# Chapter 1

# Putting Animals in their Place within a Context of Eukaryotic Innovations

*Danielle Vazquez*,[1] *Laura Wegener Parfrey*[2] and *Laura A. Katz*[1,2]

Animals represent just one of an estimated 70+ lineages of eukaryotes (Patterson 1999), and the bulk of the remaining lineages are microbial. During the past decade perspectives of the organization of eukaryotic diversity have undergone a major shift to a system that recognizes five or six high-level groupings (Adl et al. 2005, Tekle et al. 2009). While deep nodes on the eukaryotic tree of life remain elusive (reviewed in Parfrey et al. 2006), the position of animals emerging from within microbial lineages has been robustly resolved by several lines of evidence (King 2004). Animals are placed within the supergroup Opisthokonta, along with fungi and numerous microbial relatives (Adl et al. 2005, Steenkamp et al. 2006).

In this chapter we place animals in their phylogenetic context by focusing on lineages of eukaryotes defined by one or more ultrastructural identities (i.e., unique combinations of subcellular structures). Many of these clades are marked by major innovations in cell and/or genome structure at their base. Here, we focus on seven exemplary clades: foraminifera, diatoms, ciliates, apicomplexa, dinoflagellates, euglenids and kinetoplastids; we also discuss some higher-level groupings. We argue that this contrasts with animals, which lack any clear innovations at the

[1]Department of Biological Sciences, Smith College, Northampton, MA, USA.
[2]Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, MA, USA.
E-mail: lkatz@email.smith.edu

base of the grouping despite many within. Many of the eukaryotic clades originally defined by morphological and/or ultrastructural characteristics are now grouped into larger clades—the Stramenopiles, Alveolates, and Euglenozoa—that are well-supported in molecular analyses.

Here, we aim to accomplish two goals: (1) putting animals into an evolutionary context by exploring major transitions at the base of diverse microbial clades sampled across the eukaryotic tree of life; and (2) using examples from diverse eukaryotic lineages to highlight the complex evolution of features that are often believed to be fixed across the eukaryotic tree or unique to animals (as well as plants and fungi). In both sections, we aim to be exemplary rather than exhaustive and our intent is to provide a broader context for interpreting major transitions within animals, the subject of the remainder of this book.

**Part one: Understanding Innovations Across the Eukaryotic Tree of Life**

We have chosen a limited set of clades to demonstrate the types of transitions that have occurred at the base of many microbial lineages. These examples were chosen partially due to the availability of data and partially based on the authors' areas of expertise.

**Diatoms** are among the most diverse clades of eukaryotes, with an estimated 100,000 extant species, and fall within the **stramenopiles** (Fig. 1). The stramenopiles are a well-supported clade that includes kelp (Phaeophyceae), watermolds (oomycetes), slime nets (labyrinthulids) and many other lineages (Andersen 2004). Synapomorphies for diatoms include the presence of a silica shell, which gives diatoms their distinctive appearance (Fig. 2a). These shells are thought to have evolved initially as a byproduct of silica metabolism, and appear to play a protective role (Schmid 2003).

Three of our exemplar lineages—ciliates, dinoflagellates, and apicomplexa—are members of a single clade, the **alveolates** (Fig. 1). Alveolates are united by the presence of alveoli, a system of abutting sacs that form a layer immediately under the plasma membrane that is supported by microtubules (Patterson 1999). These alveolar sacs are thought to add rigidity to the cells.

**Ciliates** (Fig. 2b) are marked by numerous innovations. Morphologically, all ciliates contain rows of cilia (flagella) on the cell surface that are supported by a complex series of microtubules (Lynn 1996). The diverse patterns of ciliary organization have given this clade tremendous morphological complexity. The organization of cilia also provides numerous characters by which ciliates are described and classified, and has led to numerous monographs (e.g. Corliss 1979). Ciliates are also defined by the presence of

