

NEWS AND VIEWS

PERSPECTIVE

Mock communities highlight the diversity of host-associated eukaryotes

LAURA WEGENER PARFREY*†

*Departments of Botany and Zoology and Biodiversity Research Centre, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada V6T1Z4; †Integrated Microbial Biodiversity, Canadian Institute for Advanced Research, Toronto, ON, Canada

Host-associated microbes are ubiquitous. Every multicellular eukaryote, and even many unicellular eukaryotes (protists), hosts a diverse community of microbes. High-throughput sequencing (HTS) tools have illuminated the vast diversity of host-associated microbes and shown that they have widespread influence on host biology, ecology and evolution (McFall-Ngai *et al.* 2013). Bacteria receive most of the attention, but protists are also important components of microbial communities associated with humans (Parfrey *et al.* 2011) and other hosts. As HTS tools are increasingly used to study eukaryotes, the presence of numerous and diverse host-associated eukaryotes is emerging as a common theme across ecosystems. Indeed, HTS studies demonstrate that host-associated lineages account for between 2 and 12% of overall eukaryotic sequences detected in soil, marine and freshwater data sets, with much higher relative abundances observed in some samples (Ramirez *et al.* 2014; Simon *et al.* 2015; de Vargas *et al.* 2015). Previous studies in soil detected large numbers of predominantly parasitic lineages such as Apicomplexa, but did not delve into their origin [e.g. (Ramirez *et al.* 2014)]. In this issue of *Molecular Ecology*, Geisen *et al.* (2015) use mock communities to show that many of the eukaryotic organisms detected by environmental sequencing in soils are potentially associated with animal hosts rather than free-living. By isolating the host-associated fraction of soil microbial communities, Geisen and colleagues help explain the surprisingly high diversity of parasitic eukaryotic lineages often detected in soil/terrestrial studies using high-throughput sequencing (HTS) and reinforce the ubiquity of these host-associated microbes. It is clear that we can no longer assume that organisms detected in bulk environmental sequencing are free-living, but instead need to design studies that specifically enumerate the diversity and function of host-associated

eukaryotes. Doing so will allow the field to determine the role host-associated eukaryotes play in soils and other environments and to evaluate hypotheses on assembly of host-associated communities, disease ecology and more.

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What are these host-associated protists doing? The prevailing assumption is that they are parasites and detrimental to their host. While some protists are clearly parasitic and cause host fitness declines, many are commensal or even beneficial (Parfrey *et al.* 2011; Bogitsh *et al.* 2012). Indeed, there are many commensals nested within predominately parasitic clades in humans [e.g. *Entamoeba coli* is commensal while *E. histolytica* is a deadly parasite (Bogitsh *et al.* 2012)] and in invertebrate hosts [e.g. the commensal microsporidian *Sporanauta perivermis* lives in a marine nematode (Ardila-Garcia & Fast 2012)]. The blanket application of ‘parasites’ to host-associated eukaryotes stems from a long history of studying eukaryotes associated with animals (especially humans) in the field of parasitology. But it is unwarranted. In the absence of functional data, ‘symbiont’ is preferable as a default term for host-associated eukaryotes because it encompasses parasites, commensals and mutualists.

Geisen *et al.* (2015) use mock communities of seven common soil protists and four animals to investigate primer bias within complex communities. In addition to these 11 mock community species, they find 97 host-associated operational taxonomic units (OTUs) that are classified as protists (Fig. 1) and set out to investigate this unexpected diversity. Refreshingly, Geisen *et al.* move beyond the usual generalization that all host-associated eukaryotes are parasites. They assess the taxonomic affinity of these host-associated OTUs by building phylogenetic trees and then delve into GenBank records and the literature to assess the ecological role of close relatives. Taxonomy does not always yield an accurate picture of the host/symbiont relationship, but does a good job of predicting eukaryotes that are likely to be specifically host associated (Parfrey *et al.* 2011). Geisen *et al.* identify 20 of 97 host-associated protist OTUs as being highly similar to symbiont taxa (their table 2), representing a diverse collection of Apicomplexa, ciliates and fungi. Only a handful of these 20 OTUs are good candidates for actually being parasites of the four groups of animals in the mock community, including gregarines (Apicomplexa) and the ciliate *Anoplophrya* that infects earthworms. However, most of the OTUs detected do not appear to be specific symbionts of the mock community

