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Eco-evolutionary implications of helminth microbiomes

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Abstract

The evolution of helminth parasites has long been seen as an interplay between host resistance to infection and the parasite's capacity to bypass such resistance. However, there has recently been an increasing appreciation of the role of symbiotic microbes in the interaction of helminth parasites and their hosts. It is now clear that helminths have a different microbiome from the organisms they parasitize, and sometimes amid large variability, components of the microbiome are shared among different life stages or among populations of the parasite. Helminths have been shown to acquire microbes from their parent generations (vertical transmission) and from their surroundings (horizontal transmission). In this latter case, natural selection has been strongly linked to the fact that helminth-associated microbiota is not simply a random assemblage of the pool of microbes available from their organismal hosts or environments. Indeed, some helminth parasites and specific microbial taxa have evolved complex ecological relationships, ranging from obligate mutualism to reproductive manipulation of the helminth by associated microbes. However, our understanding is still very elementary regarding the net effect of all microbiome components in the eco-evolution of helminths and their interaction with hosts. In this non-exhaustible review, we focus on the bacterial microbiome associated with helminths (as opposed to the microbiome of their hosts) and highlight relevant concepts and key findings in bacterial transmission, ecological associations, and taxonomic and functional diversity of the bacteriome. We integrate the microbiome dimension in a discussion of the evolution of helminth parasites and identify fundamental knowledge gaps, finally suggesting research avenues for understanding the eco-evolutionary impacts of the microbiome in host–parasite interactions in light of new technological developments.

Introduction

We have long known that microbes form ecological associations with many different organisms. The first descriptions of bacteria associating with humans were done by Antonie van Leeuwenhoek in the 17th century (Finegold, 1993). More than 100 years later, microorganisms interacting with animals and plants were recognized in *A fauna and flora within living animals* (Leidy, 1853), which was followed by an increasing number of investigations characterizing microbial symbionts and their functions, particularly in human health (Savage, 2001). Fast-forward to 2023, and we are witnessing the 'microbiome revolution'. We increasingly understand that symbiotic microbes are present and perform key functions at all levels of biological organization. For example, the composition of the human microbiome has been linked to gut health, immunity modulation and disease susceptibility (Wang *et al.*, 2017; Fassarella *et al.*, 2021); the taste of wine has been linked to microbial communities in the soil (Belda *et al.*, 2017); and in conservation programmes, the health status of captivity-bred species has been linked to differences in the microbiome composition between their natural and artificial habitats (West *et al.*, 2019). Similarly, symbiotic microbes are also present in helminth parasites and their parasitized hosts, performing central roles in what was previously seen as a two-player interaction (parasite–host), with eco-evolutionary implications for all players involved (Morley, 2016; Dheilly *et al.*, 2019b; Jenkins *et al.*, 2019; Brealey *et al.*, 2022; Hahn *et al.*, 2022; Poulin *et al.*, 2022).

Much of the research involving microbiomes in parasitology has focused on the microbiome of the parasitized organism (Hayes *et al.*, 2010; Vicente *et al.*, 2016; Rapin & Harris, 2018; Rosa *et al.*, 2018; Jenkins *et al.*, 2019; Le Clec'h *et al.*, 2022). In this context, microbes can modulate the immune response against the parasite both indirectly, for example, by helping with the development of the immune system, and directly, for example, by producing toxic compounds that may kill the parasite (Dheilly *et al.*, 2015, 2017; Hahn *et al.*, 2022). However, the mere presence of parasites may alter the microbiome of a parasitized organism. This difference can be either a simple by-product of infection, as well as changes initiated by the parasitized organism as a response to the parasitic infection, or even changes induced by the parasite (Dheilly *et al.*, 2015; Hahn *et al.*, 2022).

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Among parasites, helminths are ubiquitous in the terrestrial and marine environment and are especially interesting from an evolutionary perspective, with their many different life stages requiring a combination of invertebrate and vertebrate hosts to complete a life cycle (Bennett *et al.*, 2021, 2022). Microbes in direct symbiosis with organisms parasitized by helminths have been reviewed elsewhere and perform many roles, including resistance to the parasites, heat tolerance, diet supplementation, development and immune defence (Dheilly *et al.*, 2015; Reynolds *et al.*, 2015; Brealey *et al.*, 2022). Helminths are economically relevant pathogens to vertebrates, which may be the reason why research at the parasitized organism level usually focuses on the effect of specific microbes on the susceptibility, infection, or resistance of the parasitized organism to the parasite. In short, common research topics are characterization of the host microbiome, microbiome variability among individuals hosts, species-specificity of different microbial taxa and source of microbial acquisition (Dheilly, 2014; Reynolds *et al.*, 2014, 2015; Dheilly *et al.*, 2015, 2019a; Hahn & Dheilly, 2016; Midha *et al.*, 2017; Topalovic & Vestergard, 2021; Le Clec'h *et al.*, 2022).

With the advances and increasing accessibility of metagenomics and sequencing technologies, we can target the microbiome associated with parasites and begin to understand the eco-evolutionary significance of this deeper layer of ecological interactions. Aiming to provide guidelines and advance research on the roles and implications of symbiotic microbes living within parasites, an international consortium of researchers was formed: the Parasite Microbiome Project (Dheilly *et al.*, 2017, 2019b). Recent work has revealed that the microbiome within different helminths can range from very simple (with only a few conserved taxa associated with multiple individual helminths of the same population) to highly complex (with several different taxa and significant variability in community composition among individuals) (Hahn *et al.*, 2022; Jorge *et al.*, 2022a). Helminth microbiomes can influence infection success and susceptibility to the host's immune responses (Dheilly *et al.*, 2015; Martinson *et al.*, 2020; Brealey *et al.*, 2022), and may play a role in the ability of some manipulative parasites to alter the phenotype of their animal hosts (Poulin *et al.*, 2022). A helminth species may have a geographically variable microbiome (Jorge *et al.*, 2022b), but specific microbiome components may be consistent over the many life stages of the parasite's life cycle (Jorge *et al.*, 2020). However, our understanding of these complex and dynamic microbe–helminth associations still have a long way to go, as does our knowledge about the eco-evolutionary implications of such relationships, both for helminths and for the different components of the microbiome. This is highlighted by the slowly growing body of research on helminth microbiomes compared to the rapidly growing knowledge about microbiomes in general (fig. 1). Here, we will consider the bacterial microbiome