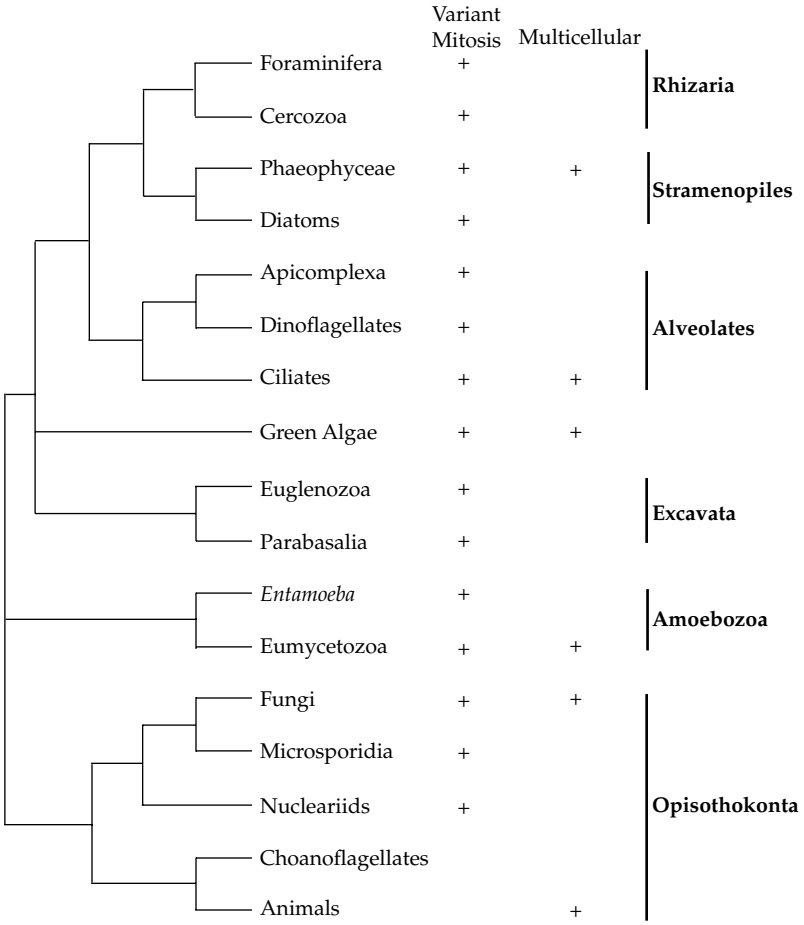


**Fig. 1.** Distribution of multicellularity and alternative types of mitosis across the eukaryotic tree of life. Variant mitosis: + denotes lineages in which mitosis deviates from the open orthomitosis (Fig. 3), the "classic" animal and plant model. Multicellar: Lineages with one or more members with multicellular life cycle stages. Tree modified from Tekle et al. 2009.

two distinct types of nuclei within each cell—the germline micronucleus and the somatic macronucleus. The genomic implications of this feature are discussed below.

**Apicomplexa**, a second major lineage within the Alveolates, include the malarial parasites *Plasmodium falciparum* and *P. vivax* (Fig. 2c). Apicomplexa are defined by the presence of an 'apical complex' at the anterior end of the cells (Morrison 2009). This structure consists of a ring of specialized microtubules and an underlying system of modified vesicles, which secrete

chemicals that can break down cell walls. The apical complex enables these parasites to invade a variety of tissues and cells, contributing to the success of this diverse clade (Morrison 2009).

**Dinoflagellates** (Fig. 2d), the third major alveolate lineage, are marked by innovations including a very unusual nucleus, called a dinokaryon. In most eukaryotes, nuclear DNA wraps around histone proteins, forming a nucleosome that can be further packaged depending on the degree of chromosome condensation. Most nuclei have condensed (heterochromatin) and decondensed (euchromatin) chromosome regions, the proportions of which vary according to transcriptional activity and life cycle stage. In contrast, the dinoflagellate nuclei are devoid of canonical histones—though histone-like proteins have been identified (Wong et al. 2003)—and their chromosomes are permanently condensed (Hackett et al. 2004). The dinoflagellate chromosome structure is proposed to be a cholesteric liquid crystal (Rizzo 2003, Costas and Goyanes 2005), but the implications of these features are not known. Dinoflagellate nuclei divide by closed mitosis (see below), with the ability to create tunnels for microtubules (Hackett et al.2004).

