An Introduction to Phylosymbiosis

Shen Jean Lim[1] and Seth R. Bordenstein[1,2,3,4]*
[1]Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA
[2]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA
[3]Vanderbilt Microbiome Initiative, Vanderbilt University, Nashville, TN, USA
[4]Vanderbilt Institute for Infection, Immunology, and Inflammation, Vanderbilt University Medical Center, Nashville, TN, USA

Corresponding Author:
Seth Bordenstein[1,2,3,4]
465 21st Ave S, Nashville, TN, 37212, USA
Email address: s.bordenstein@vanderbilt.edu

Abstract

Phylosymbiosis was recently formulated to support a hypothesis-driven framework for the characterization of an emerging trend in host-associated microbiomes. Defining phylosymbiosis as “microbial community relationships that recapitulate the phylogeny of their host”, we review the relevant literature and data in the last decade, emphasizing frequently used methods and regular patterns observed in the analyses. Quantitative support for phylosymbiosis is provided by statistical methods evaluating the distinguishability of microbiomes between hosts, topological congruency between the host phylogeny and microbiome dendrogram, and a positive association between host genetic relationships and microbiome beta diversity. Significant degrees of phylosymbiosis are prevalent in gut and surface microbiomes of plants and animals from terrestrial and aquatic habitats. Consistent with natural selection underpinning phylosymbiosis, microbiome transplant experiments demonstrate reduced host performance and/or fitness upon host-microbiome mismatches. The pervasiveness of phylosymbiosis carries several important implications for analyses of host-microbiome interactions, evolutionary biology, personalized microbiology, and conservation biology. Important future steps will be to apply evolutionary modelling for an increasingly sophisticated understanding of phylosymbiosis and to unravel the host and microbial mechanisms that contribute to the pattern. This review serves as a gateway to experimental, conceptual, and quantitative themes of phylosymbiosis and outlines opportunities ripe for investigations from a diversity of disciplines, scholars, and students.

Introduction

The last decade has brought renewed interest in the complexity of microorganisms living in association with hosts, yielding a number of new empirical results, philosophical concepts, and research opportunities (McFall-Ngai et al., 2013; Theis et al., 2016). Any discussion on the study

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27879v1 | CC BY 4.0 Open Access | rec: 30 Jul 2019, publ: 30 Jul 2019

of host-microbiome interactions must begin with clear definitions. Here, we use the terms symbiotic and symbiosis (*sym* – “together”, *bios* – “life” in Greek) to encompass associations between two or more organisms of different species and without restriction to the length of time of association or phenotypes produced by the interacting species. Since temporal and functional variation in symbiosis is context-dependent, symbiotic interactions can include a range of obligatory, facultative, transient, and permanent associations with varying degrees of specificity and functional costs and benefits.

The last two decades of technology developments and research have placed microbial symbiosis as a nexus of many biological subdisciplines. Researchers now have a full suite of tools and increased awareness of the major questions to be answered. These include holistic approaches useful for the identification of ecological (Bletz et al., 2017) and host (Ley et al., 2008; Colman, Toolson & Takacs-Vesbach, 2012; Franzenburg et al., 2013; Bost et al., 2018) drivers of microbial taxonomic and functional diversity in symbiotic systems, as well as reductionist approaches that provide mechanistic insights into transmission processes (Bright & Bulgheresi, 2010; Funkhouser & Bordenstein, 2013) and phenotypical outcomes of symbiosis (McFall-Ngai et al., 2013). The abundance of empirical and theoretical investigations on the ecology and evolution of simple symbioses also comprise fertile ground to build a foundation for the microbiome field that studies frequently complex associations between hosts and their multiple microbial associates. One emerging and new principle in this area of research is the recently defined pattern of phylosymbiosis (Brucker & Bordenstein, 2013).

Despite burgeoning research interest in phylosymbiosis and proliferating evidence of the trend in a diverse spectrum of systems, phylosymbiosis is a new topic. This review therefore aims to synthesize the topic for newcomers, students, and experts alike to focus on (a) a long-lasting definition of the term phylosymbiosis; (b) a practical guide on measuring phylosymbiosis; (c) an overview of the prevalence of phylosymbiosis in nature; (d) a discourse on the significance of phylosymbiosis; and (e) future directions in phylosymbiosis research.

What is phylosymbiosis?

We use the following quote to describe our initial and basic definition of phylosymbiosis, namely “microbial community relationships that recapitulate the phylogeny of their host” (Brucker & Bordenstein, 2013). Phylosymbiosis is first and foremost a host phylogenetic effect on host-associated microbiomes wherein “phylo” refers to host clade and “symbiosis” refers to the microbial community in or on the host. It is to be used in an analogous way to phylogeography - the study of evolutionary processes that shape geographic or ecological distributions of organisms (Avise et al., 1987; Knowles, 2009). While studying speciation in the genus of *Nasonia* parasitoid wasps, the use of the term phylosymbiosis arose from a need to distinguish a host phylogenetic effect on microbiome relationships (phylosymbiosis) from other evolutionary processes such as reciprocal evolutionary genetic changes between symbiotic organisms (coevolution) or the ancestral splitting of host and symbiont lineages (cospeciation, codivergence; Brucker & Bordenstein, 2013) . The basic reason, and a source of early confusion, was that a positive association between host phylogenetic and microbial community relationships does not a priori imply a shared and ancestral evolutionary history between hosts and their microbiomes (Brucker & Bordenstein, 2012; Brucker & Bordenstein, 2013). Rather, phylosymbiosis is an eco-evolutionary pattern observed at a snapshot in time and space, and it may or may not reflect long-term associations or co-adaptations that can be subsequently evaluated by empirical analyses.

A brief history of phylosymbiosis research

Prior to the formal definition of phylosymbiosis, examples existed in a variety of host-microbe systems. The first integrative analysis of microbiome data and host phylogeny was performed on fecal samples from humans and 59 other wild and captive mammalian species (Ley et al., 2008). The study revealed a prominent influence of diet in structuring gut bacteria-by-host associations, as well as a smaller effect of host taxonomy in shaping these associations (Ley et al., 2008). Comparisons of beta diversity-derived clusters with randomized and non-randomized mammalian phylogenies suggested a weak phylosymbiotic association that is localized to specific host clades (Ley et al., 2008). A subsequent study on fecal specimens from humans, four great ape species, and three subspecies of chimpanzees in their native habitats found topological congruency between host mitochondrial DNA (mtDNA) phylogeny and a microbial tree generated from relative abundance differences (Ochman et al., 2010).

In insects, a meta-analysis on subsets of data generated from 62 insect species spanning seven orders and nine diet categories reported statistically significant influences of host diet and host taxonomy on gut community composition (Colman, Toolson & Takas-Vesbach, 2012). However, the authors did not observe statistical congruency between topologies of the beta diversity dendrogram and insect host phylogeny (Colman, Toolson & Takacs-Vesbach, 2012). A plant-based study hypothesized a statistical correlation between rhizobacterial beta diversity and microsatellite genetic distances of ten inbred maize lines grown in a controlled greenhouse experiment (Bouffaud et al., 2012). Nonetheless, the results showed a non-linear and non-significant relationship between both components, suggested to be due to the relatively short post-domestication diversification history of maize and/or rhizobial profiling of seedling roots instead of mature plant roots (Bouffaud et al., 2012). The first functional genetic study of phylosymbiosis showed that while closely-related *Hydra* species harbor phylosymbiotic bacterial communities in freshwater and lab conditions (Fraune & Bosch, 2007), *Hydra*-microbiome specificity was altered upon knockdown of the armenin antimicrobial peptide secreted by the host (Franzenburg et al., 2013).

