Paleontological treasures among commonplace fossils: a paradigm to study evolutionary innovation

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Abstract

In the last fifteen years, preserved remnants from a number of internal organs have been described. This type of soft, non-mineralized, tissue preservation opens exciting new possibilities to understand the biology of extinct species. Information obtained from fossil organs can indicate the occupation of certain ecological niches and suggest behaviors. From a developmental standpoint, fossil organs may reveal novel ontogenetic sequences and morphogenetic events previously unexpected from phylogenetic analyses dominated by extant species. Recently, we described the first known fossilized heart. The fossil heart was found in *Rhacolepis buccalis*, an extinct basal teleost from the Santana Formation, a Cretaceous Konservat Lagerstätte in the Araripe Basin in the Northeast of Brazil (119–113 Ma). Finding the fossilized heart to be intermediate between the hearts of vigorous predators such as tunas and sedentary bottom feeders such as codfish helped to further clarify the classically-defined role of *R. buccalis* as an avid predator of shrimps – such as *Paleomattea deliciosa* – and as a small fry cannibal. The cardiac outflow tract of *R. buccalis* displayed unforeseen degrees of complexity, with multiple associated individual valves, in contrast with the single outflow tract valves of derived teleosts. These findings reaffirm that evolution does not always associate with complexity, as younger species may display morphologies that are substantially less sophisticated than those displayed by more primitive forms.

Keywords: Araripe Basin, Early Cretaceous, fossil fish, fossil heart, exquisite preservation

Introduction

The primary goal of paleontology since its inception as a discipline with a clear scientific plan has been to study all aspects of past life on Earth. Within this broad scope, the focus of the discipline has changed many times since the eighteenth century. Nevertheless, one of the strongest elements of paleontological fascination has always been the anticipated elation of bringing unknown species back to life, at least to our intellectual lives.

As the study of past life, paleontology is a historical science. The paleontological narrative on Earth results from continued interactions between evolving species and their changing environments. What is the relationship of paleontology with biology? If all extant life forms are destined to be past life sometime in the future, then biology, the study of present life is both a science of the actuality, and a direct product of the previous history of living beings in this Earth. Biology is thus as historical a discipline as paleontology. If biology is a historical science, where is it written? Biology is the substance of all living beings, but many of its current historical secrets can only be assessed through the inspection of two very special domains, the realm of fossils (the province of paleontology) and a very short window of early development (the domain of comparative developmental biology).

Fossils provide direct evidence of past life owing to the resilience of some living materials, chiefly mineralized tissues, comprising hard protective or structural elements. In special circumstances associated with quick insulation of remains within anoxic or hypoxic environments, these materials stand a good chance of avoiding the disaggregating actions of scavengers, of bacteria, and of other microorganisms (e.g. Allisson & Briggs 1991, Behrensmeyer & Kidwell 1985). Under special circumstances, fragile, non-mineralized tissues are also preserved, often in association with mass mortality events caused by, or linked to, catastrophes such flash floods, or tectonic events. The quality of the information obtained from paleontological materials is, therefore, dependent on the degree of post-mortem modification (taphonomy), as well as contingent on a particular chemistry at the microenvironment of burial sites, including preservation of biologic materials, fossil diagenesis, and their consolidation into sedimentary rocks (Martin 1999).

Although the morphology of metazoans and plants may change after sexual maturity, the most relevant differences between species are already established during their early development. Therefore, the origin of morphologic and functional diversity during evolution is intrinsically linked to the processes that control development (Erwin & Davidson 2012). To study evolution is to study descent with modification of development. In fact, the study of comparative early development is also a valuable complement to fossils, when the goal is to understand evolution of animal forms.

After the embryo grows from a cellular agglomerate to a group of cells organized in orthogonal axes of the three-dimensional space, the developing individual undergoes morphogenetic movements that place tissue and organ progenitors closer to their mature dispositions in an ontogenetic phase classically known as gastrulation (Stern 2004 and references therein). From this period on, the events of patterning, cell migration, and morphogenesis that collectively shape embryonic structures maintain relationship with the order of acquisition of structural complexity through evolutionary time. Thus, the primitive attributes appear first, while the most specialized, sometimes diagnostic, attributes of individual phyla appear only later. It is always important to state that the relationship between ontology and embryology does not follow Haeckel's long superseded view of recapitulation, in which the sequential embryonic phenotypes of derived animals represented sequential adult forms in the phylogeny. Rather, the modern view is that early embryonic forms of derived animals do recapitulate the phenotypes from embryos of more primitive animals (i.e. those that branched earlier in the phylogenetic tree). Therefore, ontogeny does provide valuable clues about the relative novelty

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of characters. For instance, elephant trunks appear much later in ontogeny than elephant limb buds, reaffirming the notion that trunks appeared much later than limbs in the phylogeny (Hautier et al. 2012). Likewise, bat wings, which are formed by a thin membrane, the patagium, appear so late in bat development, that sometimes it is difficult for the untrained eye to tell bat from mouse embryos, for example (Cretekos et al. 2008). Often, however, relationships between ontology and phylogeny are scrambled by heterochrony, when some structures may form before, or after, they would be expected to, based on the timing of the appearance of that structure in an ancestor (Gould 1977, Hall 2003).

Thus, embryonic development can be similar to a quick movie trailer, a video clip of the first steps needed to build a multicellular animal. Too much emphasis on a single movie genre can severely limit one's ability as a film critic. Likewise, excessive devotion to only a few species, or phyla, will easily mislead a paleontologist, or a developmental biologist. This is because individual developmental video clips can be quite deceiving. Sometimes, the sequence order of developmental events may be shuffled, the duration of individual events may be altered, or some combination of these two through heterochrony (Gould 1977, Hall 2003). Thus, the key to interpret the clues that development offers about the evolution of form and function is to weed out the heterochronic events from the narrative and to build a representative account that can work as an initial, generic, script. With this basic script at hand, it is possible to dissect the eccentricities that often compound the establishment of form and function during ontogeny. The latter, by their own peculiarity, constitute shared-derived characters, which will become valuable tools to label specific evolutionary lineages (i.e. a chronological series of organisms linked by descent).

Updating the synthesis: Enter developmental biology, gene regulatory networks, genomes, and the environment. The last 30 years witnessed enormous successes in studying the commonalities and similarities of development across metazoans as diverse as arthropods, chordates, mollusks, corals and comb jellies. This collective effort produced at least three major realizations. First, developmental processes display the same mechanistic bases in all multicellular life forms. Second, morphological (and functional) evolution depends on the interaction among a limited, recurrent set of chemical signals and gene products as proximate causes of developmental innovation (Held 2014). Ultimately, however, developmental changes depend on the orchestration of gene regulatory networks (Erwin & Davidson 2012) that are often in perpetual transformation due to the metastable nature of genomes. Third, genome innovation is fostered by the dynamic accumulation of changes induced by mutation, gene duplication, as well as by the agency of mobile elements such as transposons, retrotransposons and viruses (e.g. retroviruses, endogenous retroviruses), which act as continuous generators of diversity (Jacobs et al. 2014). If genomic changes influence the organism's overall survivability and/or fertility, they will be selected for, or against. Often however, genomic changes may randomly accumulate, generating specific allelic patterns in small populations, especially when gene flow is at a minimum (genetic drift). These changes are often neutral in that they do not significantly modify the fitness associated with the individual. However, these so-called neutral changes may have consequences for both morphology and function. With time, genomic changes, selectable and neutral, can accumulate to the point of leading to events of speciation.

To some, the above-mentioned achievements in our efforts to understand biology signify that crucial developmental and genomic paradigms are already quite reasonably established. This is a recognition that so much has been achieved since the beginning of the scientific revolution brought on by the discovery of DNA as an agent of heredity, that the time is ripe for efforts focused at broader levels of organization. One possible direction for this suggested program is to study the mechanisms of anatomic and functional innovation in the inclusive historic and ecologic scenarios (Jablonka & Lamb 2008, Moczek 2008, Held 2014). What we and others with similar objectives want is to work together with other pertinent disciplines to establish the

relevant physical and ecological backgrounds associated with important morpho-functional transitions and, when possible, examine the extent to which the environment may have worked as a selector, or agent, of morpho-functional innovation.

Is it possible for the environment to drive permanent changes in living beings? Indeed, there is the eloquent example of polyphenisms, when distinct phenotypes arise from the same genotype in response to changes in climate and habitat, for instance, sex determination by temperature in alligators and other reptiles (Woodward and Murray, 1993) and changes of worker to queen caste by ingestion of royal jelly (Haydak 1970). There are other proposed ways through which the environment is thought to produce permanent changes in the individual, such as the Baldwin effect and genetic assimilation, which depend on the environment selecting developmental programs that are already present in the genome as part of the genetic variability associated with one given species. Thus, the Baldwin effect and genetic assimilation are fundamentally different from the more familiar emergence of mutations and further purifying selection of mutants (Crispo 2007, Hall 1992).

In truth, it is less than firmly established that the environment operates as a proximate cause of evolution, but multiple recent lines of evidence indicate that inheritance of characters from parents (heredity) is not limited to the transmission of genes from parents to offspring. Epigenetic marks on the DNA are what first come to mind in this regard, because these changes are passed along from progenitors to progeny, but there are other relevant forms of environmental character transmission such as maternal transmission of environmentally-sensitive cellular contents, learning, and cultural inheritance (Creanza & Feldman 2016, Horsthemke 2018, Whiten 2019).

