling in vertebrates) [32–36]. These similarities raise important questions about evolutionary relationships between the *Drosophila* dorsal vessel and the hearts of vertebrates. Acknowledged in the initial contributions ([11], see also [37]), the issue of homology between the *Drosophila* dorsal vessel and the vertebrate heart has received less attention in recent years. The reasons are not difficult to conceptualize. The tremendous success in the exploitation of the *Drosophila* dorsal vessel model and the paucity of genetic information from other animals at key phylogenetic positions contributed to keep the question of homology in a box. *Drosophila* and mammals are very distantly related, since their evolutionary lines diverged around 600 million years ago (570–650 MyA) [38]. During this time, animals in the evolutionary lineages leading to arthropods and vertebrates used a set of genetic pathways inherited from a common ancestor to develop so many evolutionary adaptations in their bodies that now it is difficult to sort out ancestral from derived characters. In other words, it is still unclear whether, or perhaps more appropriately, to what extent the arthropod dorsal vessel is a homolog of the vertebrate heart.

Our lack of understanding about the evolutionary relationships between the arthropod dorsal vessel and the chambered heart of vertebrates is but a particular subset of the problems we face when we try to understand the evolution of pumping organs in animals. Also poorly understood are the relationships between these organs, the chambered hearts of molluscs and the sophisticated peristaltic pumps of annelids. This state of affairs indicates that the study of the evolutionary origins of pumping organs in animals is in need of a synthesis that establishes a common ground over which different evolutionary hypotheses can be evaluated.

Our considerations do not detract from the importance of the *Drosophila* dorsal vessel as a model for the genetic interactions that specify, determine, differentiate and organize myocytes into a coherent tridimensional pumping unit. The results obtained with the normal and diseased *Drosophila* dorsal vessel have long transcended any narrow discussion of its heuristic potential [39, 40]. Our phylogenetic analysis intends to, and hopefully will, contribute to the understanding of the position of our hearts among all other metazoan pumping organs. We argue that the search for evolutionary origins of our pumping organs will shed light on the nature of our own chordate and vertebrate ancestry and will also add yet another dimension to the still enigmatic ancestor of bilaterian animals, the protostome-deuterostome ancestor (PDA), also known as Urbilateria [41].

The concept of hearts, homology and homoplasy

Hearts have been defined in two distinct ways. For some, hearts are the chambered circulatory pumps of vertebrates. But for others, hearts are more broadly defined as any organ that propels fluid through a circulatory system [42, 43]. The first concept implies that the anatomy of all vertebrate chambered hearts is similar because they share a common origin (homology). Homology may be defined in simple terms as the occurrence of any given character in two organisms whose common ancestor also possessed the character [44].

The second concept of hearts implies that these organs are similar because they perform an analogous function (homoplasy). Homoplasy is defined as the presence of a similar character in two different animals by an independent derivation, not as the result of inheritance of that character from a common ancestor (adapted from [45]). Convergence is established most often, but not necessarily always, by different developmental mechanisms [45].

Tradition has supported the use of both homologous and homoplasic concepts, so it is perfectly reasonable to refer to the *Drosophila* dorsal vessel as a heart. However, confusion ensues when these two definitions are used interchangeably. Ignoring the duality built in the concept of hearts may lead to the automatic acceptance of homology between organs of animals whose evolutionary lines diverged hundreds of millions of years ago. During this time, animals in the lineages leading to insects and mammals had plenty of time to tinker with the complement of genetic pathways they inherited from a common ancestor to transform, create and lose genes, which ultimately had a decisive impact on their morphologies [46]. Also during this time, many anatomical structures that perform analogous functions were independently created by convergence, of which the wings of insects, pterodactyls, birds and bats are probably the most eloquent examples [47].

To appreciate the extent to which this conceptual confusion affects the field of cardiovascular development, we have only to ask ourselves why is it that most investigators are inclined to accept a convergent origin for the chambered hearts of molluscs [37, 48], while a convergent origin for the *Drosophila* dorsal vessel is seldom regarded with sympathy [49, 50], although there is no solid reason to believe that vertebrates are any closer to arthropods than to molluscs. We argue that the issue of homology between the vertebrate heart and the *Drosophila* dorsal vessel, or for that matter, between the vertebrate heart and all the other animal circulatory pumps, is far from settled.

For clarity, we will use in this review the anatomical, homologous, rather than the analogical, homoplasic, concept of hearts. Thus, we will employ the definition of hearts elaborated by Simões-Costa and colleagues [51], who consider hearts as pumps containing inflow and outflow chambers invested at some point in an animal's lifetime with myocytes. This definition is similar to the concept of chambered hearts suggested in Romer [52]; McMahon and colleagues [53]; and Farrel and colleagues [54] and clarifies the obvious differences between chambered pumps (so far identified only in vertebrates and in molluscs) and all other circulation driving devices, which will be referred here in general as circulatory pumps or pumping organs. Pumping organs and hearts of bilaterian animals are depicted in Figure 1.

Revisiting the major similarities between the *Drosophila* dorsal vessel and the vertebrate heart

A closer scrutiny of parallels between the development of the dorsal vessel and the vertebrate heart will often reveal support for analogy, rather than for homology. The theme of bilateral origin and movement towards the midline is well known. However, it is perhaps less appreciated that neither the bilateral origin nor the movement towards the midline is specific to dorsal vessel or cardiac precursors. Rather, these bilateral origins and morphogenetic movements are shared by most of the mesoderm in *Drosophila* [18] and in vertebrates [21]. Therefore, they may represent a common ancestral spatial strategy to enforce bilateral symmetry in organs that develop from the mesoderm, an embryonic layer that is formed around the blastopore and is separated into two bilateral domains by the archenteron [55]. In other words, these common features of development in *Drosophila* and vertebrates may be interpreted as ancestral characters that appeared before the advent

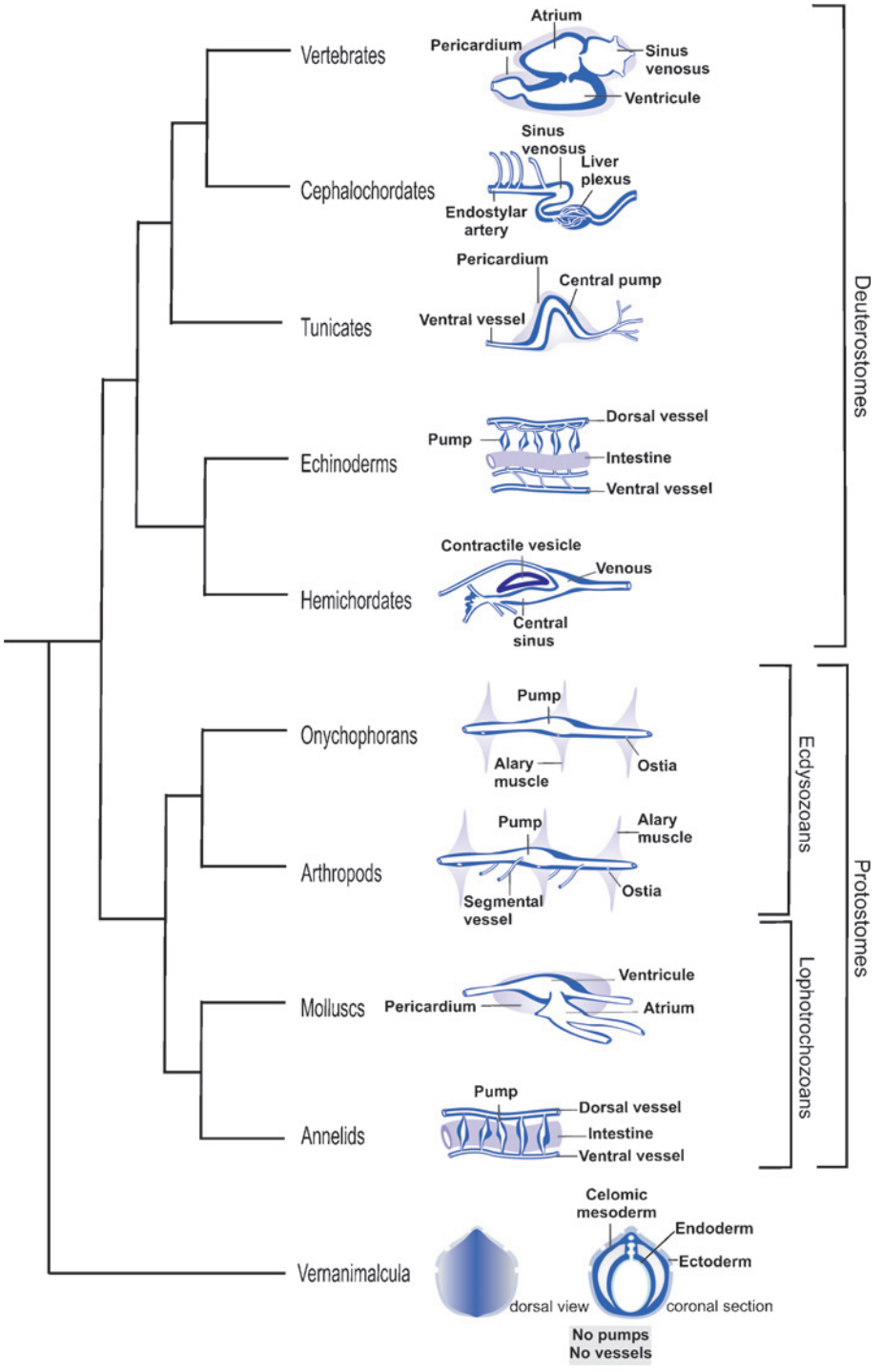

Figure 1. Bilaterian circulatory pumps. Schematic view of protostome and deuterostome pumping organs. Topology based on the molecular phylogeny. *Vernanimalcula guizhouena*, the oldest bilateral fossil found, is depicted at the base of the tree. There is little evidence for vessels or specialized pumps in this micrometer-sized fossil with an extensive coelom running its anterior-posterior axis. Deuterostome circulatory pumps based on [51], bivalve molluscan heart based on [77] and a *Vernanimalcula* scheme based on [102].

of these elaborated pumping organs in triploblastic, bilaterian animals (see discussion below).