**Foraminifera** (Fig. 1, Fig. 2e) are defined by their granular reticulopodia (Bowser and Travis 2002), a dynamic network of branching and anastomosing pseudopodia that supports bidirectional movement of particles both within and on the surface of the pseudopodia. Reticulopodia are supported by an infrastructure of microtubule bundles that support the structure and dynamic motility of the network (Bowser et al. 2002). The reticulopodia and attached particles move several orders of magnitude faster than the pseudopods of other amoebae. Unique microtubule assembly and disassembly underlain by divergent tubulins is hypothesized to enable such speed (Habura et al. 2005). Ultrastructural studies of the reticulopodia show that microtubules disassemble into helical filaments, unique tubulin polymorphs that can be stored as paracrystals and shipped out to rapidly growing points of the network (Bowser and Travis 2002). Foraminifera interact with their environment through the reticulopodia by capturing food, building shells (also called tests), and moving, and the innovation of the reticulopodia is thought to have enabled the diversification and ecological success within the clade.

The supergroup **Excavata** (Fig. 1), as proposed by Cavalier-Smith (2002), seeks to unite a number of protist lineages, including the euglenids, kinetoplastids, and parabasalids. All members are either excavate taxa (they possess a ventral feeding groove) or are thought to be descended from excavates, though the monophyly of the supergroup remains disputed (Simpson 2003). We describe a few of these lineages below.

Together, the **kinetoplastids** and eguglenids comprise the bulk of the Euglenozoa, a taxon whose monophyly appears to be robust (Simpson 2003, Parfrey et al. 2006). The kinetoplastids include marine flagellates such as *Rhynchomonas nasuta* (Fig. 2f) as well as the trypanosomes, parasites responsible for serious human illnesses such as sleeping sickness (*Trypanosoma brucei*) and leishmaniasis (e.g. *Leishmania major*) (Simpson et al. 2002). They are distinguished from other protist lineages by the presence of a single mitochondrion and a unique organization of the mitochondrial genome. Kinetoplastids have a condensed body of mitochondrial DNA called the kinetoplast that is large enough to be detected by light microscopy and is the source of group's name (Simpson et al. 2002). In trypanosomes, the kinetoplast DNA (kDNA) is arranged into interlocking maxi- and minicircles. Maxicircles are few in number and encode incomplete copies of typical mitochondrial genes, whereas the minicircles can number in the thousands (Simpson et al. 2002). Each minicircle encodes 1 to 4 small guide RNAs that mediate the extensive editing of maxicircle transcripts. Gene organization along the nuclear chromosomes of kinetoplastids also follows an unusual pattern termed absolute strand polarity. The genes are arranged in large clusters along just one strand of the DNA helix (McGrath and Katz 2004). In contrast, the genes of most other organisms are coded for on both strands.

The **euglenids** (Fig. 2g) are mostly free-living freshwater flagellates characterized by a unique pellicle made up of proteinaceous strips located beneath the cell membrane (Leedale 1967). The pellicle is further supported by a network of microtubules (Leedale 1967). While some move only using their flagella, in many euglenids the pellicle strips can slip past each other, thereby producing a distinctive inching motion called metaboly.

**Parabasalids** are a flagellate lineage composed of roughly 80 amitochondriate genera (Brugerolle and Müller 2000) such as the well-studied *Trichomonas vaginalis* and *Tritrichomonas muris* (Fig. 2h). Most members are either parasites or endosymbionts of other animals, notably termites and humans. The taxon is distinguished by their prominent Golgi bodies, which are linked to the basal bodies by parabasalid fibers (Brugerolle and Müller 2000).

Animals are members of the **Opisthokonta**, a robust clade defined by both a morphological and molecular synapomorphy. The morphological synapomorphy is the presence of a single posterior flagellum, which is found in animal sperm cells as well as choanoflagellates, the closest relatives of animals. The similarity of cell architecture and flagellar position between sponges and choanoflagellates was first noticed more than a century ago (King 2004). This character has been lost in some Opisthokont groups including the fungi. A molecular synapomorphy for

this group is the presence of an insertion in the Elongation factor 1a (EF1a) gene (Baldauf and Palmer 1993), for those taxa that have the canonical EF1a (Keeling and Inagaki 2004, Gile et al. 2006).

