

Morphostasis in alveolate evolution

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Three closely related groups of unicellular organisms (ciliates, dinoflagellates and apicomplexans) have adopted what might be the most dissimilar modes of eukaryotic life on Earth: predation, phototrophy and intracellular parasitism. Morphological and molecular evidence indicate that these groups share a common ancestor to the exclusion of all other eukaryotes and collectively form the Alveolata, one of the largest and most important assemblages of eukaryotic microorganisms recognized today. In spite of this phylogenetic framework, the differences among the groups are profound, which has given rise to considerable speculation about the earliest stages of alveolate evolution. However, we argue that a new understanding of morphostasis in several organisms is now throwing light onto the intervening history spanning the major alveolate groups. Insights into the phylogenetic positions of these morphostatic lineages reveal how documenting the distribution of character states in extant organisms, in the absence of fossils, can provide compelling inferences about intermediate steps in early macroevolutionary transitions.

Inferences about the history of life are often limited by a paleontological record that is generally incomplete and difficult to interpret [1]. However, insights about the past can also be generated from character state distributions on phylogenies of extant organisms. These insights are possible because, through time, population lineages have accumulated suites of 'quasi-independent' characters [2,3] that have endured different degrees of MORPHOSTASIS (see Glossary) and transformation. In this light, extant organisms can display extreme cases of morphostasis by retaining a nearly complete suite of character states that were present in an ancestor that existed long ago. Identifying and understanding these morphostatic lineages provides unique insights into evolutionary history.

Morphostasis is thought to arise from environmental constancy over long periods (external causes) and the intrinsic stability of functionally integrated character complexes (internal causes) [4]. The best known examples of morphostasis come from lineages of plants and animals, such as lycopods and coelocanths [5], but here we focus on poorly appreciated yet informative cases of morphostasis in unicellular eukaryotes. Free-living flagellates tend to be extremely abundant and many are cosmopolitan in their distribution, particularly those associated with stable microenvironments that extend across the Earth, such

as bodonids, jakobids and some bicosoecids [6,7]. In our view, these biogeographical patterns reduce the likelihood of lineage extinction events, especially when compared with (endemic) lineages in restricted habitats. We anticipate that a better understanding of eukaryotic microbial diversity should reveal several morphostatic lineages that will provide new inferences about early evolutionary transitions in the history of life.

This point is well illustrated by examining the early evolution of alveolates, one of the most biologically diverse

Glossary

Apical complex: a novel cell invasion apparatus positioned near the anterior end of apicomplexan cells consisting primarily of a microtubular 'conoid', a polar ring and 'rhoptries' (Fig. 2 main text). This apparatus has been used as the best synapomorphy for the Apicomplexa (from which its name is derived), but it is also found in a variety of morphostatic lineages of biflagellated alveolates, such as colpodellids and perkinsids.

Conoid: a cone-shaped cytoskeletal structure that functions as scaffolding for rhoptries of the apical complex. Conoids comprise microtubules and can be completely closed, as in apicomplexans, or open-sided, as in colpodellids and perkinsids (Fig. 2 main text, Box 1).

Extrusive organelle: diverse eukaryotic organelles that dock beneath the plasma membrane of cells and expel their contents in response to mechanical, chemical or electrical stimuli. The contents of extrusive organelles range from amorphous mucilage-like material to highly organized three-dimensional frameworks that rapidly increase in size when expelled (e.g. ejectisomes, muciferous bodies, nematocysts, rhoptries and trichocysts).

Mixotrophy: a nutritional mode whereby organisms utilize nutrients from both photosynthesis and predation. Many lineages of modern dinoflagellates are capable of mixotrophy.

Morphostasis: refers to relative stasis in morphological characteristics (also referred to as 'character stasis'). Unchanging morphological features are inferred to result from external factors, such as stabilizing selection, and internal factors, such as the intrinsic constraints on integrated character complexes.

Myzocytosis: a predatory mode whereby a cell can penetrate the cortex and draw in the cytoplasmic contents of a prey or host cell.

Phagotrophy: a mode of nutrition whereby visible particles of food (e.g. prey cells) are completely ingested and contained within a membrane-bound vesicle within the predatory cell ('phagocytosis' refers to the overall process).