The presence of so many homologous genes taking part in evidently parallel aspects of dorsal vessel and vertebrate cardiac development is striking. In fact, the roles of orthologs such as tinman/Nkx2-5 in specification, of Hox genes and RA signaling in AP patterning, of seven-up/Couptf-II in the morphogenesis of structures that control pump inflow (ostia in *Drosophila* and atria in mice) and the more recent finding of a positive, wingless-like, role of vertebrate Wnts in cardiac specification may dissuate most from questioning the homology between the dorsal vessel and the vertebrate heart [21, 30, 31, 35, 36, 56, 57].

A deeper analysis, however, will reveal inconsistencies between parallels that are drawn between dorsal

vessel and vertebrate cardiac development. For instance, as previously stated, tinman plays a major role in the assembly of the early embryonic dorsal vessel, while Nkx2-5 is not required to build the primitive mouse cardiac tube [1, 10, 11, 58]. Interestingly, Nkx2-5 cannot substitute tinman in gene-swapping experiments, since it lacks an N-terminal domain that is presumably utilized in tinman for gene interactions critical to dorsal vessel development [59, 60]. Moreover, the relationship between gene expression and dorsal vessel or cardiac fate is different for tinman and Nkx2-5. While dorsal vessel precursors express tinman, Nkx2-5 expression is initially associated with only a subset of the cells that will give rise to the left ventricle, atria and inflow tract ([61] and references therein). However, at later stages of cardiac development Nkx2-5 expression is indeed activated at nearly all cardiac segments [11], indicating that Nkx2.5 expression is delayed in a subset of mouse heart progenitor cells compared with the situation in the fly. AP patterning of the dorsal vessel and the heart by Hox genes (Ubx, Abd-a, Abd-b) and RA signaling, respectively indicates that these pumping organs separate their functional units utilizing information already encoded in the embryonic AP axis, rather than creating AP patterns *de novo* [35, 36]. Curiously, there is a definitive parallel between the patterning strategies utilized in both systems, in that the default state in the absence of either Hox function or RA signaling is an anterior phenotype, the anterior (cranial) aorta in *Drosophila* and the outflow tract/ventricle in the mouse [36, 62]. The parallels stop there, however. No cardiac phenotypes have as yet been documented in Hox gene knockout studies, which is consistent with the lack of clear AP patterns of expression for these genes in the developing heart [50]. Although a study in cultured chicken cardiac precursors indicated changes in Hox gene expression consistent with the posteriorizing effects of RA [63], and Takihara and colleagues [64] reported cardiac defects after targeted recombination of rae28, a mouse homolog of *Drosophila* polyhomeotic genes, none of these studies provided solid evidence that Hox genes play any important roles in cardiac AP patterning. In the former study, only correlative evidence is provided, while in the latter study only late cardiac defects are observed, but none related to cardiac AP patterning or to the partition of the heart into atrial and ventricular chambers.

The developmental roles of wingless in the dorsal vessel and of Wnts in the vertebrate heart have been a less than ideal material for homologies. wingless is required as a positive factor in the differentiation of the former [56], while the precursors of the latter need to be shielded from Wnt action by soluble Wnt ligands such as Crescent or Dkk [14, 65]. The more recent identification of Wnt-11 (one of the numerous vertebrate Wnt genes) as a positive factor in cardiac development has not made the case for homology any better. A necessary prerequisite of any reliable homology is a solid demonstration of orthology between candidate molecules [66], which is definitively lacking for Wnt-11, which employs non-canonical pathways [67].

Discrepancies such as the ones described above are often understood as resulting from the redundancy built in the genomes of vertebrates, which underwent repeated rounds of partial or full duplications (reviewed in [68]), or as the effect of millions of years of divergent evolution. Subfunctionalization followed by neofunctionalization of duplicated genes [68] can indeed explain the lack of evidence for a role of Hox genes in cardiac AP patterning. However, it is important to acknowledge that there are other explanations for the disparities between the genetic regulation of the *Drosophila* dorsal vessel and the vertebrate heart. These are reviewed in the next section.

Homology and genes

It has been appreciated for some time that relationships between genotype and phenotype are so plastic that homologies at the gene level are not necessarily linked to homologies at other levels such as expression patterns, developmental processes and anatomical structures ([69], reviewed in [44, 66, 70]). As a result, homologous genes can be responsible for the morphogenesis of non-homologous, homoplasic, structures (e.g. the roles of Notch in wing imaginal disc development in *Drosophila*, in the specification of the *Caenohabiditis elegans* vulva and in neuronal cell specification in chordates) ([70] and references therein). On the other hand, homologous structures may be formed by different genes [e.g. even-skipped (eve) expression is required for segmental morphogenesis in *Drosophila*, but homologous segments are normally formed without it in the nematode *Schistocerca americana* and in the arthropod *Aphidius ervi*.] (For further examples refer to [70–72]). Two common features of developmental gene regulation are at the core of this flexible relationship between genome and phenotype. First, developmental genes often have pleiotropic effects on morphogenesis (e.g. Notch, FGFs, TGFβ, BMPs, Wnts). Second, in the course of evolution, genes were frequently relieved of some of their earlier functions [45] or recruited (co-opted) for novel patterning roles (e.g. the recruitment of distalless, orthodenticle and engrailed to work on the highly derived, unique, pentaradial symmetry of echinoderms [73]. The phenomenon of co-option makes it

very difficult to distinguish a gene-to-structure relationship that was forged in a distant ancestral, from a more recent relationship established by an association with a novel developmental role, a problem compounded by the pleiotropy of developmental genes.

In summary, it is rarely justifiable to single out a given gene function and attribute it a homologous quality that will tie together genes and anatomical structures from different animals, especially when their lineages diverged a long time ago [66]. Thus, a necessary condition of any discussion on homology in development is the acknowledgement that the term should only be applied to a same category within a hierarchy of concepts, rather than freely used across levels [44, 45, 66, 70]. Thus, the presence of homologous genes working in the development of superficially similar structures is not necessarily proof that these structures are homologous [66].

Another way to look at the issues of gene conservation in developmental pathways and their relationships with morphogenesis has been championed by Eric Davidson. Davidson and colleagues [74] and Erwin and Davidson [5] proposed that homologous genes working in the development of structures such as the *Drosophila* compound eye and vertebrate eyes do not necessarily indicate homologies between these highly dissimilar, but analogous organs. Instead, they proposed that the homology lies at the level of those genetic pathways that were assembled early in evolution to give rise to the basic cell types that underlie organ function, such as photoreceptors in the eyes, neurons in ganglia or brains, enterocytes in the gut etc. Homology would thus be in differentiation, rather than in morphogenesis [5]. Of direct relevance to our discussion, the latter view suggests that the only homology warranted by the conservation of developmental roles of genes such as tinman in the dorsal vessel and of Nkx2-5 in the vertebrate heart is between the myocytes that form these organs [5].

Animal pumping organs, homology or convergence?

A glimpse at the biology of the main circulatory pumps found in animals, the hearts of vertebrates and molluscs, the arthropod dorsal vessel and some sophisticated contractile vessels of annelids, presents us with two opposing interpretations as to their origins.

One side

The parallels of design and development (e.g. the presence of different compartments, of different units specialized in reservoir or pumping function, of one-way valves, of muscular organization and of conduction systems) may persuade some that these pumping organs not only perform the same functions, but also carry with them the hallmarks of an ancestral project. When one adds to this view the surprising number of genetic circuits shared between myocytes of these organs [20, 21], there is no doubt that the view that these circulatory pumps are homologous is indeed appealing [50].

The other side

The major differences in morphology, function and use of these circulatory pumps by animals so distantly related, so diverse in morphology, development and occupying such different niches [38, 75–77] may influence others to conclude that these organs, although performing some analogous functions, were in fact independently created [5]. When one adds to this view a more critical assessment of the difficulties involved in homologizing anatomical structures on the basis of common usage of genetic circuits [66, 70], there is little doubt that the point of view that circulatory pumps of animals are convergent is also supported.

Is there anything in between?

The two alternatives discussed above represent extreme opinions on the subject. To explore the possibility that there may be alternatives in between these extremes, we will review what is known about different types of pumping organs in animals and what is known about phylogenetic relationships between animals.

Pumping organs

Circulatory pumps of animals have been classified into discrete categories according to their morphologies as chambered hearts, tubular hearts, pulsating vessels and ampullar accessory hearts [52–54]. Overall, these classifications have not employed strict and clear definitions, appealing instead for descriptions of 'typical' cases as the entrance criteria for each category. In this scheme, chambered hearts are multi-compartment pumps with single or double circuits that include one to four reservoirs (atria) and one or two main contractile compartments (ventricles). These are the hearts of vertebrates, bivalves, gastropod and cephalopod molluscs [52, 78, 79]. Tubular hearts are contractile tubes, with or without ostia, whose beating is controlled either myogenically or neurogenically. They are found in arthropods, onychophorans and annelids [53] as well as in chordates [54]. Pulsating vessels are those vascular structures that propel fluid by peristalsis and are found in annelids [53], cephalochordates and bat wing veins [54]. Ampullar hearts are defined as accessory pumps that function to boost the circulation at critical sites of high resistance or of difficult access.

