

## **The origin of animals as microbial host volumes in nutrient-limited seas**

The microbe-stuffed gut, rather than the genome, represents the most dynamic gene reservoir within complex, multicellular metazoa (animals). Microbes are known to confer increased metabolic efficiency, increased nutrient recovery, and tolerance of ocean acidity to basal taxa such as sponges, arguably the extant taxa most comparable to the first metazoan. We hypothesize that metazoan origins may be rooted in the capability to compartmentalize, metabolize, and exchange genetic material with a modulated microbiome. We present evidence that the most parsimonious adaptive response of clonal eukaryotic colonies experiencing oligotrophic (nutrient-limited) conditions that accompanied Neoproterozoic glaciation events, which were broadly contemporaneous with metazoan origins, is to evolve a morphological volume to harbor a densified microbiome. Dense microbial communities housed within a cavity would increase instances of horizontal gene transfer between microorganisms and host, accelerating evolutionary innovation at the genetic and epigenetic levels for the holobiont. The accelerated tempo of genetic exchange would continue until the host's metabolic and reproductive cells became spatially and temporally segregated from one another, at which point the process is effectively suppressed with the emergence of specialized gut and reproductive tissues. This framework may lead to new, testable hypotheses regarding metazoan evolution on Earth and a more tractable means of estimating the pervasiveness of complex, multicellular animal-like life with convergent morphologies on other planets.

# The origin of animals as microbial host volumes in nutrient-limited seas

Zachary R. Adam<sup>a,b\*</sup>, Betul Kacar<sup>c</sup>, Sanjoy M. Som<sup>d,e</sup>, Kennda L. Lynch<sup>f</sup>, Marina Walther-Antonio<sup>g</sup>, Kenneth Williford<sup>h</sup>

<sup>a</sup> Earth and Planetary Sciences Department, Harvard University, Cambridge, MA

<sup>b</sup> Lunar and Planetary Laboratories, University of Arizona, Tucson, AZ

<sup>c</sup> Departments of Molecular and Cellular Biology and Astronomy, University of Arizona, Tucson, AZ

<sup>d</sup> Blue Marble Space Institute of Science, Seattle, WA

<sup>e</sup> Exobiology Branch, NASA Ames Research Center, Moffett Field, CA

<sup>f</sup> School of Biological Sciences & School of Earth & Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA

<sup>g</sup> Department of Surgery, Department of Obstetrics & Gynecology, Microbiome Program, Center for Individualized Medicine, Mayo Clinic, Rochester, MN

<sup>h</sup> Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109 USA

\*Corresponding Author:

Zachary R. Adam

Email address: [zadam@arizona.edu](mailto:zadam@arizona.edu)

## 1 ABSTRACT

2  
3 The microbe-stuffed gut, rather than the genome, represents the most dynamic gene reservoir  
4 within complex, multicellular metazoa (animals). Microbes are known to confer increased  
5 metabolic efficiency, increased nutrient recovery, and tolerance of ocean acidity to basal taxa  
6 such as sponges, arguably the extant taxa most comparable to the first metazoan. We  
7 hypothesize that metazoan origins may be rooted in the capability to compartmentalize,  
8 metabolize, and exchange genetic material with a modulated microbiome. We present evidence  
9 that the most parsimonious adaptive response of clonal eukaryotic colonies experiencing  
10 oligotrophic (nutrient-limited) conditions that accompanied Neoproterozoic glaciation events,  
11 which were broadly contemporaneous with metazoan origins, is to evolve a morphological volume  
12 to harbor a densified microbiome. Dense microbial communities housed within a cavity would  
13 increase instances of horizontal gene transfer between microorganisms and host, accelerating  
14 evolutionary innovation at the genetic and epigenetic levels for the holobiont. The accelerated  
15 tempo of genetic exchange would continue until the host's metabolic and reproductive cells  
16 became spatially and temporally segregated from one another, at which point the process is  
17 effectively suppressed with the emergence of specialized gut and reproductive tissues. This  
18 framework may lead to new, testable hypotheses regarding metazoan evolution on Earth and a  
19 more tractable means of estimating the pervasiveness of complex, multicellular animal-like life  
20 with convergent morphologies on other planets.

## 24 INTRODUCTION

25 How did animals arise from earlier eukaryotic ancestors? The emergence of a complex,  
26 multicellular respiring organism with distinct soma and germ cell lines from unicellular (Michod  
27 2007) or colonial predecessors continues to be an active area of research (Budd and Jensen  
28 2017; Chen *et al.* 2015; dos Reis *et al.* 2015; Sperling *et al.* 2015). Investigation of such a  
29 biological innovation must link factors such as protistan-grade morphogenetic variation,  
30 environmental variability, and eukaryotic and prokaryotic ecological feedback to the emergence  
31 of the metazoan body plan (Hays 2015). A pernicious mirror to the question 'What triggered the  
32 Cambrian explosion?' also lies in plain sight: why did metazoan complexification essentially  
33 cease after the emergence of the bilaterian body plan? There are no obvious reasons why  
34 bilaterianism should represent a macroevolutionary zenith for metazoan complexification- five  
35 hundred million years of animalian structural stasis (variations on a bilaterian theme) are perhaps  
36 just as mysterious as the Cambrian explosion itself, but it is unclear if the two phenomena are  
37 linked.

38  
39 The fossil record is enigmatic regarding intermediate macroevolutionary states that link early  
40 eukaryotes to the appearance of metazoa. Preceding the "Cambrian explosion" eukaryotic  
41 fossils are mostly limited to acritarchs, common fossils interpreted as protistan-grade remains  
42 of unknown phylogenetic affinity (Peterson and Butterfield 2005). Molecular clocks tuned with  
43 paleontological data set metazoan emergence around 660 Ma (Peterson and Butterfield 2005).  
44 These dates suggest the Neoproterozoic (1000 Ma to 541 Ma) as the geological era most

1 consistent with metazoan emergence (Peterson *et al.* 2005). The Neoproterozoic fossil record is  
2 interrupted by intermittent low-latitude glaciations ('Snowball Earth' events), the first occurring  
3 around 710 Ma known as the Sturtian glaciation (Hoffman *et al.* 1998). Sedimentary features from  
4 the Trezona Formation in south Australia have been interpreted as remains of organisms thought  
5 to lie at the base of metazoa (Maloof *et al.* 2010). There is evidence for another global glaciation  
6 around 635 Ma (Marinoan), followed by a period of glacial quiescence, and another short period  
7 of glaciation (Gaskiers). At approximately 580 Ma the Ediacaran biota, with relatively large size  
8 and coordinated but enigmatic body plans, emerge (Laflamme 2014). Whether these organisms  
9 were the predecessors to bilaterian organisms (Cuthill and Morris 2017) or whether they  
10 represent 'failed experiments' in complex multicellularity that went extinct has not been  
11 conclusively resolved. What is certain is that the Ediacara represent a jump in size and  
12 complexity compared to nearly anything that came before them (Hoyal Cuthill and Conway  
13 Morris 2014), and that their demise coincides with the establishment of animalian organisms,  
14 robust predator/prey animalian behaviors and physiologies, and bioturbation of the sediment-  
15 water interface in shallow marine environments (Erwin and Valentine 2013).