Plastid: a eukaryotic cell compartment in which photosynthesis occurs and any homologous compartment in which photosynthesis has been secondarily lost (e.g. apicoplasts, Box 3). Plastids have their own genomes and can have different suites of light-harvesting pigments (e.g. those with chlorophylls a and b are most often referred to as 'chloroplasts').

Plesiomorphy: an ancestral character state as inferred from the most parsimonious distribution of character states on a specific cladogram.

Rhoptry: the primary extrusive organelle associated with the apical complex of apicomplexans, colpodellids and perkinsids. These organelles tend to be club-shaped whereby the narrower, distal ends are positioned just beneath the plasma membrane and nested within the central opening of a conoid (Fig. 2 main text). Rhoptries release a variety of proteins and lipids associated with cell penetration and invasion.

Stramenopiles: a major group of eukaryotes defined largely by novel flagellar characteristics. Most lineages are photosynthetic (e.g. diatoms and brown algae) but some are not (e.g. water molds and slime nets). Stramenopiles (with some smaller groups, such as haptophytes) are the closest sister group to alveolates.

Synapomorphy: a shared derived character state as inferred from the most parsimonious distribution of character states on a specific cladogram.

and ecologically important groups of eukaryotic microbes on Earth. Several newly discovered organisms affiliated with alveolates show a remarkable degree of morphostasis. A better understanding of the behavior, morphology (particularly the ‘feeding’ apparatus) and phylogeny of these enigmatic organisms provides insights into one of the most perplexing events in the history of life: the evolution of intracellular parasitism from free-living, photosynthetic ancestors. This example also strengthens the argument that a complete understanding of the major transitions in the history of life will come only when the biodiversity and phylogenies of eukaryotic groups other than land plants, animals and fungi are taken into account.

What are alveolates?

The Alveolata comprises three diverse groups of primarily single-celled eukaryotes: ciliates (Fig. 1a), dinoflagellates (Fig. 1b) and apicomplexans (Fig. 1c). Apicomplexans are obligate parasites and are characterized by an APICAL COMPLEX consisting of RHOPTRY-type EXTRUSIVE ORGANELLES and a microtubular CONOID, which serves as scaffolding for the rhoptries [8] (Fig. 2). The apical complex functions in host cell attachment and invasion. Apicomplexans predominantly infect animal cells, and can be found everywhere from the intestinal lumen of insects and marine worms to the erythrocytes of primates (e.g. *Plasmodium*, the causative agent of malaria). Dinoflagellates, by contrast, are very diverse in their trophic modes: many are predators or parasites, but roughly half are phototrophic, some of which rank among the most important players in the primary fixation of carbon in the oceans, whereas others are responsible for harmful (toxic) algal blooms [9]. Dinoflagellates have novel cytoskeletal and nuclear features that set them apart from other eukaryotes [10]. Lastly, ciliates are mainly dynamic predators that perform essential roles in many food chains, although some ciliates inhabit mammalian alimentary systems or invade the flesh of fish. The best

SYNAPOMORPHIES for ciliates are two heteromorphic nuclei and many cilia (short flagella) arranged in specific configurations over the cell surface.

In spite of this extreme diversity, alveolates share a distinct system of inner membranous sacs (alveoli), distinct pores (micropores), similar extrusive organelles and a variety of molecular sequence characters [11–15]. Molecular phylogenies have also shown that apicomplexans and dinoflagellates are more closely related to one another than either is to ciliates [15]. However, the phenotypic gaps between dinoflagellates, apicomplexans and ciliates are tremendous, fueling speculation about the evolutionary history spanning their separate origins.

Morphostasis in alveolates

Six of the eight organisms illustrated in Fig. 2 are alveolates, based on comparative morphology (whether *Katablepharis* and *Acrocoelus* are alveolates is less certain), but they all lack the synapomorphic features of ciliates, dinoflagellates and apicomplexans. In our view, the combination of morphological features that some of these organisms share suggests that they represent morphostatic lineages that could be instrumental in understanding transitional events in alveolate evolution. The phylogenetic position of most of these organisms within the alveolates remains untested with molecular data, but the few cases where the phylogenies are better understood have supported our conjectures about morphostasis in these lineages.

