ANNUAL REVIEWS

Annual Review of Microbiology

Inside the Host: Understanding the Evolutionary Trajectories of Intracellular Parasitism

Pavla Bartošová-Sojková,[1] Anzhelika Butenko,[1,2,3] Jitka Richtová,[1] Ivan Fiala,[1,2] Miroslav Oborník,[1,2] and Julius Lukeš[1,2]

[1]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic; email: bartosova@paru.cas.cz, jula@paru.cas.cz, obornik@paru.cas.cz

[2]Faculty of Science, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

[3]Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czech Republic

Annu. Rev. Microbiol. 2024. 78:3.1–3.21

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-041222-025305

Keywords

symbiosis, intracellular parasite, parallel evolution, adaptation, genomic features, comparative analysis

Abstract

This review explores the origins of intracellular parasitism, an intriguing facet of symbiosis, where one organism harms its host, potentially becoming deadly. We focus on three distantly related groups of single-celled eukaryotes, namely Kinetoplastea, Holomycota, and Apicomplexa, which contain multiple species-rich lineages of intracellular parasites. Using comparative analysis of morphological, physiological, and molecular features of kinetoplastids, microsporidians, and sporozoans, as well as their closest free-living relatives, we reveal the evolutionary trajectories and adaptations that enabled the transition to intracellular parasitism. Intracellular parasites have evolved various efficient mechanisms for host acquisition and exploitation, allowing them to thrive in a variety of hosts. Each group has developed unique features related to the parasitic lifestyle, involving dedicated protein families associated with host cell invasion, survival, and exit. Indeed, parallel evolution has led to distinct lineages of intracellular parasites employing diverse traits and approaches to achieve similar outcomes.

Contents

1. INTRODUCTION

Symbiosis is a fundamental aspect of life, generally defined as any sustained interaction between two or more different organisms on a continuum from pathogenic to beneficial (60). A particularly fascinating facet of symbiosis is parasitism, arguably the most species-rich form of interaction between organisms on Earth (15). Parasitic strategies stand for those that are harmful to the host. In fact, all symbioses can be understood as parasitic relationships between differently adapted hosts and symbionts at a given evolutionary stage. If we accept this view, mutualism represents a type of parasitic relationship in which the host gained dominance or even full control over the ancestrally parasitic partner. However, when in the mutualistic embrace, one partner gets the upper hand; it evolves into a parasite that exploits the other partner, with the latter becoming a host, for whom the relationship can even become deadly. Nevertheless, it seems that symbiotic relationships always begin as pathogenic or parasitic, reflecting a low level of the mutual adaptation of host and symbiont (60).

The emergence of intracellular parasitism represents a significant milestone in the history of life on Earth, showcasing the adaptability and resourcefulness of parasites in a never-ending arms race with their hosts. Intracellular parasites have evolved highly efficient strategies for invading, proliferating within, and egressing from the host cells (53). Understanding the roots of intracellular parasitism and the intimate host–parasite relationships has had, and surely will continue to have, impact on general molecular and cell biology. Moreover, it is critical for the development of a new generation of drugs and vaccines.

Intracellular parasitism evolved gradually over millions of years, many times independently in various organismal lineages (78, 133), resulting in the extant diversity of intracellular parasites. While the exact mechanisms and pathways of their evolution remain only partially understood, various scenarios, for example, from endosymbiotic theory, evolutionary arms race, horizontal gene transfer (HGT), host–parasite coevolution, and transition from commensalism, have been extensively discussed as driving forces behind the emergence of intracellular parasitism (14, 61, 67, 93, 105). Analyses of numerous parasite genomes have pinpointed the expansions of certain gene families, alongside the reductions or complete losses of other families, resulting in the (extreme) streamlining of some parasite genomes (22, 136). A parallel can be drawn between the parasites

and the domesticated microbial prokaryotes and eukaryotes used for food production, such as *Escherichia coli*, *Saccharomyces cerevisiae*, and other bacteria and fungi. Compared with their wild counterparts, these domesticated strains have restructured their metabolism to exploit a specific and abundant set of selected nutrients in the medium, losing certain genes and becoming unable to thrive in the wild (20, 121). Hence, parasitism appears to employ mechanisms reminiscent of those observed during the domestication of food-making microbes. Indeed, living inside another organism or in a rich medium is more convenient because the availability of nutrients allows for streamlining of the metabolism and the dynamic expansion, acquisition, and loss of certain gene sets. Naturally, unlike a microbe in a medium, a parasite must additionally resist the host's immune system and invent its own adaptations to be one step ahead of its host, as nicely encapsulated by the Red Queen hypothesis (120).

In this review we closely examine the origins of intracellular parasitism, drawing insights from a comparison of morphological, physiological, and genomic features found in three distantly related groups of single-celled (protistan) intracellular parasites. By comparative analyses of these traits, contrasted against their closest free-living relatives, we spotlight a set of unique adaptations of these parasites that facilitated or perhaps triggered the shift to intracellular parasitism. We focus on (*a*) Kinetoplastea, with particular attention paid to parasitic trypanosomatid flagellates, using free-living bodonids for comparative purposes; (*b*) Holomycota, with an emphasis on parasitic microsporidians and early-branching free-living nucleariids; and (*c*) Apicomplexa, represented by a mixture of heterotrophic symbionts, parasites, and mutualists placed within the apicomonads and sporozoans. Due to a substantial amount of available information, these three groups are particularly suitable for such a comparative analysis.

In the last section, we synthesize features shared among these three disparate groups, highlighting instances where their members successfully transitioned to intracellular parasitism. Our review aids in identifying knowledge gaps in this area and provides insights into the intricate evolutionary trajectories and adaptations of intracellular parasites, shedding light on their remarkable diversity and ecological versatility.

2. KINETOPLASTEA

2.1. Exploring Transitions to Intracellular Symbiosis: Kinetoplastea as a Model Group

Kinetoplastea represent a highly diverse and well-studied clade within the phylum Euglenozoa (62). They possess a plethora of features initially considered unique among eukaryotes, such as the paraflagellar rod, polycistronic transcription, ubiquitous *trans*-splicing and extremely rare *cis*-splicing, extensive circular mitochondrial DNA, and mitochondrial RNA editing, to name just the most prominent ones (77). However, these features were subsequently discovered in other lineages, including the multicellular eukaryotes (70). Divided into five orders (Eubodonida, Neobodonida, Parabodonida, Prokinetoplastida, and Trypanosomatida) (87), kinetoplastids thrive in diverse habitats, from marine and freshwater environments to deep-sea sediments and soil (37, 88, 112). They exhibit remarkable evolutionary plasticity and readily form symbiotic relationships with intracellular bacteria (51). Moreover, kinetoplastids have engaged in symbiotic relationships with eukaryotic hosts on multiple occasions, including neobodonids *Allobodo* (seaweed parasites) and *Azumiobodo* (ascidian parasites), parabodonids *Cryptobia* (invertebrate parasites) and *Trypanoplasma* (fish endoparasites), prokinetoplastids *Ichthyobodo* (fish ectoparasites) and *Perkinsela* (obligate amoeba endosymbionts), and trypanosomatids (intra- and extracellular parasites of vertebrates, invertebrates, plants, and protists) (46, 62). The sole kinetoplastid group exclusively containing free-living representatives is the monogeneric order Eubodonida (87). Here we focus primarily

on intracellular kinetoplastids and their closest relatives. For a thorough overview of other lifestyle transitions within this group, readers are referred to several comprehensive reviews (62, 69, 139).

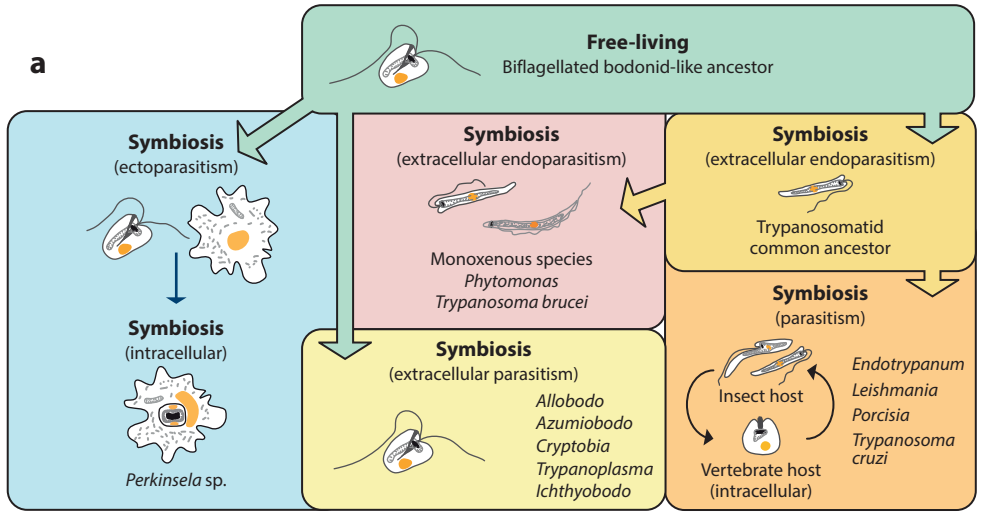
The phylogeny and lifestyles of Kinetoplastea indicate that a common ancestor of this group was a bodonid-like organism with the ability to attach to various surfaces, such as water plant leaves (12, 68) (**Figure 1*a***). It possessed a compact nuclear genome with almost no *cis*-spliced introns (12, 54, 125). This protist was a biflagellated bacterivore with gliding motility, which used a cytostome–cytopharynx complex for food intake, traits shared by many extant free-living kinetoplastids (12, 36). The ability for intracellular invasion evolved at least once within the order Prokinetoplastida (*Perkinsela*) and multiple times within the order Trypanosomatida (34, 62), including representatives of the subfamily Leishmaniinae, and the genera *Trypanosoma* and *Herpetomonas* (3, 9, 38, 62, 65). Here, we focus primarily on *Trypanosoma cruzi* (subgenus *Schizotrypanum*), *Leishmania*, and *Perkinsela*, which have been extensively studied at the molecular level.

Symbiotic relationships between two nonphotosynthetic unicellular eukaryotes are very rare. Only a few cases were reported, with some of them found in Kinetoplastea (34, 38). One example is *Perkinsela* living inside an amoeban host (34). The common ancestor of *Perkinsela* and its closest relative, *Ichthyobodo*, was likely an ectoparasite of a marine animal coinfected with an amoeba. Proximity of both interacting organisms then facilitated transition to intracellular symbiosis (139) (**Figure 1*a***). This relationship represents the earliest known case of intracellular symbiosis in Kinetoplastea, with *Perkinsela* diverging from other euglenozoans ∼500–950 Mya (12). The congruence of 18S rRNA-based phylogenies of *Perkinsela* and its hosts suggests a single ancestral switch to the intracellular lifestyle, persisting through vertical inheritance of the endosymbiont (117). In another intracellular symbiosis between two nonphotosynthetic protists, a trypanosomatid from the genus *Herpetomonas* resides inside a macronucleus of the ciliate *Euplotes encysticus*, which might have become parasitized through the consumption of an infected aquatic arthropod. This relationship severely affects the ciliate's growth, suggesting its parasitic nature (38).