Early work on bacterial 16S rRNA genes cloned and sequenced from males of three *Nasonia* parasitoid wasp species maintained in identical conditions demonstrated that the closely related sister species *N. giraulti* and *N. longicornis*, which diverged 0.4 million years ago, harbored more similar adult, pupal, and 2nd instar larval microbiomes compared to the microbiome in their outgroup species *N. vitripennis* (Brucker & Bordenstein, 2012), which diverged from the two sister species about 1.0 million years ago (Werren et al., 2010). In contrast, 1st instar larvae did not exhibit phylosymbiosis due to limited microbial diversity at this stage of development.

One hypothesis for why different host species harbor phylosymbiotic microbiomes is that hosts are adapted to the functions of their resident microbiomes and may exert an influence on the types of microbes that colonize. Microbiome transplant studies have demonstrated that resident microbes can preferentially colonize some host taxa, likely through host-specific biofilm formation (Frese et al., 2013), virulence (Sarkar et al., 2006), and colonization (Cowles & Goodrich-Blair, 2008). Likewise, a variety of host taxa have been shown to select for specific symbionts through non-immune and immune factors (Bevins & Salzman, 2011), such as oxidative signals (Damiani et al., 2016), mucus barriers (Nyholm & McFall-Ngai, 2003), and antimicrobial peptides (Franzenburg et al., 2013). Functional phylosymbiosis can be evaluated empirically in at least two ways. First, hybridization between closely related host species could disrupt host-microbiome associations in parental species and lead to hybrid maladies. Consistent with the above hypothesis, crosses between the more divergent species pair of *N. vitripennis* and *N. giraulti* produced a non-phylosymbiotic larval microbiome in F2 hybrid male 2nd instars, a hyperactive host immune response, and severe larval lethality (Brucker & Bordenstein, 2013). F2 hybrid male lethality is rescued by germ-free rearing, and conversely restored by feeding a 1:1 inoculum of the resident *Nasonia* bacteria species *Providencia rettgeri* and *Proteus mirabilis* to germ-free hybrids (Brucker & Bordenstein, 2013). This implies that non-phylosymbiotic relationships can lead to adverse functional and evolutionary consequences over time. Second, if hosts are adapted to their microbiomes, then microbiome transplant experiments between related species/lineages will lead to host fitness reductions in recipients with a non-resident microbiome relative to recipients with a transplanted resident microbiome. We discuss the performance and fitness costs of interspecific microbiome transplants in the following section.

Formalizing phylosymbiosis: from pattern to evolutionary process

With growing prominence of phylosymbiosis, we proposed an initial, methodological workflow to statistically evaluate its strength and significance in a large-scale investigation spanning 24 species across four different host clades (*Nasonia* wasps, *Drosophila* flies, mosquitoes, and *Peromyscus* deer mice; Brooks et al., 2016). Females from each clade were reared in laboratory conditions that controlled for temperature, housing, developmental stage, sex, food, and parasitic infections to minimize environmental effects on measured outcomes (Brooks et al., 2016). Analyses of bacterial 16S rRNA gene sequence data, together with a previous hominid fecal microbiome dataset (Ley et al., 2008), revealed varying degrees of phylosymbiosis in each of the five animal clades (Brooks et al., 2016). Notably, host divergence levels spanning 1-108 million years of evolution did not limit statistical detection of phylosymbiosis (Brooks et al., 2016). The strength of microbiome distinguishability between related host species of the same host clade positively associated with host divergence levels, as expected (Brooks et al., 2016). Another study on mammalian gut microbiomes demonstrated that the strength of phylosymbiosis is significant and strong within clades, but the signal decays over the course of 100 million years of mammalian evolution (Groussin et al., 2017). The consensus of both studies is that phylosymbiosis can arise early in the evolution of new species and persist to varying degrees after host ancestors split into different, but related genera.

If different host species are adapted to their phylosymbiotic microbiome, then not only will hybridization disrupt phylosymbiosis and host functions, but transfers of another species' microbiome into a recipient species will reduce host performance and/or fitness. Indeed, interspecific transplants of gut microbial communities between *Peromyscus* species decreased dry matter digestibility and increased food intake, while transplants between *Nasonia* species crucially lowered survival to adulthood by up to 43% (Brooks et al., 2016). Another study showed that reciprocal maternal symbiont transplant between two sympatric *Ontophagus* dung beetle species caused a developmental delay and elevated mortality in non-native hosts that persisted to the next generation (Parker, Dury & Moczek, 2019). Collectively, specialized host-microbe associations indicate that hosts are adapted to their native microbiomes rather than non-native microbiomes. Therefore, phylosymbiosis can arise due to natural selection as opposed to neutral evolutionary forces shaping host-microbiome associations.

What is not phylosymbiosis

Having now defined phylosymbiosis, we emphasize in this section what phylosymbiosis is not. Although various physiological and evolutionary processes may lead to and underpin phylosymbiosis, without empirical investigations, the pattern itself does not *a priori* assume that any one process has occurred. While vertical transmission of host-associated microbial communities and/or long-lasting host-microbiome associations are possible contributors ripe for investigation, phylosymbiosis does not necessarily imply exclusive vertical transmission, nor evolutionary splitting from a common ancestor via co-evolution, co-speciation, co-diversification, or co-cladogenesis (Theis et al., 2016). Early misconceptions confused the term with these evolutionary processes. Instead, phylosymbiosis first and foremost distinguishes a host phylogenetic from non-phylogenetic effect on microbiome variation. It is a testable and nullifiable observation, and it can appear at any given time and space. Once observed, more specific questions about transmission routes and evolutionary modes should be assessed. It is also important to note that phylosymbiosis outcomes can be variable and subject to temporal and spatial shifts facilitated by processes such as environmental perturbations, environmental symbiont acquisition, and host hybridization. Phylosymbiosis is unlikely to be detected in host-microbiome associations in which microbial communities are predominantly assembled stochastically from the environment.

A practical guide to studying phylosymbiosis

Investigations of phylosymbiosis vary in approach (qualitative vs quantitative), methodology, and statistical power (Mazel et al., 2018). Thus, a clear, consistent, and robust workflow to detect phylosymbiosis is desirable for newcomers and experts alike. Here, we outline an updated workflow for examining phylosymbiosis that will be discussed in detail below (Figure 1).

Host input data. To begin, we recommend identification of a host clade with at least four related lineages and an actual/hypothetical species outgroup. Studies with more lineages will increase statistical sensitivity and enable interpretations in a broader evolutionary context. Marker sequence(s) from host species can be used to generate a phylogenetic or phylogenomic tree that is confidently supported at branching nodes with bootstrap (Felsenstein, 1985a) or other measures (Anisimova & Gascuel, 2006) and across several phylogenetic inference methods (e.g. maximum likelihood (Guindon et al., 2010) and Bayesian inference (Mau & Newton, 1997)). Because an accurate host phylogenetic topology is essential for evaluating phylosymbiosis, the tree should be free from systematic artefacts such as long branch attraction. While not always possible, polytomies should be resolved in the host phylogeny. As methods used to reconstruct a host phylogeny from a sequence alignment have been extensively reviewed (Wiley & Lieberman, 2012), we will not discuss them further here. With a phylogenetic tree, pairwise host distances can also be represented as cophenetic distances, computed as the sum of branch lengths connecting a pair of terminal nodes on a phylogenetic tree (Sokal & Rohlf, 1962).