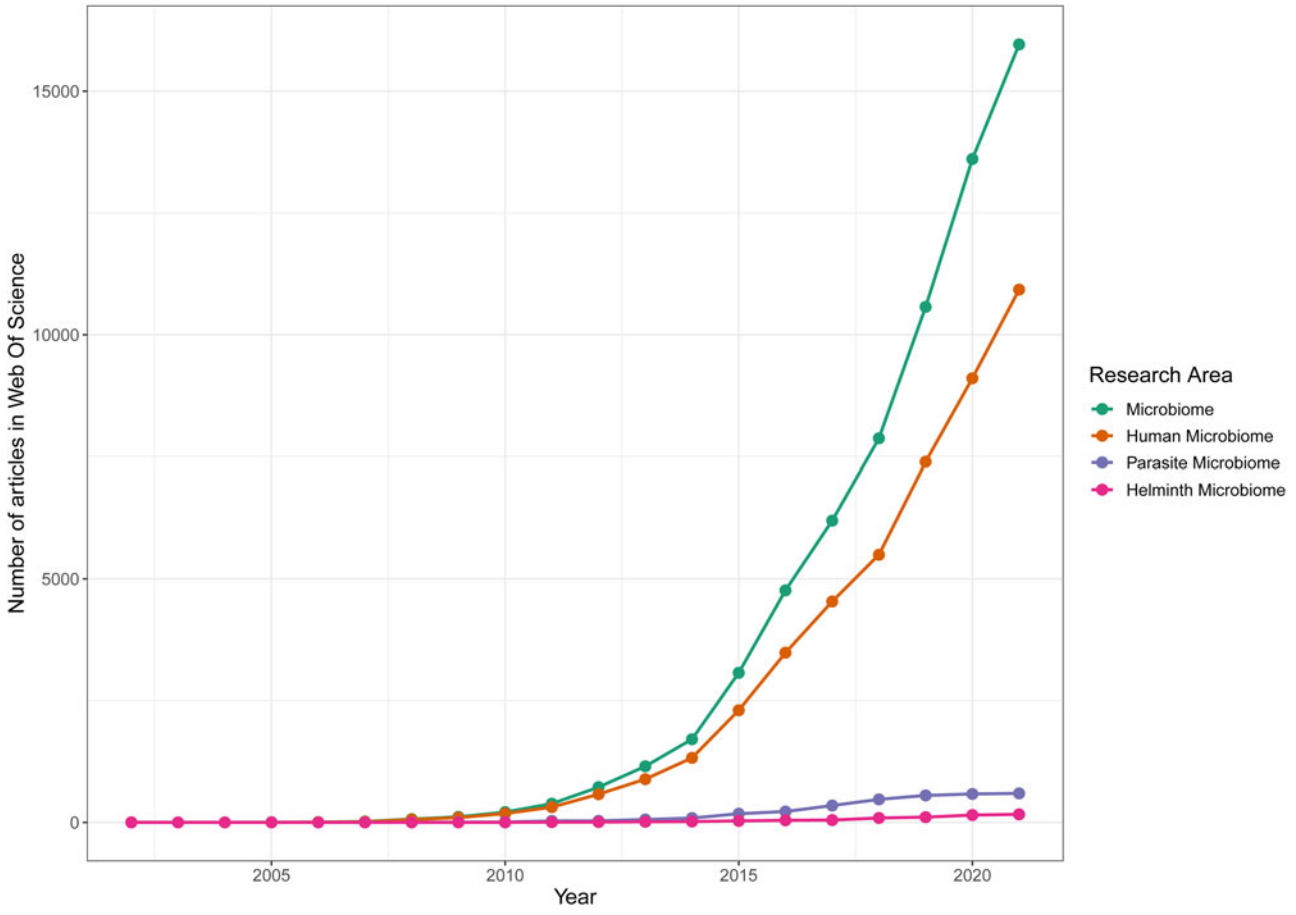


Fig. 1. Articles per year (non-cumulative) in a Web of Science search for different microbiome research areas. The search included years 2002 to 2021 and was refined to include only articles. Keywords per area: Microbiome, 'microbiome'; Human Microbiome, 'microbiome AND human'; Parasite Microbiome, 'microbiome AND parasit*'; and Helminth Microbiome, 'microbiome AND (nematod* OR cestod* OR trematod* OR monogene* OR digene* OR acanthocephal*).

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associated with helminths (excluding free living forms such as planarians) and review concepts relevant to microbial transmission, the nature of helminth–bacteria ecological interactions, the diversity in helminth bacteriomes and the eco-evolutionary impacts of such interactions. We will finish by suggesting potential avenues for future research in light of recent technological innovations.

Due to the nested nature of the ecological relationships treated in this review, we have attempted to improve clarity by hereafter referring to hosts with the meaning of a multicellular organism (animal or plant) that is parasitized by a helminth. We refer to helminths as organisms that depend on plant or animal hosts to complete their life cycle. We note that this review focuses on the bacterial community harboured by the helminths (i.e. helminth bacteriomes), for which enough literature is available to build a conceptual framework. However, the concepts discussed here likely apply to the many other microbiome components. Extremely little is known about the archaea, protozoa and fungi components of helminth microbiomes, and even though there is a growing body of work on the virome of parasites in general (Dheilly *et al.*, 2022), and unicellular eukaryotes such as microsporidians have occasionally been reported within helminths (e.g. Sokolova *et al.*, 2021), they will not be further considered in this review.

The sources: where do helminths get their bacteria from?

General research on microbial communities has reported great variability in the taxa associated with plants/animals/environment and described interesting phenomena, such as the 'founder hypothesis' (i.e. pre-existing microbial lineages that dominate recolonization), ultimately highlighting the dynamic nature of microbial symbiosis in diverse systems (Litvak & Baumler, 2019). The same seems to apply to the bacteriome of helminths. In many instances, bacterial taxa composing microbial communities are variable even among helminths parasitizing the same individual host (Jorge *et al.*, 2020, 2022b; Hahn *et al.*, 2022), and, when disrupted with antibiotics, increased abundance of founder bacteria post-disturbance may follow (Jorge *et al.*, 2022c). In other cases, there is little diversity in the bacteria composing helminth microbiomes (Brealey *et al.*, 2022). The impact of these phenomena on the fitness and evolution of helminths is still unclear.

