Parasite microbiome project: Grand challenges

Nolwenn M. Dheilly1*, Joaquín Martínez Martínez2*, Karyna Rosario3*, Paul J. Brindley4,5, Raina N. Fichorova6, Jonathan Z. Kaye7, Kevin D. Kohl6, Laura J. Knoll9, Julius LukešID,10, Susan L. Perkins11, Robert Poulin12, Lynn Schriml13,14, Luke R. Thompson14,15

1 School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York, United States of America, 2 Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine, United States of America, 3 College of Marine Science, University of South Florida, Saint Petersburg, Florida, United States of America, 4 Department of Microbiology, Immunology and Tropical Medicine, George Washington University, Washington, DC, United States of America, 5 Research Center for Neglected Diseases of Poverty, School of Medicine & Health Sciences, George Washington University, Washington, DC, United States of America, 6 Genital Tract Biology Division, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, 7 Gordon and Betty Moore Foundation, Palo Alto, California, United States of America, 8 Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, United States of America, 9 Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, 10 Institute of Parasitology, Biology Centre, Czech Academy of Sciences and Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic, 11 Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, New York, United States of America, 12 Department of Zoology, University of Otago, Dunedin, New Zealand, 13 Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, United States of America, 14 Department of Biological Sciences and Northern Gulf Institute, University of Southern Mississippi, Hattiesburg, Mississippi, United States of America, 15 Ocean Chemistry and Ecosystems Division, Atlantic Oceanographic and Meteorological Laboratory, National Oceanic and Atmospheric Administration, La Jolla, California, United States of America

* nolwenn.dheilly@stonybrook.edu (NMD); jmartinez@bigelow.org (JMM)

The first Parasite Microbiome Project (PMP) Workshop (January 9–14, 2019, Clearwater, Florida, United States) hosted researchers from across continents and disciplines to lay the foundation of the PMP consortium. The PMP vision is to catalyze scientific discourse and explorations through a systems approach, toward an integrated understanding of the microbiota of parasites and their impact on health and disease. The participants identified knowledge gaps and grand challenges in the field of host–parasite–microbe interactions summarized here. The PMP will provide an interactive centralized platform and resource for transdisciplinary collaboration to propel the field of parasitology forward by disentangling complex interactions between parasites and hosts, their respective microbiota, and microbial communities in the parasite’s direct environment (Fig 1).

Parasitism is a successful lifestyle that has evolved in virtually every clade of multicellular organisms [1–3] and protists [4, 5]. Parasitology seeks to develop the means to prevent, limit, or cure infections by parasites for the benefit of humans, agriculture, and wildlife and to understand how parasitism and parasitic disease impact not only the host but also host communities and ecosystem health. This is a challenging task, considering the diversity and complex nature of host–parasite interactions. Parasitic organisms harbor a rich tapestry of traits associated with survival and must navigate the host immune response to reproduce and be effectively transmitted to the next host.
An improved understanding of underlying molecular mechanisms and evolutionary patterns that explain interindividual, temporal, and geographic variation in the outcomes of parasitic infections is much needed [6–9]. There is an increasing recognition of the potential for host- and parasite-associated microbiota—endo- and/or ectosymbiotic archaea, bacteria, viruses, and micro-eukaryotes—to influence and shape host–parasite interactions [10]. In the past few years, the concept of individuality has given way to that of “holobiont” with the recognition that each organism is a composite of organisms [11–14] (Fig 1, Box 1). Yet we have limited insight into the nature and importance of these interactions for parasite ecology and evolution [15, 16], and not a single parasite species has its entire microbiome fully characterized.

Fig 1. The complex nature and interrelations of host–parasite–microbe interactions are illustrated using a Matryoshka (Russian nesting) doll metaphor. The PMP aims to elucidate nested interactions between a given host and parasites (e.g., helminths and protozoa) that are themselves hosts to their own symbionts and parasites (e.g., viruses and bacteria). Artwork by Meredith Brindley (http://meredithbrindley.com/). PMP, Parasite Microbiome Project.

https://doi.org/10.1371/journal.ppat.1008028.g001
The PMP

The PMP envisions a holistic understanding of host–parasite–microbe interactions by fostering global transdisciplinary explorations of the microbiomes of parasites and their direct environment (Fig 2; Box 1) [17]. The PMP will be enabled by new and existing tools, technologies, and standards developed for microbiomes and tailored for analyses of host–parasite–microbe interactions. Areas of focus will include (1) development of relevant standards for metadata collection and curation, (2) methods development for processing of parasite-associated microbes, (3) multi-omics technologies, and (4) tailoring of analytical tools for parasite-associated microbiome research. Methods and data will be shared freely with the scientific community and public using open data standards. Establishing and optimizing these methods will initially require a “test” collection of well-characterized parasite isolates/models and a comprehensive identification of parasite-associated microbes. The PMP will establish a workflow and centralized platform to maximize parasite sampling efforts and facilitate parasite microbiome research for the community at large (Fig 3; Table 1).