These are the branchial hearts of cephalopods, the lymph hearts of fishes, amphibians, reptiles and the accessory pumps of insects [52, 54, 80]. We argue that the inclusion of a category of ampullar hearts to represent the designs encountered in accessory pumps, as well as the distinction between tubular hearts and pulsating vessels are artificial and potentially misleading because they create categories that are not supported by a close examination of the morphological and physiological characteristics of these pumps. The category of ampullary accessory hearts induces the uninitiated to believe that there is a connection between pump design and its accessory nature. Instead, this category seems to have been created to include all sorts of non-conventional pumping devices. In our opinion, there is little biological justification for lumping together boosting devices that are actuated by skeletal muscles (e.g. accessory pumps of insects and caudal pumps of fishes) [80, 81], with accessory pumps such as the portal 'heart' of hagfish and the branchial 'heart' of cephalopods, which are clearly powered by the same cardiomyocytes that make up their systemic chambered hearts [79, 81]. In our view, the split between tubular hearts and pulsating vessels is unnecessary. An indication of that is the fact that pumping organs of chelicerates, crustaceans and insects, as well as those of polichaeta and oligochaeta annelids are described either as tubular hearts or pulsating vessels [53, 77], in spite of the fact that they obviously share a common, phylum-specific engineering plan, which is perhaps more obvious in arthropods than in annelids. A closer examination of the examples of tubular hearts and pulsating vessels from arthropods shows that the former are set apart from the latter because they happen to contract rapidly enough to give the impression of synchronicity, while the latter show their unmistakable peristaltic character [53, 76, 82]. Therefore, tubular hearts are a special case of pulsating vessels and constitute a clear adaptation of an original peristaltic project (see [52]). Not coincidentally, most tubular hearts are considered to be neurogenic, indicating that it is the sophisticated integrated neural control of segmental contractility, rather than their morphologies, that endows these pumps with synchronicity or near synchronicity of contraction [53, 82]. In summary, we believe the only distinction that adds a useful parameter for deep evolutionary comparisons among circulatory pumps is between those organs designed as peristaltic pumps and those designed as chambered pumps.

Hearts versus peristaltic pumps

What is a peristaltic vessel?

The etymological roots of peristalsis come from the greek *peri* (enclosing or surrounding) + stál (contraction or compression) + *sis* (suffix) and thus reflect the very general concept of a contraction that originates in the outside and compresses what is inside [42]. Peristalsis has been historically associated to the propelling movements of the gut [83], but was generalized to include the successive waves of involuntary contraction that pass along the walls of a hollow muscular structure and force its contents forwards and backwards [84]. Recently, Forouhar et al. (2006) suggested a stricter concept of peristalsis, similar to the mechanical mode of operation typical of manmade positive displacement pumps such as roller peristaltic pumps [85]. However, as stated above, the traditional concept of peristalsis is more inclusive, encompassing all kinds of propagated contractions that mix and propel, forwards and backwards, the contents of a hollow tube [42]. Here we will use the concept of peristalsis in its broad sense. Nonetheless, we believe that a consensual view of the operational definition of what a peristaltic organ is will be crucial for progress in our understanding of cardiac morphogenesis, since, as is generally accepted, the chambered hearts of vertebrates are formed from a morphogenetic elaboration of an initial peristaltic vessel, the primitive cardiac tube [51, 86, 113, 110].

The limits of peristaltic vessels

Peristaltic vessels include the majority of pumping organs in the animal kingdom, but unfortunately, the study of the mechanics of peristaltic pumping have been neglected [87]. Although peristaltic pumps are dominant, versatile and adaptable [85], there is direct and indirect evidence that they are far less prepared than the chambered hearts of vertebrates and molluscs to sustain the high rates of pumping demanded by large and/or homothermous animals [85]. Peristaltic pumps display design flaws manifested in the substantial loss of fluid energy that is incurred by backflow, distension of wall segments ahead of the stream, fluid reflections when the stream encounters constrictions and pump reversals [51, 85]. Pump reversals are not necessarily pathological and are in fact used to transform some insect hearts into bidirectional pumps [53, 80]. Most deficiencies of peristaltic design can be summed up by stating that peristaltic pumps lack effective coordination between the fluid that is entering the contractile region and the fluid that is leaving it [51]. This, of course, does not mean that peristaltic pumps can not be improved, or that backflow cannot be exploited to the animal's advantage [85, 88]. Backflow in arthropod peristaltic pumps is

often managed with the incorporation of one-way valves, while contractile coordination and avoidance of reversals can be achieved by addition of 'control units' represented in the neural control of segmental contraction by peripheral nervous system ganglia [53, 76, 82].

Hearts as solutions for the limitations of peristaltic vessels, the inflow/outflow hypothesis

In contrast to peristaltic vessels, inflow and outflow are tightly coordinated in hearts. This is achieved by a clear division of work between cardiac chambers dedicated to a reservoir (inflow) or to a pumping function (outflow). The typical backflow observed in peristaltic pumps is solved in hearts by an efficient electrical connection between chamber myocytes and by competent one-way valves [51]. Organized pathways of electrical conduction ensure an ordered and near simultaneous contraction of chamber myocytes [86, 89]. This strategy transforms flow from peristaltic to synchronous, and avoids wasting fluid energy in the distension of relaxed downstream pump segments. Moreover, efficient electric connection, with a programmed deceleration of impulse velocity at the interface of inflow and outflow chambers, works together with one-way valves to greatly reduce the chance of backflow or pump reversals [90]. The close match between the answers to the shortcomings that plague peristaltic pumps and the improvements observed in chambered hearts led Simões-Costa and colleagues [51] to propose that the latter originated from differentiation and morphogenetic reorganization of myocytes already present in the former. In this view, cardiac chambers would reflect an underlying morphogenetic principle that divides advanced circulatory pumps into units devoted to inflow or outflow [51].

Evolution has not been a concerted drive to greater complexity. Rather, the only persistent trend seems to have been adaptation [47]. Therefore, the adoption by animals of bigger bodies and/or complex behaviors has been associated with a trend to more sophisticated pumping organs than those that move the circulation in sessile, sedentary or filter-feeding animals. This trend has in fact developed independently among deuterostomes in vertebrates and among protostomes in molluscs. This suggests that the transition from peristaltic pumps towards chambered hearts was governed by common hemodynamic constraints that limited the achievement of top performance by peristaltic vessels during critical behavior. Therefore, the history of animal pumping organs can also be understood as two tales unfolding in opposing directions, a drive to achieve top performance in bigger and more active animals from specific phyla such Vertebrata and Mollusca, and a gradual simplification or complete regression in smaller, sedentary, parasitic and sessile animals from a far greater number of phyla.

What the animal phylogeny tells us about the origins of pumping organs and hearts

The advent of molecular phylogenies revolutionized the classification of animals (reviewed in [91–94]). Figure 2 displays the topology suggested by the now 'classic' molecular phylogeny in which the putative first bilaterian animal is sometimes pictured as the last common ancestor of protostomes and deuterostomes ([5], but see discussion below). In the molecular phylogeny, deuterostomes are formed by two major groups, chordates (with cephalochordates as the sister group of vertebrates and tunicates at the base) and Ambulacraria (hemichordates plus echinoderms) [95–98]. Protostomes are divided into two groups, one of animals that periodically shed their cuticles (Ecdysozoans) and another of animals that share a ciliated trocophore larvae [55], or sport adult feeding appendices, the lophophores (Lophotrochozoans) [99]. Ecdysozoans include arthropods such as the insect *Drosophila*, tardigrades, onycophorans and acoelomate nematodes such as the model species *Caenorhabditis elegans*. Lophotrochozoans include molluscs and annelids, platyhelminthes and others (Fig. 2).

Molecular topologies suggest that the basal state of the protostome-deuterostome ancestor (PDA) was not lacking in pumping organs. Therefore, the presence of circulatory pumps in most deuterostomes, in arthropod ecdysozoans and in annelid and mollusc lophotrochozoans argues in favor of the presence of an ancestral pumping organ in the PDA (see discussion below). However, the layout of the PDA suggested by molecular phylogenies has been controversial.

