2013-04

Multicellularity arose several times in the evolution of eukaryotes. (Response to DOI 10.1002/bies.201100187)

BioEssays, West Sussex, v.35, n.4, p.339-347, 2013
http://www.producao.usp.br/handle/BDPI/45022

Multicellularity arose several times in the evolution of eukaryotes (Response to DOI 10.1002/bies.201100187)

Laura Wegener Parfrey[1)] *and Daniel J. G. Lahr*[2)]*

The cellular slime mold *Dictyostelium* has cell-cell connections similar in structure, function, and underlying molecular mechanisms to animal epithelial cells. These similarities form the basis for the proposal that multicellularity is ancestral to the clade containing animals, fungi, and Amoebozoa (including *Dictyostelium*): Amorphea (formerly ''unikonts''). This hypothesis is intriguing and if true could precipitate a paradigm shift. However, phylogenetic analyses of two key genes reveal patterns inconsistent with a single origin of multicellularity. A single origin in Amorphea would also require loss of multicellularity in each of the many unicellular lineages within this clade. Further, there are numerous other origins of multicellularity within eukaryotes, including three within Amorphea, that are not characterized by these structural and mechanistic similarities. Instead, convergent evolution resulting from similar selective pressures for forming multicellular structures with motile and differentiated cells is the most likely explanation for the observed similarities between animal and dictyostelid cell-cell connections.

Keywords:
Amoebozoa; catenin; *Dictyostelium*; metazoa; microbial eukaryote; Opisthokonta; vinculin

Introduction: *Dictyostelium* multicellularity has structural and molecular similarities to animal multicellularity

Dictyostelium discoideum is a model organism that has provided insight into the origins of multicellularity, sociality, development, and cell biology [1–4]. *Dictyostelium* is a member of the dictyostelid family of amoebae, which fall within the major eukaryotic clade Amoebozoa [5]. The dictyostelids are alternatively referred to as cellular slime molds or social amoebae. There are three described genera of dictyostelids, however molecular analyses demonstrate that these intermingle, and the actual diversity within this ancient clade is much greater [4, 6]. Dictyostelid amoebae spend much of their life cycle foraging in soils and leaf litter for bacteria as single celled amoebae. In response to stress or starvation hundreds of thousands of amoebae aggregate to form a motile multicellular slug that already contains differentiated populations of cells [1]. The slug migrates until it reaches a suitable place, such as the top layer of soil, and then a multicellular fruiting body develops in a process called culmination. Culmination produces a differentiated multicellular fruiting body (sorocarp) composed of a mass of thousands of resistant spores supported by a cellulosic stalk filled with stalk cells [1]. This process is organized by stalk cells that form the tip of the slug, and later, the tip of the fruiting body. These tip cells are also the location of the cell-cell connections akin to epithelia in animals [7].

Dictyostelium is used as model for animal multicellularity because many key features are shared, such as cell adhesion, communication and signaling, differentiation, and development. Despite deep evolutionary divergence [8], many genes crucial to these processes in animals are found in *Dictyostelium* [9]. The shared processes related to multicellularity have been shown to be similar in structure, function, and underlying molecular machinery [3, 4, 10, 11]. These parallels are surprising because *Dictyostelium* multicellularity occurs by aggregation of solitary amoebae during one stage of the life cycle, which is fundamentally different from repeated division of a zygote that leads to multicellularity in animals. Further, the

DOI 10.1002/bies.201200143

[1)] Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO, USA
[2)] Department of Zoology, University of São Paulo, São Paulo, Brazil

***Corresponding author:**
Daniel J. G. Lahr
E-mail: dlahr@ib.usp.br

Both authors contributed equally to this work.

Abbreviations:
AM, aggregative multicellularity; **DM,** multicellularity by division; **ML,** maximum likelihood.

Think again

last common ancestor of dictyostelids and animals lived more than one billion years ago and they are separated by numerous unicellular and multicellular lineages (Fig. 1). There are two alternative explanations for the similarity between animals and *Dictyostelium*: homology, i.e. the common ancestor of both organismal groups already possessed many of these characteristics; or convergence, meaning that the two groups arrived at similar methods for evolving multicellularity independently. Multicellularity in different clades is commonly attributed to convergent evolution across eukaryotes [10, 12, 13]. Convergence is also presumed between animals and *Dictyostelium* [9]. In both taxa the multicellular organism is composed of motile cells that must communicate and adhere to one another. In contrast, multicellular plants and algae contain rigid, non-motile cells [10, 13]. Based on the striking similarities between metazoan and dictyostelid cell adhesion, Dickinson and colleagues hypothesize that homology is a more likely explanation and that the last common ancestor of dictyostelids and animals was multicellular [7].