Evidence from multiple gene phylogenies [14,16,17] and comparative analyses of serological epitopes [18] suggest that parasites called perkinsids (e.g. *Perkinsus* and *Parvilucifera*) are the earliest diverging sister lineage to dinoflagellates. The tiny swimming cells of perkinsids have two different flagella and, similar to apicomplexans, have an apical complex, which they also use to access the cytoplasm of animal host cells [19–21]. The conoid in perkinsids, however, differs from that of apicomplexans in being open on one side rather than completely closed (Fig. 2).

An open-sided conoid with associated rhoptries is also found in a group of biflagellated predators called colpodellids [22–26] (Fig. 1d, Fig. 2). Whereas perkinsids actually penetrate and enter their host cells, colpodellids use their apical complex to attach to prey cells and suck out their contents by MYZOCYTOSIS. Although first described in dinoflagellates [27], myzocytosis is also found in what are suspected to be the most PLESIOMORPHIC apicomplexans, the ‘archigregarines’ (e.g. *Selenidium*) [28,29] (Fig. 2). In spite of their behavioral differences in cell invasion, the overall cell morphologies of perkinsids and colpodellids are almost identical (Fig. 2).

Unexpectedly, small subunit rRNA phylogenies have shown that colpodellids are not specifically related to perkinsids, but instead are the sister group to apicomplexans [30] (Box 1). The current phylogenetic positions of perkinsids and colpodellids combined with their shared morphological characteristics provides compelling evidence for morphostasis and an ideal framework for understanding the characteristics of the last ancestor of dinoflagellates and apicomplexans (Box 1). For instance,

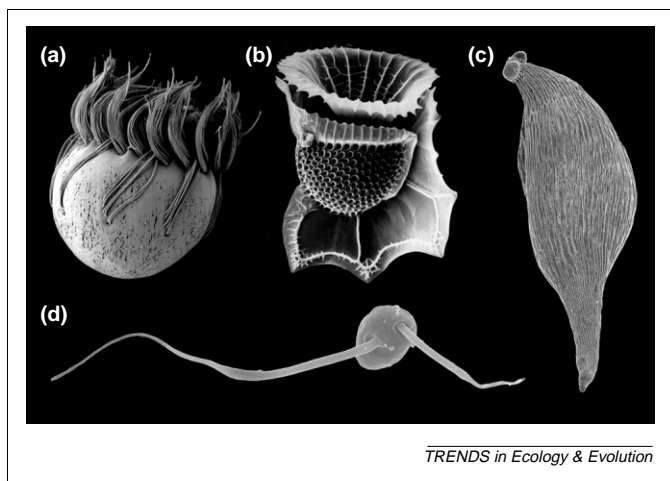
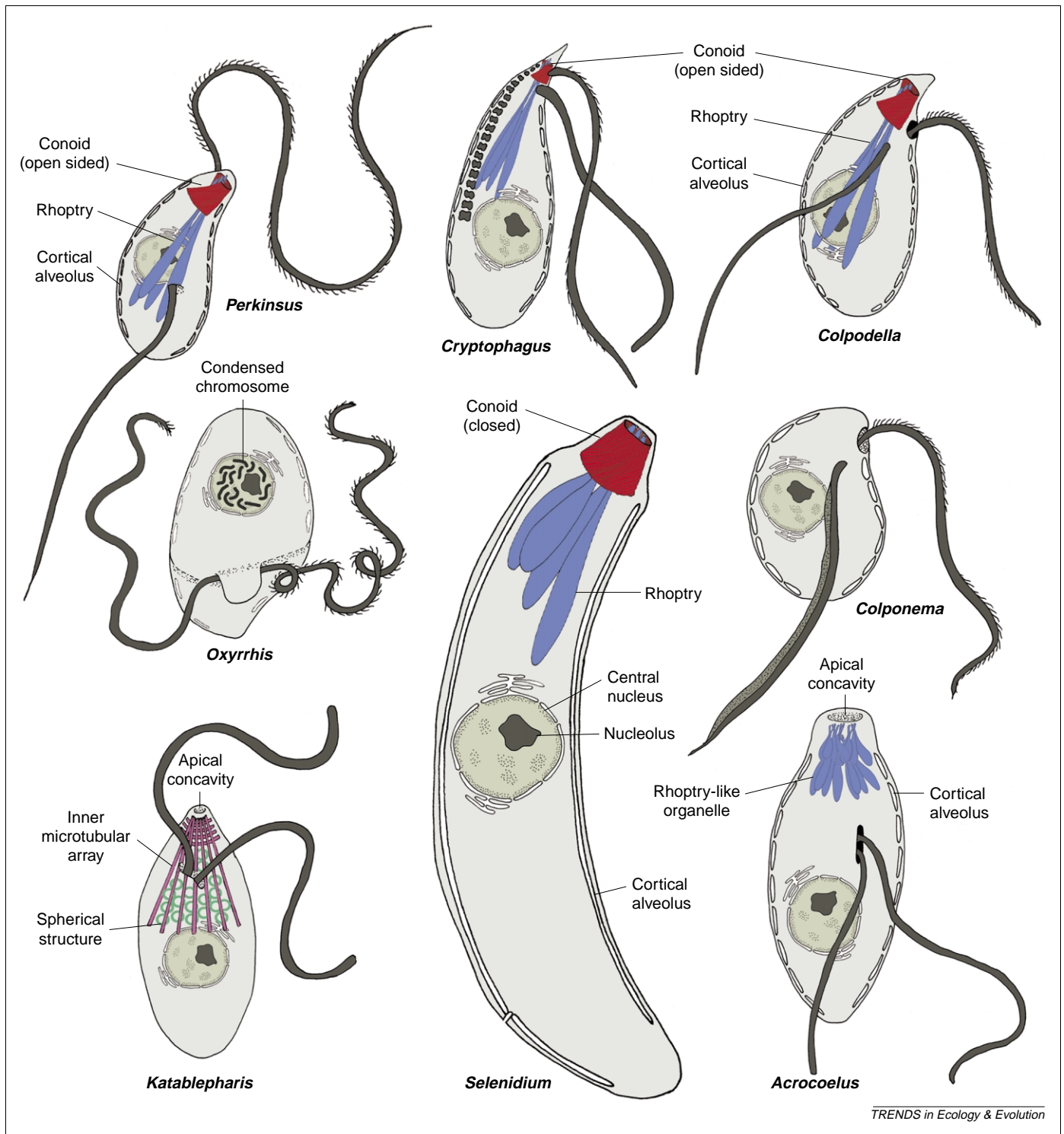


