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EVOLUTION AND ECOLOGY OF MICROBIOMES

Review

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The eukaryome: Diversity and role of microeukaryotic organisms associated with animal hosts

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Abstract

1. Awareness of the roles that host-associated microbes play in host biology has escalated in recent years. However, microbiome studies have focused essentially on bacteria, and overall, we know little about the role of host-associated eukaryotes outside the field of parasitology. Despite that, eukaryotes and microeukaryotes in particular are known to be common inhabitants of animals. In many cases, and/or for long periods of time, these associations are not associated with clinical signs of disease.
2. Unlike the study of bacterial microbiomes, the study of the microeukaryotes associated with animals has largely been restricted to visual identification or molecular targeting of particular groups. So far, since the publication of the influential Human Microbiome Project Consortium paper in 2012, few studies have been published dealing with the microeukaryotes using a high-throughput barcoding 'microbiome-like' approach in animals.
3. Nonetheless, microeukaryotes have an impact on the host physiology and lifestyle and also on the diversity and composition of the wider symbiotic community of bacteria and viruses. Beyond being parasites, microeukaryotes have many different roles in animals. For example, they directly interact with the host immune system in mammals; they have a key role on cellulose degradation, lignocellulose in xylophage termites and cockroaches; and they have an essential role in providing photosynthates to reef-building corals.
4. Certain microeukaryotic lineages have diversified within hosts more than others. These cases of co-evolution led to different forms of symbiosis: from mutualism (like *Symbiodinium* in corals or parabasalians in termites), to commensalism (*Blastocystis* in humans) or to strict parasitism (apicomplexans or microsporidians in a broad range of hosts). We will review the ecological context and the evolutionary mechanisms that ended up in these different symbiotic scenarios, across the taxonomic range of both symbionts and their metazoan hosts.
5. Host-associated microeukaryotes have impacts at many levels, from individual animal health to ecosystems and to agroeconomy. Therefore, it is crucial to have a better understanding of their diversity and roles. Novel methodologies are being developed to access the eukaryotic fraction of the microbiome using

high-throughput methods. From -omics, to imaging and barcoding approaches biased against metazoans, these novel methodologies and strategies are helping us to increase and improve our knowledge of microeukaryotes in animal-associated environments.

KEYWORDS

co-evolution, eukaryome, host–microbe interactions, microbial ecology, microbiome, microeukaryotes, parasites, symbiosis

1 | INTRODUCTION

The term eukaryome has been adopted by the protistological community (Lukeš, Stensvold, Jirků-Pomajbíková, & Parfrey, 2015) to refer to the microeukaryotic fraction of the microbiome. The term microbiome theoretically embraces all microbes associated with a host; in reality, the term almost always refers specifically and exclusively to the prokaryotic fraction of the community. Referring to these communities as the eukaryome instead of the microbiome allows us to highlight that host-associated microbial communities also contain microeukaryotes.

Microeukaryotes are important drivers of major ecosystems (Worden et al., 2015) because they play different roles in the environment, from phototrophs to saprotrophs and predators to parasites. Microeukaryotes are microscopic, nucleated and usually single-celled organisms, but form neither a monophyletic nor a functional group. This definition excludes plants, animals, macroscopic fungi and seaweeds (macroscopic algae). Eukaryotic algae, such as diatoms, green algae or pelagophytes, are the main contributors to ocean's primary production which represents half of the primary production on earth (Field, Behrenfeld, Randerson, & Falkowski, 1998). Heterotrophic flagellates are key players in trophic networks mobilizing carbon from the microbial loop to higher levels of the system (Jürgens & Massana, 2008). Parasites, many of them microeukaryotic, can have a more substantial impact on ecosystem function than predation (Kuris et al., 2008), and they play important roles in regulating and structuring natural communities (Preston, Mischler, Townsend, & Johnson, 2016). Microeukaryotic parasites also represent 17% of the World Organization for Animal Health's list of notifiable diseases of terrestrial and aquatic animals (Stentiford, Feist, Stone, Peeler, & Bass, 2014) and 95% of pathogens causing animal and plant species extinction or extirpation (Fisher et al., 2012).

