What makes an animal? The molecular quest for the origin of the Animal Kingdom

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Synopsis

What makes an animal? To find the answer we need to integrate data from disciplines such as phylogenetics, palaeontology, ecology, development, anatomy and physiology, as well as molecular biology and genomics. Knowledge of which groups branched before and after the origin of animals is essential. Recent advances in molecular phylogenetics, together with the discovery of new eukaryotic lineages, have drawn a new picture of the ancestry of animals. The nature of the early diverging animal lineages and the timing of the transition are in a state of flux. Various factors have been linked to this striking transition to multicellularity, including changes in environmental conditions and the ecological interactions between unicellular eukaryotes. The current wealth of genomic data has also shed new light on this question. The analysis of the genome of various close relatives of animals has revealed the importance that recycling of ancient genes into metazoan biological functions played into animal origins. A recent study reconstructing the genome of the last common ancestor of extant animals has unveiled an unprecedented emergence of new genes, highlighting the role of genomic novelty in the origin of metazoans.

Introduction

"*What is an animal?*" was the question asked in a seminal paper on the concept of the zootype (Slack et al. 1993), wherein the authors articulated the difficulty of finding a suitable definition for animals. Animals were studied as long as twenty five centuries ago by Aristotle in his *Historia Animālium* (Aristotle et al. 1862), were named Animalia by Carl Linneus in his *Systema Naturae* (Linnaeus 1758), and later renamed Metazoa by Ernst Haeckel (Haeckel 1874a). *Animalis* in Latin means "having breath", "having soul" or "living

being". Cavalier-Smith (Cavalier-Smith 1998) defined animals as phagotrophic multicellular eukaryotes with connective tissue, located between two dissimilar epithelia, and composed of collagen. A later definition included more characters, such as reproduction through an egg cell fertilized by a monociliated sperm cell, embryonic development (blastula followed by gastrulation), cell coordinated through signal transduction, specific mechanisms of cell adhesion (cell–cell junctions, basal lamina, and extracellular matrix), presence of ectoderm and endoderm, or the presence of sensory cells in the epithelium (Adl et al. 2012). The diversity of definitions is testament to the astonishing variety of shapes and forms of the members of the Animal Kingdom. At the same time this diversity makes animals a great model for studying the interplay between the evolution of genomes and morphologies.

The Metazoa is the eukaryotic kingdom with the largest number of described species, over 1.5 million divided into approximately 33 phyla (Zhang 2013). This is in contrast to the number of species described in plants (340,000; Larsen et al. 2017) or fungi (100,000; Blackwell 2011). Approximately 95% of this species-level animal diversity is found in only 5 phyla: arthropods (80% of the described animal species), molluscs (7.5%), chordates (4%), flatworms (2%), and nematodes (1.6%). The real number of animal species is likely much larger, with estimates ranging from 8 million species (Mora et al. 2011) to 163 million (Larsen et al. 2017). This diversity and our biased biophilia (Wilson 1984) has placed animals in a central position in natural history. Despite this, the study of many groups of marine and/or microscopic animals has been especially neglected despite their central role in ecology and evolution. Indeed, taxonomic chauvinism (Bonnet et al. 2002) led to their omission even in studies analysing biases on taxonomic research (Troudet et al. 2017). Other important features of metazoans are multicellularity, which is also found in other

eukaryotes, and the largest diversity of cell types and body plans among multicellular organisms (Rokas 2008a).

The study of the evolutionary process that preceded this surge of diversity requires the integration of multiple disciplines. All levels of biological organisation are critical, from molecules to ecosystems. The fields of molecular evolution, genetics and genomics, evolutionary developmental biology, anatomy and physiology, ecology, and the fossil record are fundamental. These areas have greatly benefited from molecular advances, such as the polymerase chain reaction (PCR) in the late twentieth century, or the current flood of omics data from next generation sequencing (NGS). Here I review the impact of advances in molecular biology and genomics on our understanding of the origin of animals.

The phylogenetic framework

To investigate a biological transition linked to a node of an evolutionary tree we must accurately identify the extant organisms branching before and after that node. Since the nineteenth century the lineages thought to flank the origin of animals have been choanoflagellates and sponges (Figure 1). Haeckel did not consider sponges (Phylum Porifera) in his first trees (Haeckel 1866), but after studying their development (Haeckel 1872) believed them essential to his Gastraea hypothesis (Haeckel 1874b). He placed poriferans as an early twig within the zoophyta ("plant animals") together with cnidarians (Haeckel 1874a). At the other side of the node, the close relationship between choanoflagellates and animals was first proposed by Dujardin (Dujardin 1841) and later by James-Clark (James-Clark 1866), based on the similarity between choanoflagellates and the choanocytes of poriferans, although their homology is still under investigation (Mah et al.