Microbiome input data. Phylosymbiosis assessment requires sequence data and microbial diversity analyses from each host species. For robust replication of phylosymbiotic host-microbe associations, we recommend sequencing at least ten samples per host lineage. Short-read sequencing of microbial phylogenetic marker genes (e.g. 16S rRNA gene) is the most common and economical method for microbial profiling. Processed sequenced reads can be analyzed by one of two current methods. First, they can be clustered into operational taxonomic units (OTUs) at different sequence cutoffs (e.g. 97% and 99%) with and/or without reference sequence database (Rideout et al., 2014; Kopylova et al., 2016). Second, they can be resolved into amplicon sequence variants (ASVs) without clustering, which may offer single-nucleotide resolution, though sequencing error rates should be accounted for (Callahan, McMurdie & Holmes, 2017). For the greatest sensitivity in phylosymbiosis assessment, meta-omics datasets are advantageous because finer-scale taxonomic and functional profiling can be achieved (Medina & Sachs, 2010).

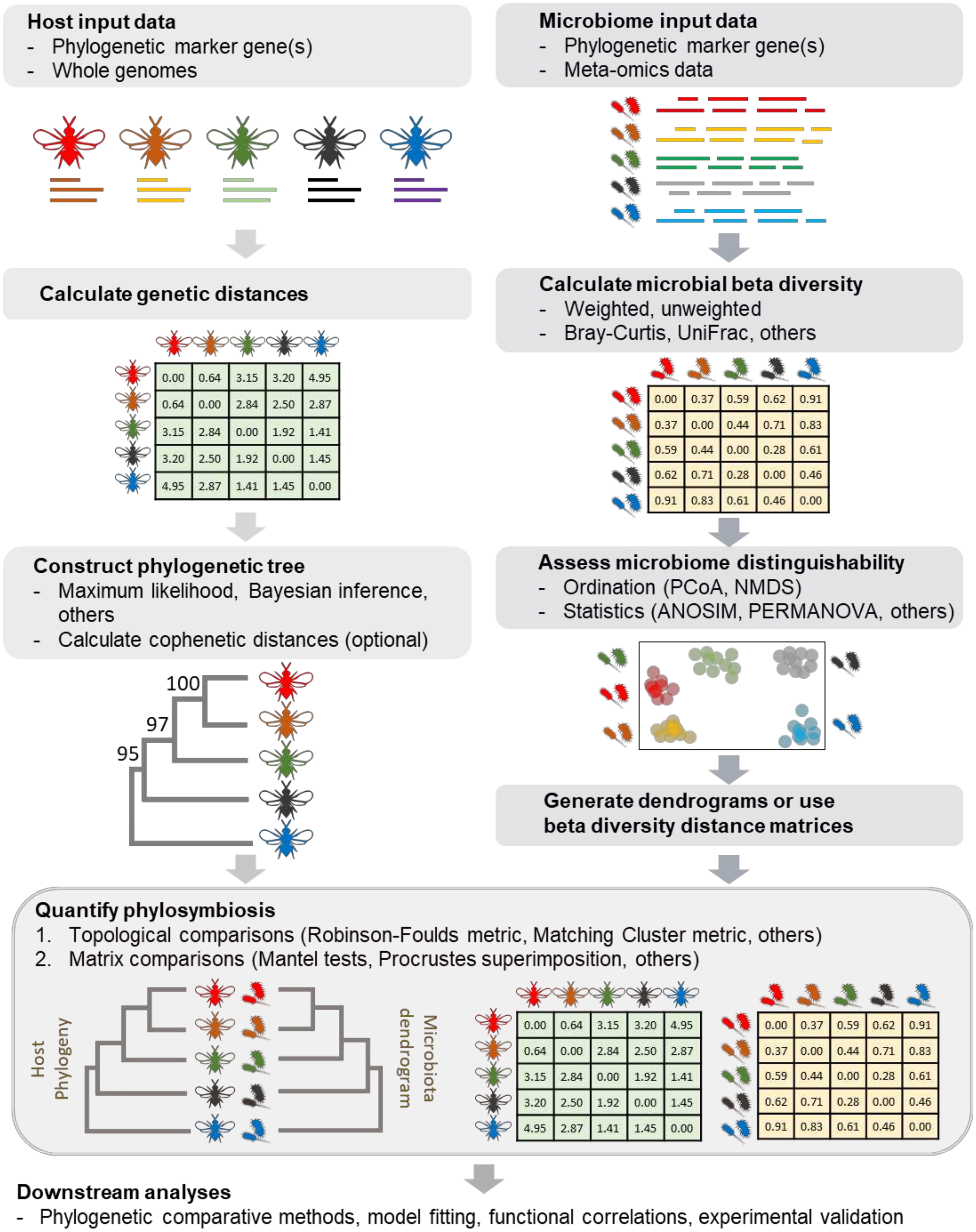

Figure 1. Overview of sequential, bioinformatics methods commonly used for phylosymbiosis analyses. Further details are provided in the text of this review.

Microbial beta diversity measures. Microbial beta diversity indices, which measure dissimilarities in microbial composition and structure across host samples, are conventionally used to measure phylosymbiosis. Compared to binary descriptors calculated based on OTU presence/absence data (e.g. Jaccard distance), quantitative descriptors of OTU abundances (e.g. Bray-Curtis dissimilarity; Bray & Curtis, 1957) and the phylogenetically informed unique fraction (UniFrac) distance (Lozupone & Knight, 2005) are preferred estimators of beta diversity.

In our 2016 study across animal clades, Bray-Curtis distance showed higher sensitivity than weighted and unweighted UniFrac distances in detecting phylosymbiosis (Brooks et al., 2016). Another study did not observe marked differences in phylosymbiosis among Jaccard, Bray-Curtis, unweighted UniFrac and weighted UniFrac measures (Mazel et al., 2018). Nevertheless, the authors noticed a slight performance advantage of weighted over unweighted UniFrac distances in detecting phylosymbiosis, and proposed that abundance-weighted measures may reduce noise arising from chance colonization of an individual OTU in a specific sample (Mazel et al., 2018). Because of this performance variability, we strongly recommend reporting results from different OTU cutoffs, ASV analyses, and beta diversity indices for phylosymbiosis detection. Our recent study on *Nasonia*-viral phylosymbiosis used metagenomic reads mapped to assembled viral contigs to calculate Bray-Curtis beta diversity (Leigh et al., 2018). As meta-omics tools and datasets become increasingly accessible, new methods of inferring microbial beta diversity from these data will improve the sensitivity of phylosymbiosis assessment.

Assessing microbiome distinguishability. In the study of phylosymbiosis, microbial beta diversity differences within and between host species are important indicators of microbiome distinguishability (e.g. Brooks et al., 2016; Pollock et al., 2018; Ross et al., 2018; Kohl et al., 2018; Kohl, Dearing & Bordenstein, 2018). Microbiome distinguishability across samples can be visualized from beta diversity data and categorical sample grouping data using ordination plots, such as principle coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) plots (Legendre & Legendre, 1998). Microbiome distinguishability can also be further statistically evaluated using typically non-parametric multivariable analyses, such as analysis of similarities (ANOSIM; Clarke, 1993) and variants of permutational multivariate analysis of variance (PERMANOVA; McArdle, Anderson, 2001). Specific pairwise comparisons of intraspecific and interspecific microbial beta diversity distances can also be performed with an appropriate non-parametric two-sample test, as implemented in our 2016 study (Brooks et al., 2016). As with beta diversity measures, we recommend reporting results of multiple statistical tests for microbiome distinguishability.