Some trypanosomatids have complex life cycles (9) and are responsible for serious human and cattle diseases, such as Chagas disease (*T. cruzi*) and leishmaniases (*Leishmania*) (32). The common ancestor of Trypanosomatidae, likely an extracellular parasite of arthropods (69), is estimated to have existed ∼450 Mya. It acquired the ability to establish transient and then obligatory symbiotic relationships with invertebrates (12) (**Figure 1*a***). Of the 20 recognized trypanosomatid genera (2), most accommodate monoxenous parasites limited to a single host, typically an insect (66). Their radiation was accompanied by an extensive expansion of the host range, including blood-sucking invertebrates, which likely facilitated transmission to vertebrates (68). These dixenous (= two-host) Trypanosomatidae (66) started circulating between the arthropod and vertebrate hosts. This is likely the case of *T. cruzi*, which circulated primarily among the reduviid bugs, being occasionally transmitted to vertebrates during a blood meal. Over time, *T. cruzi* developed a complex life cycle with multiple stages within these bugs, with only the metacyclic trypomastigotes transmitted to the vertebrate host, where they invade various types of nucleated cells (76). *Leishmania* and its closest relatives, *Porcisia* and *Endotrypanum*, followed a similar path. They have been transmitted by blood-sucking sand flies to mammals, where they reside inside erythrocytes (*Endotrypanum*); macrophages (*Leishmania*); and various tissues, such as epidermis, liver, and spleen (*Porcisia*) (62).

2.2. Evolution of Traits Associated with Intracellular Symbiosis in Kinetoplastea

Identifying specific morphological and molecular traits associated with the intracellular lifestyle is challenging. Parasites, including intracellular ones, undergo compaction of their often AT-rich nuclear and organellar genomes, metabolic streamlining, expansion of existing gene families or acquisition of new genes related to host cell invasion, survival within and exit from the host cell



b

	Morphology			Feeding		Surface proteins					Metabolic pathways			Transporters		Other	
	Flagellum	Amastigote stage*	Cytostome–cytopharynx	Endocytosis	Osmotrophy	Amastins	Mucins	MASP	*trans*-sialidases	Protease gp63	Heme biosynthesis**	Mannosyltransferase-like	Phosphomannomutase	Heme transporter LHR1/HTE	Iron transporter LIT1	Flagellum/associated genes	Protein A2
Leishmania	■	■	■	■	■	■	■	■	■	■	■	■	■	■	◪	■	◪
Endotrypanum	■	■	?	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Porcisia	■	■	?	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Novymonas	■	■	?	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Zelonia	■	■	?	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Borovskyia	■	■	?	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Crithidia	■	■	?	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Leptomonas	■	◪	?	?	■	■	■	■	■	■	■	■	■	■	◪	■	■
Strigomonadinae	■	?	?	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Herpetomonadinae	■	■	?	?	■	■	■	■	■	■	■	◪	■	◪	■	■	■
Blastocrithidiinae	■	■	?	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Blechomonadinae	■	■	?	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Trypanozoon	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Schizotrypanum	■	■	■	■	■	■	■	■	■	■	■	■	■	◪	■	■	■
Paratrypanosomatinae	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Eubodonida	■	■	■	■	?	■	■	■	■	■	■	■	■	■	■	■	■
Parabodonida	■	■	■	?	■	■	■	■	■	■	■	■	■	■	■	■	■
Neobodonida	■	◪	■	■	?	■	■	■	■	■	■	■	■	■	■	■	■
Perkinsela	■	■	■	■	?	■	■	■	■	■	■	■	■	■	■	■	■
Ichthyobodo	■	■	■	■	?	?	?	?	?	?	?	?	?	?	?	?	?
Other Prokinetoplastina	■	■	■	■	?	■	■	■	■	■	■	■	■	■	■	■	■
Diplonemea	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Intracellular — Intracellular in some species — ? Missing data — ■ Present — ■ Absent — ◪ Present in some species

(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Evolution of intracellular parasitism in Kinetoplastea. (*a*) A bodonid-like ancestor of Kinetoplastea initially attached to various surfaces, such as water plant leaves. Through evolutionary changes, it developed transient and then obligatory symbiotic relationships with invertebrates, leading to a common ancestor of Trypanosomatidae, likely an extracellular parasite of arthropods. Monoxenous Trypanosomatidae diversified, expanding their host range to blood-sucking invertebrates, enabling transmission to vertebrate hosts. The common ancestor of *Schizotrypanum* (*Trypanosoma cruzi*), as well as the ancestor of *Leishmania*, *Porcisia*, and *Endotrypanum*, acquired the ability to enter the vertebrate host cells while residing in an insect vector's extracellular milieu. *Perkinsela* sp. living inside its *Paramoeba* host is another example of intracellular parasitism having emerged in Kinetoplastea. (*b*) A cladogram of Kinetoplastea, based on data from References 2 and 62, depicting multiple independent switches to intracellular parasitism and the distribution of associated morphological, physiological, and molecular features. The transition to the intracellular lifestyle occurred in Prokinetoplastina (*Perkinsela* sp.); *T. cruzi*; and the common ancestor of *Endotrypanum*, *Leishmania*, and *Porcisia*. While most described *Herpetomonas* species are known as extracellular insect parasites, one species was found parasitizing the ciliate's macronucleus. Trait states were determined through literature research or gene analysis by standalone BLAST and hidden Markov model–based searches using HMMER version 3.4. Abbreviations: HTE, *T. cruzi* heme transport enhancer; LHR1, *Leishmania* heme response 1; LIT1, *Leishmania* iron transporter 1; MASP, mucin-associated surface protein. A single asterisk indicates the flagellum is not observed but might exist under certain conditions. Double asterisks indicate whether two or more of eight enzymes were not identified; the pathway was categorized as absent. The final three pathway enzymes are encoded in Leishmaniinae genomes, while *Phytomonas* species (Herpetomonadinae) possess only ferrochelatase. *Perkinsela* lacks uroporphyrinogen synthase, which is instead encoded in the host amoeba's genome.

(16, 45, 105). They develop specific sensory and attachment structures or alter their feeding modes by shifting toward osmotrophy, frequently encyst, and lose their flagellum sometimes even with its associated genes (16, 45, 105). Parasitic Kinetoplastea lost 1,3-β-glucan biosynthesis but expanded their repertoire of amastins, gp63 metalloproteases, subtilisins and peptidases, cell surface permeases, and transporters (**Figure 1*b***). Metabolic streamlining likely was a multistep process not directly linked to the origin of parasitism (12).

The level of integration between *Perkinsela* and host amoebae is remarkably high and exemplified by an initial consideration of the parasite to be a host organelle (34). *Perkinsela* is typically binuclear, resides directly within the host cell cytoplasm in the absence of the parasitophorous membrane, is vertically inherited, and cannot be cultivated without its host (34). Though it shares several features with other kinetoplastids, such as polycistronic transcription, spliced leader *trans*-splicing, kinetoplast DNA, and glycosome-like organelle, *Perkinsela* has lost the flagellum and flagellum-related genes (124). This highly derived lineage lacks most ancestral kinetoplastid genes, and the role of HGT to the host amoeba genome seems minimal. *Perkinsela* indeed possesses the smallest and most gene-poor genome among the studied kinetoplastids. Metabolic pathways of the symbiotic partners are intertwined, suggesting an exchange of metabolites through protein secretion by *Perkinsela* into the host cytoplasm, as well as its endocytosis (124). Although the kinetoplast genome of *Perkinsela* has been extensively reduced, it still retains all components of the complex uridine insertion/deletion RNA editing (30), and its organelle contains more DNA than that of any other known eukaryote (71). Approximately 14% of its nuclear genes encode proteins targeted to its single giant mitochondrion (124), possibly providing energy to both the kinetoplastids and its host. However, experimental evidence for this is currently lacking, and the nature of this symbiotic relationship, whether it is mutualistic, commensal, or parasitic, remains to be elucidated.

In contrast to the obligate intracellular *Perkinsela*, most dixenous trypanosomatids exhibit complex life cycles involving the intracellular stages that also vary in host cell invasion mechanisms (77). For instance, *Leishmania* primarily targets phagocytic cells, especially macrophages,

while *T. cruzi* invades virtually any nucleated cell type, mostly muscle and endothelial cells (6, 9). These trypanosomatids also employ different mechanisms of cell invasion. *Leishmania* enters cells through host cell–mediated phagocytosis, while *T. cruzi* uses lysosome-dependent or lysosome-independent pathways (6, 118). *Leishmania* amastigotes exhibit resistance to the hydrolytic environment within the phagolysosome, where they can replicate and differentiate, raising the possibility that amastigotes represent cyst-like forms that are resilient to harsh environmental conditions (118). In contrast, *T. cruzi* temporarily resides in the host-derived parasitophorous vacuole before being released into the cytoplasm for replication (9). Both *Leishmania* and *Trypanosoma* release exosomes and microvesicles into the host cells to manipulate their immune response (21). It is unclear whether there are significant differences in secretion between the intracellular and exclusively extracellular kinetoplastids. Some intracellular species, such as *T. cruzi*, retain ancestral feeding apparatus for phagocytotic nutrient uptake via the cytostome–cytopharynx complex. On the other hand, *Leishmania* has lost this structure and feeds by osmotrophy, with endocytosis occurring within the flagellar pocket (51) (**Figure 1*b***).

Intracellular trypanosomatids lack specialized morphological structures for host cell penetration, instead dedicating an array of molecular tools for this purpose. These tools include *trans*-sialidases and *trans*-sialidase-like proteins, mucins, and mucin-associated proteins in *T. cruzi* and iron transporter 1, heme transporter 1, and surface protein A2 in *Leishmania*, all of which are linked to intracellular parasitism (6, 31) (**Figure 1*b***). *Trans*-sialidases, which facilitate the transfer of sialic acid from host donor proteins to parasite mucins and contribute to host cell exit, were subject to expansion in species with the intracellular stages compared with the exclusively extracellular flagellates, such as *Trypanosoma brucei* (6, 66). Putative homologs of these proteins were identified in *Porcisia* and *Endotrypanum*; their role has yet to be determined (**Figure 1*b***). Mucins, another protein family that was subject to expansion in *T. cruzi*, act as primary sialic acid acceptors that promote internalization (31).