2014). This understanding of the evolutionary position of sponges and choanoflagellates remained stable for over 100 years and played a major role in several hypotheses on the genesis of metazoans (e.g. Hyman 1940).

At the end of the twentieth century, molecular phylogenetics dramatically rearranged the Tree of Life of the Metazoa, a true paradigm shift in our understanding of animal evolution (Figure 2; Halanych et al. 1995; Aguinaldo et al. 1997; Halanych 2004). These early phylogenies did not alter the position of choanoflagellates and sponges, although the position of some branches remained unclear (Medina et al. 2001; Carr et al. 2008). At the turn of the century, with sequencing advances, alignments of just a few genes were replaced by genome-scale datasets (phylogenomics; Delsuc et al. 2005). Early phylogenomic analyses of animals and their allies were limited in taxa – lacking sponges – but still supported the close relationship between metazoans and choanoflagellates (Philippe et al. 2004; Philippe, Delsuc, et al. 2005; Philippe, Lartillot, et al. 2005).

The enigmatic cousins of animals

Molecular phylogenetics has reshaped our understanding of the evolutionary relationships of eukaryotes (Paps et al. 2010; Adl et al. 2012; Burki 2014). The position of choanoflagellates as sister-group to animals has remained unchallenged, but the discovery of new eukaryotic lineages or the repositioning of old ones has painted a completely new picture of eukaryote evolution (Ruiz-Trillo et al. 2008; Shalchian-Tabrizi et al. 2008; Torruella et al. 2012, 2015; Paps et al. 2013; Hehenberger et al. 2017). Animals belong to the eukaryotic superclade Opisthokonta (Figure 2), eukaryotes with a posterior flagellum (lost in some groups) with a pair of centrioles, and mitochondria with flat cristae (Cavalier-Smith

1987). This group includes two major clades, the Holomycota (also named Nucletmyceta) that contains fungi and their allies, and the Holozoa (Figure 2; Paps et al. 2010). Holozoans comprise animals and choanoflagellates, but also other eukaryotic lineages essential to understanding the origin of animals, the Teretosporea and the Filastera.

Teretosporea (Torruella et al. 2015) are the sister group to the rest of holozoans. They were previously named the DRIP group (Herr et al. 1999), Mesomycetozoea (Mendoza et al. 2002), and Ichthyosporea (Cavalier-Smith 1998). The change in name from Ichthyosporea to Teretosporea was prompted by the placement of *Corallochytrium limasciporum* as sister-group to the ichthyosporeans (Torruella et al. 2015). Other analyses point to an intermediate position for *C. limasciporum* – sometimes with *Syssomonas multiformis* – between ichthyosporeans and and the group formed by filastereans, choanoflagellates, and metazoans (Paps et al. 2013; Hehenberger et al. 2017). The placement of these taxa is essential to reconstruct the evolution of locomotion (flagellum and filopodia) and feeding strategies in the ancestors of animals. The Filasterea were defined using molecular data (Shalchian-Tabrizi et al. 2008), and originally comprised two species, *Capsaspora owczarzaki* (ATCC 30864) and *Ministeria vibrans*. However two new filasterean species have been discovered recently, *Pigoraptor vietnamica* and *P. chileana* (Hehenberger et al. 2017). Interestingly, all these non-animal holozoan lineages show some type of multicellularity (see below).

Battle royale at the origin of animals: combs *v* sponges

The branching order of the earliest animals has recently become controversial. Two pioneer phylogenomic studies initiated a phylogenetic discussion that is still raging 10 years

later (Dunn et al. 2008; Hejnol et al. 2009). They independently proposed comb jellies (Phylum Ctenophora, Figure 1d) as the first-splitting lineage of animals, taking that place from sponges. Later analyses have suggested that sponges are the sister-group to all animals, and attributed the earlier results as phylogenetic artifacts caused by both long branch attraction (LBA) and poor taxon sampling (Philippe et al. 2009; Pick et al. 2010). However, the subsequent sequencing of the complete genomes of two ctenophores as well as additional transcriptomic data supported again the placement of ctenophores as the earliest-diverging metazoans (Ryan et al. 2013; Moroz et al. 2014).

Since then over a dozen studies have been published on this problem, clashing over methods, evolutionary models, LBA claims, and datasets. Some supporting sponges-first and others ctenophores-first (these are reviewed in Dunn et al. 2015, Jékely et al. 2015, and King and Rokas 2017). Andreas Hejnol (Sars Centre, Bergen) humorously coined the terms #porisis and #ctenosis to refer to the publications supporting one hypothesis or the other (Hejnol 2016). This disagreement mirrors the controversy surrounding the position of Xenoacelomorpha relative to another major transition, the origin of bilaterian animals (the War of the Worms ; Hejnol and Pang 2016; Ruiz-Trillo and Paps 2016; Telford and Copley 2016). The dispute on ctenophores-first versus sponges-first seems far from being settled; the two most recent articles still disagree on sponges (Feuda et al. 2017) or ctenophorans (Whelan et al. 2017) as sister-group to the rest of animals. Some authors have pointed that we should not be concerned about admitting our ignorance, and instead use this uncertainty to drive our research programmes (King and Rokas 2017). The evolutionary placement of early-diverging animals and non-animal holozoans at the fringes of the transition is vital, and the first step to reconstruct what makes an animal.