The extensive range of variability in the bacteriome of helminths leads to questioning the microbial sources of individual helminths in a population (Rosenberg & Zilber-Rosenberg, 2021): helminths may horizontally acquire bacteria, from their habitat, be it the external environment when they are in the infectious larval stages, or their surroundings within their host, and their diet, whatever they feed on (host tissue, or even other co-infecting parasites) (Jorge *et al.*, 2020, 2022b). Helminths may also vertically acquire bacteria, which means bacteria are transmitted among parasite generations (Jorge *et al.*, 2020, 2022b).

In cases of horizontal transmission, different generations do not share bacteria, but there is consistency in the bacteriome, for example, in populations across different geographical localities, implying a potential role of natural selection in determining the bacteria that colonize the parasite (Hahn *et al.*, 2022; Jorge *et al.*, 2022b). For example, there is geographical stability in the bacteriome of the trematode *Philophthalmus attenuatus*: parasites in different localities but at the same life stage share more bacteria than parasites of different life stages in the same locality (Jorge *et al.*, 2022b). This suggests that specific bacteria are important in each life stage, but that they are not transmitted from one generation to the other, and rather they are acquired horizontally (from the environment or the parasite's surroundings). Supporting this is the association between different bacteriomes and different genetic lineages of the cestode *Schistocephalus solidus* (Hahn *et al.*, 2022). Thus, a parasite's bacteriome is not simply a random assemblage of the pool of bacteria available in the parasite's habitat, as natural selection may restrict which bacteria will successfully colonize the helminth, although it may also depend on which bacteria were settled in before (Hahn *et al.*, 2022; Jorge *et al.*, 2022b, 2022c). Interestingly, *Eubothrium* cestodes parasitizing salmon were shown to associate with different *Mycoplasma* lineages than those found in the salmon`s microbiome, suggesting a role of divergent selection for specific *Mycoplasma* lineages in the cestode parasite and its salmon host (Brealey *et al.*, 2022). Yet, the *Mycoplasma* lineages associated with the cestode and the salmon are phylogenetically very close, suggesting shared ancestry of the specific bacterial lineages between the salmon and cestode (Brealey *et al.*, 2022). Fundamentally, in addition to consequences to the parasitized organism (and its bacteriome), the parasite also has a role in the evolution of the bacteria composing its own microbiome, which in turn may interact with the evolution of the parasite (and that of its hosts and their microbiome).

In cases of vertical transmission, if a helminth is associated with a core set of bacteria (or a core microbiome, Neu *et al.*, 2021) persistent in different habitats (e.g. different host species) and across different life stages of the helminth, then the core bacteriome and the helminth are likely responding to changes as an evolutionary unit (Jorge *et al.*, 2020). For example, *Coitocaecum parvum* trematodes have a core bacteriome that persists over different life stages through different animal hosts and environments, and the main source of these bacteria is the previous life stage (Jorge *et al.*, 2020). However, vertical transmission is imperfect, that is, only a proportion of parasite offspring inherit certain bacteria from the parent parasite (Greiman *et al.*, 2013).

From a microbial evolution perspective, the transmission mode must contribute to each bacterial lineage's persistence over evolutionary time, avoiding dead ends (Ebert, 2013; Dheilly *et al.*, 2015). Thus, there is an important correlation between the mode of bacterial transmission and the ecology of the helminth, including factors such as the helminth population density, fecundity, different life stages and habitats. Horizontal transmission is an effective transmission strategy for the bacteriome of helminths with a large population density, or that have large numbers parasitizing a single individual host, or large numbers in the same environment. In contrast, vertical transmission is a suitable strategy for bacteria persisting over patchy geographical distribution and across different life stages of the parasite. Thus, vertical bacterial transmission is tightly linked to the helminth's reproductive success (Ebert, 2013). Vertical transmission enables bacteria to persist over discrete generations of the parasite and overcome constraints such as helminths with small numbers of offspring and low success in transitioning to the next life stage in a different host species. Clearly, a strategy combining horizontal and vertical transmission enables the exploitation of a larger breadth of possibilities for bacterial persistence (Ebert, 2013) and could contribute to the large variability in the bacteriome composition of helminths. Lastly, the helminth habitat may also play a role in determining bacterial transmission strategies, given that higher vertical transmission rates are more common in terrestrial than aquatic symbiotic microbes (Russell, 2019).

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The genetic diversity of bacterial lineages and inter-specific association in allele frequencies among the helminth and bacterial alleles may help define the source of specific bacteria in helminths. In horizontal bacterial transmission, high lineage diversity in the bacteriota of a single individual is expected, as different bacterial lineages may colonize an individual helminth over several founding events (Ebert, 2013). Contrastingly, in cases of vertical transmission, specific bacterial genotypes become associated with the genotype of the individuals they inhabit, leading to inter-specific linkage disequilibrium (Ebert, 2013; Hayward *et al.*, 2021; Hahn *et al.*, 2022). Ultimately, microbes with strict vertical transmission across many helminth generations may present congruent phylogenies with the parasite (Hayward *et al.*, 2021). However, other factors unrelated to the mode of transmission can lead to interspecific linkage disequilibrium (e.g. selection and spatial structure), and for microbes with mixed transmission modes (vertical and horizontal transmission), inter-specific allelic correlation is expected to be weaker (Brandvain *et al.*, 2011; Fitzpatrick, 2014).

The nature of ecological interactions among helminths and bacteria

The variability and dynamic composition of the bacteriome of helminths reflect the complexity of the symbiotic interactions among helminths and bacteria, and broad generalizations are hardly possible. However, to better understand the ecological impacts of such interactions, it can be helpful to identify shared patterns among case studies. Following Moran *et al.*'s (2008) symbioses' classifications among microbes and insects, below we propose a system to identify characteristics of obligatory and facultative interactions among bacteria and helminths.