Additionally, mannose-metabolism-related proteins have been linked to the intracellular lifestyle in trypanosomatids (6). However, our analyses demonstrate the presence of genes encoding mannosyltransferase-like protein and phosphomannomutase also in kinetoplastids with the extracellular lifestyle. Nevertheless, it is not just the presence or absence of specific genes in the genome that matters, as the diversity of the gene repertoire is also highly relevant and is associated with the type of symbiosis (12). For example, amastins, a family of cell surface proteins crucial for the propagation of *Leishmania* amastigotes inside vertebrate macrophages, are present in all analyzed parasitic and free-living kinetoplastids except *Perkinsela*. Yet genes encoding amastins are significantly expanded in the genus *Leishmania* (12) (**Figure 1*b***). Intracellular *Endotrypanum* and *Porcisia*, which do not infect macrophages (3), exhibit a reduction in the amastin family, particularly δ-amastins. An expanded set of highly expressed repeat-rich proteins are associated with the intracellular lifestyle across various protist lineages (81). Intracellular flagellates, such as *Leishmania* with A2 proteins and *T. cruzi* with mucins, feature examples of these repetitive proteins.

3. HOLOMYCOTA

3.1. Exploring Transitions to Intracellular Parasitism: Microsporidia as a Model Group

Microsporidia, fungi, and several other lineages form a diverse clade within the Opisthokonta known as Holomycota (41). This group encompasses organisms with distinct lifestyles, including free-living, mutualistic, parasitoid, and true parasitic forms (8, 42). The earliest holomycotan lineage to emerge is the nucleariids (Nucleariida, Cristidiscoidea), represented by *Fonticula* and *Nuclearia*. These nonflagellated amoebae are primarily free-living, feeding on bacteria and green

algae through phagotrophy, similar to the ancestral holomycotan (41, 42). While some nucleariids have been found in animals, it remains unclear whether they can act as opportunistic pathogens and affect their health (35, 40, 43). As an early-diverging lineage, typically with the free-living lifestyle, nucleariids are essential for understanding the origin of intracellular parasitism in holomycotans.

The common ancestors of subsequent holomycotan lineages retained their phagotrophic feeding mode but underwent further specialization. The group Phytophagea (fungi and their sister lineage Aphelida) became endobiotic predators of cellulose-containing algae, while Opisthophagea (Microsporidia and their sister lineage Rozellida) shifted to parasitism, primarily in chitin-containing hosts. Although extant rozellids and aphelids remain phagotrophs, fungi and microsporidians switched to osmotrophy (42, 82, 127), with the former typically acting as saprotrophs or external parasites (72, 91, 138). In fungi, intracellular parasitism has been reported occasionally for sanchytrids of algae (41); chytrid parasites of plants (129); and thermally dimorphic fungi like *Blastomyces*, *Cryptococcus*, and *Histoplasma* that infect humans and other warm-blooded animals (10, 80, 89, 114). While the aphelids (e.g., *Aphelidium* and *Paraphelidium*) are usually intracellular algal parasitoids (42, 57, 82, 115, 127), rozellids (Cryptomycota or Rozellomycota) occupy mainly the cytoplasm or nucleus of other opisthokonts, such as oomycetes, fungi (*Rozella*, which also infects algae), crustaceans (*Mitosporidium*), and amoebae (*Nucleophaga*, *Morellospora*, *Paramicrosporidium*) (24, 25, 48, 106). The evolutionary line from rozellids to microsporidians is marked by several independent early-diverging microsporidian lineages, including intracellular chytridiopsids and metchnikovellids (44). Chytridiopsids (e.g., *Acarispora* and *Chytridiopsis*) parasitize mainly the gut epithelium of terrestrial arthropods like insects, myriapods, mites, and earthworms and rarely aquatic invertebrates (26, 64, 107). Metchnikovellids (e.g., *Amphiamblys* and *Metchnikovella*) are specialized hyperparasites (parasites that parasitize other parasites) of gregarines found in marine invertebrates (44, 64, 83). The remainder of holomycotans constitutes a diverse group of core (typical) Microsporidia (**Figure 2*a***). They are extremely species-rich, obligate intracytoplasmic, and rarely intranuclear parasites that infect a variety of hosts, primarily insects but also vertebrates, including humans and agriculturally important animals (130). With their unique morphological and molecular features and extensive genomic data available, microsporidians qualify as prime candidates for the study of evolution of intracellular parasitism.

3.2. Evolution of Traits Associated with Intracellular Parasitism in Holomycota

As mentioned above, intracellular parasitism often leads to genome reduction and gene content changes (105). This phenomenon is firmly fingerprinted in Holomycota and spans from relatively large unreduced genomes of nucleariids (98) and aphelid parasitoids (82) to some of the most compacted eukaryotic genomes of microsporidians (83, 106, 132). The impact of parasitism-driven reductive genome evolution is also evident in fungi, with algal parasites, the sanchytrids, having much smaller, AT-rich genomes than their saprotrophic relatives (41). However, not all parasitic fungi follow this pattern, as some dimorphic fungal pathogens have highly expanded genomes (89). Similarly, genome size also varies within Microsporidia, with the largest gene-sparse genomes surpassing in their size the smallest gene-dense genomes by at least one order of magnitude (59, 132). This variability has been attributed to the differences in gene density and the presence of overlapping genes in the extremely streamlined microsporidian genomes (29). Notably, microsporidians have lost many spliceosomal introns, although some functional long introns have been retained in their reduced genomes (134, 141). It has been proposed that such genomic diversification is linked to variations in transmission modes of Microsporidia (49). Overall, genome evolution in holomycotans is mosaic and influenced by numerous factors, such as HGT, gene losses and gains,

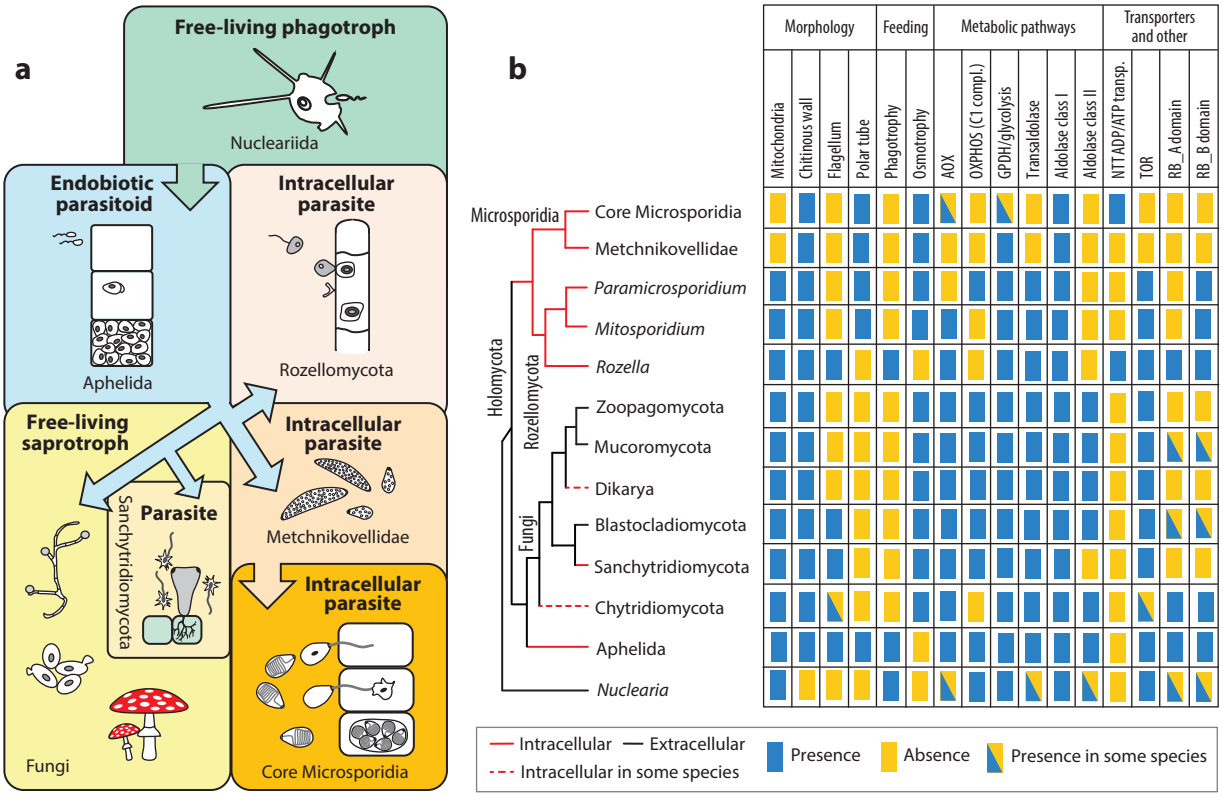


Figure 2

Evolution of intracellular parasitism in Holomycota. (*a*) The holomycotan ancestor was a free-living phagotroph, preying on bacteria and green algae. Subsequent lineages either maintained their original phagotrophic feeding mode or adopted roles as saprotrophs or parasites, targeting primarily hosts with chitinous cell walls. Aphelida and Sanchytridiomycota specialized as intracellular algal parasites, while the ancestor of Rozellomycota and Microsporidia experienced extensive diversification, expanding the host range to include amoebae (*Nucleophaga*, *Paramicrosporidium*, *Morellospora*) and opisthokont cells, initially of fungal and invertebrate hosts (*Rozella*, *Mitosporidium*, chytridiopsids, metchnikovellids, and some core Microsporidia), and later extending to vertebrate hosts (majority of core Microsporidia). (*b*) A cladogram of Holomycota based on data from References 24, 41, and 126 depicting multiple independent switches to intracellular parasitism and the distribution of associated morphological, physiological, and molecular features. The transition to the intracellular lifestyle occurred in Aphelida, Sanchytridiomycota, and the common ancestor of Microsporidia and Rozellomycota. Though most Dikarya and Chytridiomycota species function as saprotrophs or external pathogens, certain genera such as *Blastomyces*, *Cryptococcus*, *Histoplasma*, and *Synchytrium* have developed into intracellular parasites of plants and animals. The trait states were determined through literature research or by mining the genes from publicly available holomycotan genomes using BLAST implemented in the Geneious Prime software version 2019.0.4 (58); hits were verified by hmmscan in HMMER web server version 3.3.2 (104) and by reciprocal BLAST on the NCBI server. Abbreviations: AOX, alternative oxidase; GPDH, glycerol-3-phosphate dehydrogenase; NTT ADP/ATP transp., nucleotide ADP/ATP transporters; OXPHOS (C1 compl.), C1 complex of the oxidative phosphorylation machinery; RB_A, retinoblastoma A domain; RB_B, retinoblastoma B domain.

likely associated with adaptations to different parasitic strategies and pathogenesis or multiple independent transitions to parasitism (8, 89, 106).