What makes an animal?

The transition (or lack thereof) to multicellularity

Multicellularity is not exclusive to animals, plants, and fungi; it has evolved at least 25 times independently in eukaryotes (Baldauf 2003; King 2004; Rokas 2008a). Remarkably, all the opisthokont lineages show traces of multicellularity (de Mendoza et al. 2015b; Sebé-Pedrós et al. 2017). In metazoans, multicellularity is the result of development, with a single cell dividing into various differentiated cells and tissues (permanent clonal multicellularity). Some choanoflagellates show temporary clonal colonies (Fairclough et al. 2010; Dayel et al. 2011), and evidence of sexual reproduction (Carr et al. 2010; Levin and King 2013; Woznica et al. 2017). Some filastereans display aggregative colonies, while teretosporeans develop 'syncitial' (clonal) colonies (coenocytes, Sebé-Pedrós, Irimia, et al. 2013; Suga and Ruiz-Trillo 2013; Sebé-Pedrós et al. 2017). Thus, colonies formed by multiple cells were already present in opisthokonts before the origin of multicellular animals, although the taxonomic distribution is sparse and colonial stages in these taxa are always temporary.

Animals excel at making different kinds of cells, significantly surpassing fungi and plants (Rokas 2008a). The number of cell types present in a taxon has often been used as a proxy for organismal complexity (Valentine et al. 1994), although defining and counting cell types is problematic (Trapnell 2015). The assignment of cell type homology across different animal groups is also difficult, but it is an indispensable next step in reconstructing their history and improving our inferences on ancestral cell types (Ryan et al. 2013; Mah et al. 2014; Jékely et al. 2015; Ryan and Chiodin 2015). The new field of single-cell transcriptomics has great potential to refine the definition of cell types and to improve the study of their

evolution (Trapnell 2015; Marioni and Arendt 2017). Bearing these limitations in mind, it could be argued that the first animals possibly had a number of cell types similar to extant non-bilaterian animals. The last common ancestor (LCA) of Porifera probably had at least 10 cell types, with at least another 18 types emerging later in each of the different sponge classes after the diversification of the phylum (Simpson 1984). A total of 6 somatic cell types have been recently described in a placozoan (Smith et al. 2014). Ancestral metazoan cells were probably complex and multifunctional, and later subfunctionalization drove further cell diversification (Arendt 2008). Interestingly, similar numbers of cell forms are found across different life cycles of the relatives of animals: five in choanoflagellates (Dayel et al. 2011), and three in Filasterea (Sebé-Pedrós, Irimia, et al. 2013; Sebé-Pedrós et al. 2016) as well as in Teretosporea (Marshall et al. 2008; de Mendoza et al. 2015a).

All this suggests that the organisms predating the origin of the Animal Kingdom probably showed transient clonal colonies and a number of cell stages similar to the first animals (Dayel et al. 2011; Sebé-Pedrós, Irimia, et al. 2013; de Mendoza et al. 2015a; Sebé-Pedrós et al. 2016). Maybe the classical depiction of a transition from a simple unicellular eukaryote to an complex multicellular animal is not accurate, and the leap was not so immense.

Timing and triggers of the transition

The date of the origin of animals is critical to understand the paleoecological factors involved in that transition. The dating is hindered in part by the difficulty on placing fossil specimens in the Tree of Life. Complex multicellular fossils from the Cambrian are well accepted to be metazoans and, in most cases, bilaterian. But the nature of the older

Ediacaran biota is considered ambiguous by some authors (Antcliffe 2012; Antcliffe et al. 2014). A conservative reading of the fossils suggests that animals emerged during the early Cambrian period, 541 Mya (Antcliffe et al. 2014; Cunningham et al. 2017), preceding the Cambrian explosion by a short time. Alternative interpretations associate Ediacaran fossils with metazoans (Budd and Jensen 2017; Dunn et al. 2017) pushing the origin of animals further back in time. A recent study assigned fossils from 665 Mya to the Animal Kingdom (Maloof et al. 2010), and analyses of sterane biomarkers and comparative genomics suggest the presence of animals 650 Mya (Gold et al. 2016). The early applications of molecular clocks proposed exceptionally old estimates for the origin of animals (Graur and Martin 2004), but the implementation of relaxed clocks and Bayesian approaches now yields younger dates, between 833 and 650 Mya (dos Reis et al. 2015; Cunningham et al. 2017).