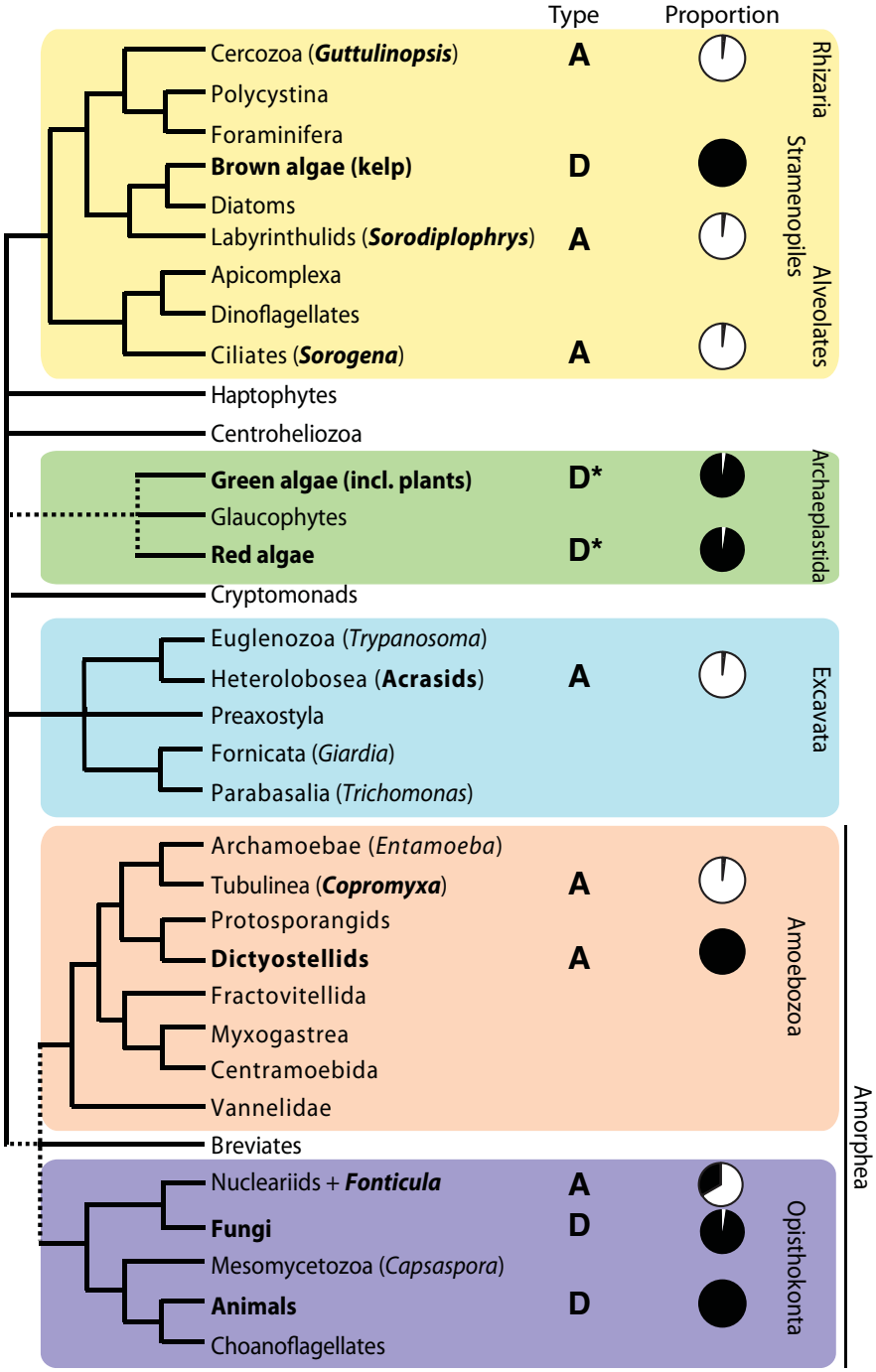

Figure 1. Tree of eukaryotes based on Parfrey et al. [39] and with Amoebozoa relationships based on Lahr et al. [5] and the placement of key multicellular lineages derived from [42, 45, 53, 58]. Bold lineages contain multicellular representatives. Type: A, Lineage contains at least one taxon with aggregative multicellularity. D, Contains at least one taxon that is multicellular by division. *Multicellularity has arisen multiple times independently in these clades. Proportion: Black represents the proportion of taxa within the clade that are multicellular.

Cell-cell junctions in *Dictyostelium* resemble animal epithelia

Central to the arguments of Dickinson et al. [7] are the similarities in the organization and cell-cell junctions between animal epithelium and the tip cells that organize the *D. discoideum* slug and then fruiting body. Epithelium is one of the major tissue types in animals and forms the lining of all free body surfaces. Epithelial cells are connected through cell junctions formed by protein complexes. Adherens junctions are one type of junction and are found throughout animals [14, 15]. In animals, adherens junctions mechanically link the actin cytoskeleton of adjacent epithelial cells to provide structural integrity [16]. Adherens junctions are formed by a protein complex that consists of transmembrane cadherin proteins that bind cadherins of other cells extracellularly. Inside the cell cadherins are connected to the actin cytoskeleton via a protein complex containing α- and β-catenin [16]. β-Catenin binds to an intracellular domain of cadherin, and α-catenin binds to β-catenin and actin, and both α-catenin and β-catenin are required for this process [16]. Catenins are essential in adherens junctions and key to animal multicellularity, hence the significance of the discovery of catenin homologs in *Dictyostelium*.

Tip cells of *D. discoideum* are interconnected by structures similar to metazoan

adherens junctions [11, 17]. *D. discoideum* has homologs of both α-catenin and β-catenin called *Dd* α-catenin and *Aardvark*, respectively [11, 18], as well as an additional member of the vinculin family [6, 9]. *Dd* α-catenin localizes to the cell surface during the multicellular phase of the life cycle (but crucially not when *Dictyostelium* is unicellular [11]) and shares biochemical properties with metazoan α-catenin, but not vinculin [11]. The other vinculin family member was not analyzed. The functional similarities of *Dd* α-catenin and metazoan α-catenin include the binding of *Dd* α-catenin to Aardvark as well as to mouse β-catenin and the ability of *Dd* α-catenin to bundle actin filaments [11]. Further, normal multicellular development of the fruiting body and the polarized epithelia-like tissue within the tip are disrupted when *Dd* α-catenin is knocked down [11]. Aardvark is also required for normal fruiting body development and tip organization [11, 19]. Aardvark knockouts are reported to disrupt the adherens junctions in *D. discoideum* in some studies [19], though not others [11]. The β-catenin homolog in *D. discoideum*, *Aardvark*, also exhibits the dual role of cell-cell adhesion and signaling [17].

Thus while the work of Dickinson et al. does support the idea of ancestral multicellularity, elucidating the evolutionary history of catenins is key to evaluating the hypothesis of ancestral multicellularity. However, this history is complicated by numerous gene duplications followed by subfunctionalization [20–22]. α- and β-catenins are not homologous as their names would suggest, instead α-catenin is a member of the vinculin gene family while β-catenin is a member of the broader catenin family [20]. Vinculins are actin-binding proteins that are involved in cell-cell adhesion at adherens junctions and integrin-mediated junctions in animals [22]. There have been numerous gene duplications and subfunctionalization of α-catenin within animals, and especially vertebrates [20, 22]. Vinculins are present in the unicellular relatives of animals, including *Capsaspora* [23] and *Dictyostelium* [6, 11], but are not found in most eukaryotes [23]. β-Catenin, on the other hand, has experienced many gene duplications, especially within animals [20, 21], and homologs of β-catenin are widely distributed across eukaryotes. β-Catenin homologs play a major role in cell adhesion in adherens junctions and in cell signaling pathways, especially *Wnt* [24]. It has been suggested that the ancestral metazoan β-catenin was a single protein that carried out both of these roles [21].