The PDA

There are two competing opinions on the morphology of the PDA. The subject has been reviewed by Erwin and Davidson ([5] and references therein) and can be summarized here as follows. One view pictures the PDA as an animal of complexity essentially similar to extant triploblastic bilaterians with a segmented body, a through gut, eyes, body appendices, a centralized, cephalized nervous system and a specialized pumping organ [50]. In this view, the presence of so many of these 'advanced' structures in the PDA is supported by roles of genes such Hox/Hom, dpp/Bmps, sog/chordin, caudal/cdx, eyeless/Pax6, Distal-less/Dll, Orthodenticle/Otx and tinman/Nkx2-5 in the development of structures thought to be homologous in protostomes and deuterostomes [50]. The alternative

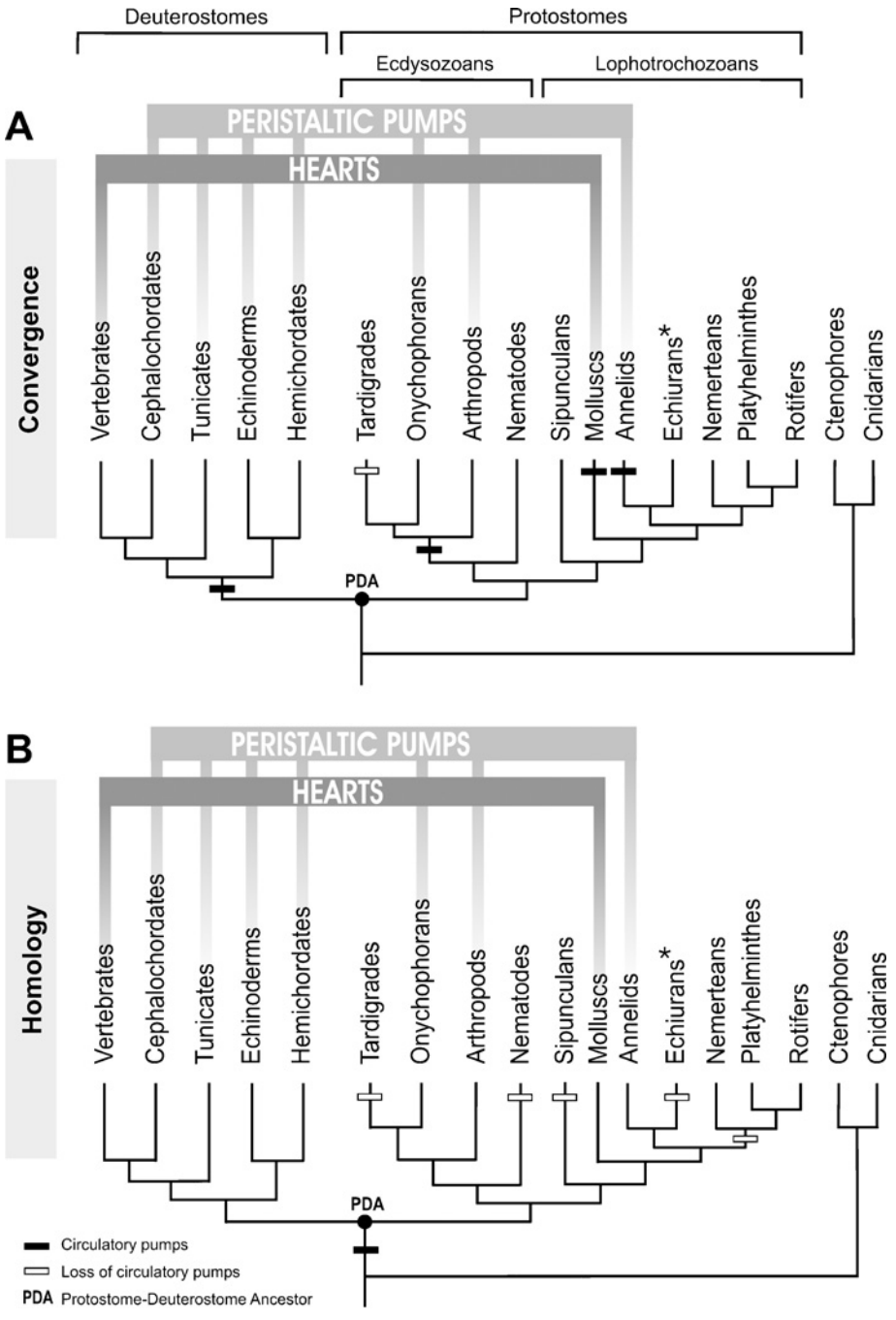

Figure 2. The origin of hearts and circulatory pumps, convergence or homology? (a) A convergent view of the origins of bilaterian (protostome and deuterostome) pumping organs. In this scenario, there is no homology of design between the hearts of vertebrates and molluscs, the dorsal vessel of arthropods and onychophorans and the peristaltic vessels of annelids. The protostome-deuterostome ancestor (PDA) is an animal that does not display specialized pumping organs, which appeared independently in the ancestors of deuterostomes, panarthropods, molluscs and annelids. (b) In this topology bilaterian pumping organs are derived from a primitive peristaltic vessel in the PDA. This scenario supports homology of all bilaterian hearts and pumping organs at the level of a primitive peristaltic vessel (see text for details). Note the multiple regression events (loss of pumping organs) in protostomes. *Despite the absence of circulatory pumps in most echiurans, a specialized pumping organ is present in the family Ikedae [76].

opinion considers this advanced PDA an unlikely proposition. This first argument is a reaction to the idea that the presence of homologous genes in protostome and deuterostome developmental pathways can be equated with homology of anatomical structures. In this view, the presence of homologous genes is interpreted as evidence for homology in the genetic circuits that give rise to differentiated cell types, not for homology in the morphogenesis of superficially similar organs that happen to fulfill analogous functions [5]. The second argument is paleontological. A complex animal like the PDA or its close descendents, such as stem group deuterostomes and protostomes, would very likely have left their traces in the fossil record either directly, or indirectly, as trace fossils. However, years of intense exploration failed to capture such fossils either in the Cambrian period (about 543–485 Mya), when the fossil record teems with representatives of the major animal groups, or before it, in the late Neoproterozoic. Erwin and Davidson [5] suggest that poor fossil representation may have resulted from the climate

in the late Neoproterozoic between 580 and 760 Mya. Simulation studies suggest very cold and inhospitable conditions for this period that may have severely limited the development of animals complex enough to leave their traces in the fossil record (for an alternative view see [93, 100] and references therein) [101]. Thus, Erwin and Davidson [5] proposed that the PDA was a rather small and simply built animal that lacked the exuberant morphological manifestations of its descendents. Although morphologically simple, this PDA would, nonetheless, have displayed the hallmarks of most tripoblast animals, such as bilateral symmetry, an AP axis, a mesoderm and a through gut [5].

Vernanimalcula as a candidate PDA

In 2004, Chen and colleagues [102] reported on phosphatized fossil specimens from a small, micrometer-size animal recovered from the Doushantuo formation in China, dated to circa 600 Mya. The animal was dubbed *Vernanimalcula* (small spring animal) in connection with the spring that followed a prolonged winter on earth [102]. *Vernanimalcula* has the distinction to be the oldest identified triploblastic animal yet. The specimens reveal an animal with an internal layer fashioned into a complete gut flanked by a distinct mouth and anus, an external layer with the suggestion of small paired sensory appendages and the very conspicuous presence of a middle layer forming an uninterrupted coelom that runs all the way through the diminutive AP axis (124—178 μm) [102].

Did *Vernanimalcula* have (or need) a heart?

Two main aspects of the body plan of *Vernanimalcula* deserve special attention. The first one is its diminutive size and the second one the presence of a continuous coelom. The micrometer size of *Vernanimalcula* places it in the same category as extant, very small, meiofaunal animals (animals that live among the water and sediment and can pass through a mesh of 1 mm, but not one of 42 μm [103]). The meiofauna includes animals such as rotifers, gastrotrichs, kinorrhynchs, nematodes and cycliophorans. Most of these animals have a through gut, excretory organs which communicate with the posterior gut and well developed reproductive organs, but nonetheless lack pumping organs and vessels [77]. Notwithstanding their cuticle, they are also thought to make full use of their external surface area for gas exchange and nutrient absorption. In summary, these animals do very well without any specialized circulatory channels or pumps, essentially because their diffusion distances are low and they can move their internal fluids by contraction of their outer muscular layers, which compress the hydrostatic skeleton formed by fluid-filled cavities [77, 104]. Likewise, the presence of a continuous coelom in *Vernanimalcula* suggests that fluids could travel essentially the length of the whole animal without the need for specialized conducting systems or a circulatory pump [105]. Therefore, an animal such as *Vernanimalcula* could survive and thrive using the combined surface area for exchange provided by the gut endoderm, the surface ectoderm and the coelomic mesoderm (Fig. 1). Meiofaunal animals have also developed efficient metabolic strategies to cope with the dramatic variations in oxygen levels in their changing environments [77, 106]. Rotifers and tardigrades excel at some of these strategies, which include synthesis of respiratory pigments for oxygen storage; switches from aerobic to anaerobic states; activation of dormant behavior; encapsulation in watertight cysts and shutdown of metabolic activity [77, 106]. In summary, at least a subset of these strategies could, conceivably, have been employed by *Vernanimalcula*, obviating the need for vessels and pumping organs.

Back to homology

The discussion on circulatory strategies, phylogenies and bilaterian origins gives us the elements to address the homology issues that we raised over the relationship between animal pumping organs. It will become apparent that, as in other systems, the issue of homology of pumping organs should be discussed only at similar levels of organization [66].

Homoplasy at the level of design

Although vertebrate and mollusc hearts share the same general principles of operation, we can safely conclude that there are no data to support their homology at the level of design. In fact, homology between these two organs requires the presence of a chambered pump with at least one inflow and one outflow compartment in the last common ancestor between vertebrate and molluscs, i.e. the PDA. As discussed above, the best PDA candidate is a tiny animal [102], similar to micrometer to millimeter size meiofaunal extant animals that do not display and do not need vessels or pumping organs. Moreover, neither morphological nor molecular phylogenies give strong support to a close kinship between vertebrates and molluscs [91, 94, 107]. Therefore, we can conclude that, when analyzed as chambered hearts, the pumping organs of vertebrates and molluscs are convergent.