Refining our views about the lives of fossil animals. Because most of the information we get from extinct animals comes from preserved hard mineralized tissues, we often turn a blind eye to opportunities for a more inclusive reconstruction of past lives based on soft tissue counterparts. There are many examples of possible inferences that we can make for the behavior of extinct animal species from even partially preserved soft tissue organs (Sansom et al. 2011, Sansom 2016). Two organs come quickly to our minds when we think about behavior: the brain and the heart. It is common to see inferences about overall intelligence, balance, visual, olfactory, and auditory acuities extracted from digital reconstructions of the brain from endocasts (Alonso et al. 2004, Zelenitsky et al. 2009, Balanoff et al. 2013, Watanabe et al. 2018) or, less often, by direct inspection of fossilized cerebral remnants (Brasier et al. 2016). These observations can provide essential detail regarding the ecological roles and behavioral as well as physiological abilities of these extinct animals. However, it is worth considering that, sometimes, brain endocasts may actually obscure, rather than illuminate functional and behavioral inferences. Chimeroid fishes, for instance, display a bizarre size discrepancy between a very large endocranial capacity and minute brain (Pradel et al. 2009), raising suspicions about the validity of cranial endocasts as surrogates for brain size in fishes (e.g. Watanabe et al. 2018).

Morphological information about hearts, or other kinds of circulatory pumps, are also highly valuable when assessing ecological niche occupation. Fishes are one example of the correlation between cardiac anatomy and niche, as it is well documented that the hearts of active predatory fishes such as tuna display robust and thickened V-shaped, pyramidal ventricles, while sedentary fishes such cod possess thin-walled, tubular ventricles (Santer et al. 1983).

Expanding a core paleontological paradigm. Until quite recently, there was little opportunity to study soft-tissue evolution with the fossil record. Indeed, for most soft-tissue organs, there was no such thing as a fossil record. Today, however, there is good evidence that organs and tissues such as vertebrate gut and gut contents (Brown et al. 2020, Martill 1990, Wilby & Martill 1992), both arthropod and vertebrate brains (Xiaoya et al. 2015, Pradel et al. 2009), umbilical cord (Long et al. 2008), coelacanth lungs (Cupello et al. 2015), and the heart (Maldanis et al. 2016) are preserved in high quality fossils. Given this emerging data, we could in theory begin to estab-

lish ontogenies (and phylogenies) for selected, well-preserved, organs, if such taxa were preserved in high-enough numbers. Frozen in time by rare combinations of mass mortality events and conducive chemical conditions, entire populations, including adults, juveniles, larvae and embryos, or at least some of these developmental stages, could be accessed. Some geological sites would be more appropriate to find specific ontological stages than others, but it should be possible to collect at least a fragmented view of organ development through the study of highly productive sites of exceptional preservation.

A case for cardiovascular paleontology

Chambered hearts as an evolutionary novelty. A major part of the reconstructed narrative associated to an extinct animal species must include, if possible, what it could, or could not do. We confront questions such as: "does this fossil suggests a sessile filter feeder, or a microbial mat grazer?" "Was this animal an ambush or a pursuit predator?" "Was this extinct fish species an aquatic, or an air breather?" "Was this dinosaur warm blooded, or cold blooded?" As discussed above, many of these questions have been traditionally addressed using extrapolation from surrogates, such as endocasts. However, the capabilities of animal circulatory pumps can provide primary data about animal lifestyles. Therefore, information from preserved heart tissues can directly suggest, corroborate, or reject hypotheses about the ecologic niche of an extinct animal.

Our hearts are sophisticated positive displacement pumps that employ two different types of chambers – the atrium and the ventricle – to segregate the functions of fluid inflow and outflow, respectively. In simpler terms, such a positive biological displacement pump generates flow by capturing a given amount of fluid and squeezing it through an outlet. The cardiac atrium receives the blood that returns to the heart from the veins and operates both as a variable capacity reservoir and as filler to the ventricle. In turn, the ventricle is a powerful variable stroke pump that rhythmically propels blood into the aorta, thus generating flow to the organs and tissues (Xavier-Neto et al. 2010). The structures of all vertebrate and mollusk hearts preserve this simple organization (Fig. 1).

From where did this mechanical arrangement come? Here it is appropriate to clarify that, in evolutionary terms, the chambered heart is an amazing oddity. This is because, as currently accepted, true chambered hearts are present in only two groups of animals: vertebrate and mollusks (Brusca & Brusca 2003). The origins of chambered hearts in these two highly interesting groups have been previously discussed (Xavier-Neto et al. 2007). From the standpoint of comparative anatomy, it is clear that the presence of chambered hearts in vertebrates and mollusks cannot be traced back to a common origin in a more primitive clade of animals (i.e. a homology due to the presence of a chambered heart in a common ancestor). Thus, there are arguments in favor of a completely independent evolution for vertebrate and mollusk hearts (evolutionary convergence), or in support of a parallel origin in a distant ancestor (parallel evolution) (Xavier-Neto et al. 2007) (Fig. 2).

Regardless of whether the chambered hearts of vertebrates and mollusks are the result of convergent or parallel evolution, there is strong indication that they both evolved from peristaltic pumps not unlike our own guts, which are primitive (in the sense of basal) displacement pumps that equip most bilaterians. Peristaltic pumps push fluids through the action of sequential waves of contraction that pass along the walls of their hollow muscular structures, driving their contents forwards and backwards (reviewed in Xavier-Neto 2010) (Fig. 3).

While currently there are no reasons to doubt that chambered hearts originated from peristaltic pumps, the actual sequence of events is not clear. There are at least three hypotheses for this crucial morpho-functional transition: the sequential hypothesis, the recruitment hypothesis, and the patterning hypothesis (Xavier-Neto et al. 2010). The sequential hypothesis finds support in comparative adult anatomy, while recruitment and patterning hypotheses derive from comparative developmental biology. These ideas are discussed below in condensed and