Reconstructing the evolutionary history of epithelial molecular machinery

We assess the evolutionary history of α- and β-catenin by constructing broadly sampled phylogenetic trees of diverse catenin sequences and use these to evaluate the hypothesis that multicellularity is ancestral to the Amorphea clade. We used the online database OrthoMCL (www.orthomcl.org) to identify homologs of α-catenin and β-catenin and then performed additional BLASTp and tBLASTn searches against each major clade and specific taxa of interest using cutoff of e−10 to increase taxon sampling. Sequences were then aligned with MAFFT version 6.882 [25] and filtered using GUIDANCE [26] in the online server (http://guidance.tau.ac.il/). The best fitting substitution matrices were determined to be LG for both α-catenin and β-catenin by the ProtTest 2.4 online server (http://darwin.uvigo.es/software/prottest2_server.html) [27], and these were used in subsequent analyses. Gene trees were estimated using (i) maximum likelihood (ML) with RAxML HPC version 7.2.8 [28] and (ii) Bayesian analysis with MrBayes version 3.1.2 [29]. We performed a thorough ML search in RAxML by generating 100 independent maximum parsimony trees as start points for optimization. The best scoring ML tree was then annotated with the results of 1,000 bootstraps performed independently with the same parameters. All analyses were performed using PROTGAMMA search algorithm. The Bayesian analysis was run for ten million generations, saving trees every 10,000 generations, with two independent MCMC runs with four chains each, and a heating parameter of 0.05. We obtained convergence after four million generations, the 400 trees before convergence were discarded as burnin and analyses used the remaining trees. Bayesian phylogenetic analyses were performed on the CIPRES Science Gateway version 3.2 [30]. Further, we tested whether a history of α-catenin consistent with the hypothesis of Dickinson and colleagues could be rejected using the Approximately Unbiased test [31, 32]. Briefly, we generated maximum likelihood reconstructions with RAxML using the same parameters as described above, but with the tree topology constrained to place dictyostelid α-catenins sister to the metazoan α-catenins (and not nested within the vinculin clades). The constrained results were then compared to the best tree found in the unconstrained RAxML analysis to calculate per-site likelihoods. The per-site likelihoods were then analyzed in CONSEL with standard parameters (including 100,000 replicates) to obtain p-values [33].

We find that the history of both α- and β-catenin supports a scenario of independent co-option for their current roles in epithelium-like connections in *Dictyostelium* and epithelia in animals, respectively (Figs. 2 and 3, and see Box 1).

Vinculin and β-catenin were independently co-opted for similar functions in cell-cell junctions

Dickinson and colleagues show through biochemical analysis that *Dd* α-catenin functions like metazoan α-catenin and not like vinculin [11]. However, our analyses demonstrate that *Dd* α-catenin is not an ortholog of metazoan α-catenin, but rather originated by a duplication of vinculin (Fig. 2). We find that dictyostelids, including *D. discoideum*, have two copies of vinculin genes that appear to have duplicated at the base of dictyostelids (Fig. 2). This duplication may have taken place earlier in the history of Amoebozoa (indicated by an intermediate branching *Acanthamoeba castellanii* vinculin), but the taxonomic sampling is not sufficient to distinguish this possibility. Additionally, while the low support values for the backbone of the tree decreases our power to distinguish among alternative topologies, we can marginally reject a single origin of animal α-catenin and dictyostelid "α-catenin" (AU-test $p = 0.034$ for monophyly of "α-catenin" and 0.031

Think again

Box 1

Innovation often arises through gene duplication

Proposed by Ohno [66] in the 1970s as a major driver of biological evolution, gene duplication is likely responsible for providing most of the basic materials for evolution to work with. Gene duplication is pervasive across all domains of life: the percentage of genes that are duplicated in organismal genomes varies from 17% in the bacterium *Helicobacter pylori* up to 65% in the plant *Arabidopsis thaliana* [67]. The rate at which genes duplicate and achieve fixation is comparable to the rate of nucleotide substitutions in mammal genomes [68]. Gene duplication can arise through multiple mechanisms, mainly unequal crossing over, retroposition, and whole-genome duplication. These have been reviewed elsewhere [67].

After duplication, the two copies present in the same genome are called paralogs and many rounds of duplication can result in gene families. Paralogous genes may undergo different evolutionary fates: because one copy of the gene is performing the original function, the other copy is free to diverge. The most common fate of duplicated genes is pseudogenization, because there are no functional constraints the gene receives mutations that render the gene non-functional [68, 69]. However, in a small percentage of cases, duplicate genes do not become pseudogenes. Some do not even diverge, due to either concerted evolution by gene conversion or very strong purifying selection for genes that produce high amounts of an essential product, like histones [70]. Those genes which do diverge may acquire new functions either by subfunctionalization or neofunctionalization, and these are an abundant source of evolutionary novelty [71].

Duplicated genes have an important consequence for phylogenetic reconstruction: ancient paralogy. This issue has been extensively explored by systematists and is still a source of error and concern in deep level phylogenies [72]. This is because genes that duplicated before the diversification of a lineage present a sampling challenge: if they have been lost in some of the derived lineages or if the experimental sampling was inadequate, the historical reconstruction will inevitably present artifactual relationships [73]. For instance, if we fail to sample the vinculins in Fig. 2, then the dictyostelid actin binding proteins will artifactually fall sister to the metazoan α-catenins.

for reciprocal monophyly of "α-catenin" and vinculin). Thus, while the ability of *Dd* α-catenin to bind Aardvark and mouse β-catenin was interpreted as conserved function, our analysis suggests that this ability is an example of molecular convergence. There is precedence for this degree of sequence level convergence, see for example protein level convergence of *Prestin* in echolocating bats and dolphins [34] and lysozyme in foregut fermenters [35].

Our phylogenetic reconstruction of β-catenin demonstrates that homologs of this protein are present in most major eukaryotic groups (Fig. 3). Consistent with previous analyses, we also recover a history rife with duplications, with much taxon-specific duplication in animals and vertebrates in particular [20, 21]. However, all of the duplications occur within taxa and none predate the origin of major eukaryotic clades (Fig. 3). The function of β-catenins varies across eukaryotes, and is unknown in many taxa. For instance, the β-catenin homolog in *A. thaliana*, named *Arabidillo* after the *Drosophila* β-catenin called *Armadillo*, promotes lateral root branching [36]. However, this role is not conserved within the plant lineage because early land plants such as *Selaginella* and *Physcomitrella* that do not exhibit elaborate root systems also have *Armadillo* homologues, indicating a more complicated history of shifting function. These β-catenin homologs (*Selagidillo* and *Physcodillo*) have been suggested to act instead in cell division and/or cell elongation in these mosses [37]. Given the broad distribution of homologs, it is likely that β-catenin was present in the common ancestor of all eukaryotes (Fig. 3). Because β-catenin homologs in most eukaryotes are not involved in adherens junctions or cell adhesion more generally, it appears likely that the functional similarity observed between *Dictyostelium* Aardvark and metazoan β-catenins has arisen through convergence and co-option of the same eukaryotic machinery.