Microeukaryotes in animals are still often intuitively associated with pathogens. Indeed, the most infamous microeukaryotes are pathogenic, causing, for instance, malaria, toxoplasmosis, giardiasis, dysentery, encephalitis, etc. (Walker, Dorrell, Schlacht, & Dacks, 2011). Much research focuses on these, as well as a small number of mutualists like the well-studied zooxanthellae in corals (Baker, 2003) or lignocellulolytic flagellates in the termite gut (Brune, 2014). But we know comparatively little about the many commensals that recent studies have identified as common members of the mammalian gut (Parfrey et al., 2014; Scanlan et al., 2014) and skin (Oh et al., 2014). This situation is analogous to the pre-microbiome era of bacteriology, when most of the bacteria associated with humans were considered pathogens, and the medical community and much of the scientific community more broadly were not aware that we are literally covered by bacteria, which in most cases are neither harmful nor beneficial (Hooper & Gordon, 2001). It is not unlikely that this is also true for microeukaryotes as well, and that many of the microeukaryotes associated with a host probably have little if any direct influence in their host biology or fitness. The field of parasitology, along with phycology and mycology, has been the main source of knowledge on microeukaryotic diversity for decades, by isolating and identifying parasites under the microscope (Kreier & Baker, 1987) or developing model systems for a more detailed study. This low-throughput, microscopy and culturing-dependent approach is by necessity targeted; scientists and physicians described what they were looking for, and most of the time they were looking for parasites for obvious reasons. It was not until the use of non-targeted, culture-independent approaches like high-throughput sequencing of genetic barcodes (Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012) that we could retrieve from an environment, not only what we might be looking for, but also everything else we did not even know that was there. This is possible because metabarcoding methods are widely available, high-throughput and relatively inexpensive. High-throughput sequencing (Taberlet et al., 2012) is awakening interest in the eukaryome, and changing the fields of parasitology, protistology and microbial ecology and pushing the community to develop theoretical frameworks and practical tools necessary to study a variety of animal systems (Ainsworth, Fordyce, & Camp, 2017; Andersen, Vedel Nielsen, & Stensvold, 2013).

2 | THE EUKARYOME WITHIN THE MICROBIOME

The vast majority of metagenomic and metabarcoding studies deal exclusively with prokaryotes. Some examine fungi, which has been called the mycobiome (Cui, Morris, & Ghedin, 2013) and only a few address the microeukaryotic community as a whole. That is not surprising because this trend extends to all environmental microbiology and is reflected in the literature and in public databases, where 80% of genomic data corresponds to bacteria and 20% to eukaryotes (mostly plants, animals and fungi). For metabarcoding, the trend is

even more pronounced: 95% of studies focuses on bacteria and only 5% on eukaryotes (Keeling & del Campo, 2017). Since the release of the human microbiome consortium paper (Huttenhower et al., 2012), thousands of papers have been published unveiling a great diversity of bacteria living within a wide range of hosts (McFall-Ngai et al., 2013). Fewer than a hundred papers have dealt with the mycobiome (Nash et al., 2017) and not more than a handful include all the other microeukaryotes in non-targeted microbiome surveys using universal primers. This is obviously a problem because the first step in understanding the ecology and putative roles of host-associated microeukaryotes is to know who they are and how they are distributed.