Fig. 1. The organization of chambered hearts. **A.** S-shaped, vertebrate heart. Inflow chambers are dorsal, outflow chambers, ventral. Red arrows indicate flow direction. **B.** In amphibians and amniotes, atrium and sinus venosus (SV) are displaced rostrally (green arrow), obscuring the original dorso/ventral disposition. **C.** Deuterostome circulatory pumps. The four-chambered vertebrate heart contrasts with the simpler peristaltic pumps of cephalochordates and tunicates (urochordates). Enteropneust hemichordates display a vesicle squeezing blood from a central sinus. Multiple pumps of the hemal system in holothuroid echinoderms. **D.** Among mollusks, the chambered hearts of gastropods are linear. Hemolymph enters the atrium symmetrically, reflecting away from the atrioventricular valve. Hemolymph ejection in the aorta produces ventricular recoil (dotted arrows) against the atrium, preventing efficient filling. The dorsal/ventral arrangement of inflow/outflow chambers in vertebrate hearts produces asymmetrical streams directed to the valve. Systolic ventricular recoil is away from the atrium and assists in flow. Figure and legends reproduced from Simões-Costa et al., 2005 with permission.
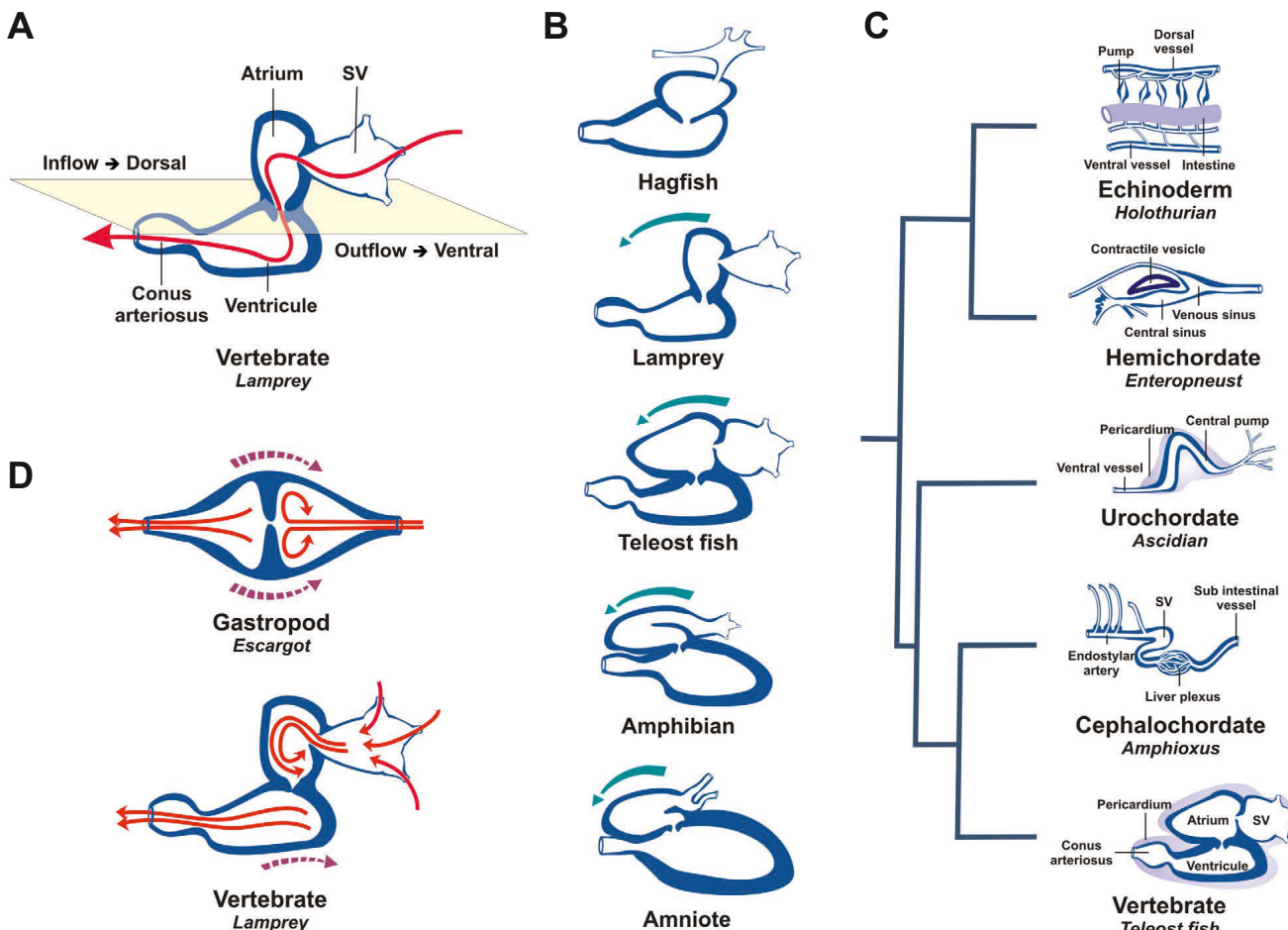
Fig. 2. The origins of animal pumping organs. **A.** Morphological metazoan phylogenies classify animals according to the presence of characters such as: true presence of tissues, embryonic layers (diploblastic and triploblastic), types of body symmetry (bilateral versus radial), and absence (acoelomate) or presence of a fluid-filled space derived from the blastocoel (pseudocoelomates) or from the mesoderm (coelomates). Triploblastic bilaterians are further divided into protostomes and deuterostomes. In these phylogenies, acoelomates and pseudocoelomates (mostly pumpless, heartless animals) are located at the base of bilaterians, suggesting that they are intermediates between diploblastic animals and coelomate triploblastic bilaterians (protostomes and deuterostomes), which display specialized pumping organs. **B.** Molecular phylogenies placed acoelomates and pseudocoelomates together with protostomes, displacing them from the basal positions they occupied in morphological phylogenies. Protostomes were divided between ecdysozoans and lophotrochozoans. Deuterostomes were reaffirmed in a natural group, but lost a few phyla to protostomes. Molecular phylogenies suggest that all bilaterian pumping organs descend from an ancestral bilaterian peristaltic pump, and that acoelomate and pseudocoelomate animals without pumping organs must have lost them secondarily. *Despite the absence of circulatory pumps in most echiurans, a specialized pumping organ is present in the family Ikedae. Figure and legends reproduced from Xavier-Neto et al., 2010 with permission.
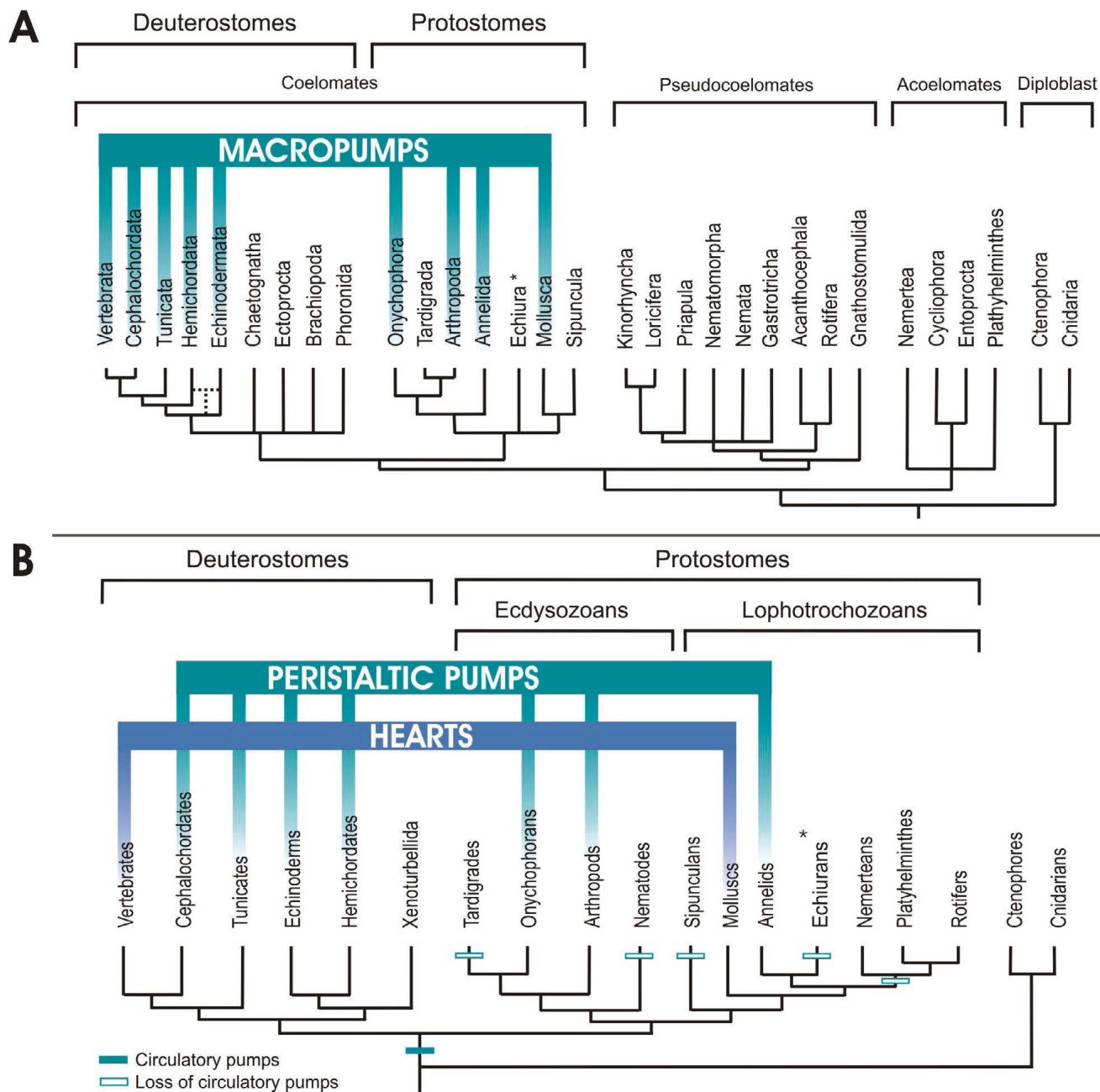
simplified fashion. The reader is directed to the original materials for more comprehensive treatments (Fishman & Olson 1997, Davidson et al. 2008, Hochgreb et al. 2003, Simões-Costa et al. 2005) (Fig. 3).

The sequential hypothesis. The sequential hypothesis simply states that the four types of recognized cardiac chambers, the sinus venosus, the atrium, the ventricle, and the conus arteriosus arose one by one during evolution (Fishman & Olson 1997, Xavier-Neto et al. 2010). One logical consequence of this hypothesis is that the transition from peristaltic pump to chambered heart necessitated the existence of a one-chambered heart. One-chambered hearts, however, are not found among the central pumps of either extant vertebrates or mollusks. However, among arthropods, there is evidence for the evolution of something akin to a possible one chambered heart from peristaltic vessels. During their embryonic and early juvenile periods, crustaceans display a lengthy peristaltic pump – the dorsal vessel – at their dorsal sides. The dorsal vessel is formed by the repetition of virtually the same units, in line with the segmental principle of arthropod body organization. However, in their adult stages, some decapods such as lobsters clearly depart from the peristaltic organization to follow a model of concentrated pumping function in the anterior part of their dorsal vessels (Wilkens 1999). How is this concentration anatomically expressed? In lobsters, it appears that multiple anterior segments of the dorsal vessel coalesced into a single compartment, in a process perhaps analogous to the collapsing of serial thin segments of a telescope into a single thicker unit to produce

a much more robust pump. It has been suggested that this conjectural "one chambered heart" is one of the evolutionary novelties that may have allowed the emergence of the largest extant crustaceans that we know, the Japanese spider crab (*Macrocheira kaempfer*), the American lobster (*Homarus americanus*), and the coconut crab (*Birgus latrus*) (Wilkens 1999). Perhaps in the near future it will be possible to do more than speculate about the types of circulatory pumps that equipped even larger, extinct arthropods such as the sea scorpions (e.g. *Jaekelopterus rhenaniae*) the giant millipede Arthropleura, or the giant dragonfly *Meganeuropsis permiana* (Fig. 4).