16  
17 Phylogenetic comparisons help fill evolutionary gaps where fossils are inconclusive or missing  
18 (Donoghue and Benton 2007). The simplest and closest common ancestor of all metazoa are  
19 choanoflagellates (King *et al.* 2008; Laflamme 2014). Choanoflagellates spend most of their life  
20 cycle as unicellular organisms, but are capable of forming multicellular colonies by failing to  
21 separate individuals at the last step of reproduction. As has long been pointed out by Haeckel  
22 (1874), and expanded upon by Cavalier-Smith (2017), individual choanoflagellates also resemble  
23 the individual choanocyte cells of sponges, the simplest metazoan organisms in modern oceans.  
24 Anatomical comparisons and phylogenetic data demonstrate that metazoans evolved from a  
25 sponge-like organism which lacked tightly controlled body symmetry or highly differentiated  
26 tissues (Müller 2003).

27  
28 Many different ideas offer insight into potential drivers for metazoan evolution, and many of these  
29 drivers are not mutually exclusive. The most widespread explanation is the 'Oxygen Control  
30 Hypothesis', namely that an increase in available dissolved oxygen drove metazoan evolution  
31 from protistan predecessors due to increased energetic availability provided by oxygen  
32 respiration (Knoll 1992). However, emerging geochemical evidence suggests that the oxygen  
33 requirements of early metazoans were met much earlier than generally thought (Mills and  
34 Canfield 2014; Zhang *et al.* 2016). As such, ample oxygen availability implies additional biological  
35 innovations, ecological pressures, or environmental constraints necessary for metazoan  
36 origination (Sebé-Pedrós *et al.* 2017). Some have put forward that salinity (Knauth 2005) or  
37 temperature (Schwartzman 2002) thresholds would also have enabled metazoans to emerge, or  
38 that initial steps in complexification evolved as a defense against ingestion by predators (Knoll  
39 and Lahr 2016; Porter 2011). Apart from external drivers, it is also possible that the evolution of  
40 metazoan complexity was contingent upon a lengthy process of morphogenetic complexification  
41 that followed its own process of genetic, epigenetic and enzymatic trial and error (Newman and  
42 Müller 2000).

1  
2 Recently-developed theories incorporate a role for holobionts in the evolution of an urmetazoan-  
3 a hypothetical last common ancestor of all animals (Müller 2001). This idea stems from noting  
4 that choanoflagellates form colonial clusters in response to chemical signals produced by certain  
5 species of Bacteroidetes (Alegado *et al.* 2012; Cavalier-Smith 2017; McFall-Ngai *et al.* 2013;  
6 McFall-Ngai 2015). This theory postulates that clades of bacteria evolved these chemical signals  
7 to reduce the predatory effectiveness of individual choanoflagellates so as to stave off their own  
8 consumption. In this view, urmetazoans evolved in response to complex ecological interactions  
9 with bacteria, presumably because the induction of colony formation presented selective  
10 advantages for choanoflagellates that were not achievable with mostly unicellular counterparts.

11  
12 Each theory indicates a possible trajectory from protists toward urmetazoans. None provide a  
13 rigorous accounting for selective pressures or adaptive pathways across intermediate  
14 physiological states. Developmental theories are essentially agnostic with respect to whether  
15 dramatic innovations in complex multicellularity relate to climatic upheavals (such as  
16 Neoproterozoic Snowball Earth events) or how selection might promote emergence of  
17 multicellular organisms of extraordinary complexity rather than mere rearrangement of different  
18 ecological relationships among existing microbial organisms.

19  
20 We address these deficiencies by outlining a new model of urmetazoan emergence. Evolutionary  
21 pressure caused by environmental change drove a subset of facultatively colonial protists to  
22 become obligate multicellular organisms with microbial symbionts prior to the evolution of a  
23 primordial gut, forming a holobiont organism. This model compares well to physiological and  
24 anatomical intermediate stages of eukaryotic organisms recently outlined by Cavalier-Smith  
25 (2017), but includes a detailed accounting of selection factors that distinguished protistan  
26 colonial aggregates from urmetazoan holobiont organisms, and provides a possible explanation  
27 for why metazoan complexification plateaued with the segregation of digestive and reproductive  
28 organs.