Obligatory mutualism: bacteria that present obligatory mutualism with helminths (also called primary symbionts) are essential to the development of the helminth, which in turn is essential to the microbe's transmission. Obligatory mutualistic bacteria are genus-specific or species-specific, meaning they are only successful in one helminth genus or species and are strictly vertically transmitted. For example, bacteria from the group *Candidatus* Symbiopectobacterium are strictly maternally transmitted among generations of the nematode *Howardula aoronymphium*, which has low success in parasitizing its *Drosophila* host when the association with the bacterium is absent (Martinson *et al.*, 2020). Some *Candidatus* Symbiopectobacterium lineages show genomic degradation, a footprint of obligatory symbiotic association due to accumulating deleterious mutations, and are phylogenetically closely related to obligate symbionts of other invertebrates (Martinson *et al.*, 2020). Few other examples of bacteria–helminth obligatory mutualism are known at present, and their 'obligatory' nature has been questioned, such as the case of *Xenorhabdus* and *Photorhabdus* gram-negative bacteria associating with Steinernematidae and Heterorhabditidae nematodes (Poinar & Thomas, 1966). These bacteria kill the nematode's insect host so that the nematode can feed on the dead insect as it reproduces and grows. The bacteria then infect the nematode juveniles, which are subsequently released to the soil in search of the next insect host (Forst & Clarke, 2002). However, even though *Xenorhabdus* and *Photorhabdus* bacteria are species-specific and vertically transmitted among the nematodes, the bacteria can be cultured in laboratory conditions free of the nematodes, which has led authors to classify the symbiotic relationship as non-obligatory mutualism (Forst & Clarke, 2002).

Facultative symbiosis: bacteria that facultatively associate with helminths (also called secondary symbionts) are not essential to the reproduction or development of the helminth and may associate with various helminth species. Thus, there is an important role for horizontal bacterial transmission. These bacteria modulate the phenotype/behaviour of the helminth in order to increase the prevalence and spread of helminth lines containing the symbiotic bacterial lineages. For example, the bacteriome of reproductive morphs of the trematode *Philophthalmus attenuatus* has been shown to differ from the bacteriome of morphs that do not reproduce (soldiers). When both morphs were treated with antibiotics within the snail host, the development of reproductives was favoured over the development of soldiers, supporting a role of the bacteriome in the formation of different morphs and indicating a potential bacteriome manipulation of the trematode reproductive strategy (increase in lines bearing the reproductive bacteriome) (Jorge *et al.*, 2022a).

There are two subcategories of facultative symbiosis:

Facultative mutualism: the phenotypic modulation induced by the bacteria causes a direct benefit to the helminth, in terms of longer life spans or protection from stress, ultimately leading to higher reproductive success. Facultative mutualism may include cases in which the bacteria help protect the helminth against their host's immune response or against other microorganisms that could compete or attack the helminth, as well as benefits in terms of dietary supplementation. For example, electron microscopy has revealed a homogeneous composition of bacteria located within cavities on the surface of two different species of tapeworm, likely providing an increase in food absorption by the worms (Caira & Jensen, 2021). Moreover, the only known function of these cavities is housing bacteria, suggesting that these structures evolved specifically because the tapeworm benefits from such relationships (Caira & Jensen, 2021).

Reproductive manipulation: the phenotypic modulation induced by the bacteria interferes with the helminth's reproduction, favouring helminth lines harbouring the bacteria. In such cases, vertical transmission is possible and would lead to increasing fecundity or reproductive success of helminth lines bearing the bacteria, as opposed to lines free from the bacteria. For example, *Neorickettsia* bacteria infecting the digenean trematode *Plagiorchis elegans* have mixed transmission (vertical and horizontal transmission) and are pathogenic to horses (Greiman *et al.*, 2013). Even though the trematode is the vector of *Neorickettsia* to the horse, the trematode cannot reproduce in the horse, thus ruling out a mutualistic relationship (Pusterla *et al.*, 2003; Greiman *et al.*, 2013). *Neorickettsia* rate of transmission during the asexual multiplication phase of *P. elegans* varies from 11–90%, confirming its imperfect vertical transmission (Greiman *et al.*, 2013). However, the effect of *Neorickettsia* on the trematode's reproductive success in its intermediate hosts (a snail and an arthropod) remains unknown.

From a bacterial evolutionary perspective, selection favouring bacteria with higher fitness does not necessarily incur benefits to the helminth with which they associate (Dheilly *et al.*, 2015; Speer *et al.*, 2020). Indeed, there are cases in which an increase in bacterial fitness may decrease the parasite's fitness, in an antagonistic dynamic. An example is *Salmonella* bacteria that are shielded from antibiotics when attached to schistosome parasites,

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however the number of trematodes in a parasitized animal is smaller in co-occurrence with *Salmonella* than when the bacteria are not associated with the schistosomes (Barnhill *et al.*, 2011; Zhu *et al.*, 2017).

Characterizing interactions among bacteria and helminths can help understand the ecological impact of the absence of certain interactions or their removal by, for example, antibiotics treatment. If obligatory mutualism is impeded, then both the bacteria and the helminth parasite in question are expected to perish or achieve greatly reduced fitness; in contrast, if an antagonist relationship is impeded, the chances of survival and success of the helminth may increase. Nevertheless, in most bacteria–helminth associations, the nature of the symbiotic relationship is fluid and can be strongly context-dependent. Microbe–microbe interactions are important in microbial communities (Proal *et al.*, 2017). There is nothing to suggest that, under different circumstances, certain bacterial lineages cannot act as beneficial agents and pathogens to the same helminth species, just as it happens in the human gut microbiome (Schubert *et al.*, 2015; Sharpton & Gaulkeb, 2015). In addition, mutualism and parasitism are but the ends of an evolutionary continuum (Drew *et al.*, 2021), and defining interactions anywhere along a continuum can be highly subjective (Leung & Poulin, 2008). Even so, identifying shared patterns among different contexts can be helpful to improve our understanding of the significance of some of these interactions for the evolution of both microbes and helminths, and this is what the aforementioned classification system can be used for.

The diversity of the bacteriome in helminths

Large variability in microbiomes is universally recognized. In humans, increasing sampling efforts inevitably correlate with a decrease in the percentage of common taxa among all people, and currently, fewer than 20 genera are shared by more than 95% of the sampled human populations (Sanna *et al.*, 2022). There is an influence of external factors on the composition of the microbiome (e.g. environment and diet), but surprisingly, the heritability of some components of the human microbiome is around 20%, suggesting a role of the genetic makeup of the individual in the composition of its microbiome (Sanna *et al.*, 2022). In helminths, both the genotype and the bacteriome of the cestode *Schistocephalus solidus* correlate with changes in the bacteriome and phenotype of its fish host (Hahn *et al.*, 2022). Further research associating the genotype of helminths and their hosts with the diversity of their bacteriome is needed to shed light on the factors underlying bacteriome variability.