A primary advantage of a centralized platform like the PMP is the collation of large aggregates of associated metadata that can be harnessed to uncover, and eventually understand, patterns of microbial diversity and ecology [40, 35]. Therefore, detailed metadata associated with each study and sample are absolutely critical to maximize the utility of each. To facilitate future research opportunities, the PMP will encourage tracking of metadata connected to both the sample and its processing and the deposition of host and parasite vouchers into museum collections to allow future analysis opportunities when new techniques and hypotheses arise [41, 42]. Any additional tissues and extracted biomolecules should also be maintained in dedicated (cryo-) collections. We will adapt practices and lessons learned by the Earth Microbiome Project (EMP) [35, 43], e.g., preparation of multiple (homogenous) aliquots of the samples to be studied [44], in a manner that is best suited for the PMP. By providing tested methods and developing standards for parasite microbiome research, the PMP foresees the following:

Box 1. Key microbiome and holobiont concepts applied to parasitology

**Direct environment:** environment of the parasite at the time of sampling (host-associated and free-living stages).

**Parasite-associated microbiome:** collection of the genomes of the microbiota (viruses, bacteria, archaea, and micro-eukaryotes) that are either chromosomally integrated or episomal, intracellular, or attached to the surface of the parasite.

**Host-associated microbiome:** collection of the genomes of the microbiota that are associated with the host, either in the direct environment of the parasite, or in a distant tissue or anatomic compartment of the host.

**Environmental microbiome:** collection of the genomes of the microbiota that are present in the direct environment of free-living (encysted or mobile) life stages of parasites.

**Holobiont:** a unit of biological organization composed of a host and its microbiota, inclusive of transient and persistent microbes.

**Hologenome:** the complete genetic content of an organism’s genome, including nuclear and organellar genomes, and its microbiome.
Elevating the integral role of the microbiome in host–parasite ecology and evolution (in a dynamic environment) to promote solution-oriented research in parasitic disease management.

Steering the larger microbiome community towards analysis of the micro-eukaryotic component of the microbiome.

Building an inclusive and transdisciplinary PMP community that catalyzes analysis of natural and model parasite systems.

Becoming a community hub that coordinates discussions fostering collaborative research to address current and future grand challenges.

Grand challenges

We encourage the scientific community to join the PMP in addressing grand challenges in the field of host–parasite–microbe interactions and designing creative experiments in a diversity of systems to explore the areas outlined next.

Identifying core and transient parasite-associated microbes

Parasite-associated microbiomes remain largely unknown, in part due to the inherent difficulties of studying the parasites themselves, e.g., challenging or nonexistent in vitro cultivation systems, complex life cycles, ethical considerations, obligate host environments that are difficult to simulate in experimental models, and national borders. Another specific challenge for investigating microbial communities associated with parasites is the necessity to isolate the parasite from the background sampled material and from host-associated microbes. The microbiota within a parasite can be divided into core microbes (intrinsic to the parasite or at least to a specific parasitic life stage) and transient microbes (temporarily acquired by the parasite from its direct environment). Comparative analyses between the parasite microbiome, the microbiome of the corresponding infected host, and a control noninfected host from the same

Fig 2. Examples conveying context-dependent usage of the microbiome and holobiont concepts in parasitology. (A) Proceroid stage of the cestode Schistocephalus solidus in the body cavity of a cyclopoid copepod. The Pam may be collected from proceroids. The De is the body cavity of the copepod. The Ham may be collected from the gut or other host tissues whereas the Em may be collected from the water. (B) Oocyst of Toxoplasma gondii that sporulated upon excretion with cat feces. The Pam may be collected from purified oocysts. Distinction between the Ham, De, and Em is difficult. (C) Trypanosoma sp. in red blood cells. The Em is not represented. The intracellular microbes potentially present within red blood cells may be considered Ham whereas microbes within the plasma can be considered in the De of the parasite. Image credits: M. Hahn; L. Knoll and J. F. Dubey; J. Lukeš. De, direct environment; Em, environmental microbiome; Ham, host-associated microbiome; Pam, parasite-associated microbiome.

https://doi.org/10.1371/journal.ppat.1008028.g002
environment will be needed to rule out potential microbial contaminants from the direct environment.

Parasite holobiont research will need to ascertain whether microbes are vertically transmitted from parasitic parent to offspring, horizontally transmitted between parasites coinfesting the same host, or transmitted between the parasite and its direct environment (the host or the external environment of the free-living stages). The objective will be to discern to what extent the parasite-associated microbiota is determined by the parasite (maintained across developmental growth, reproduction, and dispersal), by the composition of the microbiota in its direct environment, or by abiotic factors. This objective could be examined, for example, by comparing the microbiomes of parasites (1) infecting multiple host species (for generalist parasites or parasites with complex life cycles), (2) at different life stages, (3) isolated from spatially and temporally separated populations or populations with different diets, or (4) coinfesting the same host.
Understanding the roles of parasites in microbe evolution and host–microbe interactions

Parasite prevalence in a population, route(s) of parasite transmission, and interdependence between the microbe and its parasitic host will drive the evolution of the microbe’s modes of transmission. These modes are of particular interest because they will drive microbial virulence both for the parasite and its host [45]. Parasites may influence the composition of the microbiota of their hosts by diverse means. For example, parasites may (1) be vectors or

---

Table 1. Methods to tackle the grand challenges of parasite microbiome research.