Unlike free-living nucleariids, microsporidians essentially rely on host resources and use highly resistant chitinous-walled spores to persist in the external environment (48, 82). They employ specialized morphological structures and sophisticated strategies for host cell invasion (53, 123). To survive and thrive in the host, microsporidians have acquired new genes or even gene families to compensate for the loss of metabolic enzymes and regulatory pathways present in their

free-living ancestors or nonparasitic relatives (123, 132) (**Figure 2*b***). For host infection and further dissemination, core microsporidians developed in their spores a highly efficient injection apparatus represented by a typically long polar tube that rapidly discharges and actively pierces the host cell, depositing infectious sporoplasm (23, 50). Primitive microsporidia, such as metchnikovellids and chytridiopsids, have simpler injection apparatuses consisting of only a basic polar filament, the manubrium, and a short, coiled polar filament, respectively. Dissemination to new hosts requires sac-bound spores, which are ingested with food and are thus delivered close to the host cells (64). The likely ancestral strategy of rozellids is similar to that found in aphelids. In both groups, a flagellated zoospore swims toward a walled algal or water mold host on which it encysts and penetrates. Alternatively, the intranuclear (*Nucleophaga*, *Paramicrosporidium*) and intracytoplasmic (*Mitosporidium*, *Morellospora*) rozellids form an immobile walled spore that resides in the environment until it is engulfed by a phagocytic host (23). Similar to microsporidians, the rozellid spores contain a preformed polar filament and associated structures. However, their polar tube is not directly involved in host invasion, as it becomes activated only once the spore enters the phagocytic vacuole, upon which the polar tube facilitates the invasion of host cell compartments (23). Pathogenic fungi enter host cells by various mechanisms, such as complement receptor–mediated phagocytosis of macrophages (84), induced endocytosis, or active penetration of the epithelial cells (116).

To ensure efficient intracellular growth within the host, microsporidians undergo accelerated life cycles, remarkably similar to those of cancer cells (13). Already the basal metchnikovellids have lost both the A and B domains of the tumor-suppressor gene retinoblastoma (RB), a protein that in other eukaryotes participates in the conserved RB–E2F pathway, which regulates the cell cycle and cell proliferation (13). While nucleariids (except *Fonticula alba*), aphelids, and some fungi (mostly chytrids) possess the RB_A and RB_B domains (29, 48), most fungal groups lack the entire pathway and rely on alternative cell cycle regulation involving SBF/MBF transcription factors acquired through virus-mediated HGT (29, 75). Rozellids exhibit a stepwise loss of RB, with the early-branching *Rozella allomycis* still possessing both RB domains. However, more derived species, such as *Mitosporidium daphniae* and *Paramicrosporidium saccamoebae*, retain only the RB_B domain (**Figure 2*b***). It is plausible that in rozellids and microsporidians the loss of an RB domain(s) enables uncontrolled fast replication and shortening of the generation time (130).

Due to the extreme functional reduction, particularly during their intracellular development, microsporidians rely on stealing energy from their hosts (67, 128). Spores of most species, except some Enterocytozoonidae and Hepatosporidae, generate ATP through glycolysis involving the alternative oxidase and glycerol-3-phosphate shuttle (126) (**Figure 2*b***). They import host-synthesized purine nucleotides and NAD using surface-located nucleotide transporters (NTTs), which belong to the major facilitator superfamily (MFS), acquired through HGT from bacteria (67, 83, 132, 135). Moreover, microsporidians also acquired additional NTTs for the exchange and symport of host purine nucleotides such as GTP and GDP, nucleosides, and nicotinamides (29, 33, 52, 83, 103).

Energy parasitism in Holomycota varies on the basis of transporter types and mitochondrial genome complexity (**Figure 2*b***). Reconstructions of ancestral sequences indicate that acquisitions of ATP/ADP NTTs by the ancestor of rozellids and microsporidians (33) were followed by independent gene losses in metchnikovellids and some rozellids (*M. daphniae*, *P. saccamoebae*) (74) (**Figure 2*b***). Alternatively, an independent gain of these transporters may have occurred exclusively in *R. allomycis* and microsporidians (48, 82, 126). This transporter family is typically found in holomycotans with mitochondria lacking complex 1 of the oxidative phosphorylation pathway (*R. allomycis*) or (putatively) lacking the entire organellar genome (Microsporidia) (33, 55). However, exceptions exist as demonstrated by metchnikovellids and *M. daphniae*, which lack both complex 1 and ATP/ADP NTTs (106) (**Figure 2*b***).

Holomycotans lacking NTTs utilize an alternative set of MFS transporters dedicated to the import of ATP and GTP. While these transporters are not unique to holomycotans, as they are widespread in other eukaryotes as well as prokaryotes, they were already extremely diversified in the ancestor of Microsporidia and Rozellomycota (74). Additionally, similar to other parasitic eukaryotes, both groups have acquired numerous genes via HGT, such as amino acid permeases or folate-related genes, mainly involved in nucleic acid and amino acid synthesis and salvage (4, 67, 103, 106). To meet their high metabolic demands associated with massive proliferation in the host, microsporidians utilize hexokinase, which has acquired a secretion signal sequence for targeting to the host cell, enhancing the production of amino acids, lipids, and nucleotides (29). Microsporidians and their relatives have various lineage- and genus-specific adaptations that aid in host invasion and adjustment to the intracellular lifestyle (53, 123, 132) (**Figure 2*b***), but detailing them is beyond the scope of this review.

4. APICOMPLEXA

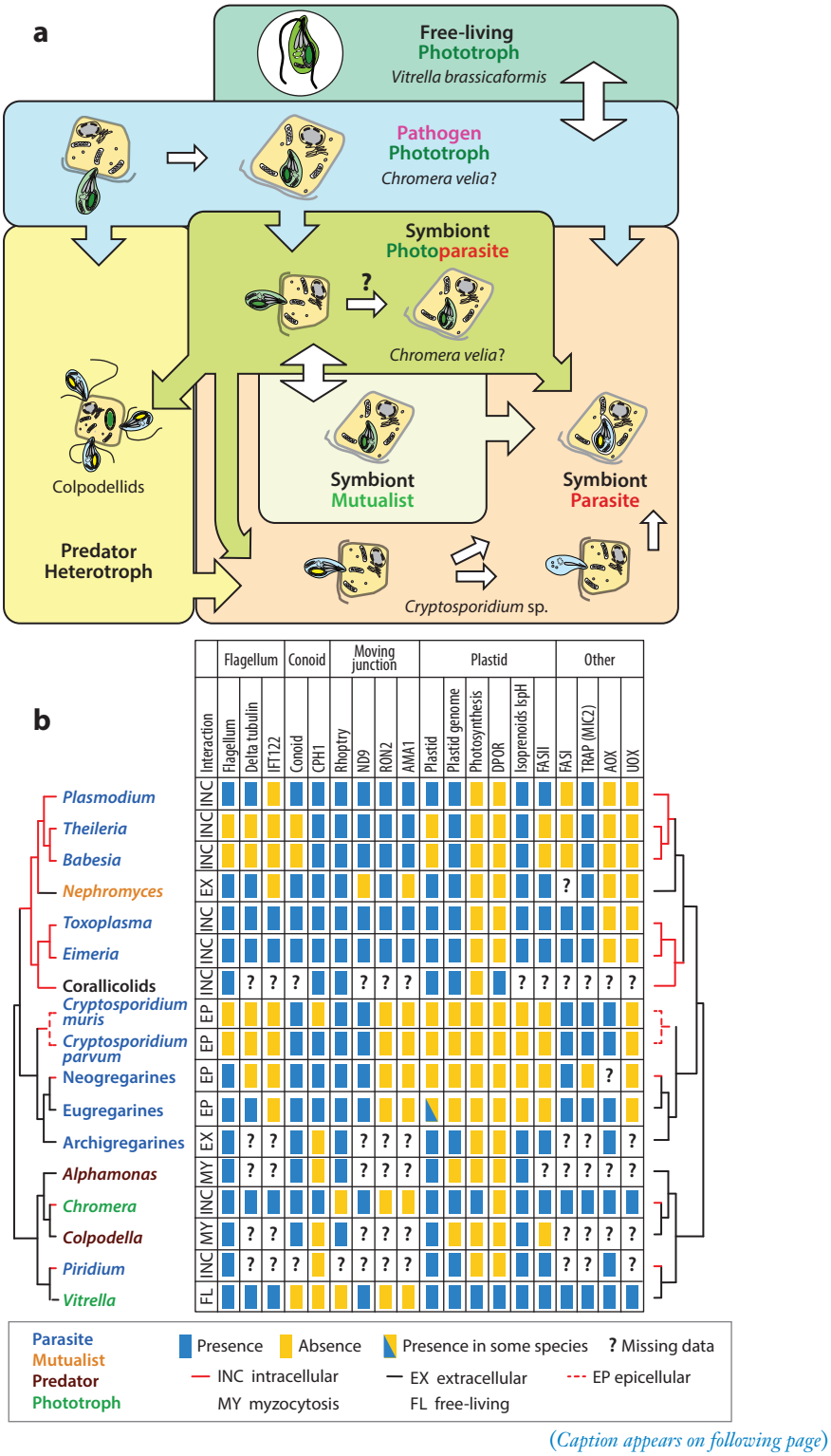
4.1. Exploring Transitions to Intracellular Symbiosis: Apicomplexa as a Model Group

Apicomplexa (as defined in 19) are a highly diverse group of protists equipped with an apical complex at the anterior apex that allows penetration of the host cell or prey. A homologous structure also occurs in Dinozoa (100), and an analogous feeding apparatus in the phylogenetically distant katablepharids has been recognized (99). Within apicomplexans, the host–parasite relationship is either evolutionarily conserved, such as the obligate parasitism in Sporozoa, or greatly varies within certain clades, as in Apicomonada (chrompodellids), that are either photosynthetic (chromerids), predatory (colpodellids), or parasitic (*Piridium sociabile*).

Sporozoa are a species-rich group predominantly encompassing obligate parasites of animals, formerly classified as Apicomplexa sensu stricto (131). They are responsible for many diseases, including malaria, toxoplasmosis, cryptosporidiosis, babesiosis, theileriosis, and eimerioses. Sporozoans are traditionally classified into Coccidia, Cryptosporidida, Gregarinida, Haemosporidia, and Piroplasmida (131), showcasing a gradual transition from extracellular (archigregarines), via epicellular (*Cryptosporidium*), to intracellular (Coccidia, Haemosporidia, and Piroplasmida) relationships with the host (**Figure 3*a***). In the much less speciose Apicomonada, parasitism evolved in parallel, as exemplified by *P. sociabile* (78), *Platyproteum* (56), and the photoparasite *Chromera velia* (94). While the apicomplexan ancestor originally relied on phototrophy, this process is retained only in chromerids (**Figure 3*a*,*b***). However, most Apicomplexa use their photosynthetic (chromerids) or nonphotosynthetic (Sporozoa and most Apicomonada) plastids to support their metabolism (56, 78, 93, 111). In most sporozoans, a plastid that lost its photosynthetic functions, termed an apicoplast, is essential for the parasite's survival and represents a prospective drug target. Hence, apicomplexans are an excellent model to study the evolutionary transition(s) from phototrophs to mixotrophs, predators, mutualists, and parasites (94). Here, we give outsized attention to Apicomonada, as they are highly relevant for understanding the evolution of intracellular parasitism.