However, the paucity of studies of host-associated microeukaryotes is not just a result of research biases but also to the serious technical issue that symbiotic microeukaryotes are evolutionarily close to their animal hosts. Among the barcoding genes used to infer the identity of micro-organisms (Hajibabaei, Shokralla, Zhou, Singer, & Baird, 2011), the most widely used is the ribosomal small subunit rRNA (SSU rRNA) gene (in bacteria called 16S rRNA). This is amplified using universal bacterial primers (Tringe & Hugenholtz, 2008). In the case of fungi, one or both internal transcriber spacer (ITS) regions of the ribosomal operon are used (Schoch et al., 2012) and for other microeukaryotes as a whole (including fungi) the SSU rRNA (in eukaryotes called 18S rRNA) is used (Pawlowski et al., 2012). These approaches have been applied again and again with success in free-living environments, but the application in host environments has not been as successful for microeukaryotes simply because universal 18S rRNA primers mostly amplify reads derived from the animal (i.e. eukaryotic) host, dominating the resulting data and underrepresenting sequences from other eukaryotes present in the sample (Parfrey et al., 2014; Wampach et al., 2017; Wilcox & Hollocher, 2018). The scientific community is actively looking for solutions to this problem, the most popular so far has been the use of blocking primers in the PCR reaction, which target the host 18S rRNA signal and preventing its extension when using universal primers (Vestheim & Jarman, 2008). The main drawback of this approach is that a specific set of blocking primers is needed for every host species or group of hosts, depending on their ribosomal diversity (Belda et al., 2017; Hino, Maruyama, & Kikuchi, 2016). This approach therefore works well for individual hosts, but limits comparisons across host species. Furthermore, it is difficult to ascertain whether amplification of sequences from eukaryotes other than the host is also being blocked. Alternatively, researchers have developed universal primers that do not amplify the SSU rRNA of hosts (Waidele et al., 2017) or primers that only amplify a particular group of microeukaryotes (Bass, Stentiford, Littlewood, & Hartikainen, 2015; Kittelmann et al., 2015; Scanlan et al., 2014). This approach is limited to investigating novel diversity within known groups to which the primers are designed, rather than unknown lineages additional to those groups. A hybrid approach is to develop primers that work on a wide taxonomic range of eukaryotes but exclude all or most metazoans. One such set of primers developed to look at parasites (Bower et al., 2004) has recently been shown to work on a wide range of microeukaryotes associated with taxonomically diverse animal hosts (del Campo et al., 2019). Extensive deployment of these methods will certainly help to identify the main questions to ask about the eukaryome, many of which are currently not even clear.

3 | EUKARYOME DIVERSITY

The role of microeukaryotes within a host covers a wide spectrum of symbiotic interactions, from parasites to mutualists (Figure 1). Most of the knowledge we currently have comes from parasitology and medical mycology studies. This bias also exists for bacteria, but it is being overcome by the relatively recent change of paradigm brought by the study of the human microbiome (Hooper & Gordon, 2001; Huttenhower et al., 2012). This bias in the case of the eukaryotes is particularly obvious when looking at the available microeukaryotic genomes in public databases, most of which are from parasites (del Campo et al., 2014), and from the literature, where parasitology papers outnumber those on any other type of microeukaryotes except fungi.

Ironically, this nevertheless represents a broad taxonomic range because parasites are widely distributed in the tree and there is no single group of eukaryotes that does not contain a parasite (Bass et al., 2015; Figure 2). Among parasitic groups, there are also biases of course, many of the best studied parasites belonging to Alveolata. Apicomplexan parasites are responsible for malaria, toxoplasmosis, and cryptosporidiosis in humans, and east coast fever and red water

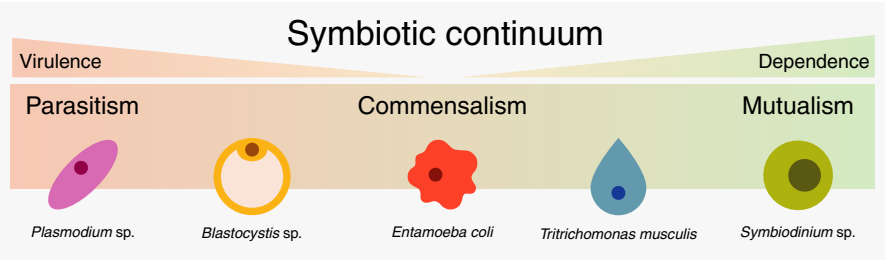

FIGURE 1 The symbiotic spectrum. Parasitism and mutualistic symbioses are often portrayed as occupying opposite ends of a scale. However, the same microbial agent can act in both capacities depending on their interactions with other symbionts, the host and environmental conditions. Therefore, a more mechanistic understanding of the interaction between hosts and their associated micro-organisms is required and will provide a more objective basis for categorizing and interpreting these interactions