Quantifying phylosymbiosis. Because phylosymbiosis is a host phylogenetic effect on microbiome variation, topological congruency tests can be used to directly compare the host phylogenetic tree topology to the microbiome dendrogram topology (e.g. Brooks et al., 2016;

Novakova et al., 2017; Leigh et al., 2018; Kohl et al., 2018; Kohl, Dearing & Bordenstein, 2018; Ross et al., 2018). Microbiome dendrograms are often hierarchically clustered from microbial beta diversity data using the unweighted pair group method with arithmetic mean (UPGMA) method (Michener & Sokal, 1957). One commonly used topological comparison metric, the Robinson-Foulds metric, computes the distance between two trees as the smallest number of operations required to convert one topology to the other (Robinson & Foulds, 1981). Another metric, the Matching Cluster distance, is more robust and sensitive than the Robinson-Foulds metric because it considers congruency at the clade level (Bogdanowicz & Giaro, 2013; Brooks et al., 2016). Using scripts available at https://github.com/awbrooks19/phylosymbiosis, statistical significance of both metrics can be evaluated by a computed p value representing the probability of obtaining a microbiota dendrogram that is topologically congruent with the host phylogenetic tree by chance (Brooks et al., 2016).Topological comparison metrics crucially do not use branch length information as there is no *a priori* reason to assume evolutionary host rates of evolution equals rates of ecological community change in the microbiome. As such, these topology tests are conservative relative to matrix correlation methods (e.g. Mantel test, see below) that directly compare host genetic divergence with microbial community dissimilarity numerically. Nevertheless, Robinson-Foulds calculations can be computationally intensive as the size of the input data increases (Pattengale, Gottlieb & Moret, 2007). Although the type I error rates of topological comparison methods in detecting phylosymbiosis have not been systematically evaluated, we recommend further scrutiny of predicted topological congruency by re-analyzing relevant subsets of large data, evaluating statistical significance, and comparing results with those produced by matrix correlation methods.

Matrix correlation methods identify phylosymbiosis by comparing the similarities between host-derived and microbial-derived distance matrices. Methods including the Mantel test (Mantel, 1967) and the more powerful Procrustean superimposition approach (Peres-Neto & Jackson, 2001) have been implemented in phylosymbiosis studies (e.g. Schottne et al., 2013; Easson & Thacker, 2014; Reveillaud et al., 2014; Sanders et al., 2014; Thomas et al., 2016; Groussin et al., 2017; Kropáčková et al., 2017; Kwong et al., 2017; Gaulke et al., 2018; Pollock et al., 2018; Javurkova et al., 2019). Partial Mantel tests (Smouse, Long & Sokal, 1986) measuring correlations between two matrices while controlling for the effects of a third variable described in another matrix have also been utilized (e.g. Easson & Thacker, 2014; Sanders et al., 2014; Thomas et al., 2016; Kwong et al., 2017). Despite their utility, the main challenge of phylosymbiosis measurement methods lies in meaningful comparisons of input data derived from different characters (Dietz, 1983) and the detection of clade-specific, non-linear relationships (Legendre & Legendre, 1998).

Emerging tools. In parallel with phylosymbiosis, research interest in evaluating phylogenetic signal, originally defined as "a tendency for related species to resemble each other more than they resemble species drawn at random from the tree" (Blomberg, S. P. & Garland Jr, 2002), from microbial diversity data is budding. Indices for measuring phylogenetic signal in univariate traits in ecology studies have been applied to examine the phylogenetic signal on alpha diversity (e.g. in sponges (Easson & Thacker, 2014; Thomas et al., 2016)). Phylogenetic signal indices like Pagel's λ (Pagel, 1999), and Blomberg's K (Blomberg, Simon P., Garland JR. & Ives, 2003) are based on a random Brownian model of trait evolution (Felsenstein, 1985b), but can also be used with more complex models. Although these methods are less commonly used on multivariable data and have not yet been applied to evaluate phylosymbiosis, they are promising alternatives for not only examining host phylogenetic signal on microbial beta diversity, but also testing evolutionary models relevant to phylosymbiosis.

Phylogenetic comparative methods, such as phylogenetic independent contrasts (Felsenstein, 1985b) and phylogenetic generalized linear mixed models (pGLMMs; Ives, Helmus, 2011), that predict the evolutionary correlation between two or more discrete or continuous traits given a known phylogeny and an evolutionary model can also be integrated into phylosymbiosis studies. pGLMMs have recently been implemented in coral microbiome (Pollock et al., 2018) and passerine feather microbiome studies (Javurkova et al., 2019) to examine the effects of latitude and colony size on coral alpha diversity, cophylogenetic coral-bacteria relationships, and relationships between alpha diversity and relative abundances of bacteriocin-producing bacteria and keratinolytic feather damaging bacteria. These methods can be useful in detecting ecological interactions, such as predator-prey relationships, mutualism, competition, and habitat filtering, as well as environmental interactions, that affect microbial community structure and possibly underpin phylosymbiosis.

Overall, as meta-omics and trait evolution analyses become more widely applicable to phylosymbiosis, one compelling direction of future phylosymbiosis investigations *in silico* is to venture beyond host phylogenetic effects on microbial diversity to encompass linkages between host phylogeny, host functions, microbial diversity, microbial functions, and environmental factors.