Multicellularity has arisen many times from unicellular ancestors across the eukaryotic tree of life

When evaluating the origin and evolution of traits in *Dictyostelium* and animals, it is useful to consider these lineages within the broader context of eukaryotic diversity. The breadth of eukaryotic diversity is comprised by more than 70 lineages that are predominantly microbial and have largely been defined by unique patterns of sub-cellular structure by electron microscopy – Patterson's "ultra-structural identity" [38]. These lineages have been grouped into a handful of major clades in recent years by evolutionary hypotheses, classifications and molecular phylogenetic analyses (Fig. 1; [39, 40, 41]). Deep relationships within eukaryotes are beginning to stabilize as taxonomic sampling of the microbial lineages has increased in large-scale phylogenetic analyses (e.g. [39, 41, 42]). The currently recognized major clades of eukaryotes are Opisthokonta, SAR, Amoebozoa, Excavata, and Archaeplastida (Fig. 1).

Multicellularity has arisen more than 25 times across the eukaryotic tree of life and in all of the major clades (Fig. 1; [12, 13]), though the majority of eukaryotic lineages are unicellular in nature [38]. Multicellularity is accomplished by two major strategies: aggregation of individual cells as in *Dictyostelium* and division of a single cell (zygote or spore) as in animals, plants and fungi [43]. Aggregative multicellularity (AM) occurs as part of the life cycle when single-celled organisms come together to produce a fruiting body [4, 44]. AM arose in all of the major eukaryotic clades with the exception of the Archaeplastida (A, Fig. 1; [45]). Multicellularity by division (DM) is also widespread, animals, fungi, and plants being the most recognizable examples (D, Fig. 1). DM is quite common in algal lineages, there being numerous origins in both red and green algae, and once at the base of the brown algae (kelps) [12, 43]. Both types of

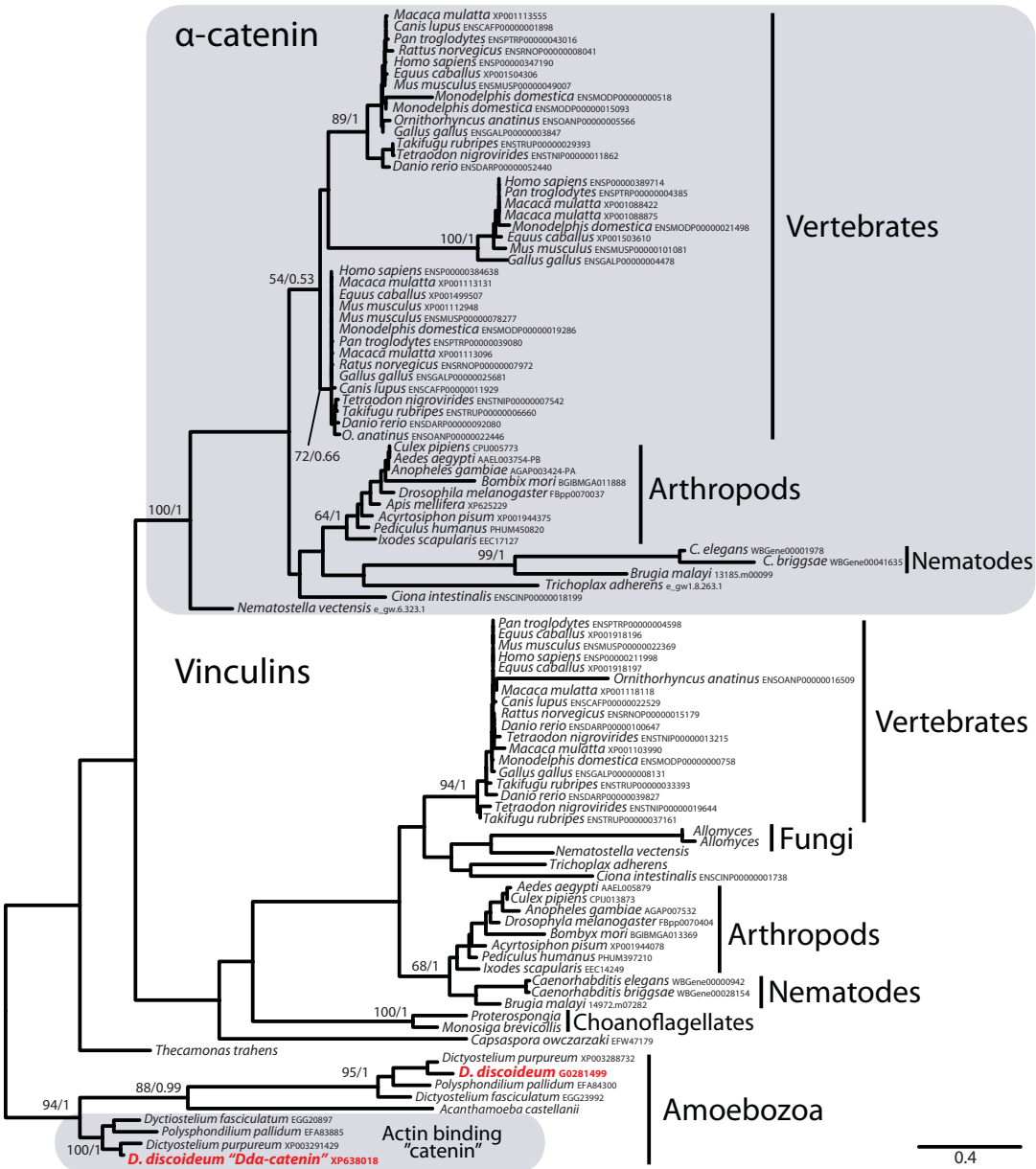

Figure 2. Phylogenetic analysis of the vinculin gene family demonstrates that α-catenin came into being by gene duplication at the base of the metazoa. The most likely phylogenetic tree shows that *Dictyostelium* α-catenin (*Dd* α-catenin) arose through a separate duplication within the dictyostelid lineage and is not orthologous to metazoan α-catenin. The tree is shown unrooted because the position of the root of the eukaryotic tree remains uncertain. Support values are maximum likelihood bootstrap followed by Bayesian posterior probabilities. The sequence for *Thecamonas trahens* was obtained from the supplement of Sebe-Pedros et al. [23] (where it was listed as *Amastigamonas* sp.).

multicellularity are thus widespread across eukaryotes and in the sister groups of animals and *Dictyostelium*.