| Method                      | Challenge and/or proposed approach                                                                 | Reference |
|-----------------------------|-----------------------------------------------------------------------------------------------------|-----------|
| **Sample collection**       |                                                                                                     |           |
| Metadata collection         | Must be complete and standardized; collect adhering to MiXs environmental package for parasite-associated samples | [18–20]  |
| Environmental parasite microbiota | Need to fractionate samples to distinguish parasite-associated microbiome from direct environment microbiome; freezing and/or preservation in ethanol or RNAlater depending on downstream processing |           |
| Laboratory parasite microbiota | Need growth conditions, in vitro animal model systems, e.g., tissue, organoids, cell lines | [21, 22] |
| **Molecular characterization** |                                                                                                     |           |
| Metagenomic DNA sequencing  | Capture whole community including prokaryotes, micro-eukaryotes, and abundant or actively replicating viruses |           |
| Amplicon DNA sequencing     | Group-specific taxonomic profiling of key groups                                                    | [20, 23–25] |
| Viral community sequencing  | Viral purification (viral metagenomes) or sequencing of vSAGs                                       |           |
| Parasite genome sequencing  | Need to supplement reference genomic databases and identify role of host genotype in shaping the interactions of resident microbes | [26, 27] |
| Transcriptomics, cDNA metagenomics | Detection of RNA viruses                                                                          | [28]     |
| Metabolomics                | Mass spectrometry (LC-MS/MS, GC-MS)                                                                |           |
| Microscopy for spatial organization | FISH and microscopy to identify localization of microbes on/inside parasite and in relation to each other; microscopy of living parasites to reveal temporal patterns | [29, 30] |
| **Data analysis**           |                                                                                                     |           |
| Data mining                 | Search existing sequence archives and parasite sequencing projects for parasite microbiomes          |           |
| Reference databases         | Build upon existing databases (e.g., EuPathDB)                                                      | https://eupathdb.org |
| Genome assembly             | Need to assemble microbial genomes from metagenomes in context of host and parasite genomic DNA; also assemble parasite genomes |           |
| Metagenomic taxonomic and functional analysis | Taxonomic composition using nucleotide composition (e.g., Kraken, Nonpareil) and marker genes (e.g., MetaPhlAn) and species-specific functional composition using nucleotide and protein databases (e.g., HUMAnN2) | [31–34] |
| Amplicon analysis           | Database curation and exact-sequence methods                                                       | [35–39]  |
| Multi-omics analysis        | Compare profiles of taxa, genes, metabolites across multi-omics methods                             |           |
| **Data sharing**            |                                                                                                     |           |
| Protocols                   | Protocols for sample collection, processing, and analysis; share on protocol-sharing service (e.g., Protocols.io) | https://protocols.io |
| Code                        | Processing and analysis code; share on GitHub repository and permanent archive (e.g., Zenodo)       | https://github.com, https://zenodo.org, https://gensc.org |
| Study metadata              | Study title, description, design, points of contact, and publication DOI; share on GitHub repository and permanent archive (e.g., Zenodo) |           |
| Sample metadata             | MiXs-compliant metadata (see above); share on GitHub repository and permanent archive (e.g., Zenodo) |           |
| Raw data                    | All raw data after collection; deposit in EBI, GenBank, and other data archives                     | https://www.ebi.ac.uk |

Abbreviations: DOI, digital object identifier; EBI, European bioinformatics institute; FISH, fluorescence in situ hybridization; GC-MS, gas chromatography-mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MiXs, Minimal Information about any Sequence; vSAG, viral single amplified genome

https://doi.org/10.1371/journal.ppat.1008028.t001
reservoirs of microbes; (2) exert pressure on the host during infection, leading to the evolution of defensive microbes; (3) compete with host microbiota for nutrients or provide metabolic and genetic reservoirs to support the growth and survival of other host microbial species; (4) modify the host environment, e.g., pH, to the benefit of other microbes; and/or (5) induce an immune response by the host that, in turn, impacts the host's microbiome.

The extent to which the host microbiome is determined by its parasites can be investigated by comparing the microbiome of individuals infected by different parasitic species and/or strains [46]. When treatments are available, they can be used to determine whether the host microbiome returns to its original state after removal of the parasite. In addition, characterizing the underlying mechanisms will be necessary to determine whether the parasite directly or indirectly interacts with the host microbiome and whether this is beneficial to the parasite or a side effect of the infection. Furthermore, by serving as vectors or reservoirs of microbes, parasites could alter the evolution of microbes by providing opportunities for host switching or novel microbe–microbe interactions that may lead to genetic exchanges. In order to gain an evolutionary perspective on host–parasite–microbe interactions, evolutionary studies encompassing microbes across host and parasite species are necessary to identify patterns of cospeciation and speciation following host shifts.

Understanding the functional role of microbes in parasite fitness and host diseases

Parasites and associated microbes can be viewed as a community of organisms that experience different selection pressures, despite the high potential for interdependence. Microbes can be either beneficial (mutualistic) or antagonistic (parasitic or with fitness conflicts) to the parasite. The nature of the interaction would lead to radically different effects of the microbes on the evolution of the holobiont and the host–parasite interaction. Similarly, microbes associated with the host may be beneficial for the parasite, as a result of selection for cooperation, or they may be detrimental due to the competition for nutrients and/or space. The nature of parasite–microbe interactions may have a critical effect on the parasite's fitness and host disease. For example, viral symbionts of parasitic protists can divert host responses toward antiviral immunity, which is inefficient in clearing the eukaryotic infection and may aid the parasite survival [21].