There is an important distinction between the core bacteriome and the transient bacteriome in helminths. The core bacteriome refers to specific bacterial lineages present throughout the helminth's life cycle, in which bacterial acquisition via vertical transmission is key (Formenti *et al.*, 2020; Jorge *et al.*, 2020; Neu *et al.*, 2021). Stable bacterial lineages across different geographical localities may also represent a core bacteriome, but in this case, horizontal transmission may tightly interact with natural selection towards keeping specific bacterial lineages associated with specific life stages of the helminth (Jorge *et al.*, 2022b; Sheehy *et al.*, 2022). For example, different lineages of *Phasmarhabditis* nematodes have a core set of bacteria even when originating from different localities and being cultured under different conditions for varying lengths of time (Sheehy *et al.*, 2022). The composition of the core bacteriome is, thus, expected to be relatively stable, probably indicating that either such bacterial lineages play a role in the helminth's ecology and evolution, or they depend on the helminth for their own transmission and survival, or both (Formenti *et al.*, 2020; Jorge *et al.*, 2020, 2022b; Sheehy *et al.*, 2022).

In comparison, transient bacteriome refers to bacterial lineages that are only present in specific life stages of the helminth, or in specific geographical localities, and can be greatly variable among individual helminths (Formenti *et al.*, 2020; Jorge *et al.*, 2020, 2022b; Hahn *et al.*, 2022). However, the transient bacteriome can still impact the helminth's biology. For example, transient bacterial lineages could correlate with differences in the pathology of virulence of helminths, or even with variability in parasite-induced manipulations of host phenotype and behaviour (Dheilly *et al.*, 2015; Poulin *et al.*, 2022). Transient bacterial lineages depend on horizontal transmission (Formenti *et al.*, 2020). However, as mentioned above, horizontally acquired bacteria do not necessarily represent a random assemblage of the bacterial pool in the helminth's environment, and host-based selective forces are relevant to determining the composition and diversity of the parasite bacteriome (Hahn *et al.*, 2022).

Many examples in the literature describe single bacterial taxa interacting with helminths and their hosts (table 1). However, conceptual complexities arise when considering the net effect of many microbial genotypes (i.e. the microbiome), involving interactions among themselves, with the helminths and with the parasitized host (and its microbiome) (Dheilly, 2014; Theis *et al.*, 2016). The great taxonomic variability in the bacteriome has led to functional investigations of individual bacterial lineages, with findings converging to the realization that the functions of many lineages are redundant (Speer *et al.*, 2020). In fact, metabolomics research has shown that microbiomes composed of different taxa may produce similar metabolites (Litvak & Baumler, 2019). Furthermore, the many microbial lineages may have a differential contribution to the microbiome (Reynolds *et al.*, 2015): a few isolated lineages could have a strong effect, and many individual lineages could have a small effect that results in a stronger combined impact on the ecology and evolution of helminths. Such considerations create a clear distinction in how microbial diversity is defined and studied: taxonomic diversity is concerned with the diversity of lineages composing the microbiome, while functional diversity characterizes the pool of functional traits in a microbiome, regardless of taxonomic diversity (Escalas *et al.*, 2019).

If knowledge about the microbiome's taxonomy in helminths is still in its infancy, the study of the microbiome's functional diversity in parasitology is even more so. However, the potential of this type of study can already be seen. For example, upon finding differences in lineages of *Mycoplasma* composing the microbiome of the cestode *Eubothrium* and its salmon host, Brealey *et al.* (2022) generated metagenome-assembled-genomes (MAGs) and performed functional annotation by comparison with previously available *Mycoplasma* genomes. Functional genomic regions coding for different metabolic pathways were present in cestode-associated *Mycoplasma* vs. salmon-associated *Mycoplasma*, suggesting adaptations of *Mycoplasma* to the different environments (i.e. adaptation to the cestode or to live within the fish gut). Nevertheless, the study was limited by the lack of available *Mycoplasma* genome assemblies in non-mammalian hosts, highlighting the need for further studies to fill this fundamental gap.

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Table 1. Selected examples of association between bacteria and helminth parasites.

	Parasite	Bacteria	Short description	Relationship	Reference
NEMATODA	Filarial nematodes (Onchocercinae and Dirofilariinae)	*Wolbachia*	bacteria are maternally transmitted among parasite generations. Treatment with antibiotics affect the worms by delaying moulting, reducing growth rates, embryonic failure and death. *Wolbachia* produce essential metabolites for the nematodes (riboflavin, haeme, glutathione and glycolytic enzymes). There is phylogenetic congruence among *Wolbachia* lineages and nematodes	obligate mutualism	Comandatore *et al.* (2013), Landmann *et al.* (2011), Sironi *et al.* (1995), Slatko *et al.* (2010), and Taylor *et al.* (2005)
	Trichuris sp.	*Escherichia coli* and *Salmonella typhimurium*; clostridia (*Preptostreptococcaceaea*); lactobacilli	bacteria are required for egg hatching, but different bacterial lineages are required for different *Trichuris* species (*Trichuris muris* vs *Trichuris suis*), suggesting adaptation to different bacterial lineages that are specific to their animal host. *Preptostreptococcaceaea* may promote egg hatching of *T. muris* and *Trichuris trichiura* in humans. *Trichuris suis* infection is associated with changes in the microbiome of the parasitized host, with increase in lactobacilli and mucolytic bacteria.	unknown	Hayes *et al.* (2010), Holm *et al.* (2015), Sargsian *et al.*, (2022), Vejzagić *et al.* (2015), and White *et al.* (2018)
	Ascaris sp.	*Pseudomonas pyocyanea* and other gram-negative bacteria	in pigs and horses, gram-negative bacteria help reduce the abundance of gram-positive bacteria that have an anti-helminthic effect, protecting *Ascaris* sp. against threatening conditions	unknown suggestion: facultative mutualism*	Emanuiloff, 1958, as cited in Morley (2016)
	Pseudocapillaria tomentosa	undefined taxa	changes in the alpha and beta diversity of the microbiome of zebrafish can be used to diagnose infection with the nematode, and are related to worm burden and infection success	unknown	Gaulke *et al.* (2019)
	Steinernematidae and Heterorhabditidae	*Xenorhabdus* and *Photorhabdus*	bacteria from the gut of juvenile worms are released to the parasitized insect host and kill the insect with toxins. The dead insect is a source of nutrition for the reproductive stages of the nematodes, and once juveniles are formed again, their guts are colonized by the bacteria before they disseminate in the ground. A specific case is that of *Photorhabdus luminescens* bacteria within *Heterorhabditis bacteriophora* nematodes that lead the	obligate mutualism (but see Forst & Clarke, 2002)	Fenton *et al.* (2011), Forst & Clarke (2002), Poinar & Thomas (1966), and Singh *et al.* (2012)