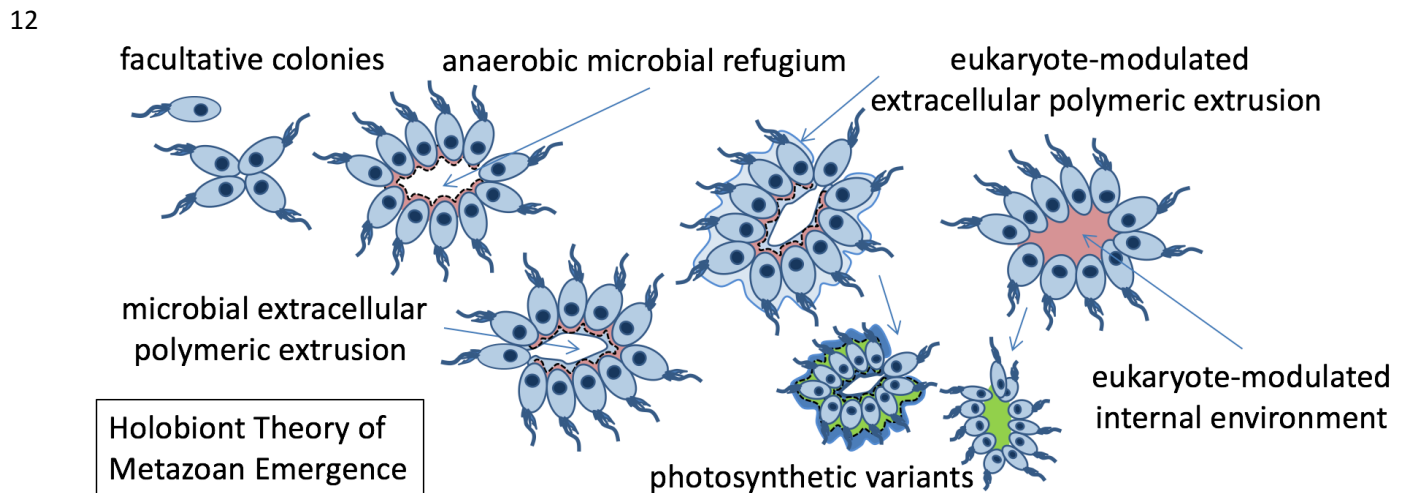
29  
30

## 31 **URMETAZOAN ORIGINS AS HOLOBIONTS IN OLIGOTROPHIC SEAS**

32  
33 Microbial symbionts perform critical roles in modern animalian ontogeny and metabolism that  
34 may have been even more important to metazoan origins. Indeed, modern animals are  
35 “metaorganisms” – macroscopic hosts in a synergistic interdependence with bacteria, archaea,  
36 fungi, and numerous other microbial and eukaryotic species (Bosch 2013). Microbial symbionts  
37 are a universal attribute of all animalia, and may be considered as a basal trait of the ancestral  
38 organism. Symbionts, owing to their sheer numbers and diversity, represent the most dynamic  
39 genetic components of all metazoa and endow them with great capacity for metabolic potential,  
40 ecological versatility and responsiveness to environmental change (Cavalier-Smith 2017; McFall-  
41 Ngai *et al.* 2013).

42

1 Adaptive versatility would seem to be all the more important during the Neoproterozoic, a period  
 2 of extreme environmental change. We hypothesize that microbial refugia, stabilized by  
 3 extracellular polymers generated by anaerobic microbes, could exist within the inner lining of  
 4 colonies of protists (Figure 1). This would have occurred during a part of a life cycle wherein adult  
 5 choanoflagellate-like organisms were in direct physical contact with a microbial film at the  
 6 sediment-water interface (Adamska 2015). These relationships were for the most part ephemeral,  
 7 opportunistic and mutual, with limited vertical inheritance of microbial species amongst the  
 8 protistan colonies. We further hypothesize that the unique oligotrophic stresses exerted by  
 9 Neoproterozoic climate fluctuations could have opened a mode of selection that favored  
 10 complex multicellular urmetazoans over equivalent ecological relationships between distinct  
 11 eukaryotic and prokaryotic populations.



13  
 14  
 15

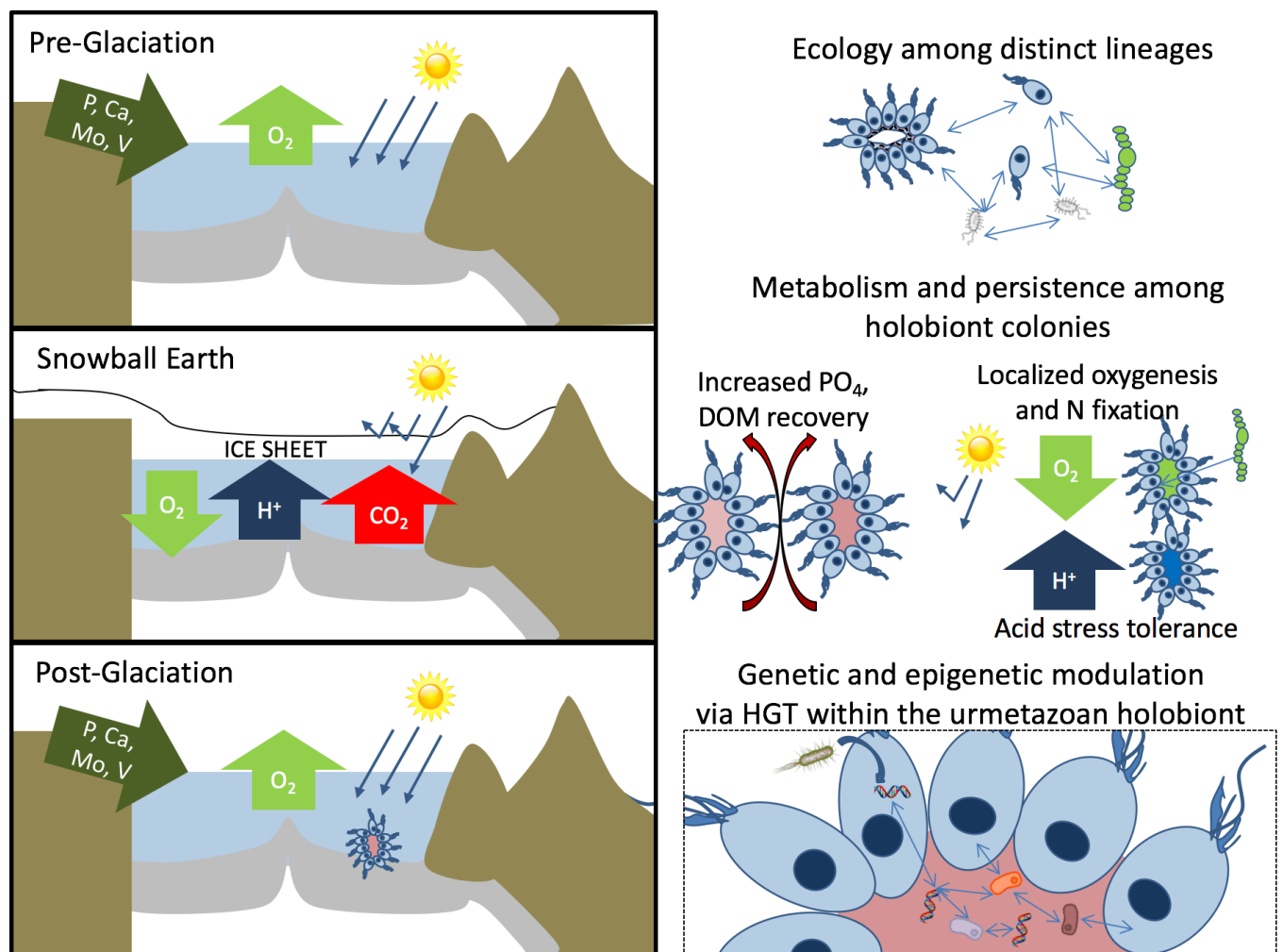
**Figure 1. Conceptual depiction of the holobiont theory of metazoan origins.**