Understanding the impact of microbes on the fitness of hosts and parasites is of relevance to epidemiological studies and is expected to provide new opportunities for therapeutic interventions. The inherent complexity of the study of host–parasite–microbe interactions necessitates the application of methods from the field of community genetics, wherein it is acceptable that the gene that governs a given phenotype resides in the genome of another species and is dependent on the environment [47]. Here, the environment of the host and parasite is the microbiome, and its impact on the evolution of the system can be tested by measuring parameters of the host and parasite fitness in the presence of different microbes. Alternatively, host–microbe interactions can be tested by considering the host as the environment.

Identifying patterns and processes of host–parasite–microbe coevolution

Interindividual variations in the outcome of a parasitic infection resulting from variations in host susceptibility, parasite virulence, and host–parasite compatibility can be better understood in the context of the geographic mosaic of coevolution [48]. Microbes also show geographic variation, and they can participate in coevolution by shifting selection pressures away from or towards either the host or the parasite [49–51]. With appropriate experimental systems, geographic variations affecting the role of microbes in host–parasite interactions can be
assessed by using a complete cross-experimental design, in which hosts from different localities are infected with parasites from their corresponding localities in the presence of either microbes isolated from the same localities or microbes from different test localities. Identification of temporal variations in selection pressures on microbes involved in host–parasite interactions would require time-shift experiments, wherein the microbes that have evolved with the host and parasite are transferred back to an ancestral host and parasite. Finally, when possible, experimental evolution of parasites and hosts in the presence or absence of the identified microbes can be used to test the effect of specific microbes on the evolution of the system and to identify mechanisms involved in parasite–microbe interaction.

Moving forward

The PMP consortium proposes a two-phase development, analogous to the Human Microbiome Project (HMP) [52]. Phase one will compile information on previously characterized parasite-associated microbes and parasite–microbe interactions (already partially reviewed in [15–16, 53]), mine genomic and transcriptomic databases to detect microbial sequences, and characterize the complete microbiome of a set of parasites representing diverse taxa and environments. A main focus during this phase will be on preparing a website and developing and sharing best practices, methods, and standards for effective sample management and integration of data. The PMP, in collaboration with the Genomic Standards Consortium (GSC; gensc.

Table 2. Representative examples of organisms for which uncovering parasite–microbe interactions is allowing major scientific advances. It is anticipated that the PMP will advance the field by facilitating similar research on diverse parasites and uncover patterns of microbial diversity and ecology that apply across phyla.

| Parasite                  | Microbe(s)                        | Significance for health, agriculture, and/or the environment                                                                 | References |
|---------------------------|-----------------------------------|-----------------------------------------------------------------------------------------------------------------------------|------------|
| Opisthorchis viverrini    | *Helicobacter pylori* and other host gut bacteria | *O. viverrini* often leads to cholangiocarcinoma. Co-infection with oncogenic bacteria that are vectored towards the liver by the fluke may contribute to cancer development | [54–56]    |
| *Trichomonas vaginalis*   | TVV 1 through 3                    | Different clinical isolates of *T. vaginalis* show variable pathogenicity to the human host cells dependent on the TVV they carry; TVV released by dying and stressed parasites can explain why antibiotic therapy fails to prevent the inflammatory sequelae of parasitic infection | [57]       |
| *Trichomonas vaginalis*   | Host vaginal microbiome            | Infection is detrimental to *Lactobacillus* and favors pathogenic bacteria associated with bacterial vaginosi            | [58]       |
| *Leishmania* spp.         | LRV1                               | LRV1-infected *Leishmania* spp. increase severity of human leishmaniasis and lead to drug treatment failures               | [59, 60]   |
| Filarial nematodes        | *Wolbachia*                       | Antibiotics, such as doxycycline and rifampicin, targeting the *Wolbachia* endosymbiont lead to loss of worm viability and fertility in human trials and increase antifilarial treatment efficacy | [61, 62]   |
| Parasitoid wasps          | Polydnaviruses and RNA viruses     | Viruses contribute to parasitoid wasps virulence by modulating host immune response, host behavior, and feeding ability         | [63–65]    |
| Ticks                     | *Coxiella*-like endosymbiont       | Symbiont codiversifies with its parasitic host and provides B vitamins missing from blood meals, enabling ticks to specialize in hematophagy | [66, 67]   |
| *Vibrio shiloi*           | Symbiotic zooxanthellae of corals  | *V. shiloi* produces toxins that target symbiotic zooxanthellae of the coral host inhibiting photosynthesis                   | [68]       |
| *Trichuris* spp.          | Host gut microbiome                | The whipworm ingests bacteria from its direct environment and favors growth of mucolytic bacteria. Bacterial attachment is required for egg hatching | [69–72]    |
| Digeneric trematodes including species of *Nanophyetes*, *Echinostoma*, *Fasciola* | *Neorickettsia* species          | Endosymbiotic bacteria within cells of the trematode. These symbionts can be transferred horizontal from the trematode to mammalian host, where they are facultative pathogens | [73, 74]   |
| *Pseudocapillaria tormentosa* | Zebrafish gut microbiota          | Abundance of some bacteria taxa predicts helminth burden and intestinal lesions in host. Gut microbiome serves as diagnostic for parasite infection. | [75]       |

Abbreviations: LRV1, *Leishmania* RNA virus 1; PMP, Parasite Microbiome Project; TVV, *Trichomonas vaginalis* virus

https://doi.org/10.1371/journal.ppat.1008028.t002
org), has initiated the development of a new parasite-associated package to be added to the Minimal Information about any Sequence (MIxS) standard [18]. This package will facilitate the collection, standardization, reporting, and integrated analyses of metadata to capture the parasite microbiome contextual information describing the host, environment, sample and sequencing data. We anticipate the MIxS-PMP to be available by the end of 2019.