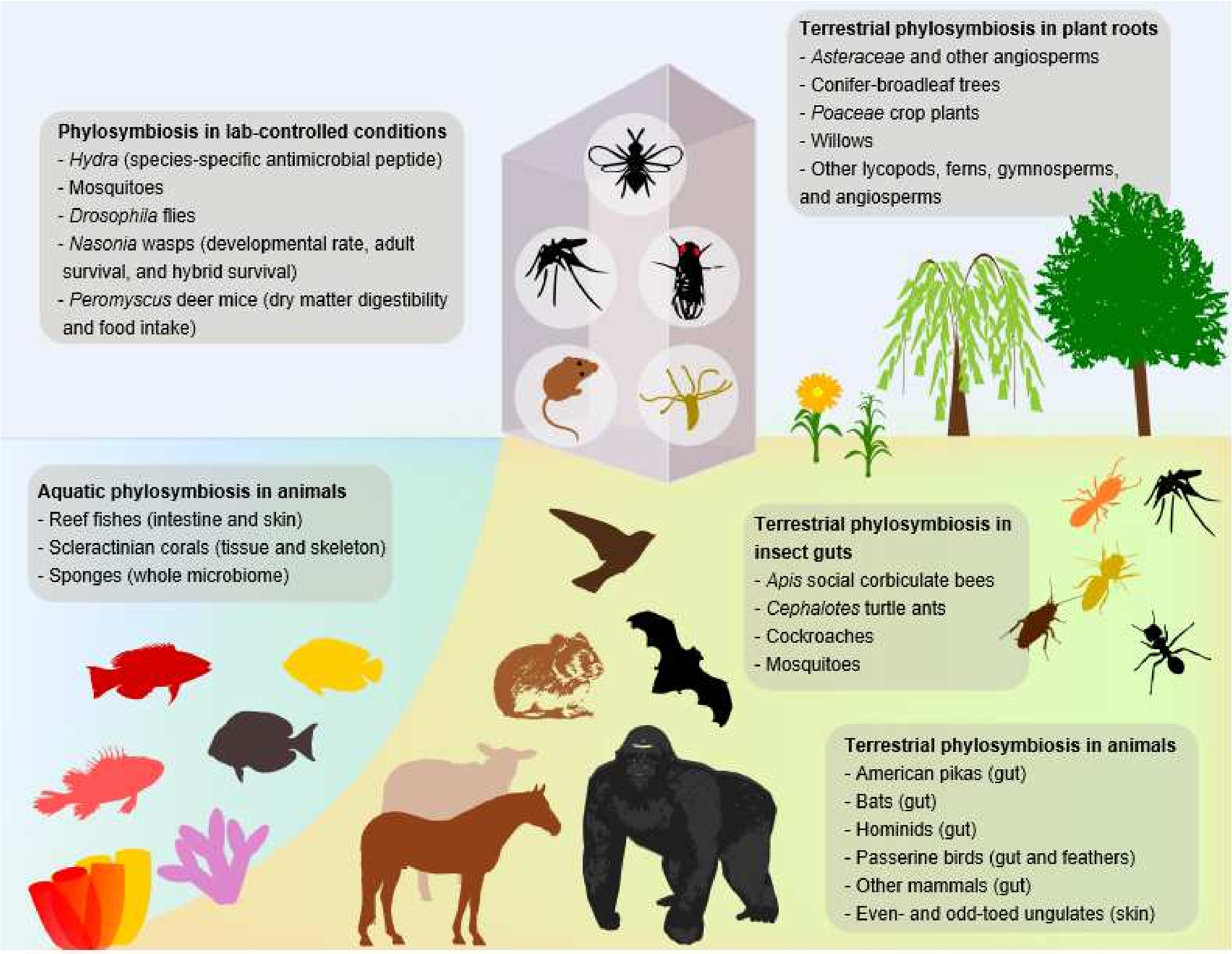

Figure 2. Representative diversity of phylosymbiosis across host species, tissues, habitats, and functions.

The prevalence of phylosymbiosis

The phylosymbiosis term has its roots in insect-microbiome studies, and investigations into the trend in insects continue to this day (Figure 2). Phylosymbiosis has also been reported in viromes of *Nasonia* parasitoid jewel wasps (Leigh et al., 2018), as well as gut microbiomes of cockroaches, lower termites, and high termites at a broad taxonomic level (Dietrich, Kohler & Brune, 2014), lab-reared (Brooks et al., 2016) and wild mosquitoes (Novakova et al., 2017), *Cephalotes* turtle ants (Sanders et al., 2014), and *Apis* social corbiculate bees (Kwong et al., 2017). In *Drosophila* flies, evidence or phylosymbiosis is mixed. The trend was not detected qualitatively in gut or whole microbiomes of two independent lab collections (Wong, Chaston & Douglas, 2013), but weakly documented in our 2016 quantitative analysis on whole microbiomes from six lab-reared *Drosophila* species that were controlled for endosymbiont status and gender (Brooks et al., 2016); five of which overlapped with species in the first lab collection in Wong, Chaston & Douglas, 2013. Recent gut microbiome analyses on wild *Drosophila* populations did not observe host trait- or species-specific partitioning of microbial beta diversity (Martinson, Douglas & Jaenike, 2017; Bost et al., 2018). Despite this, clusters of bacterial OTUs and metagenomes that covary in relative abundances across samples were shown to correlate with both microbial taxonomic order and the expression of certain host genes (Bost et al., 2018), implying functional host-microbe interactions.

The first phylosymbiosis study on mammalian gut microbiomes (Ley et al., 2008) led to similar investigations examining the effects of animal phylogeny and diet on gut microbial community dissimilarity (Ochman et al., 2010; Sanders et al., 2014; Brooks et al., 2016; Moeller et al., 2016; Groussin et al., 2017; Gaulke et al., 2018). Studies focusing on gut microbiomes of specific animal groups have confirmed phylosymbiosis in hominids (Gaulke et al., 2018), American pikas (Kohl et al., 2018), bats from 23 genera (Phillips et al., 2012), passerine birds (Kropáčková et al., 2017), and lab-maintained *Peromyscus* deer mice (Brooks et al., 2016; Kohl, Dearing & Bordenstein, 2018). In contrast, qualitative phylosymbiosis analyses in birds did not observe the trend in the gut microbiomes of 59 neotropical bird species, including passerines (Hird et al., 2015) and nine captive parrot species (Liu et al., 2019). Another quantitative study on the fecal microbiomes of 14 wild baboon populations across an African hybrid zone revealed statistically significant correlations between microbial community dissimilarity and several environmental parameters, but not host genetic distance (Grieneisen et al., 2019). Moreover, the authors did not identify any host species-specific signature on microbial abundance and composition (Grieneisen et al., 2019). Besides gut or fecal microbiomes, animal surface microbiomes have also been analyzed for phylosymbiotic associations (Ross, Rodrigues Hoffmann & Neufeld, 2019). Such associations have been confirmed in mammalian skin (Ross et al., 2018) and passerine feathers (Javurkova et al., 2019), but not in amphibian skin (Bletz et al., 2017). A meta-analysis of phylosymbiosis literature has highlighted an increased prevalence of the trend in microbiomes inhabiting internal host compartments in relation to those inhabiting external host compartments (Mazel et al., 2018). However, the finding may be inherently biased due to the larger number of studies investigating phylosymbiosis in the gut in relation to other external host compartments.

Beyond terrestrial and associated habitats, research interest in phylosymbiotic associations in aquatic habitats has been steadily growing (Figure 2). The pattern is an area of continuous study in sponges, where global microbiome surveys (Schmitt et al., 2012; Thomas et al., 2016; Lurgi et al., 2019) and taxon-specific surveys (Schottner et al, 2013; Easson & Thacker, 2014; Reveillaud et al., 201) have yielded mixed results on the extent of phylosymbiosis. Two previous studies have shown that the host phylogenetic signal on microbial beta diversity was reduced but still significant when host phylogeny is examined given host identity (Easson & Thacker, 2014; Thomas et al., 2016). In Australian scleractinian corals, phylosymbiosis was observed in tissue and skeleton compartments, but not mucus specimens that are predominantly influenced by the environment (Pollock et al., 2018). Four bacterial families exhibited co-phylogeny with corals in various compartments, implying long-term associations likely arising from coevolution or codiversification (Pollock et al., 2018). Similar to sponge-by-microbe associations (Easson &

Thacker, 2014; Thomas et al., 2016), microbial richness (alpha diversity) in all three coral compartments correlated with host phylogenetic distances (Pollock et al., 2018). In reef fishes, co-phylogeny between surgeonfishes from the Red Sea and their intestinal giant bacteria *Eplopiscium* (Miyake, Ngugi & Stingl, 2016), as well as phylosymbiosis and host dietary impacts on the skin microbiomes of 44 fish species from the Western Indian Ocean (Chiarello et al., 2018), have been reported. In contrast, phylosymbiosis was not detected in sympatric kelp species and their surface bacterial bacteriomes (Lemay et al., 2018).

Phylosymbiosis has also been assessed in plant hosts and their microbiomes, mainly to distinguish host phylogenetic effects from other soil determinants structuring plant-associated microbial communities (Figure 2). A comparative analysis of lycopods, ferns, gymnosperms, and angiosperms across a coastal tropical soil chronosequence indicated host phylogeny to be a secondary but statistically significant factor shaping root-associated bacterial community structure, after soil age (Yeoh et al., 2017). More taxonomically- and/or spatially-restricted surveys have also revealed phylosymbiosis between rhizobacterial communities and *Poaceae* crop plants (Bouffaud et al., 2014), endosphere bacterial communities and 30 plant species (Fitzpatrick et al., 2018), rhizosphere-associated fungal communities and willows from hydrocarbon-contaminated soils (Bell et al., 2014), root-associated eumycotan fungal communities and *Asteraceae* flowering plants in a dry grassland (Wehner et al., 2014), ectomycorrhizal fungal communities and conifer-broadleaf forest trees (Ishida, Nara & Hogetsu, 2007), and ectomycorrhizal fungal communities and Estonian *Salicaceae* willows (Tedersoo et al. 2013). Contrarily, qualitative incongruency between *Brassicaceae* host phylogeny and their root microbiomes has been observed (Schlaeppi et al., 2014), whereas non-statistically significant phylosymbiotic correlations have been reported between soil microbial (archaeal, bacterial, and fungal) communities and 14 *Salicaceae* species in a common garden experiment (Erlandso et al., 2018), and fungal endophyte communities and New Guinea rainforest trees (Vincent, Weiblen & May, 2016).