16

17 The breakup of the Rhodinia supercontinent (Merdith *et al.* 2017) would have increased shallow  
 18 marine oxygenated shelf volume while the deep-sea was in early stages of oxygenation (Anbar  
 19 and Knoll 2002; Canfield 1998). Increased CO<sub>2</sub> draw-down from continental breakup (Hoffman  
 20 *et al.* 1998) coupled with increased primary production as a result of oxygenation and  
 21 phosphorus availability (Horton 2015) may have contributed to the onset of global glaciation.  
 22 This “Snowball Earth” event dramatically altered the fitness landscape of eukaryotic organisms  
 23 inhabiting the shallow marine environment (Figure 2). A globe-encircling ice sheet isolated this  
 24 volume of water, simultaneously reducing solar insolation, gaseous exchange with the  
 25 atmosphere, and the flux of mineral-derived nutrients and metal cofactors from continental  
 26 weathering in the shallow marine zones that supported the majority of Earth’s eukaryotic  
 27 organisms. Despite an overall trend throughout the Neoproterozoic of stable or slightly  
 28 increasing atmospheric oxygen, the shallow marine environment would have experienced  
 29 primary productivity and oxygen level declines and suffered an encroachment of euxinic  
 30 conditions deleterious to most eukaryotic organisms.

31

1 This array of challenging environmental conditions would have placed significant environmental  
 2 stress on eukaryotic organisms. Stresses include a need to access greater amounts of oxygen,  
 3 to more efficiently use dissolved organic matter (DOM), and to scavenge for scarce nutrients and  
 4 cofactors under conditions where primary cyanobacterial and algal productivity may have  
 5 dropped. The modulation of a community of microbial symbionts is one possible adaptive  
 6 response that can simultaneously address all of these stressors, owing to the genetic and  
 7 metabolic versatility afforded by microbial symbionts. The result would have been ideal for  
 8 selection to have occurred, and fitness to have been conferred, at the level of the aggregate  
 9 holobiont, rather than for the hosts or symbionts alone (Moran and Sloan 2015). In the following  
 10 sections, we illustrate the multiple lines of evidence that link an initial facultative mutualism to  
 11 the evolution of an obligate urmetazoan holobiont.  
 12



13  
 14 **Figure 2. Depiction of environmental stresses that increased selective pressures on**  
 15 **obligate holobionts composed of multicellular eukaryotes. Phase 1: Late Meso- and early**  
 16 **Neoproterozoic environmental conditions exerted no particular stresses that favored**  
 17 **selection at the level of a holobiont organism. Phase 2: Rapid onset of global glaciation**  
 18 **events impacted shallow marine geochemical cycles in numerous ways that promoted**

1 **selection for more efficient scavenging of limited nutrients. Phase 3: Increased cell lysis**  
2 **associated with heterotrophy promoted increased HGT between symbionts and hosts.**  
3

#### 4 **PRIMARY EFFECTS: INCREASED METABOLIC EFFICIENCY AND TURNOVER**

5 Modern microbial symbionts are known to facilitate increased metazoan fitness under conditions  
6 analogous to those created by Snowball Earth events. Microbe-bearing sponges are notably  
7 adept at thriving under oligotrophic conditions in unique ways that blur the lines between fitness  
8 advantages conferred by anatomy and by those conferred by the symbionts.

9  
10 The most obvious advantage comes from improved capture of dissolved organic matter. An  
11 urmetazoan would be able to process larger volumes of water, and microbial symbionts would  
12 enable metabolism far beyond the feeding capacity of host cells alone. Microbial associates can  
13 comprise as much as 40% of modern sponge tissue volume, with densities in excess of  $10^9$   
14 microbial cells per ml of sponge tissue (Taylor *et al.* 2007a). This is several orders of magnitude  
15 higher than typical for seawater and approximately equivalent to cell densities found in microbial  
16 mats or hydrothermal vents (McKinnon *et al.* 2009). Sponges remove the same amount of DOM  
17 from the water column in 30 minutes as free-living bacteria take up in 30 days, retaining organic  
18 matter within the reef community and thereby preventing energy and nutrient losses to the open  
19 ocean (De Goeij *et al.* 2013). An urmetazoan 'presponge' could have reasonably evolved an  
20 extracellular matrix dedicated to microbial symbiont exchange (even one only fractionally  
21 efficient as a modern sponge), conferring potentially significant fitness advantages to the  
22 presponge host, particularly when subjected to oligotrophic conditions.

23  
24 Co-evolution of microbes and urmetazoa would also have conferred advantages regarding  
25 nutrient limitation. Cyanobacterial associations would have been particularly advantageous  
26 under light-limited conditions, serving as localized sources of oxygen, organic matter and  
27 biologically-available nitrogen (Sánchez-Baracaldo *et al.* 2014). Increased retention of biomass  
28 would have further enabled increased recovery of critical enzymatic cofactors that would  
29 otherwise have come from continental weathering such as Mo, Fe and V (Och and Shields-Zhou  
30 2012). An associated microbiome would also have enabled effective adaptation to oceanic  
31 acidification arising from limited gas exchange with the atmosphere and increased respiration  
32 beneath the ice sheet (Kasemann *et al.* 2010; Ribes *et al.* 2016).

33  
34 All of these specific fitness advantages would have benefited both hosts and symbionts. The  
35 emergence of a coordinated water-pumping arrangement for increased DOM recovery, however  
36 inefficient compared to modern sponges, would have direct ecological and morphological  
37 impacts on other organisms far beyond the specific colony itself. A combination of heterotrophy  
38 and eukaryovory, perhaps for the first time in Earth's history, was conducted at the organizational  
39 level of a holobiont rather than at the level of individual protists in an ecosystem. This innovation  
40 could facilitate an unprecedented tempo of trophic and genetic interactions that were entirely  
41 localized to the holobiont and mostly inaccessible to the wider ecological community.