The second phase of the project will rely on the development of experimental model systems that may be employed to prove cause-effect relationships between parasite virulence, diseases, and microbiome composition, as well as to investigate the underlying molecular mechanisms and the evolution of host–parasite–microbe interactions. Findings from initial microbiome characterizations during phase one and previously proposed experimental model systems [53] will guide the evaluation and selection of systems most suitable for addressing the scientific grand challenges identified herein.

Given the important role of parasites in ecosystems, human health, and agricultural management, propelling the field of parasitology in a coordinated way with the PMP can have an enormous payoff (Table 2). The PMP will necessitate both significant funding to conduct challenging research as well as engagement from the community to provide high-quality samples and to share detailed and accurate metadata information. Therefore, we propose constituting a community of researchers that meet annually for workshops and symposia. With this opinion article, we invite reader comments to better define grand challenges and research needs moving forward.

Acknowledgments
The authors thank Jack Gilbert for his assistance and comments to this initiative and Meredith E. Brindley for assistance with the scientific illustration.

References
1. Weinstein SB, Kuris AM. Independent origins of parasitism in Animalia. Biol Lett. 2016; 12: 20160324. https://doi.org/10.1098/rsbl.2016.0324 PMID: 27436119
2. Westwood JH, Yoder JI, Timko MP, dePamphilis CW. The evolution of parasitism in plants. Trends Plant Sci. 2010; 15: 227–235. https://doi.org/10.1016/j.tplants.2010.01.004 PMID: 20153240
3. Poulin R, Morand S. The diversity of parasites. Q Rev Biol. 2000; 75: 277–293. https://www.jstor.org/stable/2665190 PMID: 1100700
4. Baker JR. The origins of parasitism in the protists. Int J Parasitol. 1994; 24: 1131–1137. https://doi.org/10.1016/0020-7519(94)90187-2 PMID: 7729973
5. Adl SM, Bass D, Lane CE, Lukeš J, Schoch CL, Smirnov A, et al. Revisions to the classification, nomenclature, and diversity of eukaryotes. J Eukaryot Microbiol. 2019; 66: 4–119. https://doi.org/10.1111/jeu.12691 PMID: 30257078
6. Dybdahl MF, Jenkins CE, Nuìsmers SL. Identifying the molecular basis of host-parasite coevolution: merging models and mechanisms. Am Nat. 2014; 184: 1–13. https://doi.org/10.1086/676591 PMID: 24921596
7. Pulgarín-R PC, Gómez JP, Robinson S, Ricklefs RE, Cadena CD. Host species, and not environment, predicts variation in blood parasite prevalence, distribution, and diversity along a humidity gradient in northern South America. Ecol Evol. 2018; 8: 3800–3814. https://doi.org/10.1002/ece3.3785 PMID: 29721258
8. Cable J, Barber I, Boag B, Ellison AR, Morgan ER, Murray K, et al. Global change, parasite transmission and disease control: lessons from ecology. Philos Trans R Soc Lond B Biol Sci. 2017; 372: 20160088. https://doi.org/10.1098/rstb.2016.0088 PMID: 28289256
9. Arunsan P, Ittiprasert W, Smout MJ, Cochran CJ, Mann VH, Chaiyadet S, et al. Programmed knockout mutation of liver fluke granulin attenuates virulence of infection-induced hepatobiliary morbidity. Elife. 2019; 8: e41463. https://doi.org/10.7554/eLife.41463 PMID: 30644359
10. Yurchenko V, Lukeš J. Parasites and their (endo)symbiotic microbes. Parasitology. 2018; 145: 1261–1264. https://doi.org/10.1017/S0031182018001257 PMID: 30086814
11. Theis KR, Dheilly NM, Klassen JL, Brucker RM, Baines JF, Bosch TCG, et al. Getting the holobgene concept right: an eco-evolutionary framework for hosts and their microbiomes. mSystems. 2016; 1: e00028–16. https://doi.org/10.1128/mSystems.00028-16 PMID: 27822520

12. Zilber-Rosenberg I, Rosenberg E. Role of microorganisms in the evolution of animals and plants: the holobgene theory of evolution. FEMS Microb Rev. 2008; 32:723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x PMID: 18549407

13. Bordenstein SR, Theis KR. Host biology in light of the microbiome: ten principles of holobients and holobgenes. PLoS Biol. 2015; 13: e1002226. https://doi.org/10.1371/journal.pbio.1002226 PMID: 26284777

14. Dheilly NM, Bordenstein SR, Brindley PJ, Figueres C, Holmes EC, et al. Parasite microbiome. PLoS Pathog. 2015; 11: e1005039. https://doi.org/10.1371/journal.ppat.1005039 PMID: 26270819

15. Dheilly NM. Holobiont-holobiont interactions: redefining host-parasite interactions. PLoS Pathog. 2014; 10: e1004093. https://doi.org/10.1371/journal.ppat.1004093 PMID: 24992663