Fig. 1. Scanning electron micrographs of representative alveolates. The three major groups of alveolates are ciliates [(a) *Halteria*], dinoflagellates [(b) *Ornithocercus*] and apicomplexans [(c) *Monocystis*]. Colpodellids [(d) *Colpodella*] are a group of flagellates inferred to have retained a large suite of ancestral character states. (Images are not to scale). (b) reproduced with permission from Brill Academic Publishers [54].



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Fig. 2. Major cytological features of eight lineages having strong degrees of morphostasis when compared with ciliates, dinoflagellates and most apicomplexans. Each organism is unicellular as indicated by the central nucleus and large nucleolus, and all but *Katablepharis* have the diagnostic feature of alveolates, cortical alveoli. The apical complex of the biflagellated zoospores in *Perkinsus* [19–21], *Parvilucifera* [55] (not shown), *Cryptophagus* [31] and *Colpodella* [22,24–26] comprise an open-sided conoid and rhoptries. The apical complex of ‘true’ apicomplexans, such as in the sporozoite and trophozoite of the archigregarine *Selenidium* [28], comprises a completely closed conoid and rhoptries. *Oxyrrhis* [35] lacks an apical complex, has thin, permanently condensed chromosomes and is the nearest free-living sister lineage to dinoflagellates. *Katablepharis* [56,57] is an enigmatic biflagellate with a putative apical complex-like apparatus associated with an apical concavity. The inner microtubular array is indicative of a longitudinally extended conoid, and the associated spherical structures are reminiscent of rhoptries in their cytological position near the cell apex and beneath the microtubular array. *Colponema* [58] lacks obvious structures related to an apical complex, yet it is clearly a predatory alveolate that closely resembles *Colpodella* in general morphology and behavior. *Acrocoelus* [32] is a biflagellated intestinal parasite of marine worms that lacks a conoid but possesses rhoptry-like organelles associated with an apical concavity. Several enigmatic organisms with alveolate-like features have not been shown for the sake of brevity, such as *Pirsonia* [59] and *Phagodinium* [60]. (Images are not to scale).