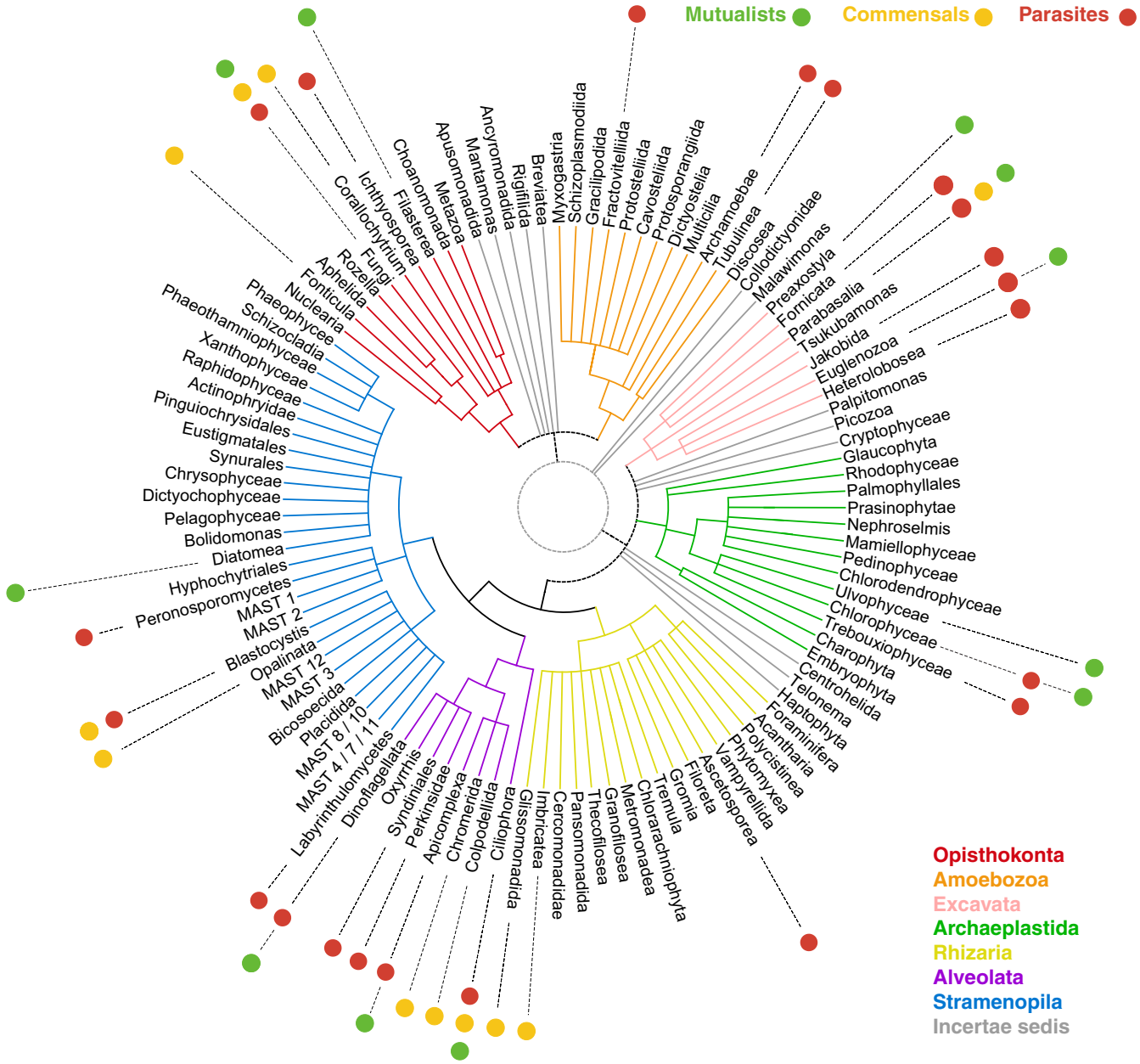

FIGURE 2 Tree of the eukaryotes showing all the groups containing known animal microeukaryotic symbionts (mutualists, parasites and commensals). Only those group organisms where a symbiotic relationship with animals has been documented are reflected in the tree. Coloured branches represent the seven main eukaryotic super groups, whereas grey branches are phylogenetically contentious taxa. Most of these relationships can be found in Walker et al. (2011) and Melo Clavijo, Donath, Serôdio, and Christa (2018). The tree is based on del Campo et al. (2014)