16. Dheilly NM, Poulin R, Thomas F. Biological warfare: microorganisms as drivers of host-parasite interactions. mSystems. 2019; 4: e00028–19. https://doi.org/10.1128/mSystems.00028-19 PMID: 30797538

17. Dheilly NM, Bolnick D, Bordenstein SR, Brindley PJ, Figueres C, Holmes EC, et al. Parasite Microbiome Project: systematic investigation of microbiome dynamics within and across parasite-host interactions. mSystems. 2017; 2: e00050–17. https://doi.org/10.1128/mSystems.00050-17 PMID: 28761932

18. Yilmaz P, Kottmann R, Field D, Knight R, Cole JR, Amaral-Zettler L, et al. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol. 2017; 35: 725–731. https://doi.org/10.1038/nbt.3893 https://www.nature.com/articles/nbt.3893#supplementary-information. PMID: 28784724

19. Roux S, Adriaenssens EM, Dutilh BE, Koonin EV, Kropinski AM, Krupovic M, et al. Minimum Information about an Uncultivated Virus Genome (MIUViG). Nat Biotechnol. 2018; 37: 29–37. https://doi.org/10.1038/nbt.4306 https://www.nature.com/articles/nbt.4306#supplementary-information. PMID: 30556814

20. Bowers RM, Kyrpides NC, Stepanauskas R, Harmon-Smith M, Doud D, Reddy TBK, et al. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol. 2017; 35: 725–731. https://doi.org/10.1038/nbt.3893 https://www.nature.com/articles/nbt.3893#supplementary-information. PMID: 28784724

21. Edwards RA, Rohwer F. Viral metagenomics. Nat Rev Microbiol. 2005; 3: 504–510. https://doi.org/10.1038/nrmicro1163 PMID: 15886693

22. Wilson WH, Gilg IC, Moniruzzaman M, Field EK, Koren S, LeCleir GR, et al. Genomic exploration of individual giant ocean viruses. ISME J. 2017; 11: 1736–1745. https://doi.org/10.1038/ismej.2017.61 PMID: 28498373

23. Martínez-Hernandez F, Forns O, Luesma Gomez M, Bolduc B, de la Cruz Pombjakova K, Wegener Parfrey L. Are human intestinal eukaryotes beneficial or commensals? PLoS Pathog. 2015; 11: e1005039. https://doi.org/10.1371/journal.ppat.1005039 PMID: 26270819

24. Spor A, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. Nat Rev Microbiol. 2011; 9: 279–290. https://doi.org/10.1038/nrmicro2540 https://www.nature.com/articles/nrmicro2540#supplementary-information. PMID: 21407244

25. Shi M, Lin X-D, Tian J-H, Chen L-J, Chen X, Li C-X, et al. Redefining the invertebrate RNA virosphere. Nature. 2016; 540: 539–543. https://doi.org/10.1038/nature20167 PMID: 27880757

26.Welch MJL, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG. Biogeography of a human oral microbiome at the micron scale. Proc Natl Acad Sci. 2016; 113: E791–E800. https://doi.org/10.1073/pnas.1522149113 PMID: 26811460

27. Martínez-Hernandez F, Forns O, Luesma Gomez M, Bolduc B, de la Cruz Pombjakova K, Wegener Parfrey L. Are human intestinal eukaryotes beneficial or commensals? PLoS Pathog. 2015; 11: e1005039. https://doi.org/10.1371/journal.ppat.1005039 PMID: 26270819

28. Jemielita M, Taormina MJ, Burns AR, Hampton JS, Rolig AS, Guillemin K, et al. Spatial and temporal features of the growth of a bacterial species colonizing the zebrafish gut. MBio. 2014; 5: e01751–14. https://doi.org/10.1128/mBio.01751-14 PMID: 25516613
31. Ondov BD, Bergman NH, Phillippy AM. Interactive metagenomic visualization in a Web browser. BMC Bioinformatics. 2011; 12: 385. https://doi.org/10.1186/1471-2105-12-385 PMID: 21961884

32. Rodriguez-R LM, Gunturu S, Tiedje JM, Cole JR, Konstantinidis KT. Nonpareil 3: fast estimation of metagenomic coverage and sequence diversity. mSystems. 2018; 3: e00039–18. https://doi.org/10.1128/mSystems.00039-18 PMID: 29657970

33. Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E, et al. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods. 2015; 12: 902–903. https://doi.org/10.1038/nmeth.3589 https://www.nature.com/articles/nmeth.3589#supplementary-information. PMID: 26418763

34. Franzosa EA, McVean LJ, Rahnavard G, Thompson LR, Weingart G, et al. Species-level functional profiling of metagenomes and metatranscriptomes. Nat Methods. 2018; 15: 962–968. https://doi.org/10.1038/s41592-018-0176-y PMID: 30377376

35. Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, et al. A communal catalogue reveals Earth's multiscale microbial diversity. Nature. 2017; 551: 457–463. https://doi.org/10.1038/nature24621 https://www.nature.com/articles/nature24621#supplementary-information. PMID: 29088705

36. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016; 13: 581–583. https://doi.org/10.1038/nmeth.3869 https://www.nature.com/articles/nmeth.3869#supplementary-information. PMID: 27214047

37. Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech Xu Z, et al. Deblur rapidly resolves single-nucleotide community sequence patterns. mSystems. 2017; 2: e00191–16. https://doi.org/10.1128/mSystems.00191-16 PMID: 28289731

38. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. Qiime allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7: 335–336. https://doi.org/10.1038/nmeth.f.303 PMID: 20383131

39. Gonzalez A, Navas-Molina JA, Kosciolek T, McDonald D, Vázquez-Baeza Y, Ackermann G, et al. Qilta: rapid, web-enabled microbiome data science using QIIME 2. Nature Biotechnol. 2019. https://doi.org/10.1038/s41587-019-0209-9 PMID: 31341288

40. Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander C, et al. Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. Mol Phylogenet Evol. 2008; 48: 369–371. https://doi.org/10.1016/j.ympev.2008.03.024 PMID: 18424089

41. Cristescu ME. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. Trends Ecol Evol. 2014; 29: 566–571. https://doi.org/10.1016/j.tree.2014.08.001 PMID: 25175416

42. Gilbert JA, Meyer F, Jansson J, Gordon J, Pace N, Tiedje J, et al. The Earth Microbiome Project: meeting report of the "1 EMP meeting on sample selection and acquisition" at Argonne National Laboratory October 6 2010. Stand Genomic Sci. 2010; 3: 249–253. https://doi.org/10.4056/aigs.1443528 PMID: 21304728

43. Klein M, Lanka S, Muller D, Knippers R. Single-stranded regions in the genome of the Ectocarpus siliculosus virus. Virology. 1994; 202: 1076–1078. https://doi.org/10.1006/viro.1994.1443 PMID: 8030215

44. Ebert D. The epidemiology and evolution of symbionts with mixed-mode transmission. Annu Rev Ecol Evol Syst. 2013; 44: 623–643. https://doi.org/10.1146/annurev-ecolsys-032513-100555

45. Kreisinger J, Bastien GR, Hauffe HC, Marchesi J, Perkins SE. Interactions between multiple helminths and the gut microbiota in wild rodents. Philos Trans R Soc Lond B Biol Sci. 2015; 370: 20140295. https://doi.org/10.1098/rstb.2014.0295 PMID: 26150661

46. Hersch-Green EI, Turley NE, Johnson MTJ. Community genetics: what have we accomplished and where should we be going? Philos Trans R Soc Lond B Biol Sci. 2011; 366: 1453–1460. https://doi.org/10.1098/rstb.2010.0331 PMID: 21444318

47. Thompson J. The Geographic Mosaic of Coevolution. Chicago, IL, USA: University of Chicago Press; 2005.

48. Ford SA, King KC. Harnessing the power of defensive microbes: evolutionary implications in nature and disease control. PLoS Pathog. 2016; 12: e1005465. https://doi.org/10.1371/journal.ppat.1005465 PMID: 27058881
51. Dennis AB, Patel V, Oliver KM, Vorburger C. Parasitoid gene expression changes after adaptation to symbiont-protected hosts. Evolution. 2017; 71: 2599–2617. https://doi.org/10.1111/evo.13333 PMID: 28841224

52. The Integrative Human Microbiome Project. Dynamic analysis of microbiome-host omics profiles during periods of human health and disease. Cell Host Microbe. 2014; 16: 276–289. https://doi.org/10.1016/j.chom.2014.08.014 PMID: 25211071

53. Hahn MA, Dheilly NM. Experimental models to study the role of microbes in host-parasite interactions. Front Microbiol. 2016; 7: 1300. https://doi.org/10.3389/fmicb.2016.01300 PMID: 27602023

54. Dengatakot R, Pinlaor S, Itthitaetarakool U, Chaitree A, Chomvarin C, Sangka A, et al. Coinfection with Helicobacter pylori and Opisthorchis viverrini enhances the severity of hepatobiliary abnormalities in hamsters. Infect Immun. 2017; 85(4): e00009–17. https://doi.org/10.1128/IAI.00009-17 PMID: 28138021.

55. Deenonpo R, Chomvarin C, Pairojkul C, Chamgramol Y, Loukas A, Brindley PJ, et al. The carcinogenic liver fluke Opisthorchis viverrini is a reservoir for species of Helicobacter. APJCP. 2015; 16 (5):1751–8. https://doi.org/10.7314/apjcp.2015.16.5.1751 PMID: 25773821.

56. Deenonpo R, Mairiang E, Mairiang P, Pairojkul C, Chamgramol Y, Rinaldi G, et al. Elevated prevalence of Helicobacter species and virulence factors in opisthorchiasis and associated hepatobiliary disease. Sci Rep. 2017; 7:42744. https://doi.org/10.1038/srep42744 PMID: 28198451

57. Fichorova RN, Lee Y, Yamamoto HS, Takagi Y, Hayes GR, Goodman RP, et al. Endobiont viruses sensed by the human host, beyond conventional antiparasitic therapy. PLoS ONE. 2012; 7(11):e48418. https://doi.org/10.1371/journal.pone.0048418 PMID: 23144878

58. Onderdonk AB, Delaney ML, Fichorova RN. The human microbiome during bacterial vaginosis. Clin microbiol rev. 2016; 29(2):223–38. Epub 02/10. https://doi.org/10.1128/CMR.00075-15 PMID: 26864580.