The significance of phylosymbiosis

Symbiosis research has arguably been revolutionized by the multi-omics era, where a deluge of data has enabled unprecedented insights into the extensive taxonomic, genetic, and functional composition of microbial communities and their associated hosts. Such large-scale accumulation of empirical and theoretical results can potentiate the development of new unifying concepts and frameworks that summarize and/or explain observations across diverse host-microbiome systems. In this vein, phylosymbiosis is maturing as a *bona fide* trend in the microbiome field spanning various kingdoms of life and their ecological niches (Figure 2).

Because phylosymbiosis assessments can be readily integrated into conventional microbiome analysis pipelines, it provides a quantitative and empirical research framework to distinguish phylogenetic effects from non-phylogenetic effects on host-microbiome associations. As such, phylosymbiosis provides testable eco-evolutionary predictions that guide hypothesis-driven experimental investigation, where the absence or presence of the trend informs more precise downstream hypotheses on ecology, evolution and functional interactions that can be further tested. This holistic view can be useful in the identification of host-by-microbe association patterns across the tree of life that could eventually illuminate intrinsic ecology, evolutionary, and physiological mechanisms shaping these interactions.

Phylosymbiosis also contributes to a growing school of thought that calls for systems-level views of host biology in light of the microbiome (Theis et al., 2016). Phenotypes of the hologenome (host and microbiome genomes) may arise from interactions within the holobiont (host and associated microbes), and these phenotypes may or may not contribute to various aspects of holobiont performance or fitness. It's important to note here that members of the microbial community contributing to holobiont function can be transient or stable, vertically or horizontally transmitted, and neutral or selected. Determining the magnitude of each of these traits and forces is an important area of future research.

Future directions

As surveys of bacterial, archaeal and/or viral communities in diverse host tissues continue to expand the knowledge inventory of phylosymbiotic relationships in natural and laboratory conditions, the next major goal in phylosymbiosis research will be to elucidate the causes and effects of this pattern with computational and laboratory approaches. Computational analyses showed that divergence in mammalian gut microbial beta diversity can be accelerated by physical barriers and, inversely, reduced by predator-prey interactions between host species (Moeller et al., 2017). In coral, coevolution or codiversification with members of the microbiome is a potential driver of phylosymbiosis (Pollock et al., 2018). Another future research area is disentangling effects of transmission routes and host vs. environmental influences on microbial community structure and functions not only *in silico* (Yeoh et al., 2017; Pollock et al., 2018), but also *in vivo*. Reductionist approaches using tractable model organism with germ-free rearing and the capacity to transplant microbiomes will be crucial to assessing functionally consequential host-microbiome interactions (Brooks et al., 2016; Parker, Dury & Moczek, 2019). Such experiments have also been successfully coupled with comparative genomics and microarray/transcriptomics methods to identify candidate host determinants affecting symbiont selection (Rawls et al., 2006), specificity (Rawls, Samuel & Gordon, 2004; Kwong et al., 2014), and hybrid lethality (Brucker & Bordenstein, 2013). As metagenomics sequencing depth increases for phylosymbiotic microbiomes and viromes (Leigh et al., 2018), integrative multi-omic approaches can also concurrently screen for microbial genes affecting host functions and fitness. Phenotypic effects of candidate host and microbial genes can be further validated using reverse genetics to determine specific cause-and-effect phylosymbiotic relationships at the genetic level. With the repertoire of computational and experimental methods currently available for the dissection of host-microbe interactions, future research possibilities in the field of phylosymbiosis and real-world applications in species conservation are copious and exciting.

Conclusions

Phylosymbiosis defines a link between host phylogeny and microbial diversity that is quantifiable and applicable across living systems. As research in this area proliferates, a definition and standardized workflow for assessing phylosymbiosis will ultimately produce and substantiate rules and themes. Future cause-and-effect validation of phylosymbiosis will bring us closer to a mechanistic understanding of the evolutionary, genetic, and molecular bases. Just as no mature theory of evolutionary genetics was possible until we understood the mode of inheritance, no mature principle of evolutionary symbiosis seems possible until we understand the mechanisms establishing host-microbiome associations.

Acknowledgements

We thank Andrew Brooks, Brittany Leigh, and Edward van Opstal for critical reviews of previous versions of the manuscript.

References

Anisimova M, Gascuel O. 2006. Approximate likelihood-ratio test for branches: a fast, accurate, ad powerful alternative. *Systematic Biology*, 55:539-552 DOI:10.1080/10635150600755453.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Negel JE, Reeb CA, Saunders NC. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, 18:489-522 DOI:10.1146/annurev.es.18.110187.002421.

Bell TH, El-Din Hassan S, Lauron-Moreau A, Al-Otaibi F, Hijri M, Yergeau E, St-Arnaud M. 2014. Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *The ISME journal*, 8:331-343 DOI:10.1038/ismej.2013.149.

Bevins CL, Salzman NH. 2011. The potter's wheel: the host's role in sculpting its microbiota. *Cellular and molecular life sciences: CMLS*, 68:3675-3685 DOI:10.1007/s00018-011-0830-3.

Bletz MC, Archer H, Harris RN, McKezie VJ, abemananjara FCE, Rakotoarison A, Vences M. 2017. Host ecology rather than host phylogeny drives amphibian skin microbial community structure in the biodiversity hotspot of Madagascar. *Frontiers in microbiology*, 8:1530 DOI:10.3389/fmicb.2017.01530.

Blomberg SP, Garland Jr T. 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *Journal of Evolutionary Biology*, 15:899-910 DOI:10.1046/j.1420-9101.2002.00472.x.

Blomberg SP, Garland JR. T, Ives AR. 2003. Testing for phylogenetic signal in comparative date: behavioral traits are more labile. *Evolution*, 57:717-745 DOI:10.1111/j.0014-3820.2003.tb00285.x.

Bogdanowicz D, Giaro K. 2013. On a matching distance between rooted phylogenetic trees. *International Journal of Applied Mathematics and Computer Science*, 23:669-684 DOI:10.2478/amcs-2013-0050.

Bost A, Martinson VG, Franzenburg S, Adair KL, Albasi A, Wells MT, Douglas AE. 2018. Functional variation in the gut microbiome of wild *Drosophila* populations. *Molecular ecology*, 27:2834-2845 DOI:10.1111/mec.14728.

Bouffaud M, Kyselkova M, Gouesnard B, Grundmann G, Muller D, Moenne-Loccoz Y. 2012. Is diversification history of maize influencing selection of soil bacteria by roots?. *Molecular ecology*, 21:195206 DOI:10.1111/j.1365-294X.2011.05359.x.

Bouffaud M, Poirier M, Muller D, Moënne-Loccoz Y. 2014. Root microbiome relates to plant host evolution in maize and other *Poaceae. Environmental microbiology*, 16:2804-2814 DOI:10.1111/1462-2920.12442.

Bray JR, Curtis JT. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecolgical Monographs*, 27:326-349 DOI:10.2307/1942268.

Bright M, Bulgheresi S. 2010. A complex journey: transmission of microbial symbionts, 8:218-230 DOI:10.1038/nrmicro2262.

Brooks AW, Kohl KD, Brucker RM, van Opstal EJ, Bordenstein SR. 2016. Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. *PLoS biology*, 14:e2000225 DOI:10.1371/journal.pbio.2000225.

Brucker RM, Bordenstein SR. 2013. The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia. Science*, 341:667-669 DOI:10.1126/science.1240659.