disease in cattle, plus myriad diseases in other vertebrates including fish and birds (Votýpka, Modrý, Oborník, Šlapeta, & Lukeš, 2016). There are parasites among other alveolate groups, including dinoflagellates and most notably the syndinians, which are widespread mostly marine parasites, perhaps the most abundant in the world (Guillou et al., 2008), and whose incompletely known host range includes various animals and many other microeukaryotes (Coats, 1999). Another alveolate parasitic group are the perkinsids, which cause disease in bivalves and frogs (Chambouvet et al., 2015), and also include the 'x-cell' fish pathogens (Freeman et al., 2017). The ciliates also include parasites such as *Ichthyophthirius*, the agent causing white spot disease in fish (Coyne et al., 2011) or parasites of invertebrates, for example, brown band agent in corals (Sweet & Bythell, 2012).

Another eukaryotic supergroup enriched in parasitic taxa are the excavates. Kinetoplastids include the agents of Chagas, leishmaniasis, Kala-azar and the African sleeping sickness (Gibson, 2016). Similarly, beaver fever (Adam, 2001), trichomoniasis (Hirt & Sherrard, 2015) or the infamous brain-eating amoeba, *Naegleria fowleri* (John, 1982) are all excavates. The third group of eukaryotes enriched in parasitic forms are the fungi. Microsporidians, which were for years considered basal eukaryotes but are now known to be among the

basal fungi (Capella-Gutiérrez, Marcet-Houben, & Gabaldón, 2012), are intracellular parasites that infect a wide range of animals from bees to humans (Stentiford et al., 2016), as well as other microeukaryotes (Bass et al., 2018). The unicellular chytrid fungi were long thought to be free-living, but have recently been found to include the genus decimating amphibian populations around the world (Fisher, Garner, & Walker, 2009). The best studied fungal parasites are, however, from the more familiar fungal groups, ascomycetes and basidiomycetes. *Candida* and *Pneumocystis* are responsible for thrush (Calderone & Fonzi, 2001) and pneumonia (Thomas & Limper, 2004) in humans. There are many others, often asymptomatic and opportunistic pathogens such as *Cryptococcus* (Kronstad et al., 2011) or *Aspergillus fumigatus* (Mccormick, Loeffler, & Ebel, 2010), affecting mostly immunocompromised individuals.

Among the other supergroups, parasites range from common to rare. Rhizaria contains two substantial parasitic radiations and one of them is the Ascetosporea infecting invertebrates (Bass, Ward, & Burki, 2019). Amoebozoan parasites are relatively common, and include two well-known human parasites, *Acanthamoeba castellanii* and *Entamoeba histolytica*. Both are typically free-living amoebae which, if they do infect humans, can cause keratitis and granulomatous encephalitis in the case of *Acanthamoeba* (Cabral & Marciano-Cabral, 2003) or dysentery in the case of *Entamoeba* (Loftus et al., 2005). Parasites have even been reported in the Archaeplastida (the group of phototrophic eukaryotes that include the land plants); green algal parasites lacking photosynthesis are known to infect insects (Tartar, Boucias, Adams, & Becnel, 2002) and vertebrates (Lass-Flörl & Mayr, 2007). It is increasingly recognized that host animals harbour a complex community of microeukaryotes, bacteria and viruses, which interact with each other and the host, in the manner of an ecosystem. Recognition of this reality has led to the development of the pathobiome concept as a basis for understanding how members of this community contribute to host health status, moving away from the one-pathogen-one-disease paradigm (Bass, Stentiford, Wang, Koskella, & Tyler, 2019).