Several lineages of eukaryotes are emerging from molecular studies that do not have morphological synapomorphies. These lineages, including Cercozoa, Amoebozoa, and Rhizaria, unite a surprising diversity of

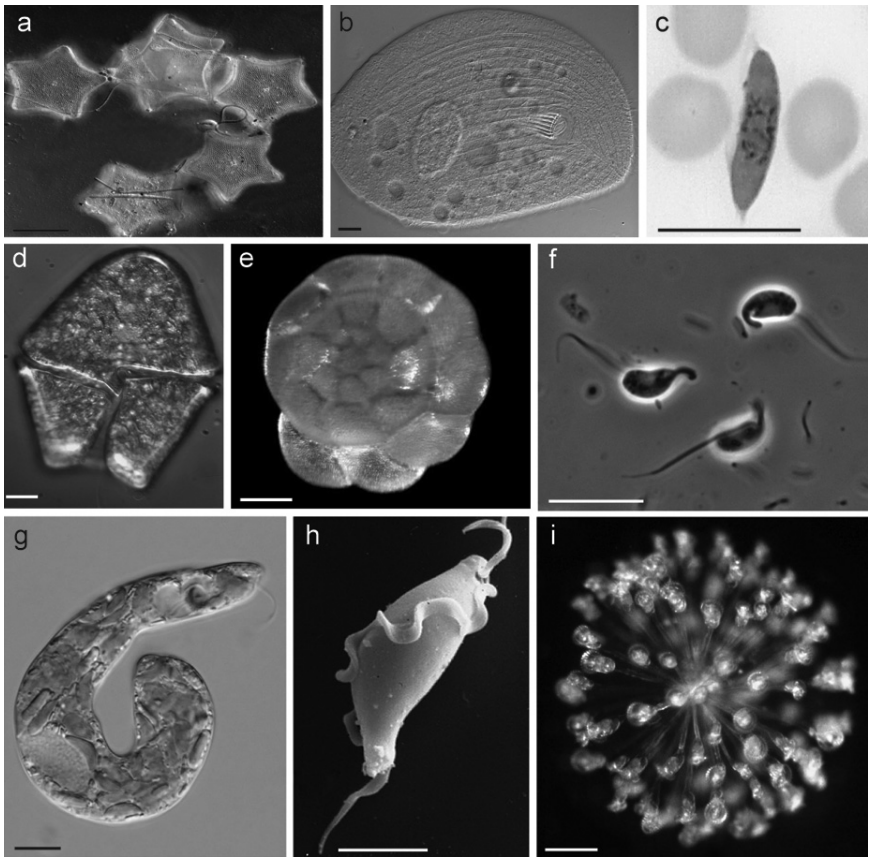


**Fig. 2.** Representative taxa from major eukaryotic lineages discussed. Stramenopiles: **(a)** Triceratium pentacrinus, a diatom; Alveolates (b-d): **(b)** Trithigmostoma cucullulus, a ciliate, **(c)** Plasmodium falciparum, an apicomplexan, and **(d)** Akashiwo sanguinea, a dinoflagellate; **(e)** Ammonia, a foraminiferan; Excavata: **(f)** Rhynchomonas nasuta, a kinetoplastid and **(g)** Euglena mutabilis, a euglenid; **(h)** Tritrichomonas muris, a parabasalid; Opisthokonta: **(i)** Conochilus, a colonial rotifer. Scale bars: b-d,f-h = 10 micrometers; a, e = 100 micrometers; i = 200 micrometers. All images are provided by micro*scope (http: //starcentral.mbl.edu/microscope/portal.php) except c, which is used under PLoS Biology's Open Access rules and can be found in Lacroix et al. (2005), credit Dr. Mae Melvin.

*Color image of this figure appears in the color plate section at the end of the book.*

morphological forms. For example, molecular studies have placed more than 15 lineages of amoebae, parasites, flagellates, and heliozoa with ultrastructural identities into the Cercozoa. These include cercomonads (gliding flagellates), and chlorarachniophytes (filose amoebae with green algal plastids), and desmothoracids (heliozoa). The lack of a discernable morphological innovation uniting these groups may be a result of the ancient age of these clades.