In addition to the arguments that are centered in the unlikely presence of sophisticated pumping organs in the PDA, a different argument can be applied to the proposed homology between the vertebrate heart and the arthropod dorsal vessel. Although sufficiently

sophisticated on its own, the arthropod dorsal vessel was initially designed as a peristaltic organ, not as a chambered pump [87, 88]. As discussed above, its blueprints reflect a fundamentally different approach to the problem of fluid propulsion than the one chosen for vertebrates and molluscs in their chambered hearts. Therefore, it is difficult to homologize these designs. It seems that arthropods took full advantage of the peristaltic model, constantly modifying it to suit the needs imposed by the numerous niches that they so successfully occupied (e.g. adding neurogenic and neuroendocrine control) and, when further challenged, created accessory pumping organs in antennas, legs, wings, circus etc., instead of pursuing new avenues of pump design [80, 87]. In this view, the resourcefulness and flexibility of the arthropod body plan conspired to keep the unmistakable identity of their dorsal vessels.

The adherence of arthropods and annelids to the peristaltic design was traditionally interpreted according to the classic idea that these animals together formed the clade of *Articulata* [92]. Therefore, the accepted view was that the peristaltic vessels of arthropods and annelids were homologous and that the latter represented the primitive state of the former [53, 82]. However, the demise of *Articulata* by molecular phylogenies argues, instead, for a convergent origin of arthropod and annelid peristaltic vessels. With the privilege of hindsight, it appears that critical differences in design, such as those represented in the segmented nature of the arthropod dorsal vessel, with its repeating pairs of ostia, its multiple valves and its suspension by allary ligaments and/or muscles, were overlooked in the face of the apparent homologies of segmentation proposed for these animals [107]. In summary, there are no compelling arguments in favor of homology of design between the pumping organs of animals such as vertebrates, arthropods, onychophorans as well as annelids and molluscs. Therefore, we have to face the possibility that all these elaborated, phylum-specific pumps are convergent at the level of design, their similarities stemming from common solutions devised for the common constraints imposed by hemodynamics [104]. This evolutionary scenario is depicted in Figure 2a.

Homology at the level of the smallest pumping unit

One interpretation that will probably provide a common ground for the supporters of homology and convergence alike is that the homology between pumping organs of bilaterian animals is observed not at the level of these sophisticated, phylum-specific, circulatory pumps, but rather at a more basal level that may reflect a common origin for all these organs in an archetypal, ancestral pump. This hypothesis proposes that the basic foundation over which all pumping organs were built was the organization of a layer of contractile myoepithelial or myocyte cells derived from the coelomic epithelium. In other words, the prototype of all circulatory pumps may have been a rather humble peristaltic vessel in which an organized layer of contractile cells presumably lined the external wall of primitive vessels such as hemal channels [108, R. Muñoz-Chapuli and J.M. Pérez-Pomares, personal communications].

The idea that all pumping organs descended from a primitive peristaltic vessel agrees with recent ideas concerning circulatory evolution in animals [109, 110]. We believe this view is consistent with the ventral origins of vertebrate organs, the dorsal origins of arthropod vessels and the commissural origins of some rather sophisticated pumps of annelids such as *Arenicola* [111, 112]. This evolutionary scenario, depicted in Figure 2b, is in line with the developmental transition observed during vertebrate ontogeny, when a primitive peristaltic tube gives rise to chambered hearts [113, 110] as well as with the perception that while the genetic circuits of pump specification and differentiation are similar in *Drosophila* and vertebrates, the morphogenetic circuits of these organs are very different [20].

The ancestral peristaltic vessel and its implications for our views on the origins of chordates, deuterostomes, protostomes and bilaterians

Placing peristaltic pumps in the chordate ancestor

Vertebrates possess chambered pumps. However, at early embryonic stages they rely on a peristaltic pump, the tubular heart [113]. Cephalochordates such as amphioxus (*Branchiostoma*) never develop chambered pumps, and their circulation is instead powered by four major ventral peristaltic vessels. Tunicates rely on a fairly sophisticated peristaltic pump that, although not chambered, shares many characters with vertebrate hearts [51]. Appendicularians (also known as larvaceans) are an exception to this rule, as they often display rudimentary peristaltic pumps or, lack pumping organs altogether, as in the Kowalevskiidae family [114]. However, it is likely that the poorly developed or missing circulatory pumps of appendicularians result from assumption of small body sizes, which in turn is often understood to be a derived character linked to the adoption of a planktonic lifestyle [115]. In summary, the presence of peristaltic pumps in embryos and/or adult chordates suggests that the ancestral chordate had a circulation driven by peristaltic pumps [51, 68].

Peristaltic pumps in protostome and deuterostome ancestors

As we described above, the chordate ancestor had a circulation powered by peristaltic pumps, perhaps similar to the ones displayed by cephalochordates. The sister group of chordates is Ambulacraria (hemichordates and echinoderms). Echinoderms have a highly derived body plan that includes multiple and unique circulatory systems such as the coelomic system, the water vascular system, the hemal system and the perihemal system [53, 77]. A detailed description and comparison of these systems is beyond the scope of this review, but it seems the hemal system of holuthuroids (sea cucumbers), with its dorsal, ventral and commissural peristaltic vessels, is similar to the circulatory layout of hemichordates and cephalochordates [116, 117] (Fig. 1). In summary, the deuterostome ancestor probably had a circulatory system powered by peristaltic pumps at least in its ventral and dorsal vessels. Likewise, the dorsal vessels of arthropods and onychophorans, the dorsal and ventral peristaltic vessels of annelids, as well as the chambered pumps of molluscs, suggest that the protostome ancestor may have displayed peristaltic pumps in the walls of its ventral and dorsal vessels (Fig. 1).

Peristaltic pumps in the PDA? What to make of **Vernanimalcula***?*

The presence of peristaltic vessels in the protostome as well as in the deuterostome ancestor argues in favor of the presence of such pumps in the PDA. However, as discussed before, this line of reasoning does not find support in the layout of *Vernanimalcula*, which is the first bilaterian animal that we know of, and also a candidate for the role of PDA (Fig. 1).

A closer look at the implications of the topologies suggested by molecular phylogenies gives us clues to solve the apparent paradox between the strong evidence that places peristaltic pumps in the PDA and the solid arguments that question the existence of these structures in *Vernanimalcula*. It is sometimes reasoned that, according to the molecular classification, the last common bilaterian ancestor is also the last common PDA [5]. However, this does not need to be so. What molecular phylogenies do tell us is that there is no living animal fitting the intermediary position between the so-called diploblastic and the triploblastic animals. Furthermore, the absence of fossil information on stem group bilaterians does not mean that there were no intermediary forms between the first triploblastic animal and the ancestors of protostomes and deuterostomes. Therefore, although *Vernanimalcula* is currently the best candidate for the bilaterian ancestor, it may not necessarily be the last common PDA [5]. For all these reasons and arguments raised here, we believe *Vernanimalcula* did not exhibit vessels or pumping organs, and as such, it may have been a stem group bilaterian or a derived offshoot of the lineage that led to protostomes and deuterostomes (Fig. 3).

Conclusions

Arguments about the homology between the *Drosophila* dorsal vessel and the vertebrate heart are not new. With the exception of the scenarios influenced by recent paleontological discoveries, all the views discussed here have, in essence, been previously voiced [5, 20, 41, 49, 50, 51, 109, 110, 118, 119]. What was not done before was to place this controversial issue where it belongs, i.e. in the more inclusive context of the evolutionary origin of all animal pumping organs. To do that, we looked at the great variety of animal circulatory pumps in search of useful parameters that could be used in deep evolutionary comparisons. We suggest that the apparent complexity of pump blueprints can be greatly reduced when we realize that there is a logical continuum between the primitive, but pervasive, peristaltic vessels and the sophisticated, but uncommon, chambered pumps [51].

Understanding the peristaltic design as the building block from which all other circulatory pumps were fashioned provides an objective foundation for a synthesis between two widely separated opinions on the evolutionary relationship between the arthropod dorsal vessel and the vertebrate heart, the proponents of homology and the supporters of convergence (Fig. 2). As indicated in our analysis, it is not possible to homologize these organs without considering together the other circulatory pumps of annelids and molluscs. When we include the latter in the analysis, the proponents of homology between the dorsal vessel and the heart (Fig. 2b) will be hard pressed to find a suitable intermediate for the chambered hearts of vertebrates and molluscs, the ostial vessels of arthropods and the peristaltic vessels of annelids, as well as to justify the presence of such an elaborate ancestral pump in a hypothetically minuscule animal (Fig. 1). As for the proponents of convergence, they will be compelled to explain how the rather un parsimonious origin of animal pumps they propose relates to the remarkable presence of the same types of ortholog genes playing roles in the development of these organs [49] (Fig. 2a).