(*Continued*)

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Table 1. (*Continued.*)

	Parasite	Bacteria	Short description	Relationship	Reference
			parasitized insect host changing colour, which deters avian predators		
	Ascaris suum	undefined taxa	bacteria supplement the limited serotonin levels of the worm	unknown suggestion: facultative mutualism*	Shahkolahi & Donahue (1993)
	Heligmosomoides polygyrus	Lactobacillacea	increase in *Lactobacillus taiwanensis* in mice is associated with susceptibility to infection by the nematode (increased abundance of Lactobacillacea bacteria promote infection by the nematode)	unknown	Reynolds *et al.* (2014)
	Xiphinema americanum	*Veruccomicrobia* and *Xiphinemobacter*	strictly vertically transmitted, maternally inherited bacteria have been proposed to reduce the number of males in the nematode populations. There is phylogenetic congruence among bacteria and nematodes	obligate mutualism	Coomans *et al.* (2000), Palomares-Rius *et al.* (2016), and Vandekerckhove *et al.* (2000)
	Elicilacunosus dharmadii and *Caulobothrium multispelaeum*	coccoid-like and bacillus-like bacteria (undefined taxa)	bacteria are associated with folds of the nematode body (fillitriches) and hypothesized to participate in diet supplementation	facultative mutualism	Caira & Jensen (2021)
TREMATODA	*Opisthorchis viverrini*	*Helicobacter pylori* and other host gut bacteria	oncogenic bacteria vectored by the parasite, in addition to alteration of the animal host microbiome, may contribute to cancer development	unknown	Deenonpoe *et al.* (2017) and Itthitaetrakool *et al.* (2016)
	Digenean trematodes (e.g. *Nanophyetes*, *Echinostoma* and *Fasciola*)	*Neorickettsia* bacteria	trematodes acquire bacteria vertically from previous generations, but functioning as disease vectors, they horizontally transfer *Neorickettsia* to mammalian hosts. *Neorickettsia* is always associated with digenean trematodes, but there is large variability in trematodes species bearing the bacteria (including among individuals of the same population)	unknown. suggestion: facultative mutualism*	Lawrence & Poulin (2016), McNulty *et al.* (2017), Pusterla *et al.* (2003), and Vaughan *et al.* (2012)
	Clinostomum marginatum	gram-negative bacteria (*Achromobacter* sp., *Edwardsiella tarda* and *Enterobacter agglomerans*)	bacteria provide active transport of glucose to the trematode, but trematodes developing in the absence of the bacteria can transport glucose via facilitated diffusion	unknown suggestion: facultative mutualism*	Aho *et al.* (1991) and Uglem *et al.* (1991)
	Schistosoma japonicum	schistosome-specific microbiome	bacteria were found associated with the tegument and gastrodermis of female schistosomes, but only with the gastrodermis of male schistosomes, suggesting that females may use the microbiome in a	unknown suggestion: reproductive manipulation*	Gobert *et al.* (2022)

(*Continued*)

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Table 1. (*Continued.*)

	Parasite	Bacteria	Short description	Relationship	Reference
			different way from males, potentially to meet egg-producing demands		
	Schistosoma mansoni	*Salmonella*	in humans, *Salmonella* bacteria attached to the worms' surface are shielded from antibiotic treatments; mice have a smaller number of worms when *Salmonella* are present	unknown	Barnhill *et al.* (2011) and Zhu *et al.* (2017)
	Philophthalmus attenuatus	*Rhodobacteraceae*	this family of bacteria was found in high prevalence in all life stages of the trematode	unknown	Jorge *et al.* (2022b)
	Coitocaecum parvum	*Streptococcus* sp.	*Streptococcus* sp. were found associated with all life stages of the trematode. The bacteria are known to perform functions related to nutrient metabolism (fermentation) and immune response	suggestion: facultative mutualism*	Jorge *et al.* (2020)
CESTODA	Pseudophyllidean and caryophyllidean	nanobacteria and bacteria (undefined taxa)	bacteria help with digestive processes of the cestodes by producing digestive enzymes, which depend on the diet of the parasitized host	unknown suggestion: facultative mutualism*	Izvekova & Komova (2005) and Korneva (2008)
	Eubothrium	*Mycoplasma*	specific mycoplasma lineages have specific adaptations for survival in the cestode and have been hypothesized to be pathogenic to salmon (*Salmo solar*), the cestode's host	unknown suggestion: facultative mutualism*	Brealey *et al.* (2022)
	Shistocephalus solidus	*Chloroflexi* family of bacteria	bacteria are prevalent in the microbiome of the cestode, and were correlated with increase in expression of proinflammatory genes (genes foxp3, tnfr1, cd97, stat6 and marco)	unknown suggestion: facultative mutualism*	Hahn *et al.* (2022)

When the nature of the symbiotic relationship was not found in the literature, a suggestion was made based on the current descriptions in the literature, and marked with * to denote that evidence is lacking and that more studies are required.

Eco-evolutionary impacts: helminths and bacteria associate, but what of it?

The evolution of parasites and their hosts has been much described within the 'evolutionary arms race' framework: individuals resistant to a parasitic infection will have better survival compared to susceptible individuals, but as natural selection benefits resistant individuals on the one hand, on the other hand it will also favour parasites with a capacity to bypass the resistance of their hosts (Buckling & Rainey, 2002). However, to incorporate the multi-dimensional nature of microbiome–parasite–host interactions, the 'evolutionary arms race' framework needs to be expanded (Rafaluk-Mohr *et al.*, 2022). In short, microbial symbionts have been described as a low-cost source of evolutionary innovation for the organism they associate with, an extra pool of genes providing diversity and a basis over which natural selection may lead to adaptation (Dheilly *et al.*, 2015; Martinson *et al.*, 2020; Poulin *et al.*, 2022). Symbiotic microbes may provide novel functions to the organisms they associate with, enabling the conquest of different niches and environments (in parasite evolution, this could translate into an increase in the diversity of hosts that can be exploited), but microbes may also manipulate the organisms they associate with (e.g. reproductive manipulation) and become essential to a helminth via evolved dependency (De Mazancourt *et al.*, 2005; Martinson *et al.*, 2020).