## Part two: Microbial Lineages Defy Many of the Long-Held Assumptions About Eukaryotes

### *Multicellularity*

Although multicellularity has often been thought of as the exclusive territory of plants, fungi, and animals, there are, in fact, a number of microbial eukaryotic lineages that exhibit the trait. The largest protists are found among the brown algae, with giant kelps like those of the genus *Macrocystis* reaching lengths of up to 60 meters (Bonner 1998). A number of brown algae also undergo extensive cell differentiation, with some producing not only stem- and leaf-like parts, but also gas-filled bladders. Furthermore, the majority of giant kelps have photosynthetic filaments at the very edges of their stalks and blades, an example of tissue differentiation. Other lineages achieve muticellularity through mechanisms that are quite different from what one finds in animals, plants, and fungi. Some of them are more colonial rather than strictly multicellular: in the ciliate genus *Zoothamnium*, individual cells form branched colonies. The cells are held together such that should one or a few cells contract, the rest of the colony will follow suit, presumably to avoid danger (Bonner 1998). The green algae genus *Volvox*, on the other hand, produces striking spherical colonies made up of a line of individual cells held together by a gelatinous glycoprotein. Colonial species are generally not considered to be truly multicellular since they do not undergo functional tissue differentiation. However, the volvocine species that form larger colonies (up to 50,000 cells) actually do experience some functional differentiation, though it is usually limited to the formation of somatic and germ cells (Herron and Michod 2008).

Perhaps the most radically different approach to multicellularity is via aggregation whereby individual cells come together (usually when conditions are poor). Unlike macrobes, however, protists that exhibit aggregative multicellularity nevertheless retain the ability to revert to their unicellular state. *Dictyostelium*, a genus of cellular slime mold, is one of the more extensively studied of the aggregative protist lineages. These amoebae have an asexual life cycle during which they feed as independent cells, interacting with each other only after their food supply

has been depleted (Bonner 1998). Cells then collectively form a fruiting body containing environmentally resistant spores. Such aggregation is characteristic of dictyostelid amoebae as well as the acrasids (the acellular "slime molds"), but it has also been found among the ciliates. *Sorogena stoianovitchae*, which lives in soil and feeds on other ciliates, is a unique ciliate species in that it forms aerial fruiting bodies in the face of nutrient scarcity (Lasek et al. 2001).

***Dynamic genomes***

Much of our understanding of the structure and function of genomes is derived from model animals, fungi, and plants. However, this macrobial bias overlooks the tremendous diversity of genome structure and variation in genome content present in microbial eukaryotes.

Data from microbial eukaryotes, in addition to emerging genome sequence data, are challenging the assumption that genomes are largely static within species. In contrast to the textbook view, there is indeed intraspecific variation in genome architecture—the content and organization of nuclear DNA—within individuals during the life cycle and among individuals in populations (Parfrey et al. 2008). In many lineages, genome content changes during the life cycle through genome processing. Genome processing is the fragmentation, amplification, and/or elimination of chromosomes or portions of chromosomes. Examples include: (1) ribosomal DNA amplification in numerous eukaryotic lineages (McGrath and Katz 2004, Zufall et al. 2005) and (2) whole genome rearrangements that occur in the somatic nuclei of ciliates and some animals (Prescott 1994, Jahn and Klobutcher 2002).

As mentioned above, every **ciliate** has two types of nuclei within a single cell: germline micronuclei and somatic macronuclei. The micronuclei go through canonical mitosis and meiosis, but are transcriptionally silent. The transcriptionally active macronuclei develop from the micronucleus through extensive genome fragmentation and amplification. In some types of ciliates this processes results in a macronucleus with ~25 million gene-sized chromosomes (Prescott 1994). Genome processing has also been shown to impact patterns of molecular evolution in ciliates, with elevated rates of protein evolution correlated with the extent of chromosomal processing. In other words, ciliates with extensively processed chromosomes have divergent proteins (Katz et al. 2004, Zufall et al. 2006).

The genomes of ***Entamoeba*** vary from a ploidy of 4N to 40N in a single population (Lohia 2003). Populations can be synchronized to 4N, but the distribution of ploidy levels is restored within hours of normal growth (Lohia 2003). Entamoebae also process their genomes by extensively amplifying the ribosomal DNA. They do not have any chromosomal

copies of rDNA; instead, it exists only as a plasmid that varies in copy number (McGrath and Katz 2004).

**Foraminifera** and some related lineages go through a "nuclear cleansing" process prior to reproduction that indicates genome processing occurred during the development of the nucleus. During this process, termed Zerfall, nuclear contents including DNA are condensed, ejected from the nucleus and degraded (Føyn 1936). The remaining nuclear material divides into hundreds to thousands of gametic nuclei (Goldstein 1997).