Two major clades of eukaryotes of particular interest here: the Opisthokonta, comprising animals, fungi and their microbial relatives; and Amoebozoa, home to 20 or so lineages of amoebae, including dictyostelids. These clades were originally joined together under the "unikont" hypothesis [46]. While the original conception of "unikonts" has since been refuted [47], the Amoebozoa + Opisthokonta relationship is widely recovered in molecular phylogenetic analyses [39, 41, 45, 48], and together with two nested unicellular lineages Breviata and Apusomonadida, is now formally recognized as clade Amorphea [42]. When evaluating character evolution within the Amorphea clade, one must consider the position of the root of eukaryotes, which has been hypothesized to fall either within Amorphea [49, 50] or between Amorphea and the remaining eukaryotes [51, 52]. The placement of the root on the eukaryotic tree of life remains an outstanding question in biology and an active area of research [47, 50]. If the root falls between Amoebozoa and Opisthokonta the position of ancestral multicellularity postulated by Dickinson et al. [7] would move

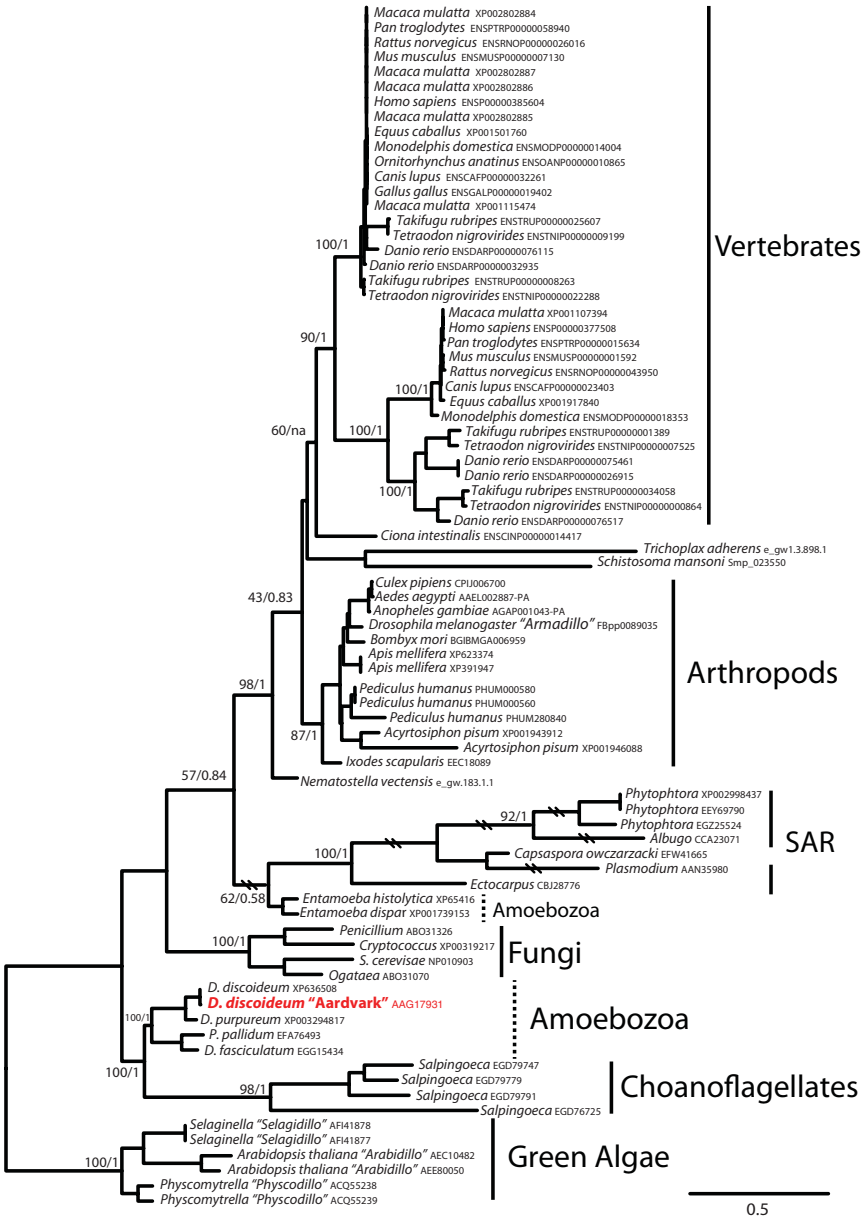

Figure 3. Phylogenetic analyses show that *Dictyostelium* Aardvark is not sister to the animal clade and demonstrates the antiquity of β-catenin. Other notes as in Fig. 2.

to the base of extant eukaryotes, necessitating a large number of losses.

Dictyostelium and animals represent two of the five multicellular lineages in Amorphea

Multicellularity is broadly distributed across the Amorphea clade, although there are numerous unicellular lineages nested within. Animals and fungi are the most diverse groups within the Opisthokonta and both achieve multicellularity by division, though the mechanisms are very different [53, 54]. Animals are entirely multicellular, but have many unicellular relatives, including the Choanoflagellates and *Capsaspora* [55]. Fungi are predominately multicellular, though early diverging lineages are largely unicellular and there have been numerous reversions to unicellularity across fungi [55, 56]. The sister group of fungi is a clade that contains the nucleariid amoebae plus *Fonticula*, an amoeboid taxon with AM [53]. Within Amoebozoa the majority of lineages are unicellular [5, 57], with two origins of AM in the dictyostelids and the genus *Copromyxa* [58]. *Copromyxa* falls within the Tubulinea, a diverse clade that includes *Amoeba proteus* of high school biology fame, and the arcellinid testate amoebae [5, 58]. The Myxogastrea, or plasmodial slime molds, are sometimes discussed as a further example of multicellularity within the Amoebozoa because they also form reproductive fruiting bodies with acellular stalks. However, myxogastrids are not an example of AM because the fruiting body does not form from aggregation of single cells. Instead, myxogastrids are giant multinucleated cells, a syncytial amoeba, that undergo cleavage during the development of the fruiting body giving rise to a mature fruiting body consisting of many cells [59]. This phylogenetic perspective highlights preponderance of unicellular lineages within the Amorphea and the differences in the nature of multicellularity that has arisen across this clade (Fig. 1), both of which make a multicellular ancestor unlikely.

Multicellularity evolves readily through convergent evolution

The broad distribution of multicellular taxa across the eukaryotic tree suggests that multicellularity is "easy" to evolve [13], and the varied mechanisms for achieving the requisites of multicellularity (e.g. cell adhesion, communication, and differentiation) found across taxa demonstrate that there are many routes to its evolution [10]. The frequent occurrence of multicellularity is likely due to strong selective pressures favoring multicellularity, few genetic changes necessary to enable the switch, or a combination of these factors [13]. Experimental evidence from several taxa support this view as multicellularity can be induced rapidly in the face of favorable selective pressure. Experiments in several diverse lineages of green algae show that predation pressure can repeatedly induce multicellularity in a few generations [13], and subjecting yeast to selective pressure for larger cells produces aggregates repeatedly [60].