4.2. Evolution of Traits Associated with Intracellular Parasitism in Apicomplexa

Chromerids, algae with a complex rhodophyte-derived plastid, represent the photosynthetic apicomplexans. The group is polyphyletic and its two described species, *C. velia* and *Vitrella brassicaformis*, differ considerably in morphology, life cycles, and genomes, challenging the determination of chromerid general characteristics (94, 96, 137). Whereas *C. velia* displays an apical complex with a reduced preconoid (97), indicating a possible symbiotic interaction with the host,



(Caption appears on following page)

Figure 3 (*Figure appears on preceding page*)

Evolution of intracellular parasitism in Apicomplexa. (*a*) The ancestor of Apicomplexa was a free-living phototroph. The need for nitrogen forced the alga to either hunt microbial prey, exist as an ectosymbiotic photoparasite, or invade a host (like *Chromera velia*). In theory, the intracellular photoparasite could shift between mutualist and parasitic states, as seen in dinoflagellate symbionts of corals. The photoparasite could lose photosynthesis due to infection of opaque host or gain organic carbon–scavenging abilities. Similarly, the mixotrophic predator could lose photosynthesis and become a typical predator, like colpodellids. Extracellular parasitism and its intracellular form may have eventually evolved from the predatory ancestor. This entire symbiotic system is dynamic, with parasitic and mutualistic forms changing in response to the level of host adaptation. (*b*) Cladograms (based on data from Reference 90), depicting two scenarios for the evolution of intracellular parasitism in Apicomplexa and the distribution of associated morphological, physiological, and molecular features. (*Left*) Intracellular parasitism arose twice in Apicomonada and twice in Sporozoa, suggesting a single origin of the intracellular lifestyle in Coccidia, Haemosporidia, and Piroplasmida and its consequent loss in *Nephromyces*. (*Right*) Alternatively, intracellular parasitism occurred three times in Sporozoa, and *Nephromyces*, an ectosymbiotic mutualist of marine polychaetes, represents an ancestral state. The trait states were determined through literature research or by mining the genes from publicly available genomes using standalone BLAST. Abbreviations: AMA1, apical membrane antigen 1; AOX, alternative oxidase; CPH1, conoid protein hub 1; DPOR, light-independent protochlorophyllide reductase; FAS, fatty acid synthase; IFT122, intraflagellar transport protein 122; IspH, isoprenoid precursor H; MIC2, microneme protein 2; ND9, nondischarged protein 9; RON2, rhoptry neck protein 2; TRAP, thrombospondin-related anonymous protein; UOX, urate oxidase.

V. brassicaformis lacks any ultrastructural evidence of this complex. Importantly, *V. brassicaformis* has a complex life cycle that, unlike that of *C. velia*, includes a sexual stage (39). Due to their isolation from corals (86), chromerids were initially considered to be mutualistic symbionts, like dinoflagellates of the genus *Symbiodinium* (101). However, *C. velia*, found intracellularly in the coral larvae (28), engages in mixotrophic photoparasitism, combining phototrophy and parasitism, a phenomenon also observed in some dinoflagellates (e.g., *Blastodinium*, *Piscinoodinium*) and parasitic plants (e.g., *Cuscuta*) (85, 94). This photoparasitic behavior was suggested to be driven by the need for nitrogen and might represent an intermediate stage in the evolution of parasitism in Apicomplexa (94).

Apicomonads have lost photosynthesis at least three times in the course of evolution (56, 78, 94). Two independent losses led to the emergence of the predatory colpodellids (18, 95), which adapted their apical complex to hunt microbes. However, the main difference between colpodellids and early-branching sporozoans is the size of their prey and hosts. Colpodellids target unicellular prey, whereas early sporozoans such as gregarines and *Cryptosporidium* invade the gut cells of their multicellular hosts (94). Consequently, some colpodellids exhibit a parasitic lifestyle in humans (140). The third loss of photosynthesis led to the emergence of an intracellular parasite, *P. sociabile*. However, while intracellular parasitic sporozoans have gradually evolved from their extracellular predecessors (94) (**Figure 3*a*,*b***), intracellular parasitic apicomonads such as *C. velia* (86) and *P. sociabile* (78) have no known extracellular symbiotic relatives.

Gregarines, subdivided into archigregarines, eugregarines, and neogregarines, typically live in symbiosis with their invertebrate hosts, including annelids, echinoderms, mollusks, hemichordates, insects, and sipunculids (1, 108, 131). Some gregarines and related *Cryptosporidium* constitute the very rare cases of secondary aplastidic organisms. Archigregarines still contain a genome-carrying plastid, whereas the organelle was entirely lost in neogregarines (79). Some eugregarines represent an intermediate state, as they have lost the plastid genome yet retained the organelle, which contains a fully imported proteome (**Figure 3*b***).

The life cycle of gregarines typically begins with the attachment of trophozoites to the host's epithelial intestinal lumen tissue. Most archigregarines do not invade the host cell, engaging in extracellular symbiosis, which most likely represents the ancestral state of Sporozoa. However,

the presence of intracellular stages in the life cycles of the archigregarine *Selenidium pygospinosis* (102) and the eugregarine *Cephaloidophora ampelisca* (92) leaves their possible gradual loss from other gregarine lineages as an alternative scenario. A type of host–symbiont interaction reminiscent of gregarines is observed in the derived cryptosporidians, although the principal difference rests in the fact that they are epicellular, penetrating the host cell without invading its cytoplasm, occupying a so-called extracytoplasmic location (7, 47, 108). The remaining Sporozoa (Coccidia, Haemosporidia, and Piroplasmida) exhibit both the extracellular and the intracellular stages in their life cycles. These parasites either directly interact with the host cytoplasm or are enclosed within the parasitophorous vacuole (122). Coccidians parasitizing warm-blooded animals and haemosporidians typically reside intracytoplasmically, whereas coccidians parasitizing cold-blooded vertebrates live extracytoplasmically, at the periphery of epithelial cells in direct contact with their cytoplasm (7, 11, 119).

The nature of the symbiotic relationship can undergo changes during the life cycle, particularly in the heteroxenous Sporozoa, where it may vary between different hosts. It appears that some sporozoans are pathogenic for their relatively recently acquired hosts, whereas they are tolerated by evolutionarily older, well-adapted hosts. Long-term coexistence and adaptation of host and symbiont thus lead to a mutualistic relationship or at least a tolerance, whereas more recent changes in host specificity tend to evolve into parasitism (109, 113). Malaria parasites are an example of this gradual adaptation: *Plasmodium* is often highly pathogenic to its mammalian hosts but is well tolerated by its definitive host, the insect vectors (17). In fact, the evolutionarily derived *Plasmodium* can kill immunonaive mammalian hosts within a few weeks. This principle seems to apply also to gregarines, which behave like mutualists in their evolutionarily older hosts (5).

Despite its phylogenetic position among the intracellular obligate parasitic Sporozoa (90), *Nephromyces* is an extracellular mutualist (110) (**Figure 3*b***) likely due to its long-term adaptation to its evolutionarily ancient hosts, the polychaetes. Its phylogenetic position between two groups of intracellular parasites, Haemosporidia and Coccidia (90), can be interpreted as a formerly intracellular symbiosis that is frozen at the extracellular stage, just before entering the host cell (see the left side of the tree in **Figure 3*b***). Alternatively, the intracellular symbiosis has evolved independently in Coccidia and Haemosporidia, with *Nephromyces* representing an ancestral state (see the right side of the tree in **Figure 3*b***). In another case, *C. velia* forms a short-term relationship with its host, behaving more like a pathogen than a parasite, causing high mortality in coral larvae (28). This suggests that the *Acropora digitifera* larvae used in experiments (28, 85) likely have become hosts either accidentally or recently and that *C. velia* has another regular host with a more stable symbiotic relationship.

The transition from phototrophic ancestors to obligate parasites required remodeling of the existing metabolic and genetic equipment to a new purpose. This evolutionary shift involved a loss of more than 3,800 genes, while only approximately 80 novel genes have been acquired (137). Losses included numerous genes related to the formation of a flagellum (e.g., δ-tubulin, intraflagellar transport protein) (122) in certain gregarines and cryptosporidians, as well as the loss of photosynthesis and related genes (e.g., photosystems, ribulose-1,5-bisphosphate carboxylase/oxygenase, chlorophyl synthesis) in all heterotrophic apicomplexans (**Figure 3*b***). In contrast, some components of the apical complex, such as apical membrane antigen 1 and rhoptry neck protein 2, were gained in Coccidia, Haemosporidia, and Piroplasmida (**Figure 3*b***). Despite the group's name, not all Apicomplexa possess apical complex and corresponding genes. The absence of this complex in *V. brassicaformis* indicates either its loss in early-branching photosynthetic apicomonads or its independent evolution in Apicomonada and Sporozoa. With the exception of mutualistic *Nephromyces*, all examined apicomplexans, including rhoptry-lacking chromerids, contain nondischarged protein 9, which is essential for rhoptry secretion (73) (**Figure 3*b***). Moreover, the conoid

protein hub1 was found in Sporozoa, including Piroplasmida that lack the conoid, but not in colpodellids, which possess the preconoid. Notably, all extracellular and epicellular apicomplexans, cryptosporidians, gregarines, and *Nephromyces* lack the apical membrane antigen 1 (27). This finding suggests that its absence may represent an ancestral state, while a less likely alternative scenario implies multiple independent losses. However, it is also possible that this protein has been ancestrally absent in gregarines and cryptosporidians and was lost only secondarily in *Nephromyces*. Indeed, the presence of some genes is hard to explain given the available information. Such is the case of light-independent protochlorophyllide reductases in the nonphotosynthetic corallicolids. Since in phototrophs these genes are typically responsible for chlorophyll synthesis in the dark (63), their function in the heterotrophic coral symbiont remains unknown.