To better understand host–parasite evolution, two main families of models have been employed, with differences in their underlying assumptions: the matching alleles model, which assumes that a lock-key specificity in alleles of parasite and host is required for infection; and the gene for gene model, which assumes that infection occurs when parasites have more virulence alleles than hosts have resistance alleles (Hamilton *et al.*, 1990; Sasaki, 2000). Natural systems do not always comply with these assumptions, and as mentioned above, more complex models are required when considering microbiomes. In particular,

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Kwiatkowski *et al.* (2012) developed a model incorporating one microbial symbiotic species that may be antagonistic or mutualistic with the parasitized host (not a component of the parasite microbiome). The model revealed that the specificity of the alleles was essential in determining the evolution of the host–symbiont–parasite system, especially for antagonistic species. While such studies are very informative, the models are highly deterministic and consider microbial transmission mostly via perfect maternal inheritance, with limited rates of horizontal transfer and genetic drift (Kwiatkowski *et al.*, 2012). Given the highly variable bacteriome of helminths, models with perfect maternal inheritance are restricted to obligate mutualistic relationships, which may have an obvious evolutionary impact, but largely exclude the dynamic nature of the bacteriome and the role it may play in host–parasite co-evolution.

The evolution of each bacterial lineage in the helminth's microbiome depends on its interactions with all other co-occurring lineages, in addition to factors such as the life-history traits of the helminth and the individual bacterium transmission strategies. The combination of all these elements in the parasite will interact with the same level of complexity in the parasitized host, creating eco-evolutionary interdependency. Ultimately, these multi-level interactions represent a paradigm shift in parasitology: the evolutionary arms race of parasites and their hosts needs to incorporate the holobiome dimension, that is, the unit formed by microbiomes and the organisms that they inhabit (Dheilly, 2014; Theis *et al.*, 2016).

Where to next?

Currently, partial 16S rRNA metagenomics is the most used approach to characterize the bacteriome of helminths; it has contributed to revealing that the composition of the bacteriome associated with helminths is different from that associated with the organisms that they parasitize (White *et al.*, 2018; Hogan *et al.*, 2019; Jorge *et al.*, 2020, 2022b, 2022c; Gobert *et al.*, 2022; Hahn *et al.*, 2022), identifying vertical and horizontal transmission of bacterial lineages among helminths (Vandekerckhove *et al.*, 2000; Vaughan *et al.*, 2012; Greiman *et al.*, 2013; Jorge *et al.*, 2020, 2022b; Hahn *et al.*, 2022), and discovering pathogenic bacteria that use helminths as vectors (Pusterla *et al.*, 2003; Greiman *et al.*, 2013; Dheilly *et al.*, 2019a). Partial 16S sequencing has been useful in finding bacteria that are strictly vertically transmitted and in mutualistic associations with helminths (Greiman *et al.*, 2013; Martinson *et al.*, 2020), defining a core bacteriome in a few helminths (Sinnathamby *et al.*, 2018; Jorge *et al.*, 2020, 2022b), and revealing great diversity in the composition and abundance of specific bacterial taxa (Palomares-Rius *et al.*, 2016; Mafuna *et al.*, 2021). However, this approach ignores the other components of the microbiome (e.g. viruses, protozoa and fungi). Even for the bacteriome, there are recognized constraints to partial 16S rRNA sequencing that mainly derive from the short size of the DNA fragment.

The development of long-range sequencing technologies such as Nanopore and PacBio has promoted and simplified full-length 16S rRNA gene sequencing (Callahan *et al.*, 2019; Johnson *et al.*, 2019). These longer DNA fragments provide improved resolution for bacterial taxonomic classification down to lineage levels, opening possibilities such as identifying lineages on the lower side of the divergence and abundance scale and undertaking phylogenetic assessments of more closely related lineages (Frank *et al.*, 2016; Johnson *et al.*, 2019; Brealey *et al.*, 2022; Luo *et al.*, 2022). However, microbiomes are composed of a number of non-bacterial organisms that are excluded by 16S amplicon-based technologies.

Amplicon-based sequencing of the internal transcribed spacer or the 18S rRNA genes can be useful to characterize the eukaryotic members of the microbiome, but is also limited in terms of taxonomic resolution, is targeted to specific components of the microbiome, and has significant challenges given the evolutionary proximity of the eukaryotic components of the microbiome with the organisms harbouring the microbiome (Hu *et al.*, 2015; Popovic *et al.*, 2018; Campo *et al.*, 2019). In the case of RNA and DNA viruses, non-amplicon-based metatranscriptomics and metagenomics are necessary, in particular for the genomic discovery and characterization of highly variable viruses in the microbiome (Dheilly *et al.*, 2022; Lee *et al.*, 2022).

Moving away from targeted sequencing, long-range sequencing methods have been facilitating the generation of lineage-resolved MAGs in complex microbial communities, with potential functional annotation of such metagenomes (Zimmermann *et al.*, 2020; Bickhart *et al.*, 2022; Jin *et al.*, 2022). Methods such as high-fidelity sequencing can result in continuous reads that are 10,000 base-pairs long, potentially spanning the full length of shorter microbial genomes (Bickhart *et al.*, 2022; Feng *et al.*, 2022), and accelerating approaches such as shotgun metagenome profiling and the generation of MAGs. However, metagenome profiling and functional characterization of helminth microbiomes are currently capped by the lack of information in databases that are directly applicable to microbial lineages in helminths (Brealey *et al.*, 2022), stressing the importance of increasing the number of studies on this specific subject. Given the potential redundant functions of different bacterial lineages (Speer *et al.*, 2020), increasing microbiome functional characterizations will lead to a better understanding of the fundamental contribution of the whole microbiome to the interaction with the parasite and with the host (fig. 2).

In parallel to functional profiling based on MAGs, metabolomics approaches can provide a snapshot of the small molecules in a system, helping characterize function and responses to experimental manipulations of the microbiome (Whitman *et al.*, 2021; Bauermeister *et al.*, 2022). Metabolomics combined with the sequencing-based characterization of the components of the microbiome can provide powerful insights into the ecological function of microbes in association with helminths and their host.