The recruitment hypothesis. The recruitment hypothesis evolved from studies of circulatory pump development in the tunicate model species *Ciona intestinalis* (Davidson et al. 2008). The organ that moves circulation in *Ciona intestinalis* operates as a classic peristaltic pump, albeit an advanced one, displaying some features such as morphological specialization (i.e. V-shaped) and encasing into a pericardial membrane (Robb 1965). During *Ciona intestinalis* development, the pump forms from a small group of four founder cells. Normally, founder cells divide asymmetrically into rostral and caudal groups. Under the force of Fibroblast Growth Factor (FGF) signaling, only the rostral daughter cells are instructed to give rise to the lineage of pump cells, which eventually organize themselves in the 3D space to form a single rounded compartment, while caudal cells will give rise to skeletal muscles. However, if the strength of FGF signaling increases through transgenesis, even caudal cells are recruited to the pump phenotype. Significantly, in a small

population of transgenic individuals, the artificially stronger FGF signal induces the formation of an additional, smaller, rounded compartment. In a few individuals, these two compartments were connected and recorded pumping fluid in concert. The experiment described above is a beautiful demonstration of how, in principle, two chambered hearts may have evolved from a peristaltic pump (Davidson et al. 2008). Moreover, the experiment demonstrates the power of developmental biology to provide testable hypothesis concerning the feasibility of crucial transitions in morphology (Fig. 3).

The patterning hypothesis. The patterning hypothesis is based on the normal mechanisms of vertebrate chambered-heart development and on the power displayed by retinoic acid signaling to modify the chamber composition of the developing vertebrate heart (Fig. 3). Indeed, by increasing the force of retinoid signaling, it is possible to induce the formation of hearts almost completely dominated by atrial chambers. Conversely, inhibiting retinoic acid leads to hearts formed essentially by ventricles (Moss et al. 1998, Rosenthal & Xavier-Neto 2000, Xavier-Neto et al. 2001, Hochgreb et al. 2003). The ability of custom retinoid signals to produce these atrialized, or ventriculized hearts is at the core of the normal developmental processes employed during cardiac chamber development. During normal vertebrate development, retinoic signaling is localized to the posterior half of the cardiac precursor domain. This well-defined location is not a fortuitous coincidence, because posterior precursors are exactly the ones destined to form the atrial chambers (which occupy a posterior position in early embryonic hearts, but curiously migrate to the cardiac top later on). Likewise, anterior cardiac precursors are distanced from retinoic acid signaling and, again, far from being a coincidence, anterior cardiac precursors will form the ventricular chambers, which occupy an anterior position in early embryonic hearts (Hochgreb et al. 2003). During early vertebrate cardiac development, both anterior and posterior cardiac precursors first organize themselves as a primordial cardiac tube (more correctly a cardiac trough, because its dorsal part is formed by the anterior gut wall) (De Ruiter et al. 1992).

The primitive cardiac trough aligns to the cranio-caudal axis, with ventricular precursors at the anterior pole and atrial precursors at the posterior pole. At this early stage of development, the primordial vertebrate pump works as a peristaltic device. This tiny peristaltic pump provides for all needs of the vertebrate embryo during a stage of exponential growth. Because of its cranio-caudal patterning, the vertebrate peristaltic pump is adjusted to propel blood from veins to the arteries. This is due to a decreasing gradient of excitability from its posterior (later atrial) to its anterior poles (later ventricular) (i. e. a higher frequency of excitation and contraction in the posterior pole, the atria, dominates all ventricular pacemakers, resulting in a net transfer of fluid to the arteries) (De Jong et al. 1992). After a series of complex tube twisting and differential growth of its myocytes, the primordial peristaltic vertebrate pump becomes a chambered heart. These developmental changes represent the basic script in all known vertebrates, and beautifully illustrate the classic idea that the study of ontogenesis does provide important clues about the origins of phylogenetically important morpho-functional transitions.

In summary, all that was learned from early vertebrate cardiac development suggests that vertebrate chambered hearts evolved directly from a peristaltic pump precursor organ without passing through a necessary one-chambered heart stage. Instead, what seems to be the norm for vertebrates is a mechanism whereby an apparent homogeneous field of cardiac precursors is first patterned in the cranio-caudal axis to establish an intermediate state pump (the patterned primordial peristaltic cardiac trough). This intermediate pump is later reshaped into an advanced pumping organ with specific chambers dedicated to inflow (atria) and outflow (ventricle) (Xavier-Neto et al. 2010) (Fig. 3).

The role of paleontology in illuminating the origin and evolution of chambered hearts. The sequential, recruitment, and patterning hypotheses for the evolution of chambered hearts illustrate well the powers of comparative anatomy and com-
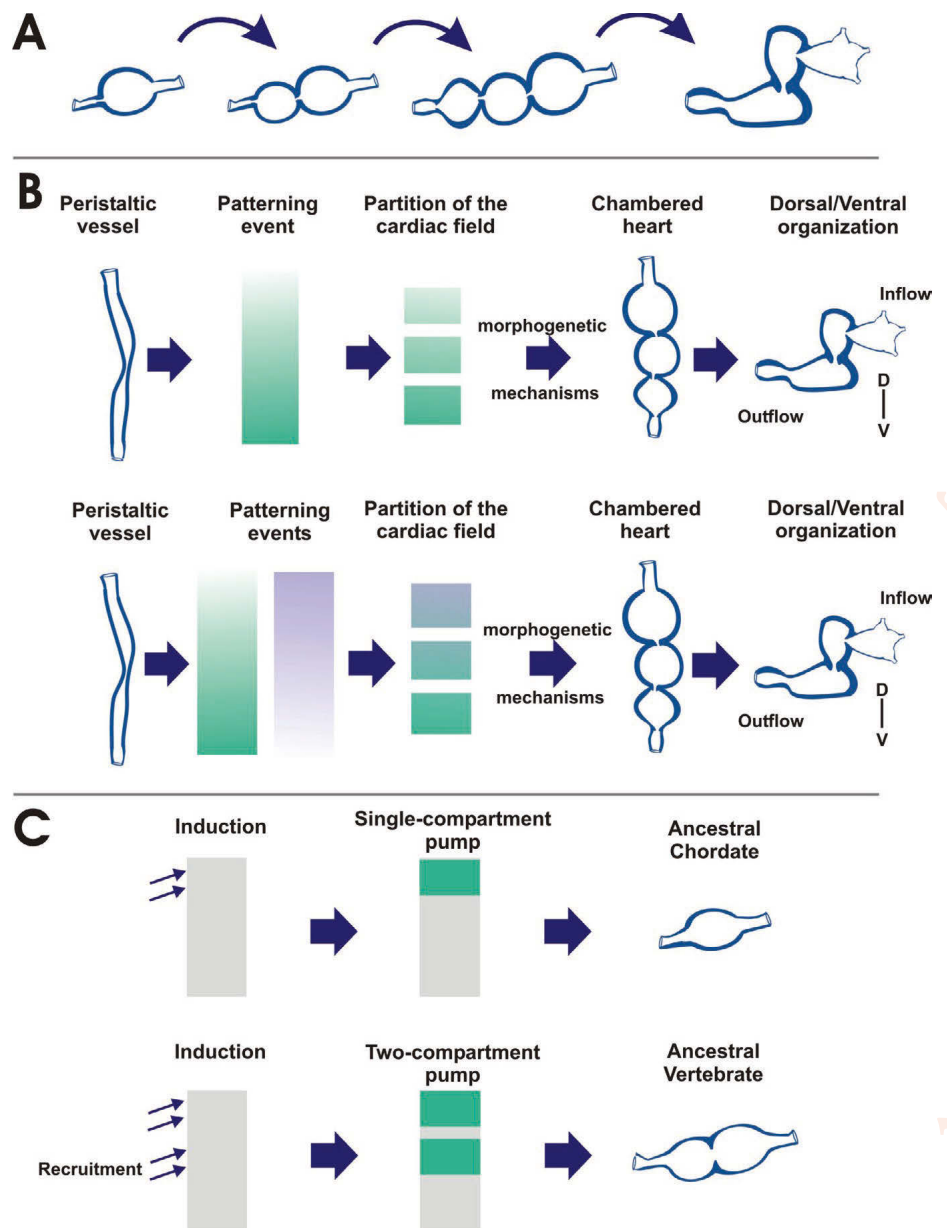

parative developmental biology for generating scenarios with explanatory potential for important morpho-functional transitions. However, it is clear that these scenarios do not provide direct evidence for what has happened historically, but rather for what may have happened.

In the end, only paleontology can provide the primary data to ascertain the true course of the few tractable ontogenetic and phylogenetic problems that we painfully identify and select for investigation. In this sense, one of the best-known examples, a critical problem in cardiac evolution, and a longstanding problem in teleost evolution since the nineteenth century, concerns the present state of the outflow tract of basal actinopterygian hearts. Derived teleosts display a minimalist arrangement of a single valve in their outflow tracts. By contrast, basal actinopterygian hearts possess an outflow decorated with a highly complicated set of one-way valves. The contrast is impressive. In one extreme there are hearts displaying dozens of outflow tract valves, as in *Polypterus*, a primitive ray-finned fish (i. e. actinopterygian) (Duran et al. 2014), while at the other extreme there are hearts showing a single valve row in the cardiac outflow tract, as in the derived actinopterygian model species *Danio rerio* (the zebrafish). This large cardiovascular anatomy spectrum constitutes an evolutionary conundrum noted by the celebrated German anatomist Karl Gegenbauer, or even before, by other earlier anatomists (see Maldanis 2016 and references therein).