## 1 **SECONDARY EFFECTS: INCREASED RATES OF HORIZONTAL GENE TRANSFER,** 2 **ADAPTATION AND EVOLUTION WITHIN THE HOLOBIONT**

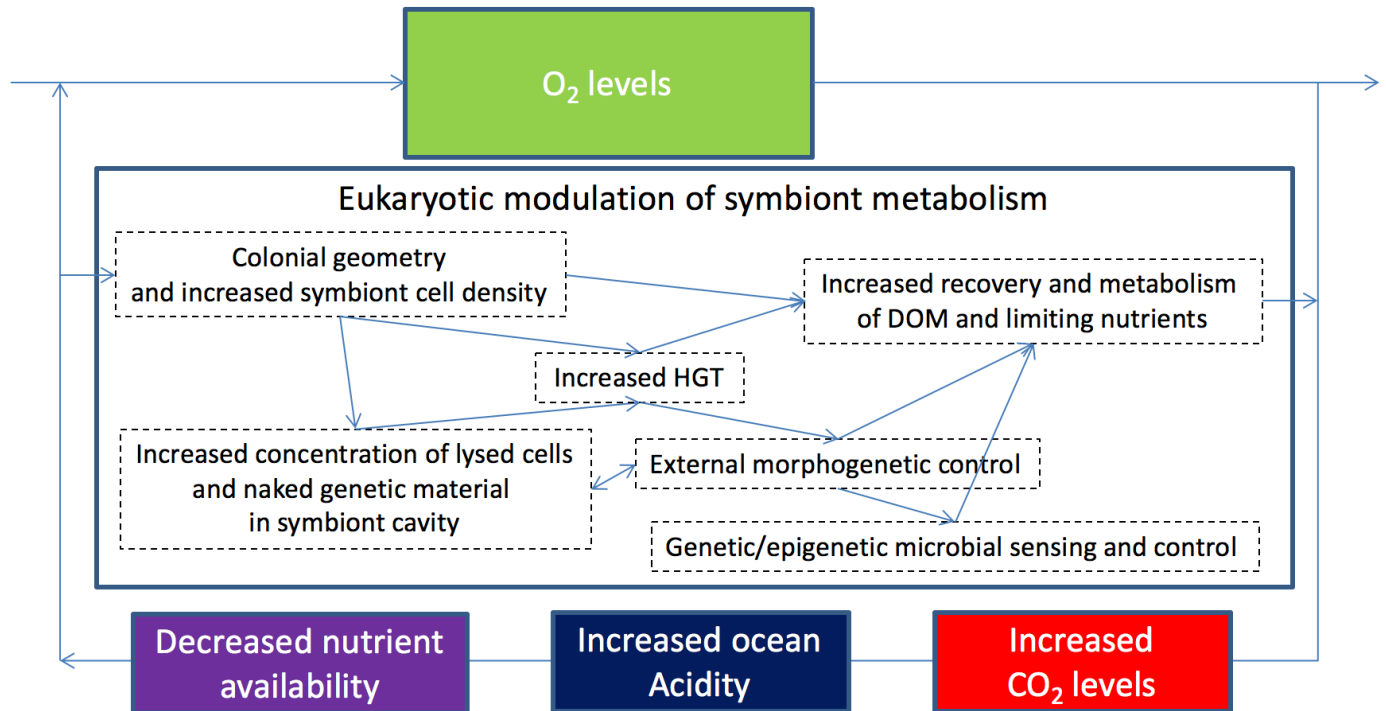
3 Eukaryotic hosts provide a unique niche for retaining and propagating the fitness consequences  
4 of horizontal gene transfer events. Large bacterial population sizes and high bacterial genetic  
5 diversity enable symbiotic relationships to evolve rapidly (Fisher 1930; Pankey *et al.* 2017)- both  
6 attributes would be expected for a densified symbiotic microbiome. However, a small eukaryotic  
7 population would propagate genetic mutations more quickly, since a reduced effective  
8 population increases vulnerability to stochastic genetic changes in the eukaryotic genome (Duret  
9 2008). In other words, a symbiotic system characterized by prolonged contact between a small  
10 (perhaps highly specific) population of eukaryotes and a larger, diverse population of prokaryotes  
11 would be predicted to lead to elevated rates of genomic change for all organisms.

12 There are multiple lines of evidence supporting this hypothetical scenario. Sponges support  
13 microbial densities comparable to microbial biofilms (McKinnon *et al.* 2009). However, unlike  
14 microbial biofilms, sponge-hosted microbes are collected as distinct groups that are isolated  
15 from the larger environment and are in constant contact with the host. Several genome-wide  
16 studies have shown that horizontal gene transfer (HGT) occurs with high frequency between  
17 microbial species if they are closely related or if they coexist in the same habitat or community,  
18 conditions which also provide more opportunities for DNA transfer from bacteria to eukaryotes  
19 (Lacroix and Citovsky 2016). As an urmetazoan evolved to process greater amounts of biomass  
20 in seawater, this increased the amount of free-floating or 'naked' genetic material and lysed cells  
21 that would have come into contact with neighboring bacterial and eukaryotic cells lining the  
22 symbiont cavity, also increasing rates of HGT via gene transformation. HGT permits fast  
23 acquisition of a new function important for species adaptation and survival (Koonin 2011). The  
24 expanded genetic repertoire, enclosed proximity, high cell density and persistent symbioses  
25 between microbes and eukaryotic host opens more frequent and consequential opportunities  
26 for ecological interaction and HGT.

27  
28 Analyses of genomic data reveal that cases of HGT from bacteria to eukaryotes are relatively  
29 common, and horizontal gene acquisitions are known drivers of adaptive evolution in eukaryotes  
30 (Schönknecht *et al.* 2014). This suggests that HGT represents a potentially pervasive adaptive  
31 response when certain conditions are met (Pankey *et al.* 2017; Sun *et al.* 2010). Most notably,  
32 the gene ratchet mechanism ("you are what you eat") is based on the observation that many  
33 protozoan eukaryotes capture other microbes as food sources (Doolittle 1998). Gene acquisition  
34 ensues when lysed DNA from food sources becomes incorporated into the host cell through  
35 recombination. Lysed cells produced by heterotrophy in an urmetazoan would release abundant  
36 bacterial DNA in close physical proximity to the urmetazoan host genome (Thomas and Nielsen  
37 2005).

38  
39 The urmetazoan thus represented the emergence of a novel, genetically malleable niche that had  
40 no prior ecological parallel for either microbial or eukaryotic species. Though external  
41 environmental factors such as O<sub>2</sub> concentration, nutrient availability and acidity changed the  
42 fitness landscape for all shallow marine organisms, a colonial eukaryote could gain acute (i.e.,

1 increased DOM recovery and metabolic efficiency) and prolonged (i.e., increased rates of HGT  
 2 and secondary morphogenetic sensing and control) fitness advantages by harboring a densified  
 3 microbiome that would not have been available to any other members of the existing ecology  
 4 (Figure 3).  
 5



6  
 7 **Figure 3. Outline of feedback elements that may have facilitated holobiont urmetazoan**  
 8 **emergence. Fitness advantages conferred on a eukaryotic organism in symbiosis with a**  
 9 **densified microbiome are nested within a broader array of external environmental drivers**  
 10 **associated with Neoproterozoic oligotrophic shallow marine settings.**  
 11

12 Elevated levels of genetic exchange would have been pervasive throughout the process of  
 13 holobiont evolution until a 'new normal' in genetic stability and metabolic efficiency was reached.  
 14 The evolution of specialized, segregated gut and reproductive tissues would have limited the  
 15 extent to which host/symbiont HGT could directly affect the rates of evolution of the holobiont  
 16 organisms, relegating eukaryote evolution to a process that occurred mostly through sexual  
 17 genetic recombination, mutation and vertical inheritance. This would possibly account for how the  
 18 pattern of Phanerozoic animal evolution that followed consisted of incremental variations on a  
 19 theme of bilaterianism.