This uncertainty makes difficult to correlate geological events with the origin of animals. For example, the rise of oxygen atmospheric concentration during the late Proterozoic has been linked to the emergence of animals (Budd and Jensen 2000), although recent studies on the physiology and genomes of extant animals challenge that idea (Planavsky et al. 2014; Mills et al. 2018). In contrast, the quick increase of calcium and phosphate in the oceans has been associated with biomineralization and early animal diversification, but these events took place during the Cambrian (Smith and Harper 2013). Ecological pressures have also been connected to the evolution of multicellularity, for example as an escape from predation by other unicellular organisms (Stanley 1973);this is supported by studies of experimental evolution (Herron et al. 2018). Finally, in addition to ecological and environmental changes, internal genomic factors have also been invoked to explain the transition to metazoans. These include biological functions such as differential

gene regulation – orchestrated by transcription factors (TF) and signalling pathways – to regulate differential gene expression in time and space (different cell types and tissues during development, but also in adults), cell adhesion (e.g. cadherins), cell type specification, control of cell cycle, and immunity (Rokas 2008b; Richter and King 2013; Brunet and King 2017; Sebé-Pedrós et al. 2017). The basis of those functions must be found in the genome.

Genomic basis of the origin of the Animal Kingdom

Something borrowed

The arrival of NGS has made available genome data for all the key taxa involved in the transition to multicellular animals, that is, non-animal opisthokonts and early-diverging animals (Ruiz-Trillo et al. 2007; King et al. 2008; Srivastava et al. 2008, 2010; Ryan et al. 2013; Suga et al. 2013; Moroz et al. 2014; Grau-Bové et al. 2017; Sebé-Pedrós et al. 2017). Comparative genomics unexpectedly found many genes previously thought to be animal-specific in the other holozoans, indicating that the emergence of these genes predates the origin of animals (Sebé-Pedrós et al. 2011, 2017; Suga et al. 2013; de Mendoza et al. 2014a; Brunet and King 2017). This earlier view was caused by the loss of many of these genes in the choanoflagellates analysed (but see Richter et al. 2017), this changed as other holozoan genomes were sequenced (Sebé-Pedrós et al. 2017).

The list of genes, pathways, and systems analysed using state-of-the-art comparative genomics is too long and complex to detail here (see recent and thorough reviews in Brunet and King 2017 and Sebé-Pedrós et al. 2017). Some relevant examples are the discovery of signalling pathways with elements shared between animals and other holozoans, such as

the receptor tyrosine kinases (Suga et al. 2014), the Hippo signalling pathway (Sebé-Pedrós et al. 2012), or some parts of G-protein coupled receptors (de Mendoza et al. 2014b). In many cases, the downstream intracellular elements of these pathways are present in holozoans, but the metazoan receptors and ligands evolved only in animals (Sebé-Pedrós et al. 2017; Paps and Holland 2018). Other signalling pathways are absent in non-animal holozoans, though; this is the case for Wnt, transforming growth factor-β (TGF-β), hedgehog, and JAK–STAT (Degnan et al. 2008; King et al. 2008; Suga et al. 2013). Similarly, some TF were unexpectedly found in holozoans (nuclear factor-κB, p53, RUNX and T-box) while others were established as animal-specific (ETS, SMAD, nuclear receptor, Doublesex, and interferon-regulatory factor; Sebé-Pedrós et al. 2011; Sebé-Pedrós, Ariza-Cosano, et al. 2013; Sebé-Pedrós and de Mendoza 2015). Finally, some parts of the cell adhesion systems also predate animals, with circa 30 proteins predicted to contain cadherin domains found in choanoflagellates (Nichols et al. 2012), integrin components in filastereans (Sebé-Pedrós et al. 2010) and choanoflagellates (Richter et al. 2017), as well as laminins, collagens and fibronectin in other holozoans (Suga et al. 2013).

Interestingly, the study of this transition is now expanding towards the non-coding elements of the genome (de Mendoza et al. 2015b; Fernandez-Valverde and Degnan 2016; Sebé-Pedrós et al. 2016; Gaiti et al. 2017). A picture of extensive genome recycling during the origin of metazoans has emerged from all these analyses. Most likely animals co-opted the machinery that is used by other holozoans to regulate gene expression at a temporary level (cells at different life stages), and repurposed it to add a spatial component (regulate differential gene expression in simultaneous cell types; Sebé-Pedrós et al. 2017). These results emphasize the role of genome tinkering in evolution. Only recently has the part that

novelty played in the origin of animals started to be revisited (Grau-Bové et al. 2017; Richter et al. 2017).