On the other side of the spectrum, some of the lineages known to contain a large number of parasites that also include many mutualistic symbionts, particularly again the alveolates and excavates (Figure 2). The dinoflagellates (alveolates) is one of the model systems for mutualistic symbiosis, the association between *Symbiodinium* (zooxanthellae) and a wide variety of invertebrates, most famously reef-building corals (Glynn, 1993). Even the often-parasitic apicomplexans have representatives that can potentially be beneficial to their host, like *Nephromyces* in the molgulid tunicates (Saffo, McCoy, Rieken, & Slamovits, 2010). In the sister lineage to the apicomplexans, there are two photosynthetic alveolates, *Chromera* and *Vitrella*, which have been proposed to be coral symbionts (Moore et al., 2008; Oborník et al., 2012), and various ciliates are also beneficial to their hosts, for example, they help in active digestion in the ruminants (Kittelmann et al., 2015). We find also a significant example of mutualistic excavate symbionts in the guts of wood-eating insects. The parabasalia oxymonads include numerous diverse gut endosymbionts (although these are best known in wood-digesting environments, they are also seen more rarely in mammals, snakes or other insects; Yamin, 1979).

Many other organisms have been reported to live in association with animals, but the nature of the relationship is less clear. Many such interactions might be loose and non-specific, and are not always associated with disease or modified health status. In animals, it can be difficult to recognize their pathogenesis. Most microeukaryotes isolated from animal hosts are labelled as parasites, and only occasionally as symbionts for which the effects on the host are unknown. Some microeukaryotes are considered to be parasites that appear instead to be commensal or even mutualistic in certain contexts, for example *Blastocystis* and *Dientamoeba*, which in some populations seem to be common and obligate members of the gut microbiome of healthy individuals but are absent in patients affected by organic bowel disease and metabolic disorders (Stensvold & van der Giezen, 2018). Another example is *Tritrichomonas*, the genus has been traditionally identified as a parasitic one but recent studies have revealed that a species infecting mice, *Tritrichomonas musculis*, is a mutualist that protects the host from infections by activating the inflammatory response (Chudnovskiy et al., 2016).

4 | THE SYMBIOTIC CONTINUUM

Despite their frequent classification as parasites, the actual life history of most host-associated microeukaryotes appears to be much broader. In fact, microeukaryotic 'parasites' are frequently recorded in surveys of free-living environments (Geisen, Laros, Vizcaíno, Bonkowski, & De Groot, 2015; Mahé et al., 2017; Simon et al., 2015; de Vargas et al., 2015), suggesting these organisms are widespread and more abundant than previously thought. It is likely that many microeukaryotes routinely detected or observed/cultured from environmental samples may also be host associated, even if transiently. Such organisms can impact the bacterial composition of the microbiome (Morton et al., 2015) or interact with and aiding the immune system to fight disease (Chudnovskiy et al., 2016). *Malassezia restricta*, the dandruff, eczema, seborrheic dermatitis and pityriasis versicolor agent (Gaitanis, Magiatis, Hantschke, Bassukas, & Velegraki, 2012), is a lipophilic yeast well adapted to live on the surface of animal skin. However, it has been shown that *Malassezia* is not only present on those patients diagnosed with one of these syndromes but also widespread on asymptomatic humans based on metagenomic data (Oh et al., 2014), and it is frequently detected in environmental sequencing studies. Most invertebrates (Field & Michiels, 2005) are infected by a group of apicomplexans related to *Cryptosporidium* named gregarines (Leander, 2008), which were thought to be gut parasites. In most cases, their presence has no negative effect on host fitness (Fredricks & Relman, 1996). Thus, it is more likely they are commensals.