Cardiac outflow tract evolution in teleosts is important because it provides evidence for the counterintuitive realization that younger species often display morphologies that are substantially less sophisticated than those displayed by earlier-diverging species. How might these drastic changes
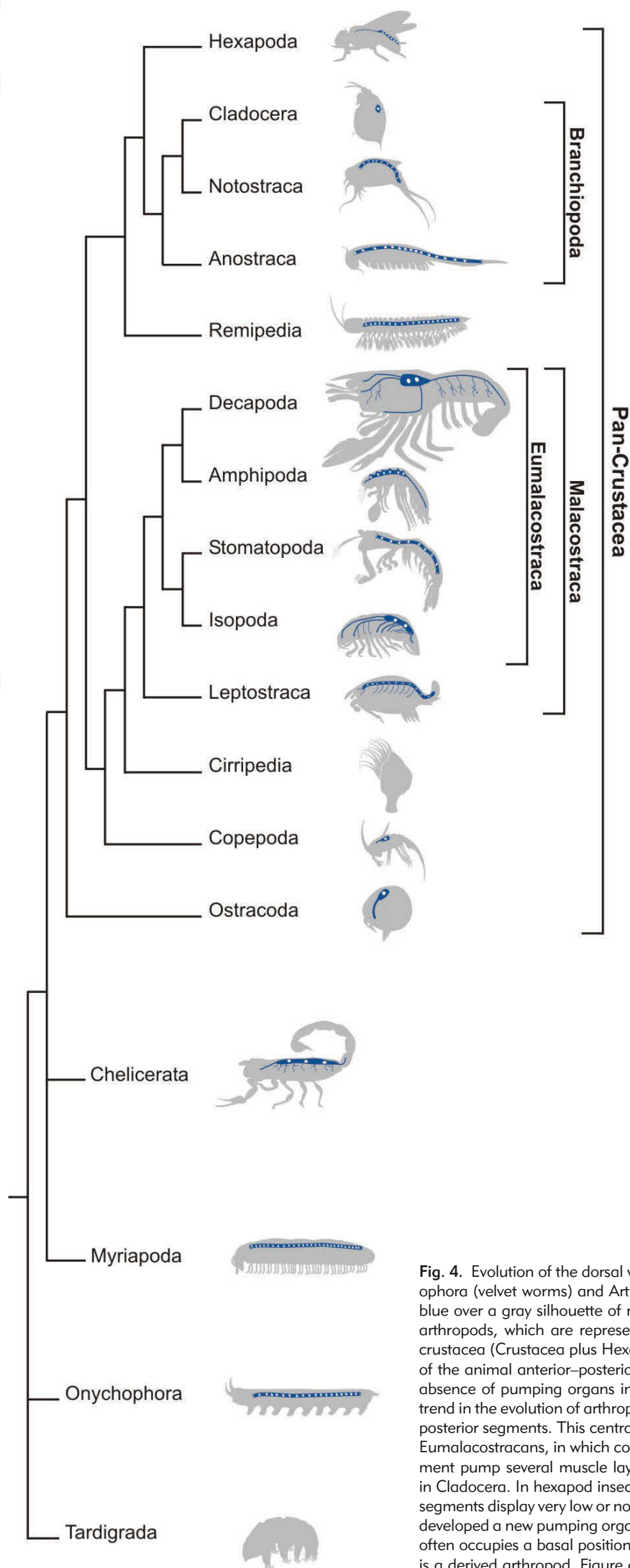
Fig. 3. Three hypotheses for the evolution of vertebrate cardiac chambers. A. The sequential hypothesis: cardiac chambers arose successively on top of a single-chambered heart from the ancestral chordate. B. The recruitment hypothesis: cardiac chambers originated when an additional progenitor domain was recruited to a pump fate, creating a two-compartment peristaltic pump that was later molded by morphogenetic and other mechanisms into inflow and outflow chambers. C. The patterning hypothesis: two or more cardiac chambers appeared simultaneously when patterning mechanism(s) divided the progenitor area of a peristaltic pump into two or more domains that were subsequently fashioned into inflow and outflow cardiac chambers by morphogenetic and other mechanisms. Figure and legends reproduced from Xavier-Neto et al., 2010 with permission.

have evolved? Was the outflow tract simplification in teleosts the result of a radical developmental event of macroevolutionary scale, or yet another garden-variety example of phyletic gradualism? Furthermore, did the changes associated with outflow tract simplification arise because of genetic drift (i.e. neutral evolution), or did they reflect a progressive adjustment in a selective environment (discussed in Maldanis et al. 2016)? If so, what were the ecologic and environmental selectors, and what were the mechanical reasons associated? Until recently, we had no idea of how such radical anatomical changes may have evolved, chiefly because we were missing suitable intermediate morphologies from a very large period of time. In summary, it is clear that the answers to the problems of cardiac chamber origins and cardiac outflow tract evolution in actinopterygians lie with the relevant fossils, because they can provide us with two invaluable clues: evidence for transitional morphologies and for the time intervals between them (Maldanis et al. 2016).

The paleontological context of the first-known fossilized heart. The pursuit of the origins of important morpho-functional transitions outlined above requires that we identify examples of tractable investigative scenarios. Here, we have argued that evolution of chambered hearts is a worthy case. Why is this so? The answer is simple: because we can find fossilized hearts.

The first indication that superabundant vertebrate fossil species can give us clues about cardiac evolution came from the famous Cretaceous site of Brazil, the Santana Formation in the Araripe Basin (Maisey 1991, Maldanis et al. 2016). The Araripe Basin contains one of the larger outcrop areas of Cretaceous rocks among the intracratonic basins of the Brazilian northeast (cratons are very old pieces of the earth's lithosphere that survived all the cycles of destruction and reformation by the merging and rifting of the continents, from their formation until now). The sedimentary area of the Araripe Basin spreads over 12200 km^2, as a result of a tectonic event that led to formation of the Equatorial and Eastern Atlantic opening. The Araripe basement is composed of magmatic and metamorphic rocks, filled with clastic and chemical rocks (Carvalho et al. 2000). During Early Cretaceous times, there were multiple ecologic and environmental changes in the Southern hemisphere, mostly due to the rupture of Gondwanaland, which allowed the emergence of new ecological spaces (Carvalho et al. 2000). These geological events are registered in the interior of Northeastern region of Brazil, especially in the Araripe Basin, in two distinct Konservatt Lagerstätten (sedimentary deposits containing numerous specimens of fossils with exceptional preservation): the Crato and Romualdo fossiliferous deposits (Selden & Nudds 2012).

The Crato Lagerstätten is in the Crato Member of the Santana Formation, which presents exquisitely preserved fossils that are ideal for detailed morphological analysis and reconstructions of past morphologies. The deposits record evidence of a mildly saline, evaporitic environment, displaying freshwater fossils and terrestrial species that presumably died near the shores of multiple interconnected lakes (Maisey 1991). Why is the Crato Member such a fine preservational space? Recent petrographic and chemical studies conducted by Catto (2015) and Catto et al. (2016) on the carbonates of the Crato Member (Aptian, Lower Cretaceous) displayed a population of autotrophic prokaryotes
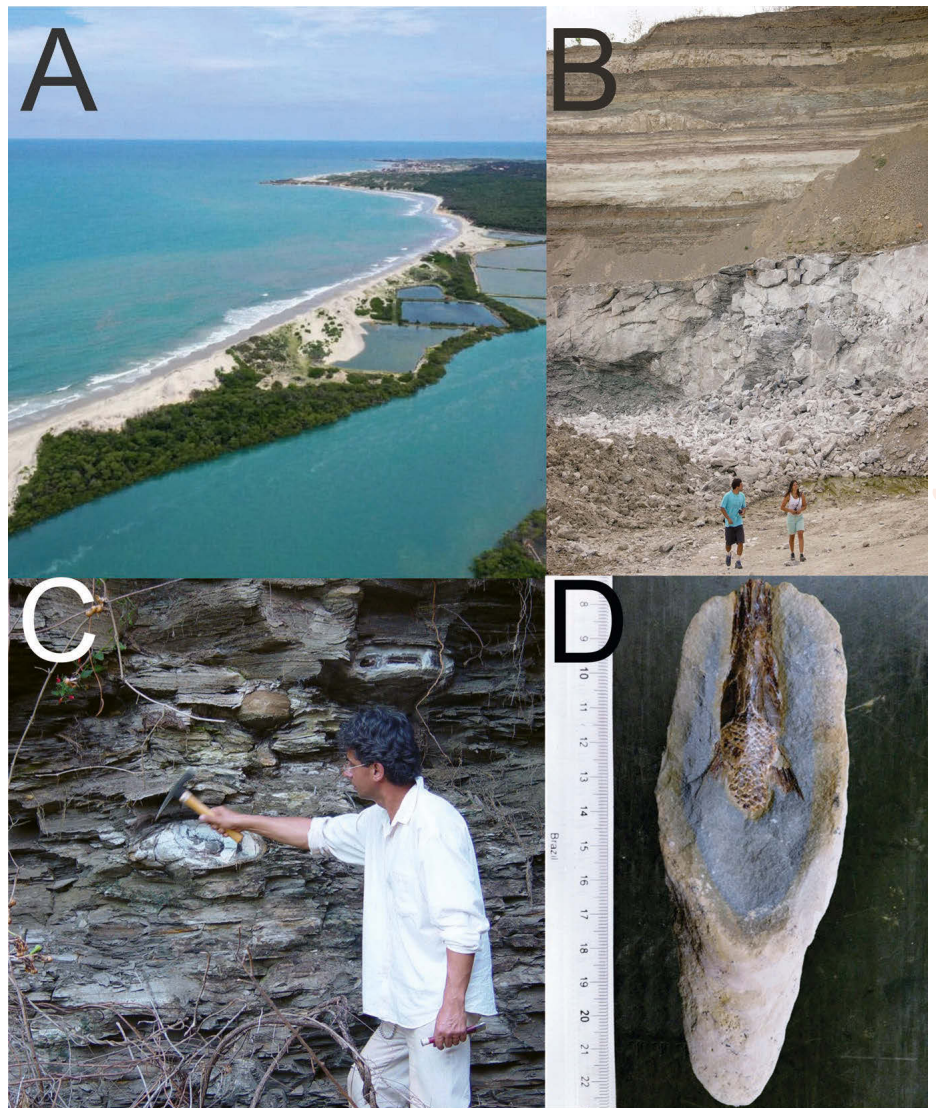
Fig. 4. Evolution of the dorsal vessel in Panarthropoda, which includes Tardigrada (water bears), Onychophora (velvet worms) and Arthropoda. Dorsal vessels and some major efferent vessels are depicted in blue over a gray silhouette of representative species. Tardigrades and onychophorans are outgroups to arthropods, which are represented by an unresolved trichotomy of Chelicerata, Myriapoda, and Pancrustacea (Crustacea plus Hexapoda). The onychophoran dorsal vessel, a tubular organ spanning most of the animal anterior–posterior axis, is thought to be similar to the ancestral arthropod condition. The absence of pumping organs in tardigrades is a potential consequence of small body sizes. One major trend in the evolution of arthropod pumping organs was the concentration of pumping work in anterior or posterior segments. This centralization may be a consequence of the fusion of body segments as seen in Eumalacostracans, in which condensation of anterior body segments created a powerful single compartment pump several muscle layers thick (decapods), or secondary to a loss of body segments, as seen in Cladocera. In hexapod insects, pumping work was concentrated in posterior segments, while anterior segments display very low or no contractility. Sessile cirripeds lost the dorsal vessel, but the class Thoracica developed a new pumping organ actuated by skeletal muscles. Remipedia, a primitive-looking arthropod, often occupies a basal position in morphological phylogenies, but recent molecular evidence suggests it is a derived arthropod. Figure and legends reproduced from Xavier-Neto et al., 2010 with permission.