Correspondence: Laura Wegener Parfrey, Fax: 604 822 6089; E-mail: lwparfrey@botany.ubc.ca

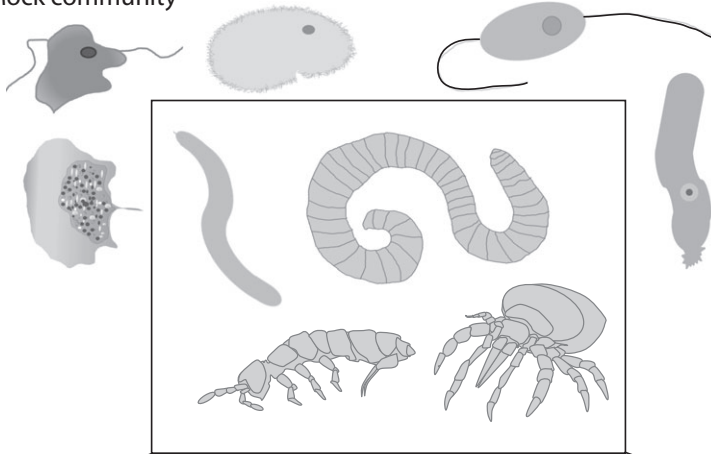
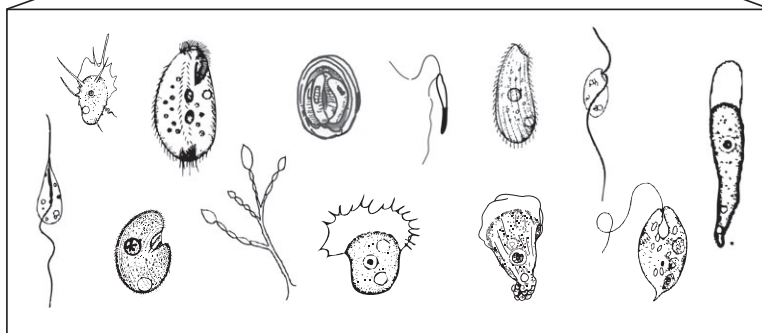
(A) Mock community**(B) Diverse animal-associated protists**

Fig. 1 Unexpected protist diversity associated with soil animals. (A) Protists and animals that comprise the mock community of Geisen *et al.* (2015). (B) Representation of the protist diversity detected on the mock community animals by sequencing. Images of mock community organisms provided by Stefan Geisen. Other images adapted from drawings on Micro*scope <http://pinkava.asu.edu/sta/central/microscope/>.

animals. Some are relatives of vertebrate parasites (e.g. *Cryptosporidium felis*), while others are plant pathogens. The majority of OTUs detected appear to belong to free-living groups of amoebae, ciliates and flagellates.

How to explain the presence of these apparently non-specific taxa? They might have been spuriously associated with mock community animals and not removed by washing, or transients within the gut either as food or on soil particles. Alternatively, they may represent previously undetected symbionts of soil animals. Geisen *et al.* took steps to minimize contamination from transient organisms, washing mock community animals and leaving them to defecate prior to DNA extraction. However, the animals were not completely sterilized and the ethanol used for washing could have precipitated DNA onto their cuticle. Differentiating between transients and taxa that specifically colonize hosts is critically important when studying host-associated taxa in general. Several methodological approaches can be employed in future studies, such as (i) thorough surface sterilization, (ii) separate processing of the gut, cuticle and other body parts of interest and (iii) inclusion of replicate host and environmental samples to enable detection of specifically associated taxa *in silico* (Venkataraman *et al.* 2015).

The findings of Geisen *et al.* raise many questions about the nature of host-associated eukaryotes, including the following.

- 1 How many protists are host-associated, free-living, or both depending on life cycle stage (amphizoic)?
- 2 How many symbionts are associated with soil animals directly, and how many represent dormant stages in the life cycle of symbionts of large vertebrates?
- 3 Are host-associated protists transmitted predominantly horizontally or vertically to their next host?
- 4 Where are individual host-associated protists localized: which are ectosymbionts versus endosymbionts living within host tissue or in the gut?
- 5 What impact do symbionts have on host biology, and how do these impacts alter ecosystem function more broadly?