### *Variations in mitosis*

Mitosis in eukaryotes assumes a diversity of forms, in contrast to the tidy, static process familiar from textbooks. In the "classic" form of mitosis—called eumitosis or open orthomitosis—the nuclear envelope breaks down completely and chromosomes are segregated by polar spindles into two daughter cells. While open orthomitosis is characteristic of animals and plants, it actually represents only one of the many variations of mitotic division found across the eukaryotic tree of life (Fig. 3 (Heath 1980, Raikov 1982)). There are a number of ways to distinguish different types of mitosis, including variations in microtubule quantity and chromatin condensation and organization (Heath 1980) as well as nuclear pore assembly (De Souza and Osmani 2007). Nevertheless, the major forms of mitosis (Fig. 3) are defined mainly by the behavior of the nuclear envelope and the symmetry of the spindles (Raikov 1994). In this section we will briefly discuss both.

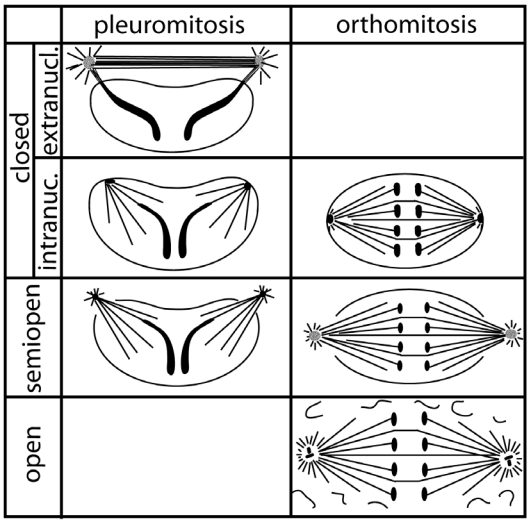


**Fig. 3.** The major forms of mitosis, as defined by the degree of nuclear envelope disintegration and spindle symmetry. Redrawn from Raikov 1994.

The terms "open," "semiopen," and "closed" describe the degree to which the nuclear envelope has disintegrated, while "orthomitosis" and "pleuromitosis" refer to the symmetry (or lack thereof) of the mitotic spindles. Thus, in both semiopen orthomitosis and semiopen pleuromitosis, the nuclear envelope only disintegrates at the polar zones, leaving just enough space through which microtubules can enter the nucleus (Raikov 1982). In some cases, though, the nuclear envelope reseals itself soon after the microtubules have passed through (Heath 1980). Semiopen pleuromitosis differs from semiopen orthomitosis in that while the latter's microtubules produce a bipolar, symmetrical spindle—the definition of orthomitosis—there are two independent half-spindles present in pleuromitosis (Raikov 1982). The half-spindles start out adjacent to one another but later move away at an angle, which initiates anaphase: there is neither a metaphase nor an equatorial plate in pleuromitosis (Raikov 1982). Semiopen pleuromitosis is typical of many apicomplexans (Raikov 1982), including the malarial parasites of the genus *Plasmodium*. Semiopen orthomitosis, meanwhile, is typical of the green flagellates Volvocales and Chloromonadida, as well as several gregarines and heliozoans (Raikov 1982).

One rare variant of "classic" open mitosis has only been observed in the gregarine genus *Stylocephalus*, in which the nuclear envelope completely disintegrates simply to reform around each individual chromosome (Heath 1980). All of the other major types of mitosis are closed—that is, the nuclear envelope does not disintegrate at all. In both closed intranuclear pleuromitosis and orthomitosis, the spindle remains entirely within the nucleus, though its positioning is different (Raikov 1982). Closed intranuclear pleuromitosis is typical of kinetoplastids, oxymonadids, foraminifera, radiolarians, as well as some green flagellates. Closed orthomitosis, on the other hand, is most typical of amoebae, ciliates, and some microsporidians (Raikov 1982). With extranuclear pleuromitosis, the spindle apparatus is found in the cytoplasm and has no direct contact with the chromosomes (Heath 1980, Ribeiro et al. 2000). Extranuclear pleuromitosis is typical of the parabasalids, such as the trichomonads (Raikov 1982) and dinoflagellates.

**Summary**

Here we have placed the evolutionary transitions leading to animals in the context of their microbial relatives on the eukaryotic tree of life. Microbial lineages make up the breadth of eukaryotic diversity, and many are marked by innovations in cell and/or genome structure at their base, which we believe contrasts with the origin of animals from their common ancestor with chonaoflagellates. An understanding of the diversity of microbial eukaryotes provides perspective in interpreting the evolution of

eukaryotic features that are typified by animals, including multicellularity, genome structure, and mitosis, as discussed here. These features highlight the variation and diversity within eukaryotes.

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