such as filamentous cyanobacteria, coccoids, and Spirulina. These bacteria are found inside microbial mats and are associated with well-preserved extracellular polymeric substance (EPS), and also preserved as carbonate, consistent with the suggested role of EPS in carbonate precipitation. Fossils from the Crato Member show micro- to nanospherical, rhombohedric, and acicular geometries. This diminutive quality of crystals associated with fossils from the Crato Member is probably one of the reasons explaining the exceptionally preserved fossils of this lithostratigraphic unit, much as the small size of grains in a photographic emulsion is responsible for its high definition. The Romualdo Lagerstätten comprises the fossils famously found as carbonate concretions in the Romualdo Member of the Santana Formation. The age of these fossils also seems to be Aptian, and they have been related to a great Aptian transgression as suggested by Arai (2014) and to further intermittent entrance of water from the Central Atlantic. These transgressive events allowed the deposition of evaporites, in a context of a restricted saltwater environment. While these observations are more or less supported by direct evidence, there are far more proposed scenarios than effectively tested hypotheses for the actual paleoenvironments associated with Romualdo concretions and fossils. Martill suggested that fossils from the Romualdo Member occurred in a context of either a lagoon environment, or as an area with restricted connections to waters of normal marine salinity (Martill 1993). On the other hand, Maisey (1991) suggested that the environment was a shallow embayment nearside a coastal region, in which episodic marine incursions allowed the mixing of salt waters and less saline waters (Fig. 5).

The literature dealing with the paleoenvironments associated with concretions from the Romualdo Member is prolific, full of sweeping syntheses and of contradictory hypotheses. No one has yet presented a strong case for any specific environment proposed, perhaps because there was never such a thing as one uniform scenario across the heterogeneous Araripe Basin at the geologic times represented in the Romualdo strata. Because it is impossible to project the enormous diversity of environments of the Araripe through time and space into a single Romualdo paleoenvironment, perhaps it is better to start by stating what it was not. The depositional environment associated with the Romualdo Member was not a stratified fresh water lake. It was neither a hypersaline body of water, nor a frankly marine environment. This is because the Romualdo Member is very poor on marine markers such as the dinoflagellate *Substilisphaera* and echinoids (echinoderms) which, excepting some brackish species, are strictly marine (Beurlen 1966, Brusca & Brusca 2003).

Recent and detailed work on the eastern side of the Araripe Basin (Serra do Mãozinha) has been shining light on many aspects of the depositional environments associated with the Romualdo Member. The Romualdo succession has been interpreted as the deposits of two cycles of marine transgression and regression (Custódio et al., 2018). The predominance of ostracods, small bivalves, and fewer gastropods is associated with conditions ranging from anoxia to dysoxia (Maisey 1991). Quite interestingly, the study of Custódio et al. (2018) suggests that high-energy events are overrepresented in the Romualdo Member. In effect, it appears that much less sediment was deposited during calm, low-energy conditions than during disrup-

Fig. 5. **A.** A modern lagoon environment close to the mouth of river Piranji, in Eastern Ceará, Brazil 4°23'12" S 37°50'14" W. Drone picture provided by Prof. Odorico de Moraes Filho, Universidade Federal do Ceará. The context is reminiscent of the transitional environment suggested for the Romualdo region, 125–113 million years ago, perhaps without the lush vegetation represented. **B.** Geologic cross section displaying the stratigraphic profile at a Gypsum Mine in the Araripe Basin, in Nova Olinda County, Ceará, Brazil. The Romualdo Member is the litostratigraphic unit above the white Gypsum layers at the bottom. Two humans as scale. **C.** Carbonatic nodules with fossil fishes from the Romualdo Member, with a human as scale. Locality of Pedra Branca, Nova Olinda County, Ceará, Brazil. **D.** Exquisitely preserved *Rhacolepis buccalis* fossil totally embedded in the concretion matrix.
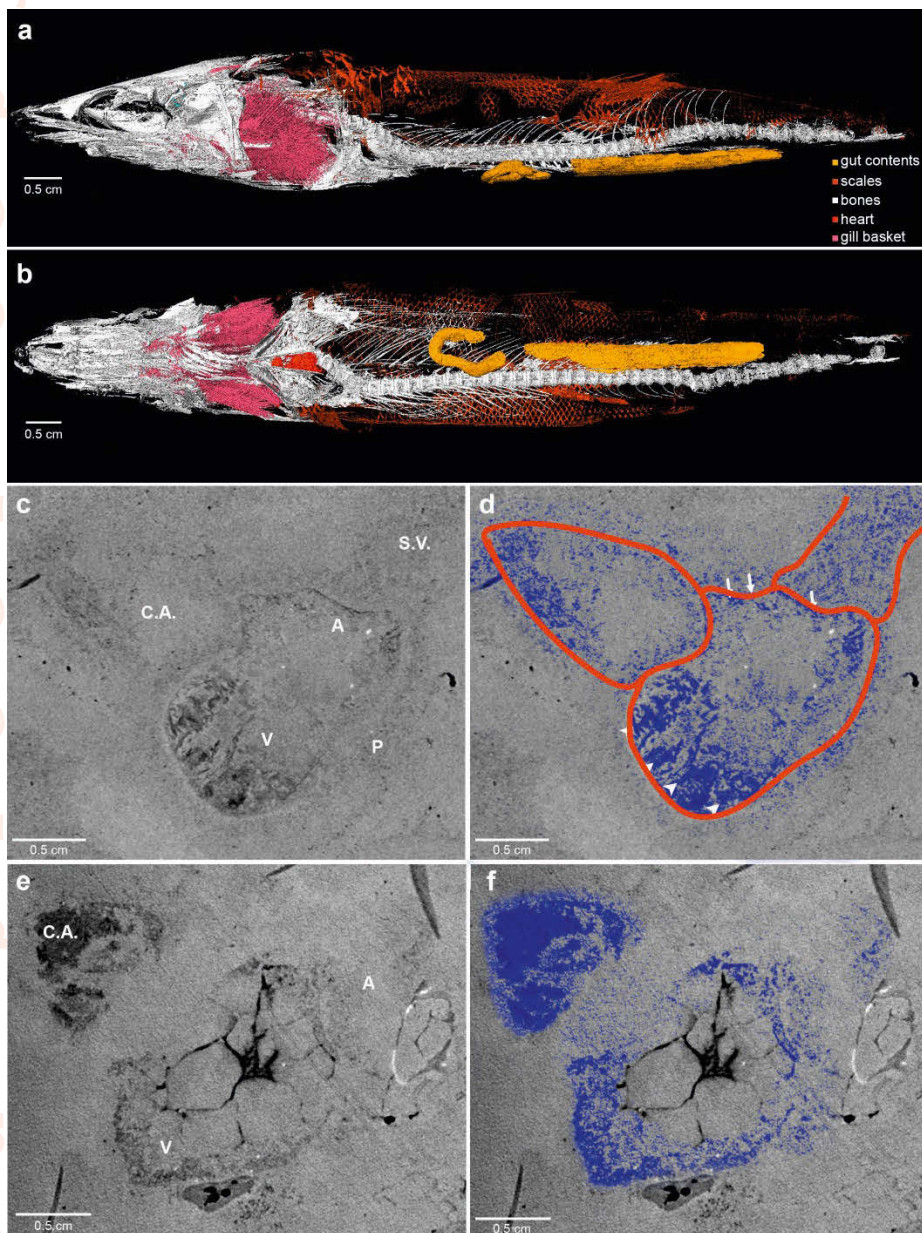
tive episodes of transgression and regression. This reasoning, if correct, may indicate that the various peaceful ecological scenarios that we traditionally imagined as associated to the Romualdo Member may not be factual (Fig. 5). Indeed, there is growing evidence that the waters in the Araripe basin were connected to an internal extension of the central Atlantic for at least one single, long-lasting interval. There are clues that, at this particular time, the Araripe was invaded by the sea through a North-West-South East axis, sequentially involving the states of Maranhão, Piauí, and Ceará (Arai 2014).