Table 1. Characteristics of aggregative multicellular organisms (slime molds)

Taxon	Description of fruiting body	Refs.
Fonticula	Spores inside a mucus matrix within stalk, and these are forced upward to form the sorocarp. Sorocarp surrounded by fibrillar material	[44]
Copromyxa	Stalk and sorocarp formed by amoebae crawling to end of fruiting body and encysting. Fruiting body and sorocarp formed by encysted amoebae	[58]
Dictyostelids	Stalk formed by excretion of cellulose matrix that may or may not contain cells. Developmental process organized by tip cells	[1]
Acrasids	Amoeboid cells within a sheath covering form the stalk and projections. Sorocarp elongates and branches so that cells are single file, and then they encyst	[44]
Sorogena	Fibrous material surrounds the spores. Acellular stalk consists of a mucilaginous matrix covered by a sheath	[74]
Sorodiplophrys	Spores of the sorocarp embedded in firm gelatinous matrix and covered with mucus	[75]
Guttulinopsis	Spores suspended in a matrix of slime. Stalk consists of amoeboid cells, cysts, and disintegrated cells within a mucus matrix	[44]

There are diverse strategies for achieving both major types of multicellularity both within Amorphea and across eukaryotes, making the view of deep ancestral multicellularity less likely.

AM arose independently in at least seven eukaryotic lineages, each with similar ecological habits [45]. These are terrestrial organisms that generally fruit on dung or plant material, and their similar habits suggest that similar selective pressures resulted in convergence of this life history strategy [45]. In each instance AM is achieved by different mechanisms (Table 1), although the molecular mechanisms have only been assessed in *Dictyostelium* and cell biology is much less studied in other AM lineages. The epithelia-like cell-cell connections described in *Dictyostelium* appear to be unique: the structure of the sorocarp does not suggest the existence of epithelia-like cell junctions in any other AM lineage. In most cases the sorocarp is held together by fibrillar material or a mass of mucus (Table 1). There are no apparent similarities in the structure or formation of the sorocarp in the Amorphea lineages with AM: *Copromyxa*, *Fonticula*, and dictyostelids (Table 1; [1, 44]). Under the scenario of a multicellular Amorphea ancestor, one would expect the same mechanisms to have been utilized in the evolution of multicellularity in these taxa. All evidence supports the widely held view that AM arose independently and through different mechanisms both within Amorphea and across eukaryotes as a result of similar selective pressures [45].

This survey of multicellularity in eukaryotes highlights the diverse modes of becoming multicellular and also the unique similarities between *Dictyostelium* and animals. In both cases, the multicellular structure consists of motile cells that are differentiated and have a complex pattern of development. Though dictyostelid multicellularity is much different than that achieved by division of a single cell in animals there are many parallels because in both systems motile cells must communicate and join together to orchestrate coordinated movement and cellular differentiation. We suggest that it is this similarity that has driven the remarkable convergence in cell adhesion as well as other aspects of cell biology.

Molecular mechanisms underlying animal multicellularity have been co-opted from unicellular ancestors

The origin of animal multicellularity has been a topic of interest for comparative biologists. For a long time the prevailing view was that key features of animal multicellularity, such as cell adhesion and signaling, must have arisen at the base of the Metazoa. However, this viewpoint has been overturned by recent genomic sequencing projects that have identified many genes involved in these processes in the unicellular relatives of animals, especially choanoflagellates, *Capsaspora*, and Mesomycetozoa [10, 23, 61–63]. As more microbial opisthokonts are sequenced it is becoming clear that the genetic toolkit for multicellularity was already present in unicellular organisms, but in case after case was co-opted to function in a multicellular context within the Metazoa [10, 61, 62, 64]. The widespread presence of genes involved in multicellularity in unicellular organisms supports the view of Dickinson and colleagues that the molecular machinery necessary for multicellular organisms is ancestral. However, it is also consistent with independent origins of multicellularity and co-option of machinery used for signaling, communication, and other processes in microbial lineages for new purposes in multicellular organisms [10, 23, 62, 65].

Conclusions

The majority of evidence supports multiple origins of multicellularity within Amorphea and eukaryotes as a whole. Overall, this analysis highlights the remarkable convergence in the cell-cell adhesion in *Dictyostelium* and animals, demonstrated by Dickinson and colleagues [7, 11]. This likely reflects the similar selective pressures faced by these two lineages that form multicellular structures from non-walled cells. Understanding the duplication events and co-option of the same molecular machinery to perform similar functions will illuminate the requirements for, and evolution of, animal multicellularity. Moving forward, it will be crucial to incorporate an array of dictyostelids that encompass the breadth of morphological forms found in the clade [3, 4], as well as other amoebozoans, in analyses aimed at elucidating the origin and evolution of dictyostelid multicellularity. These studies will in turn provide a deeper understanding of our own multicellularity. Most importantly, because metazoans are but a recent twig in an ancient tree, the diversity and complexity of unicellular eukaryotes must be acknowledged when deep evolutionary inferences are made.

Acknowledgments

Thanks to Mark Q. Martindale, Antonio C. Marques, and Mariana C. de Oliveira for insightful discussions during the development of ideas within this manuscript. We thank Jessica Metcalf, Se Jin Song, and Marcos Barbeitos for comments on the manuscript. The manuscript was improved following the comments of two anonymous reviewers and the editor. This work was conceived and partly written during the São Paulo School of Advanced Sciences—*evolution*, funded by FAPESP Grant #2011/51916-0. D.J.G.L. is funded by CNPq (501089/2011-0).