Box 1. Current phylogenetic framework for alveolates

Independent phylogenetic analyses of different molecular sequences show that ciliates diverged before the radiation of apicomplexans and dinoflagellates [14,15,30]. The earliest stages of alveolate evolution can be approached by addressing two key questions: (1) what character states were present in the most recent common ancestors of apicomplexans and dinoflagellates (Fig. 1, node a); and (2) what were the character states present in the most recent common ancestors of all alveolates (Fig. 1, lineage b)? If the earliest diverging sister lineages of apicomplexans (Fig. 1, lineage c) and dinoflagellates (Fig. 1, lineage d) turn out to be almost identical on a character state-by-state basis, then an extraordinarily confident inference could be made about the biological features of their common ancestor (Fig. 1, node a), as indicated by the blue lines (Fig. 1, Fig. 1). Molecular phylogenies suggest that colpodellids and perkinsids, which are extraordinarily similar morphologically, represent lineages c and d, respectively (Fig. 1). Both lineages possess an open-sided conoid (shown to the right) that is intermediate in form to the closed conoid of apicomplexans. The conoid homologue in dinoflagellates is unclear. Little is also known about the earliest diverging sister lineage to ciliates (Fig. 1, lineage e) and the most recent common ancestor of all alveolates (Fig. 1, node b). However, if lineage e and the inferred ancestor of apicomplexans and dinoflagellates (node a) turn out to share many character states (e.g. if *Colponema* represents lineage e), then a confident inference can be made about morphostasis in lineage e and the biological features of ancestor b (Fig. 1). The gray shaded areas in Fig. 1 indicate strong degrees of morphostasis.

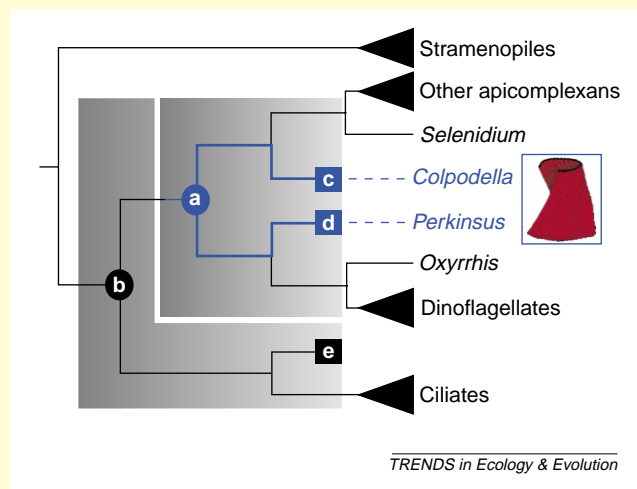


Fig. 1.

we can infer with confidence that this ancestor was a biflagellate that already had an apical complex, consisting of rhoptries and an open-sided conoid. In our view, there is little reason to doubt that this ancestor was capable of using the apical complex to penetrate and draw in the cytoplasm of its prey by myzocytosis.

Understanding these ancestral features extends our ability to infer the characteristics of the last ancestor of all alveolates (Box 1). However, inferences about the last ancestor of alveolates are also dependent on knowing the earliest stages of ciliate evolution, about which very little is currently known (Box 2).

Nonetheless, we think that the development of myzocytosis as observed in colpodellids was a precursory event in the evolution of intracellular parasitism in apicomplexans. This feeding strategy is shared by the extracellular

stages of the early diverging apicomplexans [28,29] and is quite distinct from 'true' PHAGOTROPHY. Moreover, the distinction between myzocytosis and intracellular invasion is perhaps subtler than might be first assumed, especially when comparing the feeding behaviors of other flagellates with an apical complex. For example, some 'free-living' flagellates, such as *Cryptophagus* (Fig. 2), use an apical complex to enter the cells of their algal prey and feed from the 'inside outward' [31].