There are also intermediate states between commensalism and mutualism. For example, ciliates represent a significant amount of the microbial biomass in the rumen of ruminant animals, and while they are not essential for food degradation or host survival, it does

seem that they contribute to the digestive process by degrading certain components, scavenging oxygen and changing the bacterial community structure though grazing (Kittelmann et al., 2015). Even in the best studied mutualistic systems noted above, the coral holobiont and the xylophage termite guts, there are many other complexities. In the symbiosis between *Symbiodinium* (the zooxanthellae) and corals, *Symbiodinium* uses light to produce photosynthates that the coral uses as a food source (Wooldridge, 2010). Coral polyps also feed on other organisms, but the food provided by *Symbiodinium* is essential to reef-building and coral survival (Houlbrèque & Ferrier-Pagès, 2009). This is even more obvious during episodes of thermal stress, when the zooxanthellae are expelled leaving the coral bleached. Bleaching can be fatal for the animal if it is not reversed by returning to the original thermal conditions, allowing the return of the zooxanthellae (Hughes et al., 2017). Different species or strains of *Symbiodinium* have evolved within different coral families in different areas of the world. Moreover, a single coral colony does not live with a single species of zooxanthellae, but rather a mixed community, typically dominated by one genus. As a result, changes in climate can lead to a shift of symbionts between hosts, where a dominant *Symbiodinium* genus or species gets substituted by a formerly less abundant species that is better adapted to the new conditions (Baker, 2003). This process is known as symbiont shuffling, it has been reported that, for example, in cases of high water temperatures and after bleaching events the corals shuffled their symbionts to *Durusdinium* (formerly known as *Symbiodinium* Clade D) that is known to be heat tolerant. This phenomenon of symbiont shuffling represents a mechanism of rapid adaptation to environmental perturbations and confers the corals resilience against climate change (Baker, Starger, McClanahan, & Glynn, 2004).

In corals, another symbiont also illustrates how context can shift its position in the spectrum between parasitism and mutualism. *Ostreobium quekettii* is a green alga that bores into the coral skeleton, and it has both distribution and host specificity that are similarly complex as in *Symbiodinium* (del Campo, Pombert, Šlapeta, Larkum, & Keeling, 2017). *Ostreobium* has been proposed to be mutualistic, but only during bleaching events, when *Ostreobium* provides photosynthates to the host (Fine & Loya, 2002). This can sustain the host for a while until *Symbiodinium* returns, but if *Symbiodinium* remains absent for longer periods, then *Ostreobium* overgrows the coral and ultimately kills it. For that reason, *Ostreobium* has been also considered a parasite (Verbruggen & Tribollet, 2011). So, *Ostreobium* in 'normal' conditions acts as a commensal in the skeleton, but during bleaching events it becomes a mutualist for a short term, but if the bleaching situation continues it becomes a parasite and kills its host. This is a good example of how the health status of the host defines the role of the symbiont, and that different symbiotic states are not static but dynamic.

In the other well-studied model of microeukaryotic symbiosis, xylophagous termites, the complexities of the communities are even greater. The excavates associated with xylophagous insects are essential for cellulose degradation, and all lower termites harbour flagellated parabasalians and sometimes oxymonad in their hindgut. These assemblages are specific for each host and can be very complex (with up to 19 different species of microeukaryote and perhaps 10 times that of number of bacteria), but not all these species appear to contribute to the digestion in an obvious way (Brune, 2014). Some have been well documented to encode and express lignocellulases and can be easily observed to take up large particles of wood, fitting with their perceived role in the digestion process. Other species, however, especially smaller ones, seem to have no role at all and early substitution experiments showed they were dispensable in the laboratory, although they are found in 100% of individuals in nature.

5 | GENOMIC SIGNATURES OF HOST–SYMBIONT ASSOCIATIONS

There is a somewhat vague perception that there is a molecular toolkit that allows a parasite or a mutualist to act as such, but if context defines these roles it cannot be so simple. In the past, a parasite genomic toolkit was defined for certain groups, typically including proteins involved in cellular invasion, gliding motility, division or proteins providing particular structural and functional properties like virulence to the parasite. However, recent studies have shown that in most of these cases the genes thought to be novel to the parasitic lineage were already present in the free-living relatives of those parasites (Jackson et al., 2016; Janouškovec et al., 2015; Woo et al., 2015). Probably these 'parasitic toolkits' reflect more a lack of genomic information from free-living organisms than adaptation to parasitism. The emerging picture is that parasites, instead of acquiring novel components that 'made' them parasites, have adapted to a new environment (the host) through modification of existing conserved traits (Janouškovec & Keeling, 2016).