References

1. **Kessin R.** 2001. *Dictyostelium: Evolution, Cell Biology, and the Development of Multicellularity*. Cambridge: Cambridge University Press. 284 p.
2. **Williams RS**, **Boeckeler K**, **Graf R**, **Muller-Taubenberger A**, et al. 2006. Towards a molecular understanding of human diseases using *Dictyostelium discoideum*. *Trends Mol Med* **12**: 415–24.
3. **Urushihara H.** 2008. Developmental biology of the social amoeba: history, current knowledge and prospects. *Dev Growth Differ* **50**: S277–81.
4. **Romeralo M**, **Escalante R**, **Baldauf SL.** 2012. Evolution and diversity of dictyostelid social amoebae. *Protist* **163**: 327–43.
5. **Lahr DJ**, **Grant J**, **Nguyen T**, **Lin JH**, et al. 2011. Comprehensive phylogenetic reconstruction of amoebozoa based on concatenated analyses of SSU-rDNA and actin genes. *PLoS ONE* **6**: e22780.
6. **Heidel AJ**, **Lawal HM**, **Felder M**, **Schilde C**, et al. 2011. Phylogeny-wide analysis of social amoeba genomes highlights ancient origins for complex intercellular communication. *Genome Res* **21**: 1882–91.
7. **Dickinson DJ**, **Nelson WJ**, **Weis WI.** 2012. An epithelial tissue in *Dictyostelium* challenges the traditional origin of metazoan multicellularity. *BioEssays* **34**: 833–40.
8. **Parfrey LW**, **Lahr DJ**, **Knoll AH**, **Katz LA.** 2011. Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proc Natl Acad Sci USA* **108**: 13624–9.
9. **Eichinger L**, **Pachebat JA**, **Glockner G**, **Rajandream MA**, et al. 2005. The genome of the social amoeba *Dictyostelium discoideum*. *Nature* **435**: 43–57.
10. **Abedin M**, **King N.** 2010. Diverse evolutionary paths to cell adhesion. *Trends Cell Biol* **20**: 734–42.
11. **Dickinson DJ**, **Nelson WJ**, **Weis WI.** 2011. A polarized epithelium organized by beta- and alpha-catenin predates cadherin and metazoan origins. *Science* **331**: 1336–9.
12. **Knoll AH.** 2011. The multiple origins of complex multicellularity. *Annu Rev Earth Pl Sci* **39**: 217–39.
13. **Grosberg RK**, **Strathmann RR.** 2007. The evolution of multicellularity: a minor major transition? *Annu Rev Ecol Evol Syst* **38**: 621–54.
14. **Leys SP**, **Nichols SA**, **Adams ED.** 2009. Epithelia and integration in sponges. *Integr Comp Biol* **49**: 167–77.
15. **Magie CR**, **Martindale MQ.** 2008. Cell-cell adhesion in the cnidaria: insights into the evolution of tissue morphogenesis. *Biol Bull* **214**: 218–32.
16. **Niessen CM.** 2007. Tight junctions/adherens junctions: basic structure and function. *J Invest Dermatol* **127**: 2525–32.
17. **Grimson MJ**, **Coates JC**, **Reynolds JP**, **Shipman M**, et al. 2000. Adherens junctions and [beta]-catenin-mediated cell signalling in a non-metazoan organism. *Nature* **408**: 727–31.
18. **Coates JC**, **Harwood AJ.** 2001. Cell-cell adhesion and signal transduction during *Dictyostelium* development. *J Cell Sci* **114**: 4349–58.
19. **Coates JC**, **Grimson MJ**, **Williams RS**, **Bergman W**, et al. 2002. Loss of the beta-catenin homologue aardvark causes ectopic stalk formation in *Dictyostelium*. *Mech Dev* **116**: 117–27.
20. **Zhao Z-M**, **Reynolds A**, **Gaucher E.** 2011. The evolutionary history of the catenin gene family during metazoan evolution. *BMC Evol Biol* **11**: 198.
21. **Schneider SQ**, **Finnerty JR**, **Martindale MQ.** 2003. Protein evolution: structure-function relationships of the oncogene beta-catenin in the evolution of multicellular animals. *J Exp Zool B Mol Dev Evol* **295**: 25–44.
22. **Kobielak A**, **Fuchs E.** 2004. Alpha-catenin: at the junction of intercellular adhesion and actin dynamics. *Nat Rev Mol Cell Biol* **5**: 614–25.
23. **Sebe-Pedros A**, **Roger AJ**, **Lang FB**, **King N**, et al. 2010. Ancient origin of the integrin-mediated adhesion and signaling machinery. *Proc Natl Acad Sci USA* **107**: 10142–7.
24. **Wikramanayake AH**, **Hong M**, **Lee PN**, **Pang K**, et al. 2003. An ancient role for nuclear [beta]-catenin in the evolution of axial polarity and germ layer segregation. *Nature* **426**: 446–50.
25. **Katoh K**, **Toh H.** 2008. Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinform* **9**: 286–98.
26. **Penn O**, **Privman E**, **Ashkenazy H**, **Landan G**, et al. 2010. GUIDANCE: a web server for assessing alignment confidence scores. *Nucleic Acids Res* **38**: W23–8.
27. **Abascal F**, **Zardoya R**, **Posada D.** 2005. ProtTest: selection of best-fit models of protein evolution. *Bioinformatics* **21**: 2104–5.
28. **Stamatakis A**, **Hoover P**, **Rougemont J.** 2008. A rapid bootstrap algorithm for the RAxML Web-servers. *Syst Biol* **57**: 758–71.
29. **Ronquist F**, **Huelsenbeck JP.** 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–4.
30. **Miller MA**, **Pfeiffer W**, **Schwartz T.** 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov 2010, New Orleans, LA pp. 1–8.
31. **Shimodaira H.** 2002. An approximately unbiased test of phylogenetic tree selection. *Syst Biol* **51**: 492–508.
32. **Shimodaira H.** 2004. Approximately unbiased tests of regions using multistep-multiscale bootstrap resampling. *Ann Stat* **32**: 2616–41.
33. **Shimodaira H**, **Hasegawa M.** 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* **17**: 1246–7.
34. **Liu Y**, **Cotton JA**, **Shen B**, **Han X**, et al. 2010. Convergent sequence evolution between echolocating bats and dolphins. *Curr Biol* **20**: R53–4.
35. **Kornegay JR**, **Schilling JW**, **Wilson AC.** 1994. Molecular adaptation of a leaf-eating bird: stomach lysozyme of the hoatzin. *Mol Biol Evol* **11**: 921–8.
36. **Coates JC**, **Laplaze L**, **Haseloff J.** 2006. Armadillo-related proteins promote lateral root development in *Arabidopsis*. *Proc Natl Acad Sci USA* **103**: 1621–6.
37. **Moody L**, **Saidi Y**, **Smiles E**, **Bradshaw S**, et al. 2012. ARABIDILLO gene homologues in basal land plants: species-specific gene duplication and likely functional redundancy. *Planta* **236**: 1927–41.
38. **Patterson DJ.** 1999. The diversity of eukaryotes. *Am Nat* **154**: S96–124.
39. **Parfrey LW**, **Grant J**, **Tekle YI**, **Lasek-Nesselquist E**, et al. 2010. Broadly sampled multigene analyses yield a well-resolved eukaryotic tree of life. *Syst Biol* **59**: 518–33.
40. **Adl SM**, **Simpson AGB**, **Farmer MA**, **Andersen RA**, et al. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J Eukaryot Microbiol* **52**: 399–451.
41. **Burki F**, **Okamoto N**, **Pombert JF**, **Keeling PJ.** 2012. The evolutionary history of haptophytes and cryptophytes: phylogenomic evidence for separate origins. *Proc Biol Sci* **279**: 2246–54.
42. **Adl SM**, **Simpson AGB**, **Lane CE**, **Lukeš J**, et al. 2012. The revised classification of eukaryotes. *J Euk Microbiol* **59**: 429–514.
43. **Bonner JT.** 1998. The origins of multicellularity. *Integrative Biol* **1**: 27–36.
44. **Blanton RL.** 1990. Phylum acrasea. In Margulis L, Corliss JO, Melkonian M, Chapman DJ, eds; *Handbook of Protoctista*. Boston, MA: Jones and Bartlett. p. 75–87.
45. **Brown MW**, **Kolisko M**, **Silberman JD**, **Roger AJ.** 2012. Aggregative multicellularity evolved independently in the eukaryotic supergroup Rhizaria. *Curr Biol* **22**: 1123–7.
46. **Cavalier-Smith T.** 2003. Protist phylogeny and the high-level classification of Protozoa. *Euro J Protistol* **39**: 338–48.
47. **Roger AJ**, **Simpson AGB.** 2009. Evolution: revisiting the root of the eukaryote tree. *Curr Biol* **19**: R165–7.
48. **Katz LA**, **Grant J**, **Parfrey LW**, **Gant A**, et al. 2011. *Subulatomonas tetraspora* nov. gen. nov. sp. is a member of a previously unrecognized major clade of eukaryotes. *Protist* **162**: 762–73.
49. **Stechmann A**, **Cavalier-Smith T.** 2002. Rooting the eukaryote tree by using a derived gene fusion. *Science* **297**: 89–91.
50. **Katz LA**, **Grant JR**, **Parfrey LW**, **Burleigh JG.** 2012. Turning the crown upside down: gene tree parsimony roots the eukaryotic tree of life. *Syst Biol* **61**: 653–60.
51. **Stechmann A**, **Cavalier-Smith T.** 2003. The root of the eukaryote tree pinpointed. *Curr Biol* **13**: R665–6.
52. **Derelle R**, **Lang F.** 2012. Rooting the eukaryotic tree with mitochondrial and bacterial proteins. *Mol Biol Evol* **29**: 1277–89.
53. **Brown MW**, **Spiegel FW**, **Silberman JD.** 2009. Phylogeny of the ''forgotten'' cellular slime mold, *Fonticula alba*, reveals a key evol-