5. CONCLUDING REMARKS

The three analyzed groups of protists developed unique adaptations that allowed them successful expansions as intracellular parasites. The emerging pattern is compatible with an independent origin of these colorful adaptations that occurred multiple times throughout evolution. As a result, distinct lineages of intracellular parasites employ different morphological, physiological, and molecular features to achieve similar outcomes. Such trait divergence, genome size, and content variation are not surprising, given the considerable phylogenetic distance among these organisms and differences in both the age of their parasitism and the hosts' biology. Certain features, such as the loss of a flagellum, the shift to osmotrophy, the formation of resistant cyst-like stages, and the presence of diverse transporters, appear characteristic for, if not common denominators of, intracellular parasites, regardless of their evolutionary origin. However, specific traits linked one way or another to the emergence of intracellular parasitism vary significantly not only across the three compared groups but also within them. These distinctive traits frequently involve gene expansion or horizontal acquisitions of proteins associated with nucleic acid and amino acid synthesis and salvage, coupled with genetic rearrangements facilitating rapid proliferation and extensive diversification. The highly effective mechanisms for host acquisition and exploitation have enabled intracellular parasites to thrive in numerous habitats, reflecting their remarkable adaptability.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors acknowledge support from the Czech Science Foundation (22-01026S to J.L., 19-28399X to P.B.S. and I.F., 21-03224S to M.O., 23-06203S to J.R., and 23-07695S to A.B.) and the Gordon and Betty Moore Foundation (GBMF #9354 to J.L.).

LITERATURE CITED

1. Adl SM, Bass D, Lane CE, Lukeš J, Schoch CL, et al. 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. *J. Eukaryot. Microbiol.* 66:4–119
2. Albanaz ATS, Carrington M, Frolov AO, Ganyukova AI, Gerasimov ES, et al. 2023. Shining the spotlight on the neglected: new high-quality genome assemblies as a gateway to understanding the evolution of Trypanosomatidae. *BMC Genom.* 24:471
3. Albanaz ATS, Gerasimov ES, Shaw JJ, Sádlová J, Lukeš J, et al. 2021. Genome analysis of *Endotrypanum* and *Porcisia* spp., closest phylogenetic relatives of *Leishmania*, highlights the role of amastins in shaping pathogenicity. *Genes* 12:444

4. Alexander WG, Wisecaver JH, Rokas A, Hittinger CT. 2016. Horizontally acquired genes in early-diverging pathogenic fungi enable the use of host nucleosides and nucleotides. *PNAS* 113:4116–21
5. Arcila F, Meunier J. 2020. Friend or foe? The apparent benefits of gregarine (Apicomplexa: Sporozoa) infection in the European earwig. *Int. J. Parasitol.* 50:461–69
6. Bartholomeu DC, de Paiva RM, Mendes TA, DaRocha WD, Teixeira SM. 2014. Unveiling the intracellular survival gene kit of trypanosomatid parasites. *PLOS Pathog.* 10:e1004399
7. Bartošová-Sojková P, Oppenheim RD, Soldati-Favre D, Lukeš J. 2015. Epicellular apicomplexans: parasites "on the way in." *PLOS Pathog.* 11:e1005080
8. Bass D, Czech L, Williams BAP, Berney C, Dunthorn M, et al. 2018. Clarifying the relationships between Microsporidia and Cryptomycota. *J. Eukaryot. Microbiol.* 65:773–82
9. Batista MF, Najera CA, Meneghelli I, Bahia D. 2020. The parasitic intracellular lifestyle of trypanosomatids: parasitophorous vacuole development and survival. *Front. Cell Dev. Biol.* 8:396
10. Buchanan KL, Murphy JW. 1998. What makes *Cryptococcus neoformans* a pathogen? *Emerg. Infect. Dis.* 4:71–83
11. Burrell A, Tomley FM, Vaughan S, Marugan-Hernandez V. 2020. Life cycle stages, specific organelles and invasion mechanisms of *Eimeria* species. *Parasitology* 147:263–78
12. Butenko A, Hammond M, Field MC, Ginger ML, Yurchenko V, Lukeš J. 2021. Reductionist pathways for parasitism in euglenozoans? Expanded datasets provide new insights. *Trends Parasitol.* 37:100–16
13. Cao L, Peng B, Yao L, Zhang X, Sun K, et al. 2010. The ancient function of RB-E2F pathway: insights from its evolutionary history. *Biol. Direct* 5:55
14. Capewell P, Cooper A, Clucas C, Weir W, MacLeod A. 2015. A co-evolutionary arms race: trypanosomes shaping the human genome, humans shaping the trypanosome genome. *Parasitology* 142(Suppl. 1):S108–19
15. Carlson CJ, Dallas TA, Alexander LW, Phelan AL, Phillips AJ. 2020. What would it take to describe the global diversity of parasites? *Proc. Biol. Sci.* 287:20201841
16. Casadevall A. 2008. Evolution of intracellular pathogens. *Annu. Rev. Microbiol.* 62:19–33
17. Castillo JC, Ferreira ABB, Trisnadi N, Barillas-Mury C. 2017. Activation of mosquito complement antiplasmodial response requires cellular immunity. *Sci. Immunol.* 2:eaal1505
18. Cavalier-Smith T. 1993. Kingdom Protozoa and its 18 phyla. *Microbiol. Rev.* 57:953–94
19. Cavalier-Smith T. 2018. Kingdom Chromista and its eight phyla: a new synthesis emphasising periplastid protein targeting, cytoskeletal and periplastid evolution, and ancient divergences. *Protoplasma* 255:297–357
20. Chacón-Vargas K, McCarthy CO, Choi D, Wang L, Yu JH, Gibbons JGG. 2021. Comparison of two *Aspergillus oryzae* genomes from different clades reveals independent evolution of alpha-amylase duplication, variation in secondary metabolism genes, and differences in primary metabolism. *Front. Microbiol.* 12:691296
21. Coakley G, Maizels RM, Buck AH. 2015. Exosomes and other extracellular vesicles: the new communicators in parasite infections. *Trends Parasitol.* 31:477–89
22. Corradi N, Slamovits CH. 2011. The intriguing nature of microsporidian genomes. *Brief. Funct. Genom.* 10:115–24
23. Corsaro D. 2022. Insights into Microsporidia evolution from early diverging Microsporidia. In *Microsporidia*, Vol. 114, ed. LM Weiss, AW Reinke, pp. 71–90. Cham, Switz.: Springer
24. Corsaro D, Walochnik J, Venditti D, Hauroeder B, Michel R. 2020. Solving an old enigma: *Morellospora saccamoebae* gen. nov., sp. nov. (Rozellomycota), a *Sphaerita*-like parasite of free-living amoebae. *Parasitol. Res.* 119:925–34
25. Corsaro D, Walochnik J, Venditti D, Steinmann J, Müller K-D, Michel R. 2014. Microsporidia-like parasites of amoebae belong to the early fungal lineage Rozellomycota. *Parasitol. Res.* 113:1909–18
26. Corsaro D, Wylezich C, Venditti D, Michel R, Walochnik J, Wegensteiner R. 2019. Filling gaps in the microsporidian tree: rDNA phylogeny of *Chytridiopsis typographi* (Microsporidia: Chytridiopsida). *Parasitol. Res.* 118:169–80
27. Cova MM, Lamarque MH, Lebrun M. 2022. How Apicomplexa parasites secrete and build their invasion machinery. *Annu. Rev. Microbiol.* 76:619–40

28. Cumbo VR, Baird AH, Moore RB, Negri AP, Neilan BA, et al. 2013. *Chromera velia* is endosymbiotic in larvae of the reef corals *Acropora digitifera* and *A. tenuis*. *Protist* 164:237–44
29. Cuomo CA, Desjardins CA, Bakowski MA, Goldberg J, Ma AT, et al. 2012. Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. *Genome Res.* 22:2478–88
30. David V, Flegontov P, Gerasimov E, Tanifuji G, Hashimi H, et al. 2015. Gene loss and error-prone RNA editing in the mitochondrion of *Perkinsela*, an endosymbiotic kinetoplastid. *mBio* 6:e01498-15
31. de Castro Neto AL, da Silveira JF, Mortara RA. 2021. Comparative analysis of virulence mechanisms of trypanosomatids pathogenic to humans. *Front. Cell. Infect. Microbiol.* 11:669079
32. De Rycker M, Wyllie S, Horn D, Read KD, Gilbert IH. 2023. Anti-trypanosomatid drug discovery: progress and challenges. *Nat. Rev. Microbiol.* 21:35–50
33. Dean P, Sendra KM, Williams TA, Watson AK, Major P, et al. 2018. Transporter gene acquisition and innovation in the evolution of Microsporidia intracellular parasites. *Nat. Commun.* 9:1709
34. Dyková I, Fiala I, Lom J, Lukeš J. 2003. *Perkinsiella* amoebae-like endosymbionts of *Neoparamoeba* spp., relatives of the kinetoplastid *Ichthyobodo*. *Eur. J. Protistol.* 39:37–52
35. Dyková I, Veverková M, Fiala I, Macháčková B, Pecková H. 2003. *Nuclearia pattersoni* sp n. (Filosea), a new species of amphizoic amoeba isolated from gills of roach (*Rutilus rutilus*), and its rickettsial endosymbiont. *Folia Parasitol.* 50:161–70
36. Etheridge RD. 2022. Protozoan phagotrophy from predators to parasites: an overview of the enigmatic cytostome-cytopharynx complex of *Trypanosoma cruzi*. *J. Eukaryot. Microbiol.* 69:e12896
37. Flegontova O, Flegontov P, Londoño PAC, Walczowski W, Šantić D, et al. 2020. Environmental determinants of the distribution of planktonic diplonemids and kinetoplastids in the oceans. *Environ. Microbiol.* 22:4014–31
38. Fokin SI, Schrallhammer M, Chiellini C, Verni F, Petroni G. 2014. Free-living ciliates as potential reservoirs for eukaryotic parasites: occurrence of a trypanosomatid in the macronucleus of *Euplotes encysticus*. *Parasites Vectors* 7:203
39. Füssy Z, Masařová P, Kručinská J, Esson HJ, Oborník M. 2017. Budding of the alveolate alga *Vitrella brassicaformis* resembles sexual and asexual processes in apicomplexan parasites. *Protist* 168:80–91
40. Gabaldón T, Völcker E, Torruella G. 2022. On the biology, diversity and evolution of nucleariid amoebae (Amorphea, Obazoa, Opisthokonta). *Protist* 173:25895
41. Galindo LJ, López-García P, Torruella G, Karpov S, Moreira D. 2021. Phylogenomics of a new fungal phylum reveals multiple waves of reductive evolution across Holomycota. *Nat. Commun.* 12:4973
42. Galindo LJ, Torruella G, López-García P, Ciobanu M, Gutiérrez-Preciado A, et al. 2022. Phylogenomics supports the monophyly of aphelids and fungi and identifies new molecular synapomorphies. *Syst. Biol.* 72:505–15
43. Galindo LJ, Torruella G, Moreira D, Eglit Y, Simpson AGB, et al. 2019. Combined cultivation and single-cell approaches to the phylogenomics of nucleariid amoebae, close relatives of fungi. *Philos. Trans. R. Soc. B* 374:20190094
44. Galindo LJ, Torruella G, Moreira D, Timpano H, Paskerova G, et al. 2018. Evolutionary genomics of *Metchnikovella incurvata* (Metchnikovellidae): an early branching microsporidium. *Genome Biol. Evol.* 10:2736–48
45. Garvetto A, Murúa P, Kirchmair M, Salvenmoser W, Hittorf M, et al. 2023. Phagocytosis underpins the biotrophic lifestyle of intracellular parasites in the class Phytomyxea (Rhizaria). *New Phytol.* 238:2130–43
46. Goodwin JD, Lee TF, Kugrens P, Simpson AGB. 2018. *Allobodo chlorophagus* n. gen. n. sp., a kinetoplastid that infiltrates and feeds on the invasive alga *Codium fragile*. *Protist* 169:911–25
47. Guérin A, Roy NH, Kugler EM, Berry L, Burkhardt JK, et al. 2021. *Cryptosporidium* rhoptry effector protein ROP1 injected during invasion targets the host cytoskeletal modulator LMO7. *Cell Host Microbe* 29:1407–20.e5
48. Haag KL, James TY, Pombert J-F, Larsson R, Schaer TMM, et al. 2014. Evolution of a morphological novelty occurred before genome compaction in a lineage of extreme parasites. *PNAS* 111:15480–85
49. Haag KL, Pombert JF, Sun YK, de Albuquerque NRM, Batliner B, et al. 2020. Microsporidia with vertical transmission were likely shaped by nonadaptive processes. *Genome Biol. Evol.* 12:3599–614
50. Han B, Takvorian PM, Weiss LM. 2020. Invasion of host cells by Microsporidia. *Front. Microbiol.* 11:172