Here we took the middle ground, because this scenario presents the best option to integrate what we know about pumping organs with the recent paleontological findings that we discussed. We reject

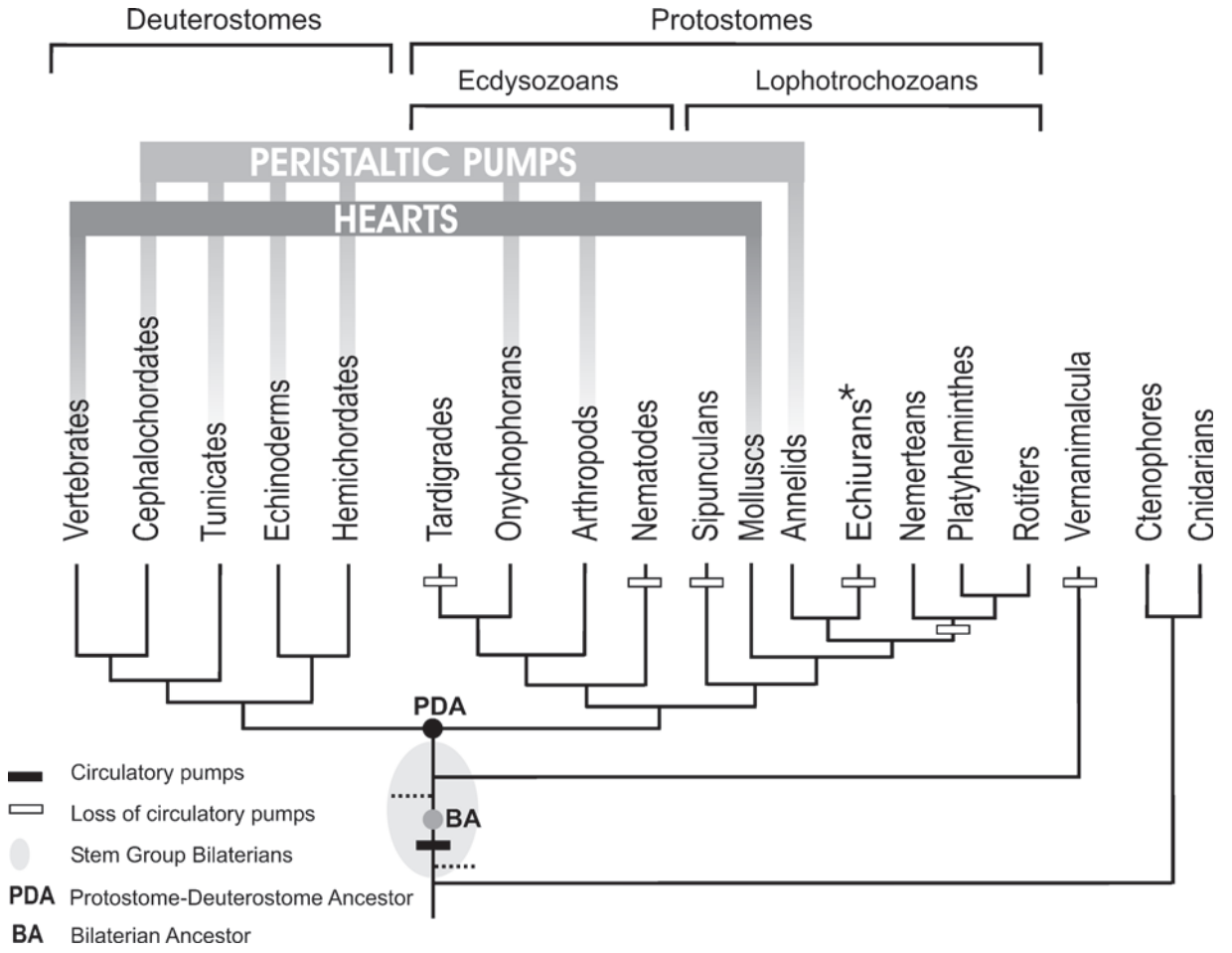

Figure 3. The phylogenetic position of *Vernanimalcula*. We propose that *Vernanimalcula* guizhouena, the oldest bilateral fossil found is a derived offshoot of a bilaterian ancestor that already had a primitive peristaltic vessel. In this view, the ancestral pump was lost in *Vernanimalcula*, but was independently modified in protostome and deuterostome lineages. The gray shading represents stem group bilaterian ancestors.

the idea that the designs of the arthropod (and onychophoran) dorsal vessels, the chambered hearts of vertebrates and molluscs and the peristaltic vessels of annelids are homologous (i.e. the designs are convergent). However, we also do not support the view that these organs were created entirely independently (i.e. there must have been a common origin for all those pumps at a lower hierarchical level such as the primitive peristaltic vessel already discussed). Accordingly, most shared genetic circuits between animal pumps would reflect the common origins of cell type or tissue, rather than homology between the designs, which are clearly specific to phyla or superphyla [5].

Evolutionary parallelism has been defined as the development of features in lineages that are more closely related than those that show convergence [45], so that the concept is usually employed in closely related animals. However, it is tempting to propose that the origin of pumping organs constitutes a case of evolutionary parallelism in the sense that the dissimilar pump designs of bilaterians could have been independently developed from an ancient, but still homologous feature [47].

Going back to the points argued in the introduction, we conclude that the dorsal vessel of *Drosophila* and the vertebrate heart, as well as the other bilaterian pumping organs, are indeed homologous, but only at a very deep level that reflects their origins in a primitive peristaltic organ present in a common ancestral that lived more than 600 Mya. These ancient origins are presumably the source of a common genetic circuitry for the concerted operation of their myocytes, while parallel, but independent changes forced by common physical constraints may be responsible for superficial similarities that are best understood as functional analogies. These evolutionary considerations offer a balanced paradigm to interpret the similarities and differences that may become apparent through the examination of an increasing number of phylogeneti-

cally relevant species and emerging models. They also impact on how we interpret the use of animal models in the study of cardiac development by stressing the need to understand the roles played by physical, hemodynamic forces in cardiovascular development and function [120, 121].

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1 Bodmer, R. (1993) The gene tinman is required for specification of the heart and visceral muscles in Drosophila. Development 118, 719–729.
2 Lyons, I., Parsons, L.M., Hartley, L., Li, R., Andrews, J.E., Robb, L. and Harvey, R.P. (1995) Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2–5. Genes Dev. 9, 1654–1666.
3 Tanaka, M., Chen, Z., Bartunkova, S., Yamasaki, N. and Izumo, S. (1999) The cardiac homeobox gene Csx/Nkx2.5 lies genetically upstream of multiple genes essential for heart development. Development 126, 1269–1280.
4 Schott, J.J., Benson, D.W., Basson, C.T., Pease, W., Silberbach, G.M., Moak, J.P., Maron, B.J., Seidman, C.E. and Seidman, J.G. (1998) Congenital heart disease caused by mutations in the transcription factor NKX2–5. Science 281, 108–111.
5 Erwin, D.H. and Davidson, E.H. (2002) The last common bilaterian ancestor. Development 129, 3021–3032.
6 Lassar, A.B., Paterson, B.M. and Weintraub, H. (1986) Transfection of a DNA locus that mediates the conversion of 10T1/2 fibroblasts to myoblasts. Cell 47, 649–656.
7 McGrew, M., Xavier-Neto, J., Pourquie, O. and Rosenthal, N. (1999) Molecular genetics of skeletal muscle development. In, Heart Development, pp. 493–517, Harvey, R.P. and Rosenthal, N. (eds.), Academic Press, San Diego.
8 Bodmer, R., Jan, L.Y. and Jan, Y.N. (1990) A new homeobox-containing gene, msh-2, is transiently expressed early during mesoderm formation of Drosophila. Development 110, 661–669.
9 Kim, Y. and Nirenberg, M. (1989) Drosophila NK-homeobox genes. Proc. Natl. Acad. Sci. USA 86, 7716–7720.
10 Komuro, I. and Izumo, S. (1993) Csx, a murine homeobox-containing gene specifically expressed in the developing heart. Proc. Natl. Acad. Sci. USA 90, 8145–8149.
11 Lints, T.J., Parsons, L.M., Hartley, L., Lyons, I. and Harvey, R.P. (1993) Nkx-2.5, a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. Development 119, 419–431.
12 Eisenberg, L.M. (2002) Belief vs. scientific observation, the curious story of the precardiac mesoderm. Anat. Rec. 266, 194–197.
13 Schultheiss, T.M., Xydas, S. and Lassar, A.B. (1995) Induction of avian cardiac myogenesis by anterior endoderm. Development 121, 4203–4214.
14 Marvin, M.J., Di Rocco, G., Gardiner, A., Bush, S.M. and Lassar, A.B. (2001) Inhibition of Wnt activity induces heart formation from posterior mesoderm. Genes Dev. 15, 316–327.
15 Tzahor, E. and Lassar, A.B. (2001) Wnt signals from the neural tube block ectopic cardiogenesis. Genes Dev. 15, 255–260.
16 Alsan, B.H. and Schultheiss, T.M. (2002) Regulation of avian cardiogenesis by Fgf8 signaling. Development 129, 1935–1943.
17 Bodmer, R. (1995) Heart development in Drosophila and its relationship to vertebrates. Trends Cardiovasc. Med. 5, 21–28.
18 Bodmer, R. and Frasch, M. (1999) Genetic determination of Drosophila heart development. In, Heart Development, pp. 65–90, Harvey, R.P. and Rosenthal, N. (eds.), Academic Press, San Diego.
19 Frasch, M. (1999) Intersecting signalling and transcriptional pathways in Drosophila heart specification. Semin. Cell Dev. Biol. 10, 61–71.
20 Cripps, R.M. and Olson, E.N. (2002) Control of cardiac development by an evolutionarily conserved transcriptional network. Dev. Biol. 246, 14–28.
21 Zaffran, S. and Frasch, M. (2002) Early signals in cardiac development. Circ. Res. 91, 457–469.
22 Gajewski, K., Fossett, N., Molkentin, J.D. and Schulz, R.A. (1999) The zinc finger proteins Pannier and GATA4 function as cardiogenic factors in Drosophila. Development 126, 5679–5688.
23 Arceci, R.J., King, A.A., Simon, M.C., Orkin, S.H. and Wilson, D.B. (1993) Mouse GATA-4, a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. Mol. Cell Biol. 13, 2235–2246.
24 Kelley, C., Blumberg, H., Zon, L.I. and Evans, T. (1993) GATA-4 is a novel transcription factor expressed in endocardium of the developing heart. Development 118, 817–827.
25 Nguyen, H.T., Bodmer, R., Abmayr, S.M., McDermott, J.C. and Spoerel, N.A. (1994) D-mef2, a Drosophila mesoderm-specific MADS box-containing gene with a biphasic expression profile during embryogenesis. Proc. Natl. Acad. Sci. USA 91, 7520–7524.
26 Lilly, B., Galewsky, S., Firulli, A.B., Schulz, R.A. and Olson, E.N. (1994) D-MEF2, a MADS box transcription factor expressed in differentiating mesoderm and muscle cell lineages during Drosophila embryogenesis. Proc. Natl. Acad. Sci. USA 91, 5662–5666.
27 McDermott, J.C., Cardoso, M.C., Yu, Y.T., Andres, V., Leife, D., Krainc, D., Lipton, S.A. and Nadal-Ginard, B. (1993) hMEF2C gene encodes skeletal muscle- and brain-specific transcription factors. Mol. Cell Biol. 13, 2564–2577.
28 Martin, J.F., Schwarz, J.J. and Olson, E.N. (1993) Myocyte enhancer factor (MEF) 2C, a tissue-restricted member of the MEF-2 family of transcription factors. Proc. Natl. Acad. Sci. USA 90, 5282–5286.
29 Frasch, M. (1995) Induction of visceral and cardiac mesoderm by ectodermal Dpp in the early Drosophila embryo. Nature 374, 464–467.
30 Pereira, F.A., Qiu, Y., Zhou, G., Tsai, M.J. and Tsai, S.Y. (1999) The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. Genes Dev. 13, 1037–1049.
31 Gajewski, K., Choi, C.Y., Kim, Y. and Schulz, R.A. (2000) Genetically distinct cardial cells within the Drosophila heart. Genesis 28, 36–43.
32 Ponzielli, R., Astier, M., Chartier, A., Gallet, A., Therond, P. and Semeriva, M. (2002) Heart tube patterning in Drosophila requires integration of axial and segmental information provided by the Bithorax Complex genes and hedgehog signaling. Development 129, 4509–4521.
33 Lovato, T.L., Nguyen, T.P., Molina, M.R. and Cripps, R.M. (2002) The Hox gene abdominal-A specifies heart cell fate in the Drosophila dorsal vessel. Development 129, 5019–5027.
34 Lo, P.C., Skeath, J.B., Gajewski, K., Schulz, R.A. and Frasch, M. (2002) Homeotic genes autonomously specify the anteroposterior subdivision of the Drosophila dorsal vessel into aorta and heart. Dev. Biol. 251, 307–319.
35 Lo, P.C. and Frasch, M. (2003) Establishing A-P polarity in the embryonic heart tube, a conserved function of Hox genes in Drosophila and vertebrates? Trends Cardiovasc. Med. 13, 182–187.
36 Hochgreb, T., Linhares, V.L., Menezes, D.C., Sampaio, A.C., Yan, C.Y., Cardoso, W.V., Rosenthal, N. and Xavier-Neto, J.