## 20 PHYLOGENOMIC INSIGHTS FROM CHOANOFLLAGELLATES

21 Choanoflagellates have served as a model organism for urmetazoan multicellularity. Their  
 22 genetics reflect elements of the basal metazoan unicellular organism (Adamska 2015; Cavalier-  
 23 Smith 2017). Phylogenomic analyses have identified more than 100 genes of possible algal origin  
 24 in *Monosiga*, a unicellular species of choanoflagellates that is perhaps the closest protozoan

1 relative of animals (Bosch 2013). Genes of possible algal origin in *Monosiga* represent a relatively  
2 high percentage (1.12%) of the organism's total genome, approaching values typically exhibited  
3 by prokaryotes (Schönknecht *et al.* 2014). Several genes involved in carbohydrate metabolism  
4 appear to have been transferred independently to *Monosiga*. Considering that only about 14%  
5 (588/4,300) of genes with annotated functions in the *Monosiga* genome are related to  
6 carbohydrate or amino acid metabolism, the enrichment of such genes in the identified algal  
7 genes is significant (Sun *et al.* 2010). At a minimum, this demonstrates that the feeding habit of  
8 choanoflagellates may be strongly implicated with increased occurrence of HGT (Doolittle 1998;  
9 Keeling and Palmer 2008). If a unicellular choanoflagellate ancestor were driven by oligotrophic  
10 conditions to undertake a colonial feeding habit with microbial symbionts, the rate of HGT (and  
11 thus adaptation at genetic or epigenetic levels) would be predicted to increase commensurately.  
12

13 The choanoflagellate *S. rosetta* responds to sulfonolipids produced by Bacteroidetes by forming  
14 colonial rosettes, triggering eukaryotic morphogenesis (Alegado *et al.* 2012). This has been  
15 interpreted as a response evolved by bacteria to reduce the probability of consumption by  
16 individual choanoflagellates. Alternatively, we hypothesize that it is also possible that  
17 choanoflagellates preserve traces of a triggered effect that lead to a holobiont organism.  
18 Bacteroidetes are regarded as specialists for the degradation of high molecular weight organic  
19 matter, i.e., proteins and carbohydrates (Taylor *et al.* 2007b; Thomas *et al.* 2011), which would  
20 be logical if the relationship evolved in response to DOM limitations in oligotrophic shallow marine  
21 environments.  
22

23 Choanoflagellates may serve as a direct vehicle for exploring urmetazoan origins. For example,  
24 some of the genes responsible for the integrin-mediated adhesion complex (IMAC) are missing  
25 from existing choanoflagellates (Brown *et al.* 2013; King *et al.* 2008). The IMAC helps facilitate  
26 cell signaling, motility and adhesion to the extracellular matrix. Engineering modern  
27 choanoflagellates with the modern or phylogenetically inferred ancestral (Kacar *et al.* 2017) IMAC  
28 systems and other associated genes responsible for sensing and modulating an external volume  
29 of a colony may regenerate traces of the phenotype that gave rise to urmetazoan intermediates.

### 30 **ASTROBIOLOGICAL IMPLICATIONS: THE UBIQUITY OF ANIMAL-LIKE LIFE?**

31 The relationship between metazoa and the primary energy reservoir that enables metazoan  
32 metabolism (e.g., the oxygen content of the atmosphere on Earth) is of profound importance.  
33 Our proposed holobiont hypothesis of metazoan emergence provides a new framework to  
34 discuss the emergence of animal-like life on other planets. Oxygen seems to be needed for  
35 complexification (Catling *et al.* 2005), but may not by itself be a predictive factor for the  
36 emergence of metazoan-grade organisms. Selection requirements may include a period of  
37 nutrient limitation long enough to prompt innovation for colonial eukaryotes to form a densified  
38 holobiont organism. Accordingly, we posit that metazoan emergence is correlated to the  
39 emergence of a mutually beneficial, eukaryote-curated microbiome that temporarily accelerates  
40 gene transfer events. The need for environmental stress to exert selection at the level of a  
41 holobiont suggests that planetary environments conducive to the origin of life may not  
42 necessarily lead to complex life. Animal-like life in the universe may be limited to planets that

1 experience dramatically changing environmental conditions over time periods long enough to  
2 enable holobiont selection.

3  
4

## 5 ACKNOWLEDGMENTS

6

7 This work is the product of the S.T.E.A.K. Working Group discussion held during the 2017 NASA  
8 Astrobiology Science Conference in Mesa, Arizona. This work was supported by a grant from  
9 the Simons Foundation (494291, Z.A.), by John Templeton Foundation (58562, B.K.) and NASA  
10 Astrobiology Institute (NNA17BB05A, B.K.). M.W.A. was supported by CTSA Grant Number KL2  
11 TR002379 from the National Center for Advancing Translational Science (NCATS). Its contents  
12 are solely the responsibility of the authors and do not necessarily represent the official views of  
13 the NIH. K.H.W. acknowledges the support of a grant from the National Aeronautics and Space  
14 Administration for work performed at the Jet Propulsion Laboratory, California Institute of  
15 Technology. The opinions expressed in this publication are those of the authors and do not  
16 necessarily reflect the views of any organization. The funders had no role in study design, data  
17 collection and interpretation, or the decision to submit the work for publication.