Brucker RM, Bordenstein SR. 2012. The roles of host evolutionary relationships (genus: *Nasonia*) and development in structuring microbial communities. *Evolution*, 66:349-362 DOI:10.1111/j.1558-5646.2011.01454.x.

Callahan BJ, McMrdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME journal*, 11:2639-2643 DOI:10.1038/ismej.2017.119.

Chiarello M, Auguet JC, Bettarel Y, Bouvier C, Claverie T, Graham NAJ, Rieuvilleneuve F, Sucre E, Bouvier T, Villeger S. 218. Skin microbiome of coral reef fish is highly variable and driven by host phylogeny and diet. *Microbiome*, 6:147-018-0530-4 DOI:10.1186/s40168-018-0530-4.

Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure.*Ausralian Journal of Ecology*, 18:117-143 DOI:10.1111/j.1442-9993.1993.tb00438.x.

Colman DR, Toolson EC, Takacs-Vesbach C. 2012. Do diet and taxonomy influence insect gut bacterial communities?. *Molecular ecology*, 21:5124-5137 DOI:10.1111/j.1365-294X.2012.05752.x.

Cowles CE, Goodrich-Blair H. 2008. The *Xenorhabdus* nematophila *nilABC* genes confer the ability of *Xenorhabdus* spp. to colonize *Steinernema carpocapsae* nematodes. *Journal of Bacteriology*, 190:4121-4128 DOI:10.1128/JB.00123-08.

Damiani I, Pauly N, Puppo A, Brouquisse R, Boscari A.2016. Reactive oxygen species and nitric oxide control early steps of the legume-*Rhizobium* symbiotic interaction. *Frontiers in plant science*, 7:454 DOI:10.3389/fpls.2016.00454.

Dietrich C, Kohler T, Brune A. 2014. The cockroach origin of the termite gut microbiota patterns in bacterial community structure reflect major evolutionary events. *Applied and Environmental Microbiology*, 80:2261-2269 DOI:10.1128/AEM.04206-13.

Dietz EJ. 1983. Permutation tests for association between two distance matrices. *Systematic zoology*, 32:21-26 DOI:10.2307/2413216.

Easson CG, Thacker RW. 2014. Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges. *Frontiers in microbiology*, 5:532 DOI:10.3389/fmicb.2014.00532.

Erlandson S, Wei X, Savage J, Cavender-Bares J, Peay K. 2018. Soil abiotic variables are more important than *Salicaceae* phylogeny or habitat specialization in determining soil microbial community structure. *Molecular ecology*, 27:2007-2024 DOI:10.1111/mec.14576.

Felsenstein J. 1985a. Confidence limits on phylogenies: an approach using the bootstrap, 39:783-791.

Felsenstein J. 1985b. Phylogenies and the comparative method. *The American Naturalist*, 125:1-15 DOI:10.1086/284325.

Fitzpatrick CR, Copeland J, Wang PW, Guttman DS, Kotanen PM, Johnson MTJ. 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences of the United States of America*, 11:E1157-E1165 DOI:10.1073/pnas.1717617115.

Franzenburg S, Walter J, Kunzel S, Wang J, Baines JF, Bosch TCG, Fraune S. 2013. Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proceedings of the National Academy of Sciences of the United States of America*, 110:E3730 DOI:10.1073/pnas.1304960110.

Fraune S, Bosch TCG. 2007. Long-term maintenance of species-specific bacterial microbiota in the basal metazoan *Hydra*. *Proceedings of the National Academy of Sciences of the United States of America*, 104:13146-13151 DOI:10.1073/pnas.0703375104.

Frese SA, MacKenzie DA, Peterson DA, Schmaltz R, Fangman T, Zhou Y, Zhang C, Benson AK, Cody LA, Mulholland F, Juge N, Walter J. 2013. Molecular characterization of host-specific biofilm formation in a vertebrate gut symbiont. *PLoS Genetics*, 9:e1004057 DOI:10.1371/journal.pgen.1004057.

Funkhouser LJ, Bordenstein SR. 2013. Mom knows best: the universality of maternal microbial transmission. *PLoS biology*, 1:e1001631 DOI:10.1371/journal.pbio.1001631.

Gaulke CA, Arnold HK, Humphreys IR, Kembel SW, O'Dwyer JP, Sharpton TJ. 2018. Ecophylogenetics clarifies the evolutionary association between mammals and their gut microbiota. *mBio*, 9:e01348-18 DOI:10.1128/mBio.01348-18.

Grieneisen LE, Charpentier MJ, Alberts SC, Ran B, Gideon B, Jenny T, Archie EA. 2019. Genes, geology and germs: gut microbiota across a primate hybrid zone are explained by site soil properties, not host species. *Proceedings of the Royal Society B: Biological Sciences*, 286 DOI:10.1098/rspb.2019.0431.

Groussin M, Mazel F, Sanders JG, Smillie CS, Lavergne S, Thuiller W, Alm EJ. 2017. Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nature communications*, 8:14319 DOI:10.1038/ncomms14319.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordij W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59:307-21 DOI:10.1093/sysbio/syq010.

Hird SM, Sanchez C, Carstens BC, Brumfield RT. 2015. Comparative gut microbiota of 59 neotropical bird species. *Frontiers in microbiology*, 6:1403 DOI:10.3389/fmicb.2015.01403.

Ishida TA, Nara K, Hogetsu T. 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. *New Phytologist*, 174:430-440 DOI:10.1111/j.1469-8137.2007.02016.x.

Ives AR, Helmus MR. 2011. Generalized linear mixed models for phylogenetic analyses of community structure. *Ecological Monographs*, 81:511-525 DOI:10.1890/10-1264.1.

Javurkova V, Kreisinger J, Prochazka P, Pozgayova M, Sevcikova K, Brlik V, Adamik P, Heneberg P, Porkert J. 2019. Unveiled feather microcosm: feather microbiota of passerine birds is closely associated with host species identity and bacteriocin-producing bacteria. *The ISME journal* DOI:10.1038/s41396-019-0438-4.

Knowles LL. 2009. Statistical phylogeography. *Annual Review of Ecology, Evolution, and Systematics*, 40:593-612 DOI:10.1146/annurev.ecolsys.38.091206.095702.

Kohl KD, Dearing MD, Bordenstein SR. 2018. Microbial communities exhibit host species distinguishability and phylosymbiosis along the length of the gastrointestinal tract. *Molecular ecology*, 27:1874-1883 DOI:10.1111/mec.14460.

Kohl KD, Varner J, Wilkening JL, Dearing MD. 2018. Gut microbial communities of American pikas (*Ochotona princeps*): Evidence for phylosymbiosis and adaptations to novel diets. *The Journal of animal ecology*, 87:323-330 DOI:10.1111/1365-2656.12692.

Kopylova E, Navas-Molina JA, Mercier C, Xu ZZ, Mahé F, He Y, Zhou H, Rognes T, Caporaso JG, Knight R. 2016. Open-source sequence clustering methods improve the state of the art. *mSystems*, 1:e00003-15 DOI:10.1128/mSystems.00003-15.

Kropáčková L, Těšický M, Albrecht T, Kubovčiak J, Čížková D, Tomášek O, Martin J, Bobek L, Králová T, Procházka P, Kreisinger J. 2017. Codiversification of gastrointestinal microbiota and phylogeny in passerines is not explained by ecological divergence. *Molecular ecology*, 26:5292-5304 DOI:10.1111/mec.14144.

Kwong WK, Engel P, Koch H, Moran NA. 2014. Genomics and host specialization of honey bee and bumble bee gut symbionts. *Proceedings of the National Academy of Sciences of the United States of America*, 111:11509-11514 DOI:10.1073/pnas.1405838111.