Going back to our goal of finding direct evidence for relevant vertebrate cardiac morpho-functional transitions in the fossil record, we argued for the study of well-known and prolific species as an appropriate strategy to find fossil hearts. In this regard, what better resources to explore in the Araripe basin than *Rhacolepis*, a basal teleost fish from the Pachyrhizodontoidei clade (related to Elopomorpha) (Fig. 5D), the most common fossil fish from the Romualdo Member, Santana Formation?

Rhacolepis

Rhacolepis is preserved in three dimensions, usually displaying a fusiform body enveloped by a robust-looking mesh of cycloid scales (Voltani 2016) (Fig. 5D). The fine preservation of the *Rhacolepis* exterior frequently tricks the examiner into hopes of similar internal preservation. However, there is no such correlation. When opened, the best-looking fossils more often than not display only a few scattered vertebrae in the midst of amorphous crystalline materials. In fact, our experience indicates that internal preservation is linked not to external appearances, but to one of the three lithologies described by Maisey for the Romualdo Member concretions (Maisey 1991). In Maisey's view, the variance associated with Romualdo Member

concretions could be tentatively described with at least three categories loosely associated with fossil provenance: Santana concretions, Jardim concretions, and Missão Velha concretions. Santana concretions are ovoid and elongated, formed by a poorly laminated, coarsely granular, beige limestone matrix essentially devoid of clay and pyrite (Maisey 1991). In the original description, Santana concretions do not harbor *Rhacolepis* fossils, a notion that was recently supported by controlled excavations of the Romualdo Member southwest of the city of Santana do Cariri (Faraa et al., 2005). The most promising *Rhacolepis* fossils as far as internal preservation is concerned are those classified by Maisey as typical from the Jardim lithology. The *Rhacolepis*

Fig. 6. Phase contrast synchrotron microtomography of teleost fossil hearts. 3D reconstructions. **A.** Left lateral view. **B.** Ventral view. **C–F.** Sagittal sections of two specimens. Blue masks in **D** and **F** highlight fossil cardiac chambers and pericardium in the two specimens. Note that thin trabeculae are associated to the atrium (arrows) and that thick trabeculae are typical of the ventricle (arrowheads). Abbreviations: A, atrium; C.A., conus arteriosus; P, pericardium; S.V., sinus venosus; V, ventricle. Figure and legends reproduced from Maldanis et al., 2016 with permission.

only the most common fossils arising from a given locality across the Occidental-Oriental axis of the Araripe basin, rather than the only possible fossilization mode there. In effect, it is not difficult to find the typical pyrite-poor Jardim fossils in the municipality of Jardim less than 10 km apart from fossils that reek intensely of petrol in the Romualdo Member. That such different fossilization environments can coexist in the same acknowledged stratum is, no doubt, a reflection of the four-dimensional nature of the diverse taphonomic and diagenetic processes that acted upon the remains of fishes from the region in a given time period.

Enter the stone heart. The discovery of the fossilized heart was largely the product of a developmental biology program initiated in the late 1990's (Moss et al. 1998), when one of us (J.X.N.) established that signaling by the morphogen retinoic acid was key to giving rise to atrial, rather than to ventricular, cardiac chambers in amniotes (Hochgreb et al. 2003). From then on, we set out to inquire whether retinoid signaling was a potential ancestral mechanism of chamber formation in vertebrates (Simões-Costa et al. 2005) and, if so, whether its operations could suggest how chambered hearts evolved from peristaltic pumps. Ideally, this was a matter to be decided by paleontology, by close examination of the fossil record of hearts, more precisely with a focus on samples from Cambrian, or Pre-Cambrian sites of exceptional preservation, perhaps such as the Burgess Shale in Canada 520–512 million years ago (Ma), or the Doushantuo Formation in China (635–551 Ma).

Regrettably, there was no such a thing as a fossil record for chambered hearts. Nonetheless, rather than a completely void area, the field of fossil hearts had a few highly interesting non-conclusive findings and at least a high-profile disappointment in Willo, the dinosaur. Willo was a *Thescelosaurus* and its bones were found preserved in sandstone with a presumptive cardiac remnant lying in a very provocative region, i.e. protected by the pectoral girdle and behind the sternum (Fischer et al. 2000), which is the typical position of a bona fide amniote heart (Kardong, 2009). The 3D analysis of Willo's putative heart revealed some fascinating similarities with a vertebrate heart, prompting the authors to suggest that the dinosaur heart was perhaps closest in structure to an avian than to a lizard's heart (Fischer et al. 2000). These conclusions were received with strong skepticism, chiefly because sandstone is not a documented vehicle for large masses of soft tissue preservation outside the protective influence of bone structure (Rowe et al. 2000). Indeed, when reassessed several years later, Willo's putative heart appeared to be composed of cell-like material of vegetal, rather than of animal, origin, suggesting that Willo's putative heart was actually an iron-rich concretion, perhaps formed by sand aggregates and some organic material under water (Cleland et al. 2011).

Given the state of affairs with the cardiac fossil record, in 2005 we were convinced that it was appropriate to take a few steps back from the initial goal to find clues about the origins of chambers in Cambrian and Pre-Cambrian fossils and instead try to find evidence that hearts fossilize. The exquisitely preserved fossils from the Santana formation proved to be more than equal to this task. After a brief stint with the magnificent fish fossils of the Crato Member, we quickly concentrated on selecting suitable specimens of *Rhacolepis* for invasive analyses using classic acid preparations (Maisey 1991). In a stroke of luck, we found a suitable heart candidate (yet to be described) in the second specimen analyzed. The distinguishing feature that called our attention was a cone-shaped structure lying squeezed between the bones of the pectoral girdle. Further acid dissolution revealed the outlines of a complete fish heart, from its typical conus arteriosus anterior (the cone-shaped structure that drew our attention), to a succession of chambers such as ventricle, atrium, sinus venosus and Cuvier ducts. We spent the next 11 years in search of further fossilized hearts and in an investigation of the taphonomic conditions associated with cardiac fossilization.

The unfortunate experience with the putative Willo heart told us that we needed to amass unequivocal evidence for cardiac fossilization before publication of our original findings. For that, we reasoned that we would require multiple fossil hearts, prefer-
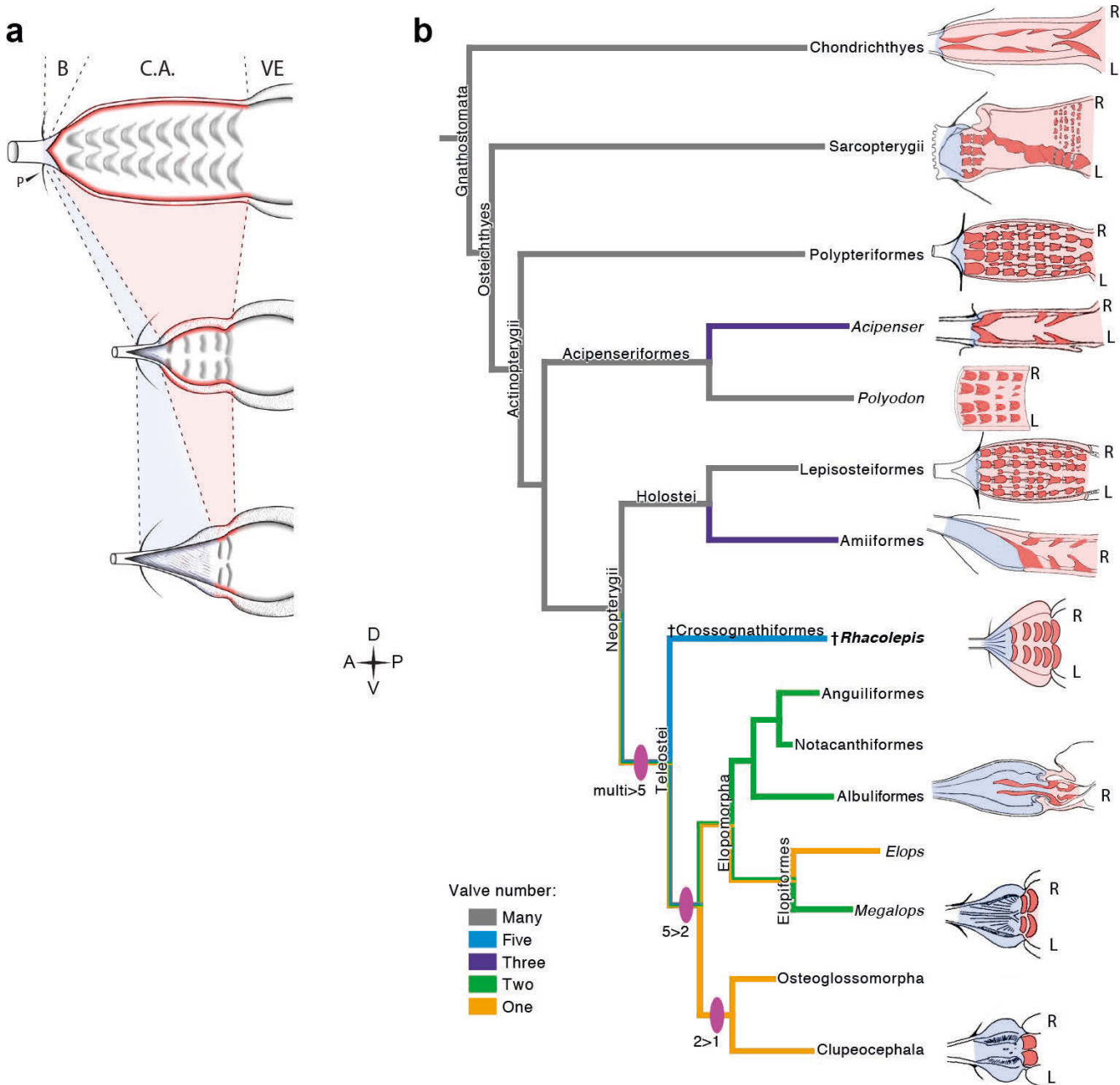
fossils from the Jardim lithology range from those laterally compressed, or platy, to three-dimensional forms. The latter include fusiform types, highly bloated fish, and some specimens with a curious triangular cross section. As originally described, the fossil matrix is well laminated and contains observable amounts of clay. Iron oxides (oxihydroxides) are common and pyrite is present in minute quantities, if at all, in the matrix. Recrystallized calcite is often found inside the fish associated with large body cavities, which may have been produced by necrosis and gas production. Septarian crack formations are also common in these fossils (Maisey 1991). *Rhacolepis* fossils from the Missão Velha lithology are perhaps the most beautiful externally, displaying finely preserved scales. The matrix is typically dark green and abundant, so that relatively small fossils are associated with brick-size rocks. Importantly, the pyrite content of Missão Velha fossils is high and they are rich in clay (Maisey 1991). However, *Rhacolepis* fossils from the Missão Velha lithology often disappoint the examiner hoping for good internal preservation in specimens with finely preserved scales, as they frequently contain very few conserved internal features among large amounts of chalky materials. In sum, from the perspective of internal conservation, *Rhacolepis* fossils with a Jardim lithology (Fig. 5D) are the ones to be tried first.