Something old

A recent article has focused on genomic novelty in the origin of the Animal Kingdom (Paps and Holland 2018). These analyses use a new bioinformatics pipeline that has been also used successfully to analyse the origin of placental mammals (Dunwell et al. 2017). The study compared 62 genomes (approximately 1.5 million proteins) belonging to 13 animal phyla and 8 eukaryotic outgroups, with particular consideration to taxon sampling, representative selection of outgroups, and the assignment of gene homology. For the latter, reciprocal sequence comparisons with BLAST (Altschul et al. 1990) combined with Markov clustering (Enright et al. 2002) were used, in contrast with other approaches that use a limited taxon sampling and one-way BLAST (Domazet-Lošo et al. 2007; Moyers and Zhang 2015). This approach defines gene homology groups (HG), which in some cases contain well-known gene families (e.g. *Irx*), in others gene classes (e.g. POU class of homeobox genes), or superfamilies (e.g. Wnt ligands). The evolutionary emergence of a HG is determined based on the HG occupancy in all members of a phylogenetic group instead of using a single species as an anchor. A novel bioinformatic tool was developed to that end (Phylogenetic Aware Parsing Script; GitHub). This pipeline was used to reconstruct all the HG present in the genome of the LCA of animals (Ancestral HG), as well as in the genome of other holozoan ancestors. The reconstructions assessed different evolutionary trees (e.g. sponges-first vs ctenophores-first) to accommodate phylogenetic uncertainties. The Ancestral HG set consists of 6,331 HG, whose predominant functions are related to gene regulation (e.g. nucleic acid binding proteins, TF), metabolism (e.g. hydrolases, transferases, etc), and signalling pathways (e.g. Wnt, TGF-beta, cadherin, integrin, etc.). Remarkably, a similar

distribution of biological functions is also predicted in the older LCA as well as in modern metazoan genomes (human and fruit fly).

Something new

Novel Homology Groups is the subset of Ancestral HG that are present in the ingroup but absent in the outgroup. Therefore, they are new HG that emerge in the LCA of a group. Novel genes are most likely product of gene duplication events followed by quick evolutionary divergence (Holland et al. 2017). The number of Novel HG for each LCA shows a 4-fold increase in the LCA of animals compared to older nodes. There are 1189 Novel HG in animals, 19% of the total HG in the first metazoan, compared to only 8-10% Novel HGs found in the older nodes inspected. These animal Novel HGs present a higher percentage of regulatory functions compared to the animal Ancestral HG set (e.g. 23% vs 6% transcription factors, 11% vs 4% signalling), and has fewer enzymes. Moreover, the number of genes performing some biological functions — nucleic acid binding, TF, and signalling molecules — shows a peak in the LCA of animals in contrast with older nodes. Thus, not only did many new genes emerge in the first animal genome, but they were also associated with functions essential to multicellularity.

Another interesting set is the Novel Core Homology Groups. These are novel HG that are highly retained, that is, present in almost all of the members of the ingroup. It is assumed that new HG with essential functions will be refractory to gene loss during the evolution of metazoans. A total of 25 Novel Core HG are found in the LCA of animals (Table 1), a 5-fold increase compared to other holozoan Novel Core HG. This implies that not only was novelty common in the first animal genome, but the level of retention of these novel genes were also higher than in other ancestors. The functions of these genes are crucial to

multicellularity. Seven Corel New HG comprise TF, eight HG include signalling pathways, and the rest show functions related to cell adhesion, cell cycle, receptors, exocytosis, and regulation of protein translation. Remarkably, they include many of the animal receptors and ligands missing in non-animal holozoans. Many of these genes were previously thought to be associated with the emergence of animals, but additional new genes are here, for the first time, linked to the transition. The Lost HG are also analysed; their values are similar across the different ancestors, including metazoans. It is difficult to identify the biological function of Lost HG, as these are missing in model organisms. Most Lost HG in animals code for enzymes, and the list is lacking in developmentally relevant genes.

Tempo and mode of genomic evolution

Paps and Holland (2018) unveil the inferred genome of the first animal, and show that overall it is similar to the genomes of other holozoan ancestors and modern animals. However, compared to other holozoans it has a higher proportion of novel genes and higher percentage of these is retained. Different scenarios can explain these observations, based on the length of the branch leading to the metazoan LCA – how long it took for the last common ancestor of all animals to evolve – and/or the relative rate of gene birth and death (Figure 3). The first scenario presupposes a constant rate of gene birth over time, with a branch leading to the first metazoan longer than others (Figure 3a). During this 'stew' time, the molecular components of animal biology were 'cooking' in the genome of the animal ancestor. However, phylogenetic analyses of opisthokonts do not show longer branches leading to animals (e.g. Torruella et al. 2015), as expected under this scenario. The second scenario assumes similar branch lengths across all nodes, but with novel genes quickly popping up during a short 'popcorn' phase. This popcorn effect could be caused by either

lower rates of gene death and/or higher rates of gene birth (Figure 3b and 3c). Lower rates of gene death would increase the number of novel genes being fixed; the rapid recruitment of new genes into key gene regulatory networks could make them refractory to gene loss (Figure 3b). However, our analyses show similar levels of Lost HG across all nodes, including the animal one. The genomic popcorn could be also produced by higher gene birth rate, speculatively caused by multiple segmental duplications, whole genome duplications, environmental factors increasing mutation rates, etc. (Figure 3c).