(2003) A caudorostral wave of RALDH2 conveys anteroposterior information to the cardiac field. Development 130, 5363–5374.
37 Harvey, R.P. (1996) NK-2 homeobox genes and heart development. Dev. Biol. 178, 203–216.
38 Peterson, K.J., Lyons, J.B., Nowak, K.S., Takacs, C.M., Wargo, M.J. and McPeek, M.A. (2004) Estimating metazoan divergence times with a molecular clock. Proc. Natl. Acad. Sci. USA 101, 6536–6541.
39 Bier, E. and Bodmer, R. (2004) Drosophila, an emerging model for cardiac disease. Gene 342, 1–11.
40 Wolf, M.J., Amrein, H., Izatt, J.A., Choma, M.A., Reedy, M.C. and Rockman, H.A. (2006) Drosophila as a model for the identification of genes causing adult human heart disease. Proc. Natl. Acad. Sci. USA 103, 1394–1399.
41 De Robertis, E.M. and Sasai, Y.A. (1996) A common plan for dorsoventral patterning in Bilateria. Nature 380, 37–40.
42 The American Heritage Dictionary of the English Language (2000) 4th edn., Houghton Mifflin, Boston, MA.
43 Encycloplaedia Britannica (2006). Encycoplaedia Britannica Premium Service. http,//www.britannica.com/eb/article-9039718.
44 Baguna, J. and Garcia-Fernandez, J. (2003) Evo-Devo, the long and winding road. Int. J. Dev. Biol. 47, 705–713.
45 Hall, B.K. (2003) Descent with modification, the unity underlying homology and homoplasy as seen through an analysis of development and evolution. Biol. Rev. Camb. Philos. Soc. 78, 409–433.
46 Duboule, D. and Wilkins, A.S. (1998) The evolution of 'bricolage'. Trends Genet. 14, 54–59.
47 Simpson, G.G. (1965) The Meaning of Evolution. A Study of the History of Life and of Its Significance to Man, Yale University Press, New Haven and London.
48 Fishman, M.C. and Chien, K.R. (1997) Fashioning the vertebrate heart, earliest embryonic decisions. Development 124, 2099–2117.
49 Scott, M.P. (1994) Intimations of a creature. Cell 79, 1121–1124.
50 Veraksa, A., Del Campo, M. and McGinnis, W. (2000) Developmental patterning genes and their conserved functions, from model organisms to humans. Mol. Genet. Metab. 69, 85–100.
51 Simoes-Costa, M.S., Vasconcelos, M., Sampaio, A.C., Cravo, R.M., Linhares, V.L., Hochgreb, T., Yan, C.Y., Davidson, B. and Xavier-Neto, J. (2005) The evolutionary origin of cardiac chambers. Dev. Biol. 277, 1–15.
52 Romer, A.S. (1962) The Vertebrate Body, 3rd edn., pp. 386–434, W.B. Saunders, Philadelphia, PA.
53 McMahon, B.R., Wilkens, J.L. and Smith, P.J.S. (1997) Invertebrate circulatory systems. In, Handbook of Physiology, sect. 13, vol. 2, pp. 931–1008, Dantzler, W.H. (ed.), American Physiological Society, Oxford University Press.
54 Farrell, A.P. (1997) Evolution of cardiovascular systems, insights into ontogeny. In, Development of Cardiovascular Systems, molecules to organisms, pp. 101–113, Burggren, W.W. and Keller, B.B. (eds.), Cambridge University Press, Cambridge.
55 Hyman, L.H. (1951) Introduction to the bilateria. In, The Invertebrates, Platyhelminthes and Rhynchocoela. The Acoelomate Bilateria, Vol. 2, pp. 1–51, McGraw-Hill Book Company, New York.
56 Wu, X., Golden, K. and Bodmer, R. (1995) Heart development in Drosophila requires the segment polarity gene wingless. Dev. Biol. 169, 619–628.
57 Park, M., Wu, X., Golden, K., Axelrod, J.D. and Bodmer, R. (1996) The wingless signaling pathway is directly involved in Drosophila heart development. Dev. Biol. 177, 104–116.
58 Azpiazu, N. and Frasch, M. (1993) tinman and bagpipe, two homeo box genes that determine cell fates in the dorsal mesoderm of Drosophila. Genes Dev. 7, 1325–1340.
59 Park, M., Lewis, C., Turbay, D., Chung, A., Chen, J.N., Evans, S., Breitbart, R.E., Fishman, M.C., Izumo, S. and Bodmer, R. (1998) Differential rescue of visceral and cardiac defects in Drosophila by vertebrate tinman-related genes. Proc. Natl. Acad. Sci. USA 95, 9366–9371.
60 Ranganayakulu, G., Elliott, D.A., Harvey, R.P. and Olson, E.N. (1998) Divergent roles for NK-2 class homeobox genes in cardiogenesis in flies and mice. Development 125, 3037–3048.
61 Kelly, R.G. (2005) Molecular inroads into the anterior heart field. Trends Cardiovasc. Med. 15, 51–56.
62 Perrin, L., Monier, B., Ponzielli, R., Astier, M. and Semeriva, M. (2004) Drosophila cardiac tube organogenesis requires multiple phases of Hox activity. Dev. Biol. 272, 419–431.
63 Searcy, R.D. and Yutzey, K.E. (1998) Analysis of Hox gene expression during early avian heart development. Dev. Dyn. 213, 82–91.
64 Takihara, Y., Tomotsune, D., Shirai, M., Katoh-Fukui, Y., Nishii, K., Motaleb, M.A., Nomura, M., Tsuchiya, R., Fujita, Y., Shibata, Y. et al. (1997) Targeted disruption of the mouse homologue of the Drosophila polyhomeotic gene leads to altered anteroposterior patterning and neural crest defects. Development 124, 3673–3682.
65 Schneider, V.A. and Mercola, M. (2001) Wnt antagonism initiates cardiogenesis in Xenopus laevis. Genes Dev. 15, 304–315.
66 Abouheif, E., Akam, M., Dickinson, W.J., Holland, P.W., Meyer, A., Patel, N.H., Raff, R.A., Roth, V.L. and Wray, G.A. (1997) Homology and developmental genes. Trends Genet. 13, 432–433.
67 Pandur, P., Lasche, M., Eisenberg, L.M. and Kuhl, M. (2002) Wnt-11 activation of a non-canonical Wnt signalling pathway is required for cardiogenesis. Nature 418, 636–641.
68 Schubert, M., Escriva, H., Xavier-Neto, J. and Laudet, V. (2006) Amphioxus and tunicates as evolutionary model systems. Trends Ecol. Evol. 21, 269–277.
69 de Beer, G.R. (1971) Homology, An Unresolved Problem. Oxford University Press. Oxford.
70 Wray, G.A. and Abouheif, E. (1998) When is homology not homology? Curr. Opin. Genet. Dev. 8, 675–680.
71 Patel, N.H., Ball, E.E. and Goodman, C.S. (1992) Changing role of even-skipped during the evolution of insect pattern formation. Nature 357, 339–342.
72 Rudel, D. and Sommer, R.J. (2003) The evolution of developmental mechanisms. Dev. Biol. 264, 15–37.
73 Lowe, C.J. and Wray, G.A. (1997) Radical alterations in the roles of homeobox genes during echinoderm evolution. Nature 389, 718–721.
74 Davidson, E.H., Peterson, K.J. and Cameron, R.A. (1995) Origin of bilaterian body plans, evolution of developmental regulatory mechanisms. Science 270, 1319–1325.
75 Martin, A.W. and Johansen, K. (1965) Adaptations of the circulation in invertebrate animals. In, Handbook of Physiology, sect. 2, vol. 3, pp. 2545–2581, Hamilton, W.F. and Dow, P. (eds.), American Physiological Society, Washington, DC.
76 Maynard, D.M. (1960) Circulation and heart function. In, The Physiology of Crustacea. Metabolism and Growth, vol 1, pp. 161–226, Waterman, T.H. (ed.), Academic Press, New York.
77 Brusca, R.C. and Brusca, G.J. (2003) Invertebrates. 2nd edn., Sinauer Associates, Sunderland, MA.
78 Jones, H.D. (1983) The circulatory systems of gastropods and bivalves. In, The Mollusca, vol 5, Physiology, part 2, pp. 189–238, Saleuddin, A.S.M. and Wilbur, K.M. (eds.), Academic Press, New York.
79 Wells, M.J. (1983) Circulation in cephalopods. In, The Mollusca, vol 5, Physiology, part 2, pp. 239–290, Saleuddin, A.S.M. and Wilbur, K.M. (eds.), Academic Press, New York.
80 Pass, G. (2000) Accessory pulsatile organs, evolutionary innovations in insects. Annu. Rev. Entomol. 45, 495–518.
81 Satchell, G.H. (1991) Physiology and Form of Fish Circulation, Cambridge University Press, Cambridge.
82 Clark, A.J. (1927) Comparative Physiology of the Heart, Macmillan. New York.
83 Spencer, N.J., Smith, C.B. and Smith, T.K. (2001) Role of muscle tone in peristalsis in guineapig small intestine. J. Physiol. 530, 295–306.