The same may be true of mutualistic symbionts, but genomic studies are less common than for parasites, though sets of genes related to mutualism have nevertheless been defined. For example, in *Symbiodinium* genes associated with light reactions, ion and metabolite transport, and stress responses have all been linked to adaptation to the needs of the symbiotic lifestyle and maintaining the regulation with its host. Genes involved in amino acid and glycoprotein biosynthesis have also been positively selected for, seemingly to provide nutrients that the host cannot synthetize by itself and are considered essential for the establishment of the symbiosis (Lin et al., 2015; Liu et al., 2018). These observations are the result of the genome analysis of zooxanthellae and represent an enrichment of certain gene families in their genomes, but we lack a clear comparison with close, free-living relatives, so we do not know whether they represent mutualist innovations or whether they are exaptation as has been observed in parasite evolution. In the case of the termite gut flagellates, some of the genes involved in cellulose degradation appear to be have been acquired by horizontal gene transfer from bacteria that also inhabit the gut (Todaka et al., 2010), suggesting the parabasalians moved into this environment for other reasons and adapted in such a way as to become beneficial to the host. A similar process of horizontal gene transfer

from bacteria has been observed in the commensal *Blastocystis*, where genes associated with carbohydrate use and anaerobic lifestyle are derived from bacteria have been reported in its genome (Eme, Gentekaki, Curtis, Archibald, & Roger, 2017). While the genomic mechanisms and evolutionary history of some parasites are well studied, this is not the case for mutualists and commensals. To fully understand the genomic basis and the evolution of the eukaryome, we need to increase our efforts to sequence and analyse the genomes of non-pathogenic microeukaryotes. In addition to characterizing eukaryome–host interactions according to virulence or (inter-) dependence on the host, we suggest that a more fundamental measure and understanding of the relationships can be based on the nature of the interaction with the host (Bass, Ward, et al., 2019). At extremes, the interaction will be very tight, for example, the metabolic complementarity shown by some microsporidia or the interdependence of the *Symbiodinium*–coral relationship, whereas entirely commensal relationships shown by a wide diversity of microeukaryotes in the gut or skin flora may be understood as more akin to hitchhiking (Figure 1).

6 | CONCLUSIONS

Compared with the study of host-associated bacterial communities, our understanding of animal-associated microeukaryotes is in its infancy. The impact of microeukaryotes on animal health, as a common inhabitant of the gut or other parts of the body, is potentially as relevant as the impact that prokaryotes have, but we know very little about it. To move forward, the first thing we need to do is to identify the main players, so we need more metabarcoding-based studies to characterize the eukaryome diversity. However, it is crucial not to make the same mistake twice, we should not consider the microeukaryotic fraction of the microbiome as an isolated compartment. Microeukaryotes are constantly interacting not only with the host but also with the prokaryotic microbes (and viruses) that share the same environment. We need to consider all the members of the microbiome as a whole and study their interactions. One very good model for this approach is the termite gut ecosystem. Although studying the eukaryome at the community level is important, working at the organismal level is also critical to fully understand the functional roles played by individual microeukaryotes, with a particular emphasis on the commensals, where the functional relationship is not obvious. The use of genomic approaches will help us to infer the evolutionary mechanisms involved in the establishment of the symbiosis, and comparative approaches that have been informative for the origin and evolutionary adaptations of parasites should be extended to mutualists and commensals. Finally, it is also important to consider and treat the host as another environment for microbes; animals as an environment may have some peculiarities, but they are not fundamentally different from oceans or soils. Changing a host-centric vision of symbiosis, based on the positive of negative effect that the micro-organisms have on the host, to a microbe-centric one based on the roles played by the micro-organisms in the system and how they interact and adapt to changes and perturbations, will help us to break the constrains of the simplistic and static view that we nowadays have on the symbiotic associations.

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J.d.C. conceived the idea and decided the content of this review. J.d.C., D.B. and P.J.K. wrote the manuscript. J.d.C. prepared the figures.

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There is no archived data associated with this manuscript.

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