51. Harmer J, Yurchenko V, Nenarokova A, Lukeš J, Ginger ML. 2018. Farming, slaving and enslavement: histories of endosymbioses during kinetoplastid evolution. *Parasitology* 145:1311–23
52. Heinz E, Hacker C, Dean P, Mifsud J, Goldberg AV, et al. 2014. Plasma membrane-located purine nucleotide transport proteins are key components for host exploitation by microsporidian intracellular parasites. *PLOS Pathog.* 10:e1004547
53. Horta MF, Andrade LO, Martins-Duarte ÉS, Castro-Gomes T. 2020. Cell invasion by intracellular parasites – the many roads to infection. *J. Cell Sci.* 133:jcs232488
54. Jackson AP, Otto TD, Aslett M, Armstrong SD, Bringaud F, et al. 2016. Kinetoplastid phylogenomics reveals the evolutionary innovations associated with the origins of parasitism. *Curr. Biol.* 26:161–72
55. James TY, Pelin A, Bonen L, Ahrendt S, Sain D, et al. 2013. Shared signatures of parasitism and phylogenomics unite Cryptomycota and Microsporidia. *Curr. Biol.* 23:1548–53
56. Janouškovec J, Paskerova GG, Miroliubova TS, Mikhailov KV, Birley T, et al. 2019. Apicomplexan-like parasites are polyphyletic and widely but selectively dependent on cryptic plastid organelles. *eLife* 8:e49662
57. Karpov SA, Mamkaeva MA, Aleoshin VV, Nassonova E, Lilje O, Gleason FH. 2014. Morphology, phylogeny, and ecology of the aphelids (Aphelidea, Opisthokonta) and proposal for the new superphylum Opisthosporidia. *Front. Microbiol.* 5:112
58. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–49
59. Keeling PJ, Fast NM, Corradi N. 2014. Microsporidian genome structure and function. In *Microsporidia: Pathogens of Opportunity*, ed. LM Weiss, JJ Becnel, pp. 221–29. Oxford, UK: Wiley-Blackwell
60. Keeling PJ, McCutcheon JP. 2017. Endosymbiosis: The feeling is not mutual. *J. Theor. Biol.* 434:75–79
61. Kishore SP, Stiller JW, Deitsch KW. 2013. Horizontal gene transfer of epigenetic machinery and evolution of parasitism in the malaria parasite *Plasmodium falciparum* and other apicomplexans. *BMC Evol. Biol.* 13:37
62. Kostygov AY, Karnkowska A, Votýpka J, Tashyreva D, Maciszewski K, et al. 2021. Euglenozoa: taxonomy, diversity and ecology, symbioses and viruses. *Open Biol.* 11:200407
63. Kwong WK, del Campo J, Mathur V, Vermeij MJA, Keeling PJ. 2019. A widespread coral-infecting apicomplexan with chlorophyll biosynthesis genes. *Nature* 568:103–7
64. Larsson JIR. 2014. The primitive Microsporidia. In *Microsporidia: Pathogens of Opportunity*, ed. LM Weiss, JJ Becnel, pp. 605–34. Oxford, UK: Wiley-Blackwell
65. Lee YF, Cheng CC, Chen JS, Lin NN, Hung YW, et al. 2013. Evidence of intracellular stages in *Trypanosoma* (*Megatrypanum*) *theileri* in non-phagocytic mammalian cells. *Vet. Parasitol.* 191:228–39
66. Lukeš J, Butenko A, Hashimi H, Maslov DA, Votýpka J, Yurchenko V. 2018. Trypanosomatids are much more than just trypanosomes: clues from the expanded family tree. *Trends Parasitol.* 34:466–80
67. Lukeš J, Husník F. 2018. Microsporidia: A single horizontal gene transfer drives a great leap forward. *Curr. Biol.* 28:R712–15
68. Lukeš J, Kachale A, Votýpka J, Butenko A, Field MC. 2022. African trypanosome strategies for conquering new hosts and territories: the end of monophyly? *Trends Parasitol.* 38:724–36
69. Lukeš J, Skalický T, Týč J, Votýpka J, Yurchenko V. 2014. Evolution of parasitism in kinetoplastid flagellates. *Mol. Biochem. Parasitol.* 195:115–22
70. Lukeš J, Speijer D, Zíková A, Alfonzo JD, Hashimi H, Field MC. 2023. Trypanosomes as a magnifying glass for cell and molecular biology. *Trends Parasitol.* 39:902–12
71. Lukeš J, Wheeler R, Jirsová D, David V, Archibald JM. 2018. Massive mitochondrial DNA content in diplonemid and kinetoplastid protists. *IUBMB Life* 70:1267–74
72. Lutzoni F, Nowak MD, Alfaro ME, Reeb V, Miadlikowska J, et al. 2018. Contemporaneous radiations of fungi and plants linked to symbiosis. *Nat. Commun.* 9:5451
73. Mageswaran SK, Guérin A, Theveny LM, Chen WD, Martinez M, et al. 2021. In situ ultrastructures of two evolutionarily distant apicomplexan rhoptry secretion systems. *Nat. Commun.* 12:4983
74. Major P, Sendra KM, Dean P, Williams TA, Watson AK, et al. 2019. A new family of cell surface located purine transporters in Microsporidia and related fungal endoparasites. *eLife* 8:e47037

75. Manickavinayaham S, Dennehey BK, Johnson DG. 2021. Direct regulation of DNA repair by E2F and RB in mammals and plants: core function or convergent evolution? *Cancers* 13:934
76. Martin-Escolano J, Marín C, Rosales MJ, Tsaousis AD, Medina-Carmona E, Martin-Escolano R. 2022. An updated view of the *Trypanosoma cruzi* life cycle: intervention points for an effective treatment. *ACS Infect. Dis.* 8:1107–15
77. Maslov DA, Opperdoes FR, Kostygov AY, Hashimi H, Lukeš J, Yurchenko V. 2019. Recent advances in trypanosomatid research: genome organization, expression, metabolism, taxonomy and evolution. *Parasitology* 146:1–27
78. Mathur V, Kolísko M, Hehenberger E, Irwin NAT, Leander BS, et al. 2019. Multiple independent origins of apicomplexan-like parasites. *Curr. Biol.* 29:2936–41.e5
79. Mathur V, Salomaki ED, Wakeman KC, Na I, Kwong WK, et al. 2023. Reconstruction of plastid proteomes of apicomplexans and close relatives reveals the major evolutionary outcomes of cryptic plastids. *Mol. Biol. Evol.* 40:msad002
80. McBride JA, Gauthier GM, Klein BS. 2019. Turning on virulence: mechanisms that underpin the morphologic transition and pathogenicity of *Blastomyces*. *Virulence* 10:801–9
81. Mendes TA, Lobo FP, Rodrigues TS, Rodrigues-Luiz GF, daRocha WD, et al. 2013. Repeat-enriched proteins are related to host cell invasion and immune evasion in parasitic protozoa. *Mol. Biol. Evol.* 30:951–63
82. Mikhailov KV, Karpov SA, Letcher PM, Lee PA, Logacheva MD, et al. 2022. Genomic analysis reveals cryptic diversity in aphelids and sheds light on the emergence of Fungi. *Curr. Biol.* 32:4607–19.e7
83. Mikhailov KV, Simdyanov TG, Aleoshin VV. 2017. Genomic survey of a hyperparasitic microsporidian *Amphiamblys* sp. (Metchnikovellidae). *Genome Biol. Evol.* 9:454–67
84. Mittal J, Ponce MG, Gendlina I, Nosanchuk JD. 2019. *Histoplasma capsulatum*: mechanisms for pathogenesis. In *Fungal Physiology and Immunopathogenesis*, Vol. 422, ed. ML Rodrigues, pp. 157–91. Berlin: Springer
85. Mohamed AR, Cumbo VR, Harii S, Shinzato C, Chan CX, et al. 2018. Deciphering the nature of the coral–*Chromera* association. *ISME J.* 12:776–90
86. Moore RB, Oborník M, Janouškovec J, Chrudimský T, Vancová M, et al. 2008. A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* 452:959–63
87. Moreira D, López-García P, Vickerman K. 2004. An updated view of kinetoplastid phylogeny using environmental sequences and a closer outgroup: proposal for a new classification of the class Kinetoplastea. *Int. J. Syst. Evol. Microbiol.* 54:1861–75
88. Mukherjee I, Hodoki Y, Okazaki Y, Fujinaga S, Ohbayashi K, Nakano SI. 2019. Widespread dominance of kinetoplastids and unexpected presence of diplonemids in deep freshwater lakes. *Front. Microbiol.* 10:2375
89. Muñoz JF, Gauthier GM, Desjardins CA, Gallo JE, Holder J, et al. 2015. The dynamic genome and transcriptome of the human fungal pathogen *Blastomyces* and close relative *Emmonsia*. *PLOS Genet.* 11:e1005493
90. Muñoz-Gómez SA, Durnin K, Eme L, Paight C, Lane CE, et al. 2019. *Nephromyces* represents a diverse and novel lineage of the Apicomplexa that has retained apicoplasts. *Genome Biol. Evol.* 11:2727–40
91. Naranjo-Ortiz MA, Gabaldón T. 2019. Fungal evolution: major ecological adaptations and evolutionary transitions. *Biol. Rev.* 94:1443–76
92. Nowlin N, Smith I. 1917. The intracellular development of a gregarine *Frenzelina ampelisca* n. sp. *J. Parasitol.* 4:83–88
93. Oborník M. 2019. Endosymbiotic evolution of algae, secondary heterotrophy and parasitism. *Biomolecules* 9:266
94. Oborník M. 2020. Photoparasitism as an intermediate state in the evolution of apicomplexan parasites. *Trends Parasitol.* 36:727–34
95. Oborník M, Kručinská J, Esson H. 2016. Life cycles of chromerids resemble those of colpodellids and apicomplexan parasites. *Perspect. Phycol.* 3:21–27
96. Oborník M, Lukeš J. 2015. The organellar genomes of *Chromera* and *Vitrella*, the phototrophic relatives of apicomplexan parasites. *Annu. Rev. Microbiol.* 69:129–44