In addition to microbiome functional descriptions, differential abundances of individual taxa within microbial communities are relevant to the net effect of the microbiome in the parasite–host interaction (Reynolds *et al.*, 2015; Gaulke *et al.*, 2019; Poulin *et al.*, 2022). Increasing the number of quantitative microbiome characterizations with techniques such as quantitative polymerase chain reaction, flow cytometry and microbiome profiling poses its own challenges (Galazzo *et al.*, 2020), but is essential to advancing our understanding of the differential prevalence and contribution of microbial lineages to the eco-evolutionary dynamics of parasite–host interactions. The use of fluorescence *in situ* hybridization, immunofluorescence and electron microscopy to visualize and localize larger microbial symbionts associated with helminths is also very informative, leading to a better understanding of the nature of the microbe–parasite association and mode of transmission (Plotnikov & Korneva, 2008; Tropini *et al.*, 2017; Jenkins *et al.*, 2019; Caira & Jensen, 2021).

As sequencing costs decrease and bioinformatic resources are further developed, a considerable methodological challenge to

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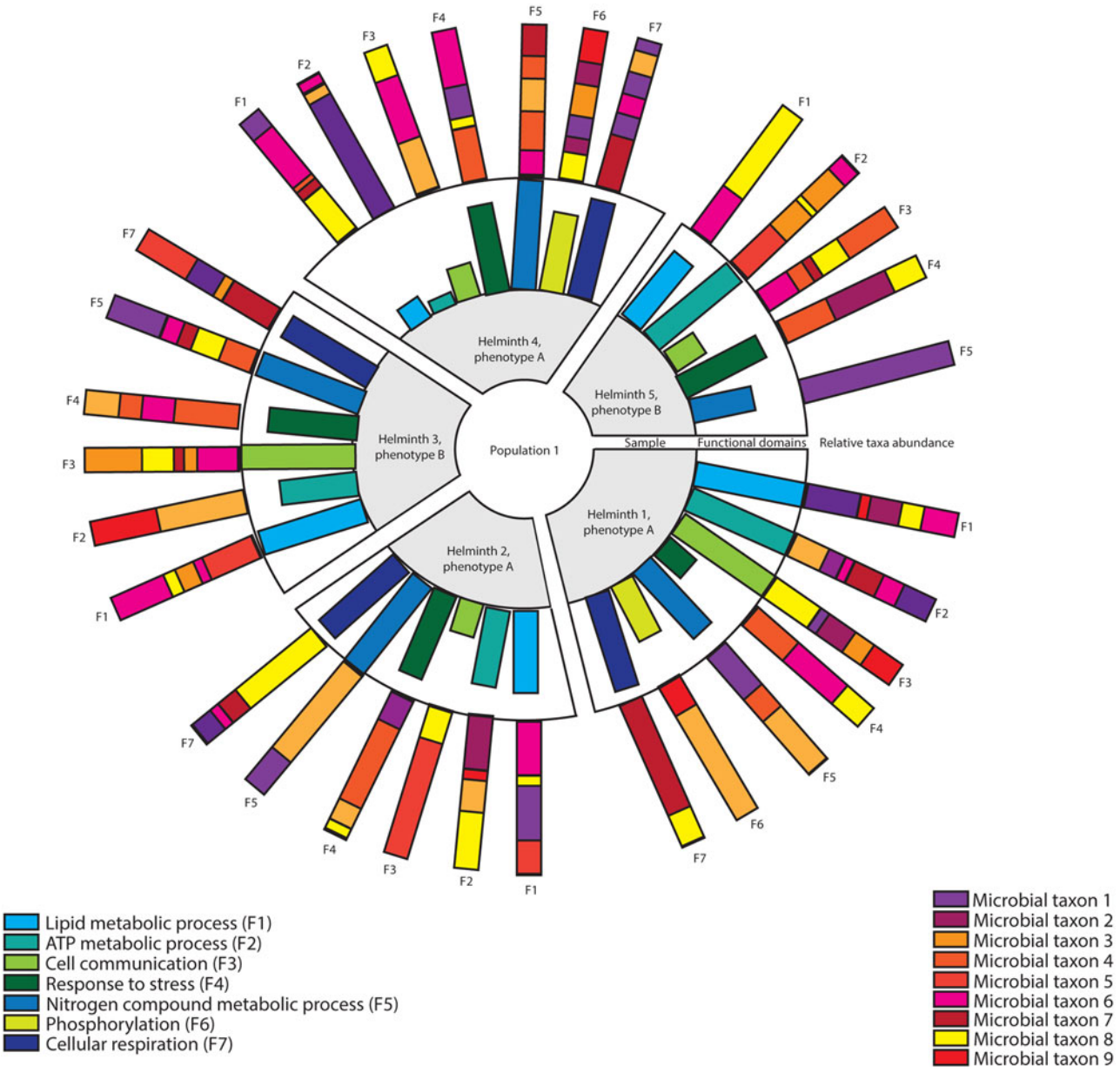


Fig. 2. Circular plot representing the diversity of functional domains and microbial taxa associated with different individual helminths of the same species in a population. From the centre out: first layer represents data associated with the helminths (e.g. phenotypic data, here represented by arbitrary letters – A and B); second layer represents the relative abundance of major functional domains (or gene ontology terms, such as in Ugarte *et al.*, 2018), with different domains represented by bars with different colours (from F1 to F7), and arbitrary functional domains named in the bottom left legend. Not all domains are necessarily present/detected in all individual helminths, and their relative effect in each individual can be different (different height of bars); third layer represents the relative abundance of microbial taxa (different coloured sections within a bar, arbitrarily represented in the bottom right legend) associated with each functional domain in each individual (from F1 to F7). There is diversity in the taxa associated with each functional domain among helminth individuals, due to functional redundancy.

advancing research in the microbiome of helminths lies in the input DNA requirements, in terms of quality and quantity of DNA per sample (Petrone *et al.*, 2022). Most new approaches do not rely on polymerase chain reactions, which eliminates the issue of amplification bias (McLaren *et al.*, 2019; Petrone *et al.*, 2022), and facilitates the inclusion of non-bacterial components of the microbiome. However, due to the nature of the samples and the fact that not all microbial lineages can be cultured, obtaining large volumes of biological material may not be viable. Thus, for research on the microbiomes of helminths to benefit from deeper sequencing methods and MAGs, it will be necessary to optimize and benchmark laboratory protocols to improve the DNA/RNA quality and quantity retained. Developing and following best-practice guidelines, such as the recommendations of the Parasite Microbiome Project (Dheilly *et al.*, 2017, 2019b; Formenti *et al.*, 2020), will be essential to both be able to embark on these extraordinary research avenues and to form an active community to share experiences and move the field forward.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X23000056

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Data accessibility. Data associated with fig. 1 is provided as supplementary information.

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