Most unusual of all is the recently described *Acrocoelus* (Fig. 2), an intestinal parasite of hemichordate worms that has two flagella and rhoptry-like extrusive organelles, but appears to lack conoid-like scaffolding. Instead, it has an anterior concavity that appears to function as a conoid [32]. The phylogenetic position of *Acrocoelus* is unknown, but the absence of a conoid in the presence of rhoptries could indicate that either *Acrocoelus* has lost a conoid or it represents an intermediate stage in the evolution of the apical complex (perhaps providing the initial morphogenetic template for conoid evolution).

Altogether, the distinction between free-living predation (by myzocytosis) and intracellular parasitism is blurred near the divergence of apicomplexans and dinoflagellates. In this context, we can identify the key innovations associated with the subsequent evolution of both lineages.

The first dinoflagellates

The suites of character states in two genera of marine heterotrophic flagellates, *Perkinsus* and *Oxyrrhis* (Fig. 2, Box 1), shed considerable light onto the earliest stages in dinoflagellate evolution. For instance, we would suggest that the apical complex of perkinsids is an ancestral, morphostatic feature that was either lost or radically modified early in dinoflagellate evolution. Homologous remnants of the apical complex in dinoflagellates are unclear, but certain features of dinoflagellates are suggestive of the apical complex, such as the microtubular baskets of some feeding tentacles [33,34] and the 'apical pore complex' (a collection of extrusive organelles near the anterior end of cells) [9].

The most distinctive innovation in dinoflagellate evolution, however, is the association between a coiled transverse flagellum and a transverse groove called the 'cingulum' [10]. This functional unit, which drives the cell forward during swimming, is absent in perkinsids and intermediately developed in *Oxyrrhis* [35]. This inference is supported by multiple protein phylogenies that show *Oxyrrhis* as the earliest sister lineage to dinoflagellates following the divergence of perkinsids [14] (Box 1). *Oxyrrhis* also provides insights into the evolution of dinoflagellate chromosomes, which remain condensed in interphase and that, apparently, lack histones (highly conserved proteins found in the nucleus of all other known groups of eukaryotes). *Oxyrrhis* appears to have an intermediate state in the development of dinoflagellate chromosomes, because histone-like proteins appear to be present in association with thin, but permanently condensed chromosomes [36]. Histones are also thought to be present in other potentially early diverging dinoflagellates, such as syndinians [37]. Whether these nuclear

Box 2. The early evolution of ciliates

The most distinctive innovations of ciliates are two functionally autonomous nuclei (macronucleus and micronucleus) [61] and a complex cytoskeleton, called the 'kinetome', comprising multiple short flagella or cilia arranged in rows along the longitudinal axis of the cell [62]. The history of these characteristics is largely speculative, because few lineages show intermediate states for either heteromorphic nuclei or the kinetome. However, 'karyorelictids', as the name implies, have plesiomorphic features, such as a nondividing macronucleus, a repeated cortical unit comprising two flagella (dikinetids) and unusual extrusive organelles [63,64]. Serial replication of the flagellar apparatus of an ancestral biflagellate is thought to have given rise to the numerous flagella and associated longitudinal patterning of modern ciliates [62,65]. This cytoskeletal pattern has occurred independently in several derived dinoflagellates, such as *Polykrikos* and possibly *Haplozoon* [62,66]; these organisms are essentially chains of fused cells with a longitudinal row of biflagellar apparatuses. An analogous evolutionary trajectory is suspected to have preceded the modern kinetome of ciliates [62,65].

Enigmatic alveolates, such as *Colponema* and perhaps *Katablepharis*, could provide additional clues about early ciliate evolution. *Colponema* is a biflagellate alveolate that engulfs eukaryotic prey and is, in general terms, very similar to colpodellids. However, *Colponema* lacks obvious rhoptry-like and conoid-like structures (Fig. 2, main text). It also lacks the diagnostic features of dinoflagellates and ciliates. Molecular determination of its phylogenetic position will demonstrate whether the absence of an apical apparatus in *Colponema* was the result of secondary loss or represents the morphostatic character state indicative of the last ancestor of all alveolates.