These scenarios are compatible with previous paleontological models of early animal evolution (Antcliffe 2012), which rest on the nature of Ediacaran fossils. The stew hypothesis is consistent with the 'slow-burning fuse' model, in which Ediacarans are assumed to be metazoans and they emerged ~1,000 Mya, although they didn't diversify until the Cambrian (Antcliffe 2012). This would be supported by some molecular clocks (Erwin et al. 2011; dos Reis et al. 2015), and the missing genomes of Ediacaran fauna would break the long branch leading to the ancestor of the metazoan crown-group (Figure 3a). In contrast, the popcorn scenario is compatible with the 'evolutionary Big Bang' model (animals originated during early Cambrian and diversified quickly after) and the 'shallow fuse' model (animals emerged in late Ediacaran, then radiated in the Cambrian; Antcliffe 2012). However, a better understanding of the rates of gene birth/death and the phylogenetic placement of the Ediacaran fossils is needed to discriminate between these scenarios and models.

Conclusions

Our view on animal evolution has changed dramatically in the last 25 years. New phylogenies based on molecular data have restructured the Tree of Life and our

understanding of organismal evolution. The wealth of genomic data from NGS is democratising the study of organisms, offering a window to the hidden biology of the taxa closest to the dawn of metazoans. There has never been a more stimulating time to study the origin of the Animal Kingdom, and future data and techniques can only refine the current picture further. During the transition to animal multicellularity, old holozoan functions were recycled but also new ones emerged. Further work is needed to elucidate the pace of that change, and to reveal whether these patterns are specific to the metazoans, or if similar convergent patterns can be seen in plants, fungi and other multicellular lineages.

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Figure captions

Figure 1. A. Drawing of a feeding choanoflagellate (Kent 1880). **B.** A colony of choanoflagellates (Metchnikoff 1886). **C.** Plate 5 from *Kunstformen der Natur* (Haeckel 1904) on calcarean sponges (Phylum Porifera). **D.** Plate 27 from *Kunstformen der Natur* (Haeckel 1904) on comb jellies (Phylum Ctenophora).

Figure 2. Current understanding of the phylogeny of opisthokonts, animals and their closest relatives. Based on different publications (Ruiz-Trillo et al. 2008; Shalchian-Tabrizi et al. 2008; Torruella et al. 2012, 2015; Paps et al. 2013; Budd and Jensen 2017; Hehenberger et al. 2017). Drawings from phylopic.org and the author.

Figure 3. Tempo and mode of genome evolution at the origin of the Animal Kingdom. **A.** Stew model, with animals taking longer time to emerge. **B.** Popcorn model with lower gene death in the transition to multicellular animals. **C.** Popcorn model with higher gene birth in the dawn of metazoans.