18  
19

## 20 REFERENCES

21

- 22 Adamska M. (2015) Developmental signalling and emergence of animal multicellularity. In:  
23 *Evolutionary Transitions to Multicellular Life*, Springer, pp 425-450.
- 24 Alegado R. A., Brown L. W., Cao S., Dermenjian R. K., Zuzow R., Fairclough S. R., Clardy J., and  
25 King N. (2012) A bacterial sulfonolipid triggers multicellular development in the closest  
26 living relatives of animals. *eLife*, 1: e00013.
- 27 Anbar A. D., and Knoll A. (2002) Proterozoic ocean chemistry and evolution: a bioinorganic  
28 bridge? *science*, 297: 1137-1142.
- 29 Bosch T. C. G. (2013) Cnidarian-Microbe Interactions and the Origin of Innate Immunity in  
30 Metazoans. *Annual Review of Microbiology*, 67: 499-518.
- 31 Brown M. W., Sharpe S. C., Silberman J. D., Heiss A. A., Lang B. F., Simpson A. G. B., and Roger  
32 A. J. (2013) Phylogenomics demonstrates that breviate flagellates are related to  
33 opisthokonts and apusomonads. *Proceedings of the Royal Society B: Biological  
34 Sciences*, 280.
- 35 Budd G. E., and Jensen S. (2017) The origin of the animals and a 'Savannah' hypothesis for early  
36 bilaterian evolution. *Biological Reviews*, 92: 446-473.
- 37 Canfield D. E. (1998) A new model for Proterozoic ocean chemistry. *Nature*, 396: 450.
- 38 Catling D. C., Glein C. R., Zahnle K. J., and McKay C. P. (2005) Why O<sub>2</sub> Is Required by Complex  
39 Life on Habitable Planets and the Concept of Planetary" Oxygenation Time".  
40 *Astrobiology*, 5: 415-438.
- 41 Cavalier-Smith T. (2017) Origin of animal multicellularity: precursors, causes, consequences—  
42 the choanoflagellate/sponge transition, neurogenesis and the Cambrian explosion.  
43 *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372.

- 1 Chen X., Ling H.-F., Vance D., Shields-Zhou G. A., Zhu M., Poulton S. W., Och L. M., Jiang S.-  
2 Y., Li D., and Cremonese L. (2015) Rise to modern levels of ocean oxygenation coincided  
3 with the Cambrian radiation of animals. *Nature communications*, 6: 7142.
- 4 Cuthill J. F. H., and Morris S. C. (2017) Nutrient-dependent growth underpinned the Ediacaran  
5 transition to large body size. *Nature ecology & evolution*, 1: 1201.
- 6 De Goeij J. M., Van Oevelen D., Vermeij M. J., Osinga R., Middelburg J. J., de Goeij A. F., and  
7 Admiraal W. (2013) Surviving in a marine desert: the sponge loop retains resources within  
8 coral reefs. *Science*, 342: 108-110.
- 9 Donoghue P. C., and Benton M. J. (2007) Rocks and clocks: calibrating the Tree of Life using  
10 fossils and molecules. *Trends in Ecology & Evolution*, 22: 424-431.
- 11 Doolittle W. F. (1998) You are what you eat: a gene transfer ratchet could account for bacterial  
12 genes in eukaryotic nuclear genomes. *Trends in Genetics*, 14: 307-311.
- 13 dos Reis M., Thawornwattana Y., Angelis K., Telford M. J., Donoghue P. C., and Yang Z. (2015)  
14 Uncertainty in the timing of origin of animals and the limits of precision in molecular  
15 timescales. *Current Biology*, 25: 2939-2950.
- 16 Duret L. (2008) Neutral theory: the null hypothesis of molecular evolution. *Nature Education*, 1:  
17 803-806.
- 18 Erwin D. H., and Valentine J. W. (2013) *The Cambrian Explosion: The Construction of Animal*  
19 *Biodiversity*. W. H. Freeman & Company.
- 20 Fisher R. A. (1930) *The genetical theory of natural selection: a complete variorum edition*. Oxford  
21 University Press.
- 22 Haeckel E. (1874) *Memoirs: The Gastraea-Theory, the Phylogenetic Classification of the Animal*  
23 *Kingdom and the Homology of the Germ-Lamellæ*. *Quarterly Journal of Microscopical*  
24 *Science*, s2-14: 142-165.
- 25 Hays L. E. (2015) *NASA Astrobiology Strategy 2015*. National Aeronautics and Space  
26 Administration.
- 27 Hoffman P. F., Kaufman A. J., Halverson G. P., and Schrag D. P. (1998) A Neoproterozoic  
28 Snowball Earth. *Science*, 281: 1342-1346.
- 29 Horton F. (2015) Did phosphorus derived from the weathering of large igneous provinces fertilize  
30 the Neoproterozoic ocean? *Geochemistry, Geophysics, Geosystems*, 16: 1723-1738.
- 31 Hoyal Cuthill J. F., and Conway Morris S. (2014) Fractal branching organizations of Ediacaran  
32 rangeomorph fronds reveal a lost Proterozoic body plan. *Proceedings of the National*  
33 *Academy of Sciences*, 111: 13122-13126.
- 34 Kacar B., Guy L., Smith E., and Baross J. (2017) Resurrecting ancestral genes in bacteria to  
35 interpret ancient biosignatures. *Phil. Trans. R. Soc. A*, 375: 20160352.
- 36 Kasemann S. A., Prave A. R., Fallick A. E., Hawkesworth C. J., and Hoffmann K.-H. (2010)  
37 Neoproterozoic ice ages, boron isotopes, and ocean acidification: Implications for a  
38 snowball Earth. *Geology*, 38: 775-778.
- 39 Keeling P. J., and Palmer J. D. (2008) Horizontal gene transfer in eukaryotic evolution. *Nature*  
40 *Reviews Genetics*, 9: 605-618.