Katablepharis (Fig. 2, main text) is a biflagellate predator that might have ties to alveolates. This organism has, at one time or another, been allied to cryptomonads, chlorophytes and suctorian ciliates based on the presence of ejectosome-type extrusive organelles, surface scales and distinct patterns of microtubules [57,67]; thus, the relationship of *Katablepharis* to other eukaryotes is far from clear. The phylogenetic position of *Katablepharis* could shed significant light on the evolution of the apical complex. For instance, if it were shown that *Katablepharis* is a close sister lineage to alveolates, then the origin of an apical complex-like apparatus could be pushed back before the divergence of ciliates. This would imply that certain features of ciliates, such as the microtubule arrays in the tentacles of suctorians [67], are homologous to the apical complex of other alveolates.

features represent intermediate states in the evolution of dinoflagellate chromosomes or derived states for the respective lineages has yet to be unambiguously demonstrated with molecular phylogenies.

The first apicomplexans

The ancestors of modern apicomplexans adapted to their obligate intracellular parasitic lifestyle in several ways, including the loss of flagella (except in some microgametes) and the development of gliding motility [38,39]. Perhaps the most notable innovations of apicomplexans are complex life cycles comprising a cell-invasion stage (sporozoite) with a well conserved, closed conoid. Phylogenetic and morphological data demonstrate that the closed conoids of apicomplexans are derived from open-sided conoids, similar to those of colpodellids and perkinsids (Fig. 2, Box 1), although some authors have argued against this conclusion [17].

The most infamous apicomplexans (e.g. *Plasmodium* and *Babesia*) depend on two hosts, with vertebrates being the definitive hosts, and so have received much attention

from the agricultural and medical research communities. However, one major but neglected subgroup of apicomplexans, the gregarines, is thought to infect only invertebrates (and perhaps foraminiferans) and is suspected to have maintained many ancestral characteristics of apicomplexans [40]. For instance, they complete their life cycle in a single host, are common in marine environments, and are usually found in extracellular cavities, such as the coelom or gut lumen. Gregarines, however, show extensive diversity in cytoskeletal morphology and behavior [41]. Molecular phylogenies corroborate the early divergence of these organisms from other apicomplexans, but these studies have also suggested that an important terrestrial vertebrate parasite, *Cryptosporidium*, descended from the gregarine lineage [42–44]. This surprising result highlights the poor state of knowledge regarding gregarines and the importance of documenting the actual diversity of a group before it is collectively deemed plesiomorphic.

Predators, parasites and plastids

As discussed, a mixture of myzocytotic predators and parasites diverge near the nexus of dinoflagellates and apicomplexans, suggesting that the common ancestor of all of these lineages employed a similar mode of life. However, many dinoflagellates and apicomplexans have PLASTIDS, some of which are actively involved in photosynthesis. No trace of a plastid has been detected in ciliates, but many members of the closest sister group to alveolates, the STRAMENOPILES, also contain plastids. Currently, the strongest evidence suggests that the plastids of stramenopiles and alveolates arose from a common endosymbiosis with a red algal prey cell, indicating that the ancestor of all alveolates also contained a plastid of red algal origin [45,46]. If so, then an important question remains. Did these ancestors possess a photosynthetic plastid as in many dinoflagellates or a highly reduced, vestigial plastid such as those found in apicomplexans [47] (Box 3)?

Even if the ancestor of apicomplexans and dinoflagellates was photosynthetic, it was also capable of myzocytosis as suggested by the current phylogenetic framework (Box 1). This dual nutritional mode has been called MIXOTROPHY, which occurs in some modern dinoflagellates [48]. If the common ancestor was mixotrophic, then photosynthesis must have been lost many times in unrelated lineages, a pattern that is well within the boundaries of what has been inferred about the history of dinoflagellates [49]. However, if loss of photosynthesis was rampant, it is difficult to explain why photosynthesis persisted at all along the predatory stem lineage of all alveolates. Conversely, if photosynthesis was lost and the plastid was 'downgraded' to something similar to the apicoplast early in alveolate evolution, then it would be unnecessary to invoke the presence of a photosynthetic plastid in the colpodellid–perkinsid-like ancestor of apicomplexans and dinoflagellates. This fits well with our current understanding of these morphostatic lineages, but leaves the major problem of how roughly half of dinoflagellates became photosynthetic. One possible explanation is that an ancestral dinoflagellate contained a nonphotosynthetic apicoplast-like organelle that was

Box 3. Plastids in human parasites

All known apicomplexans are obligate parasites of other eukaryotes, and most of these are intracellular parasites of animals. It was unexpected, therefore, to find that apicomplexans contain a plastid, the organelle responsible for photosynthesis in plants and algae.