utionary branch within Opisthokonta. *Mol Biol Evol* **26**: 2699–709.
54. **Medina M**, **Collins A**, **Taylor J**, **Valentine J**, et al. 2003. Phylogeny of Opisthokonta and the evolution of multicellularity and complexity in Fungi and Metazoa. *Int J Astrobiol* **2**: 203–11.
55. **Torruella G**, **Derelle R**, **Paps J**, **Lang BF**, et al. 2012. Phylogenetic relationships within the Opisthokonta based on phylogenomic analyses of conserved single-copy protein domains. *Mol Biol Evol* **29**: 531–44.
56. **Hibbett DS**, **Binder M**, **Bischoff JF**, **Blackwell M**, et al. 2007. A higher-level phylogenetic classification of the Fungi. *Mycol Res* **111**: 509–47.
57. **Smirnov AV**, **Chao E**, **Nassonova ES**, **Cavalier-Smith T.** 2011. A revised classification of naked lobose amoebae (Amoebozoa: lobosa). *Protist* **162**: 545–70.
58. **Brown MW**, **Silberman JD**, **Spiegel FW.** 2011. ''Slime molds'' among the Tubulinea (Amoebozoa): molecular systematics and taxonomy of Copromyxa. *Protist* **162**: 277–87.
59. **Frederick L.** 1990. Phylum plasmodial slime molds, class Myxomycota. In Margulis L, Corliss JO, Melkonian M, Chapman DJ, eds; *Handbook of Protoctista*. Boston: Jones and Bartlett. p. 467–83.
60. **Ratcliff WC**, **Denison RF**, **Borrello M**, **Travisano M.** 2012. Experimental evolution of multicellularity. *Proc Natl Acad Sci USA* **109**: 1595–600.
61. **Nichols SA**, **Roberts BW**, **Richter DJ**, **Fairclough SR**, et al. 2012. Origin of metazoan cadherin diversity and the antiquity of the classical cadherin/beta-catenin complex. *Proc Natl Acad Sci USA* **109**: 13046–51.
62. **Suga H**, **Dacre M**, **de Mendoza A**, **Shalchian-Tabrizi K**, et al. 2012. Genomic survey of Premetazoans shows deep conservation of cytoplasmic tyrosine kinases and multiple radiations of receptor tyrosine kinases. *Sci Signal* **5**: ra35.
63. **Sebe-Pedros A**, **de Mendoza A**, **Lang BF**, **Degnan BM**, et al. 2011. Unexpected repertoire of metazoan transcription factors in the unicellular holozoan *Capsaspora owczarzaki*. *Mol Biol Evol* **28**: 1241–54.
64. **Sebe-Pedros A**, **Ruiz-Trillo I.** 2010. Integrin-mediated adhesion complex: cooption of signaling systems at the dawn of Metazoa. *Commun Integr Biol* **3**: 475–7.
65. **Mikhailov KV**, **Konstantinova AV**, **Nikitin MA**, **Troshin PV**, et al. 2009. The origin of Metazoa: a transition from temporal to spatial cell differentiation. *BioEssays* **31**: 758–68.
66. **Ohno S.** 1970. *Evolution by Gene Duplication*. New York: Spinger-Verlag.
67. **Zhang J.** 2003. Evolution by gene duplication: an update. *Trends Ecol Evol* **18**: 292–8.
68. **Lynch M**, **Conery JS.** 2000. The evolutionary fate and consequences of duplicate genes. *Science* **290**: 1151–5.
69. **Walsh JB.** 1995. How often do duplicated genes evolve new functions? *Genetics* **139**: 421–8.
70. **Nei M**, **Rogozin IB**, **Piontkivska H.** 2000. Purifying selection and birth-and-death evolution in the ubiquitin gene family. *Proc Natl Acad Sci USA* **97**: 10866–71.
71. **De Smet R**, **Van de Peer Y.** 2012. Redundancy and rewiring of genetic networks following genome-wide duplication events. *Curr Opin Plant Biol* **15**: 168–76.
72. **Qiu H**, **Yang EC**, **Bhattacharya D**, **Yoon HS.** 2012. Ancient gene paralogy may mislead inference of plastid phylogeny. *Mol Biol Evol* **29**: 3333–43.
73. **Maddison WP.** 1997. Gene trees in species trees. *Syst Biol* **46**: 523–36.
74. **Olive LS**, **Blanton RL.** 1980. Aerial sorocarp development by the aggregative ciliate *Sorogena stoianovitchae*. *J Protozool* **27**: 293–9.
75. **Dykstra MJ**, **Olive LS.** 1975. *Sorodiplophrys* – unusual sorocarp producing protist. *Mycologia* **67**: 873–9.