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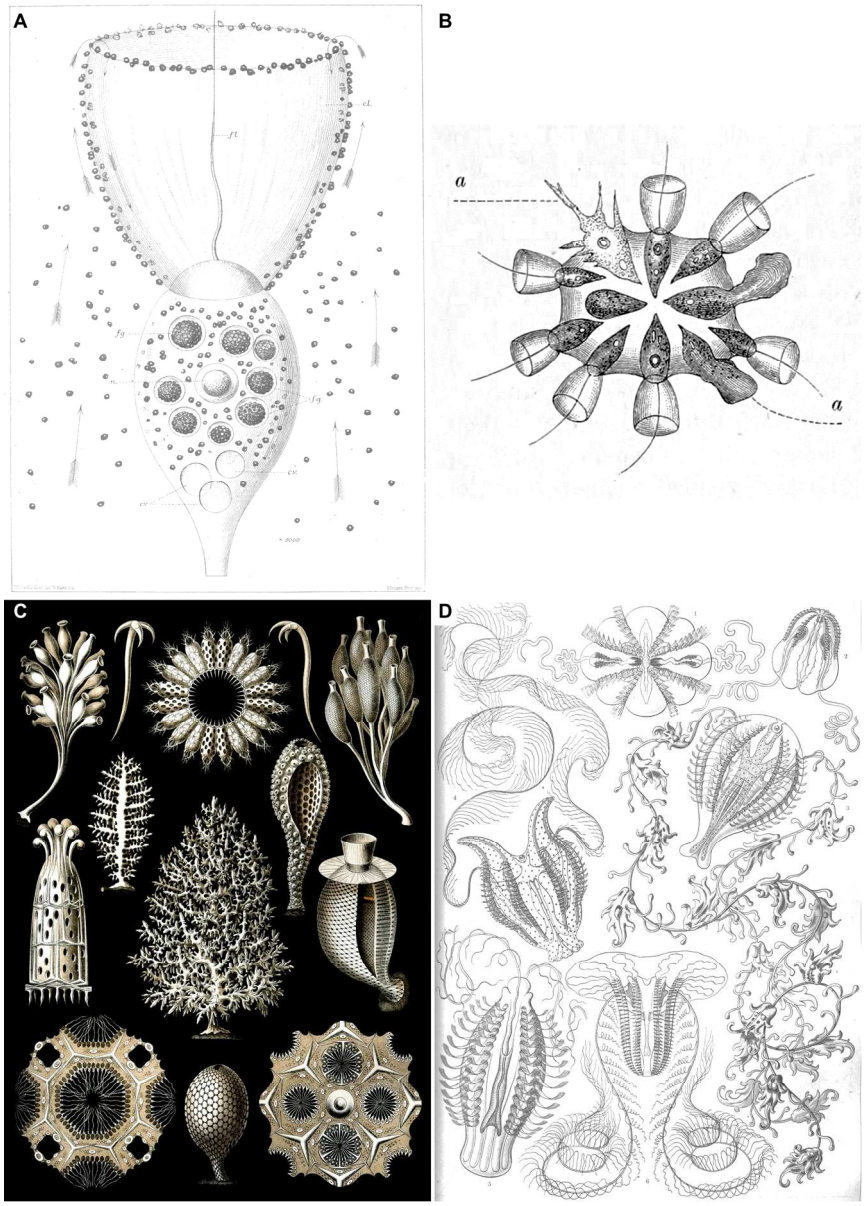

Figure 1. A. Drawing of a feeding choanoflagellate (Kent 1880). B. A colony of choanoflagellates (Metchnikoff 1886). C. Plate 5 from Kunstformen der Natur (Haeckel 1904) on calcarean sponges (Phylum Porifera). D. Plate 27 from Kunstformen der Natur (Haeckel 1904) on comb jellies (Phylum Ctenophora).

500x700mm (96 x 96 DPI)