97. Oborník M, Vancová M, Lai D-H, Janouškovec J, Keeling PJ, Lukeš J. 2011. Morphology and ultrastructure of multiple life cycle stages of the photosynthetic relative of Apicomplexa, *Chromera velia*. *Protist* 162:115–30
98. Ocaña-Pallarès E, Williams TA, López-Escardó D, Arroyo AS, Pathmanathan JS, et al. 2022. Divergent genomic trajectories predate the origin of animals and fungi. *Nature* 609:747–53
99. Okamoto N, Inouye I. 2005. The katablepharids are a distant sister group of the Cryptophyta: A proposal for Katablepharidophyta divisio nova/Kathablepharida phylum novum based on SSU rDNA and beta-tubulin phylogeny. *Protist* 156:163–79
100. Okamoto N, Keeling PJ. 2014. The 3D structure of the apical complex and association with the flagellar apparatus revealed by serial TEM tomography in *Psammosa pacifica*, a distant relative of the Apicomplexa. *PLOS ONE* 9:e84653
101. Okamoto N, McFadden GI. 2008. The mother of all parasites. *Future Microbiol.* 3:391–95
102. Paskerova GG, Miroliubova TS, Diakin A, Kováčiková M, Valigurová A, et al. 2018. Fine structure and molecular phylogenetic position of two marine gregarines, *Selenidium pygospionis* sp. n. and *S. pherusae* sp. n., with notes on the phylogeny of Archigregarinida (Apicomplexa). *Protist* 169:826–52
103. Pombert J-F, Selman M, Burki F, Bardell FT, Farinelli L, et al. 2012. Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. *PNAS* 109:12638–43
104. Potter SC, Luciani A, Eddy SR, Park Y, Lopez R, Finn RD. 2018. HMMER web server: 2018 update. *Nucleic Acids Res.* 46:W200–4
105. Poulin R, Randhawa HS. 2015. Evolution of parasitism along convergent lines: from ecology to genomics. *Parasitology* 142:S6–15
106. Quandt CA, Beaudet D, Corsaro D, Walochnik J, Michel R, et al. 2017. The genome of an intranuclear parasite, *Paramicrosporidium saccamoebae*, reveals alternative adaptations to obligate intracellular parasitism. *eLife* 6:e29594
107. Radek R, Kariton M, Dabert J, Alberti G. 2015. Ultrastructural characterization of *Acarispora falculifera* n. gen., n. sp., a new microsporidium (Opisthokonta: Chytridiopsida) from the feather mite *Falculifer rostratus* (Astigmata: Pterolichoidea). *Acta Parasitol.* 60:200–10
108. Rueckert S, Betts EL, Tsaousis AD. 2019. The symbiotic spectrum: Where do the gregarines fit? *Trends Parasitol.* 35:687–94
109. Sachs JL, Wilcox TP. 2006. A shift to parasitism in the jellyfish symbiont *Symbiodinium microadriaticum*. *Proc. Biol. Sci.* 273:425–29
110. Saffo MB, McCoy AM, Rieken C, Slamovits CH. 2010. *Nephromyces*, a beneficial apicomplexan symbiont in marine animals. *PNAS* 107:16190–95
111. Salomaki ED, Kolisko M. 2019. There is treasure everywhere: reductive plastid evolution in Apicomplexa in light of their close relatives. *Biomolecules* 9:378
112. Schoenle A, Hohlfeld M, Hermanns K, Mahé F, de Vargas C, et al. 2021. High and specific diversity of protists in the deep-sea basins dominated by diplonemids, kinetoplastids, ciliates and foraminiferans. *Commun. Biol.* 4:501
113. Seal S, Dharmarajan G, Khan I. 2021. Evolution of pathogen tolerance and emerging infections: a missing experimental paradigm. *eLife* 10:e68874
114. Sebghati TS, Engle JT, Goldman WE. 2000. Intracellular parasitism by *Histoplasma capsulatum*: fungal virulence and calcium dependence. *Science* 290:1368–72
115. Seto K, Matsuzawa T, Kuno H, Kagami M. 2020. Morphology, ultrastructure, and molecular phylogeny of *Aphelidium collabens* sp. nov. (Aphelida), a parasitoid of a green alga *Coccomyxa* sp. *Protist* 171:125728
116. Sheppard DC, Filler SG. 2015. Host cell invasion by medically important fungi. *Cold Spring Harb. Perspect. Med.* 5:a019687
117. Sibbald SJ, Cenci U, Colp M, Eglit Y, O'Kelly CJ, Archibald JM. 2017. Diversity and evolution of *Paramoeba* spp. and their kinetoplastid endosymbionts. *J. Eukaryot. Microbiol.* 64:598–607
118. Sibley LD. 2011. Invasion and intracellular survival by protozoan parasites. *Immunol. Rev.* 240:72–91
119. Sokol-Borrelli SL, Coombs RS, Boyle JP. 2020. A comparison of stage conversion in the coccidian apicomplexans *Toxoplasma gondii*, *Hammondia hammondi*, and *Neospora caninum*. *Front. Cell. Infect. Microbiol.* 10:608283

120. Sole R. 2022. Revisiting Leigh Van Valen's "A New Evolutionary Law" 1973. *Biol. Theory* 17:120–25
121. Somerville V, Berthoud H, Schmidt RS, Bachmann HP, Meng YH, et al. 2022. Functional strain redundancy and persistent phage infection in Swiss hard cheese starter cultures. *ISME J.* 16:388–99
122. Suss-Toby E, Zimmerberg J, Ward GE. 1996. *Toxoplasma* invasion: The parasitophorous vacuole is formed from host cell plasma membrane and pinches off via a fission pore. *PNAS* 93:8413–18
123. Tamim El Jarkass H, Reinke AW. 2020. The ins and outs of host-microsporidia interactions during invasion, proliferation and exit. *Cell. Microbiol.* 22:e13247
124. Tanifuji G, Cenci U, Moog D, Dean S, Nakayama T, et al. 2017. Genome sequencing reveals metabolic and cellular interdependence in an amoeba-kinetoplastid symbiosis. *Sci. Rep.* 7:11688
125. Tikhonenkov DV, Gawryluk RMR, Mylnikov AP, Keeling PJ. 2021. First finding of free-living representatives of Prokinetoplastina and their nuclear and mitochondrial genomes. *Sci. Rep.* 11:2946
126. Timofeev S, Tokarev Y, Dolgikh V. 2020. Energy metabolism and its evolution in Microsporidia and allied taxa. *Parasitol. Res.* 119:1433–41
127. Torruella G, Grau-Bové X, Moreira D, Karpov SA, Burns JA, et al. 2018. Global transcriptome analysis of the aphelid *Paraphelidium tribonemae* supports the phagotrophic origin of fungi. *Commun. Biol.* 1:231
128. Tsaousis AD, Kunji ERS, Goldberg AV, Lucocq JM, Hirt RP, Embley TM. 2008. A novel route for ATP acquisition by the remnant mitochondria of *Encephalitozoon cuniculi*. *Nature* 453:553–56
129. van de Vossenberg BTLH, Warris S, Nguyen HDT, van Gent-Pelzer MPE, Joly DL, et al. 2019. Comparative genomics of chytrid fungi reveal insights into the obligate biotrophic and pathogenic lifestyle of *Synchytrium endobioticum*. *Sci. Rep.* 9:8672
130. Vávra J, Lukeš J. 2013. Microsporidia and 'the art of living together.' *Adv. Parasitol.* 82:253–319
131. Votýpka J, Modrý D, Oborník M, Šlapeta J, Lukeš J. 2017. Apicomplexa. In *Handbook of the Protists*, ed. JM Archibald, AGB Simpson, CH Slamovits, pp. 567–624. Cham, Switz.: Springer
132. Wadi L, Reinke AW. 2020. Evolution of microsporidia: an extremely successful group of eukaryotic intracellular parasites. *PLOS Pathog.* 16:e1008276
133. Weinstein SB, Kuris AM. 2016. Independent origins of parasitism in Animalia. *Biol. Lett.* 12:20160324
134. Whelan TA, Lee NT, Lee RCH, Fast NM. 2019. Microsporidian introns retained against a background of genome reduction: characterization of an unusual set of introns. *Genome Biol. Evol.* 11:263–69
135. Williams BAP, Hirt RP, Lucocq JM, Embley TM. 2002. A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. *Nature* 418:865–69
136. Wolf YI, Koonin EV. 2013. Genome reduction as the dominant mode of evolution. *Bioessays* 35:829–37
137. Woo YH, Ansari H, Otto TD, Klinger CM, Kolisko M, et al. 2015. Chromerid genomes reveal the evolutionary path from photosynthetic algae to obligate intracellular parasites. *eLife* 4:e06974
138. Wu BJ, Hao WL, Cox MP. 2022. Reconstruction of gene innovation associated with major evolutionary transitions in the kingdom Fungi. *BMC Biol.* 20:144
139. Yazaki E, Ishikawa SA, Kume K, Kumagai A, Kamaishi T, et al. 2017. Global Kinetoplastea phylogeny inferred from a large-scale multigene alignment including parasitic species for better understanding transitions from a free-living to a parasitic lifestyle. *Genes Genet. Syst.* 92:35–42
140. Yuan CL, Keeling PJ, Krause PJ, Horak A, Bent S, et al. 2012. *Colpodella* spp.–like parasite infection in woman, China. *Emerg. Infect. Dis.* 18:125–27
141. Žárský V, Karnkowska A, Boscaro V, Trznadel M, Whelan TA, et al. 2023. Contrasting outcomes of genome reduction in mikrocytids and microsporidians. *BMC Biol.* 21:137