84 Merrian-Webster's Medical Dictionary. (2002) Merrian-Webster, Springfield, MA.
85 Forouhar, A.S., Liebling, M., Hickerson, A., Nasiraei-Moghaddam, A., Tsai, H.J., Hove, J.R., Fraser, S.E., Dickinson, M.E. and Gharib, M. (2006) The embryonic vertebrate heart tube is a dynamic suction pump. Science 312, 751–753.
86 Moorman, A.F. and Christofells, V.M. (2003) Cardiac chamber formation, development, genes, and evolution. Physiol. Rev. 83, 1223–1267.
87 Wilkens, J.L. (1999) Evolution of the cardiovascular system in Crustacea. Am. Zool. 39, 199–214.
88 Hertel, W., Wirkner, C.S. and Pass, G. (2002) Studies on the cardiac physiology of Onychophora and Chilopoda. Comp. Biochem. Physiol. A Mo.l Integr. Physiol. 133, 605–609.
89 Kardong, K.V. (2002) Vertebrates, Comparative Anatomy, Function, Evolution, McGraw-Hill, Boston.
90 Guyton, A.C. and Hall, J.E. (2000) Textbook of Medical Physiology, 10th edn., W.B. Saunders, Philadelphia, PA.
91 Adoutte, A., Balavoine, G., Lartillot, N. and de Rosa, R. (1999) Animal evolution: The end of the intermediate taxa? Trends Genet. 15, 104–108.
92 Adoutte, A., Balavoine, G., Lartillot, N., Lespinet, O., Prud'homme, B. and de Rosa, R. (2000) The new animal phylogeny, reliability and implications. Proc. Natl. Acad. Sci. USA 97, 4453–4456.
93 Blair, J.E. and Hedges, S.B. (2005) Molecular phylogeny and divergence times of deuterostome animals. Mol. Biol. Evol. 22, 2275–2284.
94 Philippe, H., Lartillot, N. and Brinkmann, H. (2005) Multigene analyses of bilaterian animals corroborate the monophyly of Ecdysozoa, Lophotrochozoa, and Protostomia. Mol. Biol. Evol. 22, 1246–1253.
95 Wada, H. and Satoh, N. (1994) Details of the evolutionary history from invertebrates to vertebrates, as deduced from the sequences of 18S rDNA. Proc. Natl. Acad. Sci. USA 91, 1801–1804.
96 Halanych, K.M. (1995) The phylogenetic position of the pterobranch hemichordates based on 18S rDNA sequence data. Mol. Phylogenet. Evol. 4, 72–76.
97 Cameron, C.B., Garey, J.R. and Swalla, B.J. (2000) Evolution of the chordate body plan, new insights from phylogenetic analyses of deuterostome phyla. Proc. Natl. Acad. Sci. USA 97, 4469–4474.
98 Swalla, B.J., Cameron, C.B., Corley, L.S. and Garey, J.R. (2000) Urochordates are monophyletic within the deuterostomes. Syst. Biol. 49, 52–64.
99 Aguinaldo, A.M., Turbeville, J.M., Linford, L.S., Rivera, M.C., Garey, J.R., Raff, R.A. and Lake, J.A. (1997) Evidence for a clade of nematodes, arthropods and other moulting animals. Nature 387,489–493.
100 Hyde, W.T., Crowley, T.J., Baum, S.K. and Peltier, W.R. (2000) Neoproterozoic 'snowball Earth' simulations with a coupled climate/ice-sheet model. Nature 405, 425–429.
101 Hoffman, P.F., Kaufman, A.J., Halverson, G.P. and Schrag, D.P. (1998) A neoproterozoic snowball earth. Science 281, 1342–1346.
102 Chen, J.Y., Bottjer, D.J., Oliveri, P., Dornbos, S.Q., Gao, F., Ruffins, S. et al. (2004) Small bilaterian fossils from 40 to 55 million years before the cambrian. Science 305, 218–222.
103 Higgins, R.P. and Thiel, H. (1988) Introduction to the Study of Meiofauna, Smithsonian Institution Press, Washington, DC.
104 LaBarbera, M. and Vogel, S. (1982) The design of fluid transport systems in organisms. Am. Sci. 70, 54–60.
105 Ruppert, E.E. and Carle, K.J. (1983) Morphology of the metazoan circulatory systems. Zoomorphology 103, 193–208.
106 Pigon, A. and Weglarska, B. (1955) Rate of metabolism in tardigrades during active life and anabiosis. Nature 176, 121–122.
107 Davis, G.K. and Patel, N.H. (1999) The origin and evolution of segmentation. Trends Cell Biol. 9, M68–72.
108 Ruppert, E.E.. (1997) Aschelminthes. In, Microscopic Anatomy of Invertebrates, Harrison, F.W. and Woollacott, R.M. (eds.), Wiley-Liss, Wilmington, DE.
109 Bishopric, N.H. Evolution of the heart from bacteria to man. Ann. N. Y. Acad. Sci. 1047, 13–29.
110 Olson, E.N. (2006) Gene regulatory networks in the evolution and development of the heart. Science 313, 1922–1927.
111 Martin, A.W. (1980) Some invertebrate myogenic hearts: the hearts of worms and molluscs. In, Hearts and Heart-Like Organs, Comparative Anatomy and Development, vol. 1, pp. 1–39, Bourne, G.H. (ed.), Academic Press, New York.
112 Milne-Edwards, H. (1982) Recherches pour server a l'histoire de la circulation du sang chez les annelids. Ann. Sci. Nat. Zool. Biol. Anim. Ser. 10, 193–221.
113 de Jong, F., Opthof, T., Wilde, A.A., Janse, M.J., Charles, R., Lamers, W.H. et al. (1992) Persisting zones of slow impulse conduction in developing chicken hearts. Circ. Res. 71, 240–250.
114 Alldredge, A. (1976) Appendicularians. Sci. Am. 235, 94–102.
115 Zeng, L. and Swalla, B.J. (2005) Molecular phylogeny of the protochordates, chordate evolution. Can. J. Zool. 83, 24–33.
116 Ruppert, E.E. (2005) Key characters uniting hemichordates and chordates, homologies or homoplasies? Can. J. Zool. 83, 8–23.
117 Herried, C.F., LaRussa, V.F. and DeFesi, C.R. (1976) Blood vascular system of the holothuroid Stichopus moebi. J. Morphol. 150, 423–452.
118 Carroll, S.B., Grenier, J.K. and Weatherbee, S.D. (2001) From DNA to diversity: Molecular Genetics and the Evolution of Animal Design. Blackwell Science, Malden, MA.
119 Gilbert, S.F. (2006) Developmental Biology. Sinauer Associates, Sunderland, MA.
120 Hove, J.R., Koster, R.W., Forouhar, A.S., Acevedo-Bolton, G., Fraser, S.E. and Gharib, M. (2005) Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. Nature 421, 172–177.
121 Topper, J.N. and Gimbrone, M.A. (1999) Blood flow and vascular gene expression, fluid shear stress as a modulator of endothelial phenotype. Mol. Med. Today 5, 40–46.