The plastid was observed and documented with electron microscopy on several occasions in different apicomplexans [68], but its significance was not recognized because it lacked the ultrastructural hallmarks of plant plastids; it instead resembled a simple bag surrounded by multiple membranes. In the 1970s, a small circular genome was observed in the malaria parasite *Plasmodium* and, simultaneously, was logically attributed to the mitochondrion [69]. However, when sequences were characterized from this element, they were found to be prokaryotic, but not mitochondrial (the mitochondrial genome was shown to be a 6-kbp linear element) [70]. Eventually, evidence that the circular element was a 35-kbp plastid genome accumulated through sequence comparisons and phylogenetic analyses [70], and this genome has now been shown to localize to a simple organelle bound by four membranes [47], the so-called 'apicoplast'.

Apicomplexan parasites no longer use this organelle for photosynthesis, so what does it do and why was it retained? Plant and algal plastids are much more than photosynthesis machines, being responsible for a variety of other biosynthetic pathways. In apicomplexans, the plastid has been shown to contain plastid-derived enzymes for fatty acid, isoprenoid and heme biosynthetic pathways [71,72]. These activities almost certainly account for the retention of the organelle as the host became increasingly adapted to parasitism.

The discovery of the apicoplast led to a debate as to whether it originated from the plastids of green or red algae. The unique split structure of a nuclear-encoded mitochondrion-targeted gene (*cox2*) found in both apicomplexans and green algae has recently been used as indirect evidence supporting a green algal origin for the apicoplast [73]. However, the strongest molecular evidence based on the order and content of apicoplast genes [70] and the characterization of a plastid (*GAPDH*) gene replacement [46] suggests that the apicoplast is derived from the red algal lineage. This inference is consistent with the phylogenetic position of apicomplexans among other eukaryotes, where apicomplexans are nested within photosynthetic lineages having plastids clearly derived from red algae, such as dinoflagellates, haptophytes and stramenopiles.

replaced by a photosynthetic plastid, derived ultimately from red algae, sometime after their divergence from apicomplexans [50]. This process is called 'plastid replacement', and has occurred several times in certain dinoflagellates [49,51,52]. Distinguishing between these two scenarios is challenging [53] and will require significantly more insight into the nature of dinoflagellate plastids, which are presently among the most poorly understood eukaryotic organelles.

Conclusions

Insights into the phylogenetic positions of organisms such as colpodellids and perkinsids, with near identical swimming cells possessing an open-sided conoid and rhoptries, are vital for inferring the earliest steps toward intracellular parasitism in apicomplexans. Molecular phylogenetic data suggest that colpodellids are the free-living sister group to apicomplexans (all of which are obligate parasites), whereas perkinsids are the parasitic sister group to dinoflagellates (most of which are free-living flagellates). Thus, we can confidently infer that the common ancestor of apicomplexans and dinoflagellates

already had an apical complex involved in the acquisition of nutrients from the cytoplasm of prey cells. The presence and potential role of a plastid in this ancestor is less clear, but remains one of the more interesting questions in alveolate evolution. The discovery and characterization of plastid genomes in diverse alveolates, particularly gregarines, should dramatically improve our current understanding of the enigmatic endosymbiotic history of the apicoplast.

Reconstruction of historical events using the distribution of character states in extant taxa requires synthetic phylogenies based on comparative morphology and sequences from multiple, vertically inherited genes. Robust evolutionary inferences also depend on a comprehensive understanding of the 'true' diversity within extant groups of organisms. Continued research on poorly understood groups of eukaryotic microbes should provide significant insights about contemporary ecosystems and the early history of life. This research depends, in large part, upon explorations into diverse natural environments and the discovery and isolation of the microorganisms. In the case of alveolates, our new understanding of species diversity and phylogenetic relationships is providing a compelling framework for demonstrating morphostasis and major evolutionary transformations. Paleontological data are important assets in these endeavors, but are by no means a necessity. Nonetheless, a more satisfying degree of confidence in the evolutionary scenarios outlined awaits additional morphological details and molecular phylogenetic studies, particularly using protein sequence data, of all of the organisms and organelles mentioned here.

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