The significance of concretion lithologies for the preservation of *Rhacolepis* structures. Maisey's 1991 description of the three Romualdo Member lithologies spanning West to East region of the Araripe basin from Santana do Cariri, Jardim and Missão Velha represented a useful overview for subsequent studies on the mechanisms of fossilization from this rich horizon. At that time, it was already clear that the three lithologies represented

Fig. 7. The *Rhacolepis buccalis* conus arteriosus is morphologically intermediate in actinopterygian cardiac outflow tract evolution. **A.** Hypothetical transition from a character state composed of an array of multiple valve rows in the conus arteriosus of basal actinopterygians, such as Polypteriformes (top), to a derived state characterized by the dominance of the valveless bulbus arteriosus in living teleosts (here represented by a generalized elopomorph at the bottom), through an intermediate state represented in the conus arteriosus of fossilized *R. buccalis* hearts (middle). Anterior to left. **B.** Cladogram depicting phylogenetic relationships among early and derived gnathostomes and their corresponding morphologies of the cardiac outflow region. Drawings represent either the inner sides of right (R) and left (L) counterparts, or only the inner right side of the cardiac outflow tract. Blue and pink coloring highlight, respectively, bulbus and conus arteriosus (and respective valves) in extant species. A parsimony ancestral character state reconstruction was performed for the number of conal valves, following the color code in terminals. Genera illustrating the conal condition in each branch are: *Squalus* for Chondrichthyes; *Neoceratodus* for Sarcopterygii; *Polypterus* for Polypteriformes; *Lepisosteus* for Lepisosteiformes; *Amia* for Amiiformes; *Pterothrissus* for Albuliformes; Gadus for Clupeocephala. Abbreviations: B, bulbus; C.A., conus arteriosus; L, left side; P, pericardium; R, right side; VE, ventricle. Figure and legends reproduced from Maldanis et al., 2016 with permission.

ably displayed through non-invasive methods. At first, we tried conventional computed tomography (CT). However, CT proved of limited value for direct visualization of cardiac remains. In our hands, CT was only useful for selecting specimens with a better likelihood of displaying internal conservation. This is probably due to the small radiographic contrast between fossil and matrix and to the limited resolution of conventional CT.

High quality, non-invasive data displaying in situ *Rhacolepis* hearts were eventually obtained through collaboration with Vincent Fernandez and Paul Tafforeau from the ESRF in Grenoble, France. Using phase-contrast X-ray synchrotron microtomography, we showed hearts from three *Rhacolepis* specimens which, collectively, revealed convincing evidence that exceptional cardiac preservation is possible (Fig. 6).

What we learned from the first-known fossilized heart. The fossil heart of *Rhacolepis* highlights some important concepts. The most fundamental concept is easy enough to understand: today it is perfectly possible, but not necessarily simple, to search for the origins of vertebrate organs using a combination of paleontology, developmental biology and evolutionary approaches. Moreover, finding a heart for *Rhacolepis* helped to refine our views about the life of this iconic Cretaceous species.

The place of *Rhacolepis buccalis* in the trophic chain. The *Rhacolepis* heart is of the so-called saccular nature, intermediate between the athletic hearts of vigorous predators, which display very thick ventricles and typical pyramidal forms, and the hearts of sedentary fishes, which exhibit thin ventricles shaped as elongated tubes (Santer 1983). The fossilized *Rhacolepis*

heart's intermediate, saccular morphology was found to be in agreement with the ecological role and position in the trophic chain as hypothesized twenty five years ago, when Maisey and de Carvalho (1995) used acid preparations to investigate gut contents in fossils and found within *Rhacolepis* juveniles substantial numbers of the shrimp *Paleomattea deliciosa* – described in holotype from these gut contents (Maisey & de Carvalho 1995). Incidentally, there are few species so aptly named as the sergestid shrimp *Paleomattea deliciosa*. This small shrimp was probably a favorite delicacy (mattea in Latin) for *R. buccalis*, who ate it with voracity, and perhaps gusto, hence the name "deliciosa" (delicious, in Portuguese). Curiously, and unbeknownst to Maisey and de Carvalho (1995) at the time, mattea also stands for "a gift of God" (more specifically from Yahweh) in Greek, so, after all, perhaps *Paleomattea deliciosa* was indeed a God-given delicacy for *Rhacolepis buccalis*, who devoured it to the point of filling its entire gut, as revealed by inspection of preserved acid-prepared gut contents (Maisey & de Carvalho 1995), or by three-dimensional reconstitution of phase contrast synchrotron radiation microtomographic sections (unpublished). Therefore, the fossil heart of *Rhacolepis* supports the ecological role of this species as mid-level in the trophic chain Maisey 1991, Maisey & de Carvalho 1995).

The heart of *Rhacolepis* illuminates the process of outflow tract simplification in teleosts. Finding a fossilized heart was fortunate enough, but in a second strike of luck, we found that *Rhacolepis* hearts were exquisitely preserved and allowed a detailed examination of their anatomic structure, including a

highly detailed depiction of the valvar apparatus associated with its outflow tract. The truncus arteriosus of *Rhacolepis* contains five rows of valves, while more basal actinopterygians such as *Polypterus* display nine valve rows, and higher actinopterygians sport only one valve. Clearly, the valvar apparatus of *R. buccalis* represents a long-lost intermediate morphology, a snapshot of evolution in the making taken at the Aptian/Albian border, 113–119 Ma in the Cretaceous.

With the long-lost morphology of the *R. buccalis* heart recovered, it was possible to tentatively answer at least one of the questions raised. Was the outflow tract simplification in teleosts the result of a more punctuational macroevolutionary event, or the outcome of phyletic gradualism? The provisional answer is that the scenario is more compatible with phyletic gradualism. This is because there were at least three steps of valvar simplification in teleosts: multiple valve rows to five (e. g. from *Polypterus* to *R. buccalis*), five valve rows to two (from *R. buccalis* to living elopomorphs), and two valve rows to one single valve row retained in all other teleosts (Maldanis et al. 2016).

Because there are basal extant actinopterygian species still living with morphologies that changed very little for millions of years when compared to their fossils, it was possible for us to suggest that valvar reduction in actinopterygians was more likely the result of gradual phyletic changes, than the result of a punctuational event (Fig. 7).

In a review of our findings, Long (2016) warns us that we have but one data point, meaning that we have the very first cardiac fossil and, therefore, that it may be premature to exclude any hypothesis. The point is well taken. However, our reasoning was that, as stated before, most of the morphologic transitions in the actinopterygian cardiac outflow are documented in very old animals that are still living and, therefore, are accessible for detailed study of their organs (our interpretation assumes that cardiac anatomy in these animals did not change much). Long's argument would thus be particularly relevant if, sometime in the future, we or others could find cardiac fossils from extant derived teleosts displaying the ancestral multivalvar arrangement, rather than their current derived, monovalvar, condition (e. g. cypriniformes with multiple valves rows in the conus arteriosus). This is an interesting proposition, which illustrates how far we are from clarifying many relevant patterns of evolution. In any event, it is great that we live in a fascinating time in which we can pose these ambitious questions that may potentially be answered using a paleontological perspective.

Acknowledgements

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