- 1 King N., Westbrook M. J., Young S. L., Kuo A., Abedin M., Chapman J., Fairclough S., Hellsten  
2 U., Isogai Y., Letunic I. and others. (2008) The genome of the choanoflagellate *Monosiga*  
3 *brevicollis* and the origin of metazoans. *Nature*, 451: 783.
- 4 Knauth L. P. (2005) Temperature and salinity history of the Precambrian ocean: implications for  
5 the course of microbial evolution. *Palaeogeography, Palaeoclimatology, Palaeoecology*,  
6 219: 53-69.
- 7 Knoll A. H. (1992) Biological and biogeochemical preludes to the Ediacaran radiation. In: *Origin*  
8 *and early evolution of the metazoa*, Springer, pp 53-84.
- 9 Knoll A. H., and Lahr D. J. (2016) Fossils, feeding, and the evolution of complex multicellularity.  
10 *Multicellularity, Origins and Evolution, The Vienna Series in Theoretical Biology: Boston,*  
11 *Massachusetts Institute of Technology:* 1-16.
- 12 Koonin E. V. (2011) The logic of chance: the nature and origin of biological evolution. FT press.
- 13 Lacroix B., and Citovsky V. (2016) Transfer of DNA from Bacteria to Eukaryotes. *MBio*, 7: e00863-  
14 16.
- 15 Laflamme M. (2014) Modeling morphological diversity in the oldest large multicellular organisms.  
16 *Proceedings of the National Academy of Sciences*, 111: 12962-12963.
- 17 Maloof A. C., Rose C. V., Beach R., Samuels B. M., Calmet C. C., Erwin D. H., Poirier G. R., Yao  
18 N., and Simons F. J. (2010) Possible animal-body fossils in pre-Marinoan limestones from  
19 South Australia. *Nature Geosci*, 3: 653-659.
- 20 McFall-Ngai M., Hadfield M. G., Bosch T. C. G., Carey H. V., Domazet-Lošo T., Douglas A. E.,  
21 Dubilier N., Eberl G., Fukami T., Gilbert S. F. and others. (2013) Animals in a bacterial  
22 world, a new imperative for the life sciences. *Proceedings of the National Academy of*  
23 *Sciences*, 110: 3229-3236.
- 24 McFall-Ngai M. J. (2015) Giving microbes their due – animal life in a microbially dominant world.  
25 *The Journal of Experimental Biology*, 218: 1968-1973.
- 26 McKinnon W., Pappalardo R., and Khurana K. (2009) Europa: Perspectives on an ocean world.  
27 *Europa, Edited by Robert T. Pappalardo, William B. McKinnon, Krishan K. Khurana; with*  
28 *the assistance of René Dotson with 85 collaborating authors. University of Arizona Press,*  
29 *Tucson, 2009. The University of Arizona space science series ISBN: 9780816528448, p.*  
30 *697: 697.*
- 31 Merdith A. S., Collins A. S., Williams S. E., Pisarevsky S., Foden J. D., Archibald D. B., Blades  
32 M. L., Alessio B. L., Armistead S., and Plavsa D. (2017) A full-plate global reconstruction  
33 of the Neoproterozoic. *Gondwana Research*, 50: 84-134.
- 34 Michod R. E. (2007) Evolution of individuality during the transition from unicellular to multicellular  
35 life. *Proceedings of the National Academy of Sciences*, 104: 8613-8618.
- 36 Mills D. B., and Canfield D. E. (2014) Oxygen and animal evolution: Did a rise of atmospheric  
37 oxygen “trigger” the origin of animals? *BioEssays*, 36: 1145-1155.
- 38 Moran N. A., and Sloan D. B. (2015) The hologenome concept: helpful or hollow? *PLoS biology*,  
39 13: e1002311.
- 40 Müller W. E. (2001) How was metazoan threshold crossed? The hypothetical Urmetazoa.  
41 *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*,  
42 129: 433-460.

- 1 Müller W. E. (2003) The origin of metazoan complexity: Porifera as integrated animals. *Integrative*  
2 *and Comparative Biology*, 43: 3-10.
- 3 Newman S. A., and Müller G. B. (2000) Epigenetic mechanisms of character origination. *Journal*  
4 *of Experimental Zoology Part A: Ecological Genetics and Physiology*, 288: 304-317.
- 5 Och L. M., and Shields-Zhou G. A. (2012) The Neoproterozoic oxygenation event: Environmental  
6 perturbations and biogeochemical cycling. *Earth-Science Reviews*, 110: 26-57.
- 7 Pankey M. S., Foxall R. L., Ster I. M., Perry L. A., Schuster B. M., Donner R. A., Coyle M., Cooper  
8 V. S., and Whistler C. A. (2017) Host-selected mutations converging on a global regulator  
9 drive an adaptive leap towards symbiosis in bacteria. *eLife*, 6.
- 10 Peterson K. J., and Butterfield N. J. (2005) Origin of the Eumetazoa: testing ecological predictions  
11 of molecular clocks against the Proterozoic fossil record. *Proceedings of the National*  
12 *Academy of Sciences*, 102: 9547-9552.
- 13 Peterson K. J., McPeck M. A., and Evans D. A. (2005) Tempo and mode of early animal evolution:  
14 inferences from rocks, Hox, and molecular clocks. *Paleobiology*, 31: 36-55.
- 15 Porter S. (2011) The rise of predators. *Geology*, 39: 607-608.
- 16 Ribes M., Calvo E., Movilla J., Logares R., Coma R., and Pelejero C. (2016) Restructuring of the  
17 sponge microbiome favors tolerance to ocean acidification. *Environmental microbiology*  
18 *reports*, 8: 536-544.
- 19 Sánchez-Baracaldo P., Ridgwell A., and Raven J. A. (2014) A neoproterozoic transition in the  
20 marine nitrogen cycle. *Current Biology*, 24: 652-657.
- 21 Schönknecht G., Weber A. P., and Lercher M. J. (2014) Horizontal gene acquisitions by  
22 eukaryotes as drivers of adaptive evolution. *Bioessays*, 36: 9-20.
- 23 Schwartzman D. (2002) Life, temperature, and the Earth: the self-organizing biosphere. Columbia  
24 University Press.
- 25 Sebé-Pedrós A., Degnan B. M., and Ruiz-Trillo I. (2017) The origin of Metazoa: a unicellular  
26 perspective. *Nature Reviews Genetics*, 18: 498.
- 27 Sperling E. A., Knoll A. H., and Girguis P. R. (2015) The ecological physiology of Earth's second  
28 oxygen revolution. *Annual Review of Ecology, Evolution, and Systematics*, 46: 215-235.
- 29 Sun G., Yang Z., Ishwar A., and Huang J. (2010) Algal Genes in the Closest Relatives of Animals.  
30 *Molecular Biology and Evolution*, 27: 2879-2889.
- 31 Taylor M. W., Hill R. T., Piel J., Thacker R. W., and Hentschel U. (2007a) Soaking it up: the  
32 complex lives of marine sponges and their microbial associates. *The ISME Journal*, 1:  
33 187-190.
- 34 Taylor M. W., Radax R., Steger D., and Wagner M. (2007b) Sponge-associated microorganisms:  
35 evolution, ecology, and biotechnological potential. *Microbiology and molecular biology*  
36 *reviews*, 71: 295-347.
- 37 Thomas C. M., and Nielsen K. M. (2005) Mechanisms of, and Barriers to, Horizontal Gene  
38 Transfer between Bacteria. *Nature Reviews Microbiology*, 3: 711.
- 39 Thomas F., Hehemann J.-H., Rebuffet E., Czjzek M., and Michel G. (2011) Environmental and  
40 Gut Bacteroidetes: The Food Connection. *Frontiers in Microbiology*, 2: 93.

- 1 Zhang S., Wang X., Wang H., Bjerrum C. J., Hammarlund E. U., Costa M. M., Connelly J. N.,
- 2 Zhang B., Su J., and Canfield D. E. (2016) Sufficient oxygen for animal respiration 1,400
- 3 million years ago. *Proceedings of the National Academy of Sciences*, 113: 1731-1736.

4