These findings also shed new light on open questions in ecology. For example, the disease ecology of marine systems is hypothesized to differ from that in terrestrial ecosystems in fundamental ways because marine pathogens are thought to spread primarily through horizontal transmission (via resistant stages/cysts/spores acquired from the environment), while vertical or vector transmission is thought to be the rule in terrestrial systems (McCallum *et al.* 2004; de Vargas *et al.* 2015). In support of this hypothesis, de Vargas *et al.* (2015) report high numbers and diversity of host-associated taxa and parasites in global marine samples both in the tiny size fractions when they are outside a host, and in the larger size fractions when they are infecting a host. However, the high portion of symbiotic

taxa detected in bulk soil by other studies (Ramirez *et al.* 2014) and references in Geisen *et al.* (2015) suggests that the perception of this dichotomy may arise from a focus on large vertebrates in terrestrial ecosystems and the paucity of comprehensive studies of soils prior to HTS data. Indeed, many of the eukaryotic organisms (*Entamoeba*, worms, *Blas-tocystis*) that inhabit the mammalian gut are spread horizontally: they are passed as cysts and are acquired by new hosts from environmental reservoirs (Bogitsh *et al.* 2012). There may be fewer differences between marine and terrestrial ecosystems than expected.

Moving forward it will be important to distinguish between free-living and host-associated microbes to illuminate the diversity of understudied symbiotic lineages, but also to better understand functioning of soil and aquatic ecosystems more generally. In soil, biogeochemical processes vary across microhabitats, including between host-associated versus free-living niches (Drake & Horn 2007). Microbes associated with animal hosts likely have a different functional role than those associated with fungi, or with plants, or free-living within the soil matrix. For example, host-associated microbes may be particularly important in nitrogen fixation – earthworm guts and plant roots are enriched in nitrogen fixing bacteria (Drake & Horn 2007). Host-associated pathogens and mutualists also have the ability to shift host function, causing changes that may cascade through communities (McFall-Ngai *et al.* 2013). Thus, detecting pathogens or symbionts of key species (i.e. earthworms) may provide broader insight into soil health and ecosystem function. Turning attention to microbes may also highlight further ecological damage in the disturbed soils of agricultural and forestry systems, which are generally depauperate in their soil animal communities. The consequences of diversity loss may extend beyond loss of animal species per se to the biogeochemical functions once performed by their associated microbes.

Taken as a whole, the findings of Geisen *et al.* are noteworthy not because they point to a new and rare phenomenon, but because they demonstrate in a clear and convincing way that host-associated microbes must be reckoned with if we are to develop a complete understanding of ecosystem diversity and function.

References

- Ardila-Garcia AM, Fast NM (2012) Microsporidian infection in a free-living marine nematode. *Eukaryotic Cell*, **11**, 1544–1551.
- Bogitsh B, Carter C, Oelmann T (2012) *Human Parasitology*, 4th edn. Elsevier, San Diego.
- Drake HL, Horn MA (2007) As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. *Annual Review of Microbiology*, **61**, 169–189.
- Geisen S, Laros I, Vizcaino A, Bonkowski M, de Groot GA (2015) Not all are free-living: high-throughput DNA metabarcoding reveals a diverse community of protists parasitizing soil metazoa. *Molecular Ecology* **24**, 4556–4569.
- McCallum HI, Kuris A, Harvell CD *et al.* (2004) Does terrestrial epidemiology apply to marine systems? *Trends in Ecology & Evolution*, **19**, 585–591.
- McFall-Ngai M, Hadfield MG, Bosch TC *et al.* (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 3229–3236.
- Parfrey LW, Walters WA, Knight R (2011) Microbial eukaryotes in the human microbiome: ecology, evolution, and future directions. *Frontiers in Cellular and Infection Microbiology*, **2**, 1–6.
- Ramirez KS, Leff JW, Barberan A *et al.* (2014) Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. *Proceedings Biological Sciences/The Royal Society*, **281**, 1–9.
- Simon M, Lopez-Garcia P, Deschamps P *et al.* (2015) Marked seasonality and high spatial variability of protist communities in shallow freshwater systems. *The ISME Journal*, 1–13. doi: 10.1038/ismej.2015.6.
- de Vargas C, Audic S, Henry N *et al.* (2015) Ocean plankton. Eukaryotic plankton diversity in the sunlit ocean. *Science*, **348**, 1261605.
- Venkataraman A, Bassis CM, Beck JM *et al.* (2015) Application of a neutral community model to assess structuring of the human lung microbiome. *mBio*, **6**, 1–9.

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