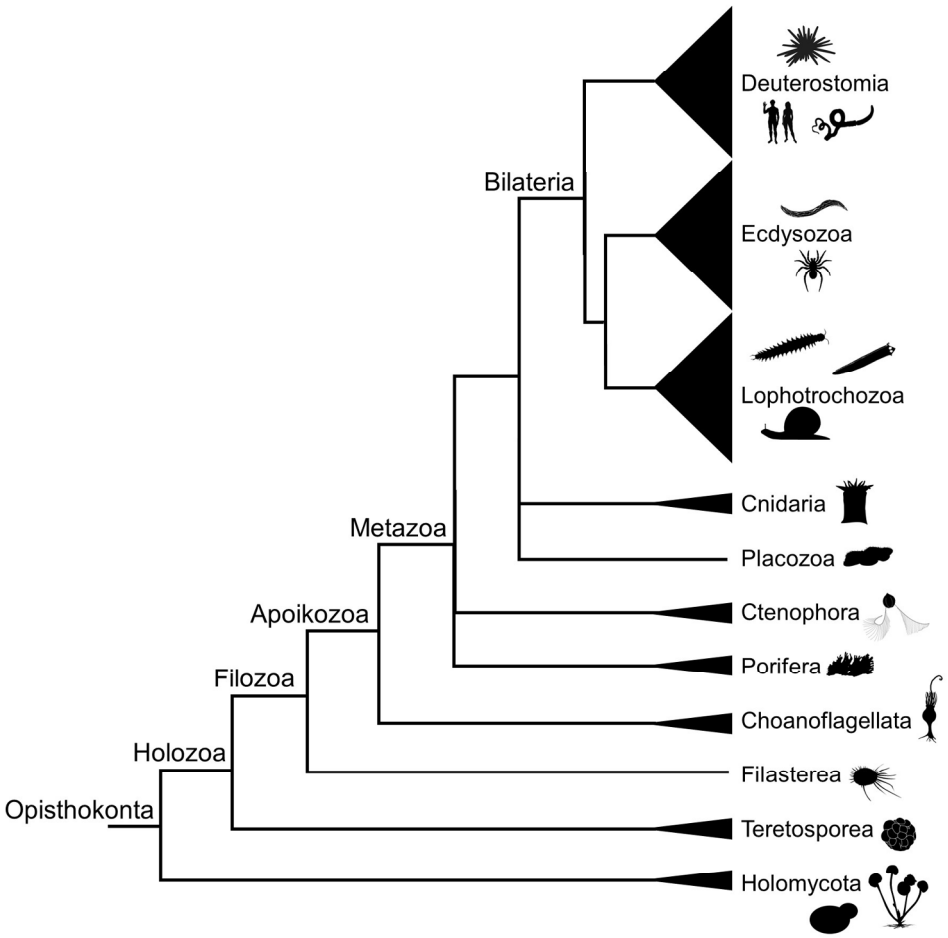

Figure 2. Current understanding of the phylogeny of opisthokonts, animals and their closest relatives. Based on different publications (Ruiz-Trillo et al. 2008; Shalchian-Tabrizi et al. 2008; Torruella et al. 2012, 2015; Paps et al. 2013; Budd and Jensen 2017; Hehenberger et al. 2017). Drawings from phylopic.org and the author.

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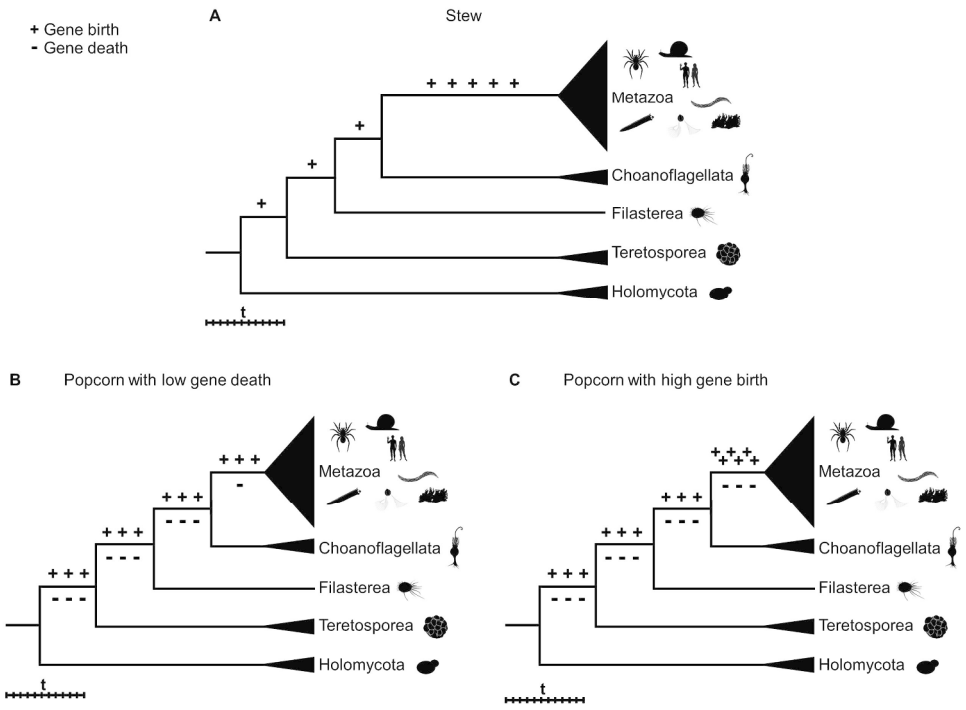

Figure 3. Tempo and mode of genome evolution at the origin of the Animal Kingdom. A. Stew model, with animals taking longer time to emerge. B. Popcorn model with lower gene death in the transition to multicellular animals. C. Popcorn model with higher gene birth in the dawn of metazoans.

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Table 1. Novel Essential Genes in the Animal Kingdom. List of the 25 novel HG that are highly retained in the genomes of the Animal Kingdom. Adapted from Paps & Holland (2018).

Transcription Factors	
Homeobox	NKL subclass
	SIX Class
	POU Class
bHLH	hes/hairy
	bHLH-PAS
	twist/hand
ETS	

Signalling pathways	
Wnt	Wnt
	Frizzled
	pangolín/TCF-LEF
	armadillo/beta-catenin
TGF-Beta	TGF-Beta/BMP
	SMAD
	TFG-Beta Receptor
	JNK pathway interaction

Transcripts polyadenilation
Cytoplasmic Polyadenylation Element Binding Protein (CPEB)

Cell adhesion
Fermitin
Liprin
Alpha-catenin

Cell cycle
RUN (after RaP2 interacting protein 8, UNC-14 and NESCA)
MAP kinase-activating death domain (MADD/GEF)

Receptors
Nuclear Hormone Receptors
Neurotransmitter Receptors

Synaptic exocytosis
Calcium activated protein for secretion (CADPS)
Rab3-interacting molecules (RIM)