59. Ives A, Ronet C, Prevel F, Ruzzante G, Fuertes-Marraco S, Schutz F, et al. Leishmania RNA virus controls the severity of mucocutaneous Leishmaniasis. Science. 2011; 331(6018):775–8. https://doi.org/10.1126/science.1199326 PMID: 21310223

60. Adaui V, Lye L-F, Akopyants NS, Zimic M, Llanos-Cuentas A, Garcia L, et al. Association of the endobiont double-stranded RNA virus LRV1 with treatment failure for human Leishmaniasis caused by Leishmania braziliensis in Peru and Bolivia. J Infect Dis. 2015; 213(1):112–21. https://doi.org/10.1093/infdis/jiv354 PMID: 26123965

61. Landmann F, Voronin D, Sullivan W, Taylor MJ. Anti-filarial activity of antibiotic therapy is due to extensive apoptosis after Wolbachia depletion from filarial nematodes. PLoS Pathog. 2011; 7(11):e1002351. https://doi.org/10.1371/journal.ppat.1002351 PMID: 22072969

62. Slatko BE, Taylor MJ, Foster JM. The Wolbachia endosymbiont as an anti-filarial nematode target. Symbiosis. 2010; 51(1):55–65. Epub 06/05. https://doi.org/10.1007/s13199-010-0067-1 PMID: 20730111.

63. Gauthier J, Drenzen J-M, Herniou EA. The recurrent domestication of viruses: major evolutionary transitions in parasitic wasps. Parasitol. 2017; 145(6):122–131. Epub 05/23. https://doi.org/10.1191/0031182017700018 PMID: 28534462

64. Dheilly NM, Maure F, Ravallee M, Galinier R, Doyon J, Duval D, et al. Who is the puppet master? Replication of a parasitic wasp-associated virus correlates with host behaviour manipulation. Proc Roy Soc B. 2015; 282(1803). https://doi.org/10.1098/rspb.2014.2773 PMID: 25673981

65. Tan C-W, Peiffer M, Hoover K, Rosa C, Acevedo FE, Felton GW. Symbiotic polynoduirus of a parasite manipulates caterpillar and plant immunity. Proc Nat Acad Sci. 2016; 113(20):5199. https://doi.org/10.1073/pnas.1517934115 PMID: 29712862

66. Gottlieb Y, Lalzar I, Klasson L. Distinctive Genome Reduction Rates Revealed by Genomic analyses of two Coxella-like endosymbionts in ticks. Genome Biol Evol. 2010; 5(6):1779–96. Epub 10/23. https://doi.org/10.1093/gbe/evq016 PMID: 20625560.

67. Smith TA, Driscoll T, Gillespie JJ, Raghavan R. A Coxella-like endosymbiont is a potential vitamin source for the Lone Star tick. Genome Biol Evol. 2015; 7(3):831–8. https://doi.org/10.1093/gbe/evv016 PMID: 25618142.

68. Banin E, Khare SK, Naider F, Rosenberg E. Proline-rich peptide from the coral pathogen Vibrio shiloi that inhibits photosynthesis of zooxanthellae. App Env Microbiol. 2001; 67(4):1536. https://doi.org/10.1128/AEM.67.4.1536–1541.2001

69. Hayes KS, Bancroft AJ, Goldrick M, Portsmouth C, Roberts IS, Gencics RK. Exploitation of the intestinal microbiota by the parasitic nematode Trichuris muris. Science. 2010; 328(5984):1391. https://doi.org/10.1126/science.1187703 PMID: 20538949

70. Holm JB, Sorobeta D, Kilerich P, Ramayo-Caldas Y, Estellé J, Ma T, et al. Chronic Trichuris muris infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of...
71. Li RW, Wu S, Li W, Navarro K, Couch RD, Hill D, et al. Alterations in the porcine colon microbiota induced by the gastrointestinal nematode Trichuris suis. Infection and Immunity. 2012; 80(6):2150. https://doi.org/10.1128/IAI.00141-12 PMID: 22493085

72. Ramanan D, Bowcutt R, Lee SC, Tang MS, Kurtz ZD, Ding Y, et al. Helminth infection promotes colonization resistance via type 2 immunity. Science. 2016; 352(6285):608. https://doi.org/10.1126/science.aaf3229 PMID: 27080105

73. Vaughan JA, Tkach VV, Greiman SE. Chapter 3—Neorickettsial endosymbionts of the digenea: diversity, transmission and distribution. In: Rollinson D, Hay SI, editors. Adv Parasitol. 79: Academic Press; 2012. p. 253–97.

74. McNulty SN, Tort JF, Rinaldi G, Fischer K, Rosa BA, Smircich P, et al. Genomes of Fasciola hepatica from the Americas reveal colonization with Neorickettsia endobacteria related to the agents of potomac horse and human sennetsu fevers. PLoS Genet. 2017; 13(1):e1006537. https://doi.org/10.1371/journal.pgen.1006537 PMID: 28060841

75. Gaulke CA, Martins ML, Watral VG, Humphreys IR, Spagnoli ST, Kent ML, et al. A longitudinal assessment of host-microbe-parasite interactions resolves the zebrafish gut microbiome’s link to Pseudocapillaria tomentosa infection and pathology. Microbiome. 2019; 7(1):10. https://doi.org/10.1186/s40168-019-0622-9 PMID: 30678738