Kwong WK, Medina LA, Koch H, Sing KW, Soh EJY, Ascher JS, Jaffe R, Moran NA. 2017. Dynamic microbiome evolution in social bees. *Science advances*, 3:e1600513 DOI:10.1126/sciadv.1600513.

Legendre P, Legendre L. 1998. *Numerical Ecology*. Amsterdam: Elsevier Science B.V.

Leigh B, Bordenstein SR, Brooks AW, Mikaelyan A, Bordenstein SR. 2018. Finer-scale phylosymbiosis: insights from insect viromes. *mSystems*, 3:e00131-18 DOI:10.1128/mSystems.00131-18.

Lemay MA, Martone PT, Keeling PJ, Burt JM, Krumhansl KA, Sanders RD, Wegener Parfrey L. 2018. Sympatric kelp species share a large portion of their surface bacterial communities. *Environmental microbiology*, 20:658-670 DOI:10.1111/1462-2920.13993.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI. 2008. Evolution of mammals and their gut microbes. *Science*, 320:1647-1651 DOI:10.1126/science.1155725.

Liu H, Chen Z, Gao G, Sun C, Li Y, Zhu Y. 2019. Characterization and comparison of gut microbiomes in nine species of parrots in captivity. *Symbiosis*, 78:241 DOI:10.1007/s13199-019-00613-7.

Lozupone C, Knight R. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, 71:8228-8235 DOI:10.1128/AEM.71.12.8228-8235.2005.

Lurgi M, Thomas T, Wemheuer B, Webster NS, Motoya JM. 2019. Modularity and predicted functions of the global sponge-microbiome network. *Nature communications*, 10:992-019-08925-4 DOI:10.1038/s41467-019-08925-4.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer research*, 27:209-220.

Martinson VG, Douglas AE, JaenikeJ. 2017. Community structure of the gut microbiota in sympatric species of wild *Drosophila. Ecology Letters*, 20:629-639 DOI:10.1111/ele.12761.

Mau B, Newton MA. 1997. Phylogenetic inference for binary data on dendograms using Markov Chain Monte Carlo. *Journal of Computational and Graphical Statistics*, 6:122-11 DOI:10.1080/10618600.1997.10474731.

Mazel F, Davis KM, Loudon A, Kwong WK, Groussin M, Parfrey LW. 2018. Is host filtering the main driver of phylosymbiosis across the tree of life?. *mSystems*, 3:e00097-18 DOI:10.1128/mSystems.00097-18.

McArdle BH, Anerson MJ. 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, 82:290-297 DOI: 10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2.

McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Loso T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleber S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ. 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 DOI:10.1073/pnas.1218525110.

Medina M, Sachs JL. 2010. Symbiont genomics, our new tangled bank. *Genomics*, 95:129-137 DOI:10.1016/j.ygeno.2009.12.004.

Michener CD, Sokal RR. 1957. A quantitative approach to a problem in classification. *Evolution*, 11:130-162 DOI:10.2307/2406046.

Miyake S, Ngugi DK, Stingl U. 2016. Phylogenetic diversity, distribution, and cophylogeny of giant bacteria (*Epulopiscium*) with their surgeonfish hosts in the Red Sea. *Frontiers in microbiology*, 7:285 DOI:10.3389/fmicb.2016.00285.

Moeller AH, Caro-Quintero A, Mjungu D, Georgiev AV, Lonsdorf EV, Muller MN, Pusey AE, Peeters M, Hahn BH, Ochman H. 2016. Cospeciation of gut microbiota with hominids. *Science*, 353:380-382 DOI:10.1126/science.aaf3951.

Moeller AH, Suzuki TA, Lin D, Lacey EA, Wasser SK, Nachman MW. 2017. Dispersal limitation promotes the diversification of the mammalian gut microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 114:13768-13773 DOI:10.1073/pnas.1700122114.

Novakova E, Woodhams DC, Rodriguez-Ruano SM, Brucker RM, Leff JW, Maharaj A, Amir A, Knight R, Scott J. 2017. Mosquito microbiome dynamics, a background for prevalence and seasonality of West Nile Virus. *Frontiers in microbiology*, 8:52 DOI:10.3389/fmicb.2017.00526.

Nyholm SV, McFall-Ngai M. 2003. Dominance of *Vibrio fischeri* in secreted mucus outside the light organ of *Euprymna scolopes*: the first site of symbiont specificity. *Applied and Environmental Microbiology*, 69:3932-3937 DOI:10.1128/AEM.69.7.3932-3937.2003.

Ochman H, Worobey M, Kuo CH, Ndjango JB, Peeters M, Hahn BH, Hugenholtz P. 2010. Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS biology*, 8:e1000546 DOI:10.1371/journal.pbio.1000546.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature*, 401:877-884 DOI:10.1038/44766.

Parker ES, Dury GJ, Moczek AP. 2019. Transgenerational developmental effects of species-specific, maternally transmitted microbiota in *Onthophagus* dung beetles. *Ecological Entomology*, 44:274-282 DOI:10.1111/een.12703.

Pattengale ND, Gottlieb EJ, Moret BME. 2007. Efficiently computing the Robinson-Foulds metric. *Journal of Computational Biology*, 14:724-735 DOI:10.1089/cmb.2007.R012.

Peres-Neto P, Jackson DA. 2001. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia*, 129:169-178 DOI:10.1007/s004420100720.

Phillips CD, Phelan G, Dowd SE, McDonough MM, Ferguson AW, Delton Hanson J, Siles L, Ordonez-Garza N, San Francisco M, Baker RJ. 2012. Microbiome analysis among bats describes influences of host phylogeny, life history, physiology and geography. *Molecular ecology*, 21:2617-2627 DOI:10.1111/j.1365-294X.2012.05568.x.

Pollock FJ, McMinds R, Smith S, Bourne DG, Willis BL, Medina M, Thurber RV, Zaneveld JR. 2018. Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nature Communications*, 9:4921 DOI:10.1038/s41467-018-07275-x.

Rawls JF, Mahowald MA, Ley RE, Gordon JI. 2006. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. *Cell*, 127:423-433 DOI:S0092-8674(06)01226-8.

Rawls JF, Samuel BS, Gordon JI. 2004. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 101:4596-4601 DOI:10.1073/pnas.0400706101.

Reveillaud J, Maignien L, Murat Eren A, Huber JA, Apprill A, Sogin ML, Vanreusel A. 2014. Host-specificity among abundant and rare taxa in the sponge microbiome. *The ISME journal*, 8:1198-1209 DOI:10.1038/ismej.2013.227.

Rideout JR, He Y, Navas-Molina JA, Walters WA, Ursell LK, Gibbons SM, Chase J, McDonald D, Gonzalez A, Robbins-Pianka A, Clemente JC, Gilbert JA, Huse SM, Zhou HW, Knight R, Caporaso JG. 2014. Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. *PeerJ*, 2:e545 DOI:10.7717/peerj.545.

Robinson DF, Foulds LR. 1981. Comparison of phylogenetic trees. *Mathematical Biosciences*, 53:131-147 DOI:10.1016/0025-5564(81)90043-2.

Ross AA, Muller KM, Weese JS, Neufeld JD. 2018. Comprehensive skin microbiome analysis reveals the uniqueness of human skin and evidence for phylosymbiosis within the class Mammalia. *Proceedings of the National Academy of Sciences of the United States of America*, 115:E5786-E5795 DOI:10.1073/pnas.1801302115.

Ross AA, Rodrigues Hoffmann A, Neufeld JD. 2019. The skin microbiome of vertebrates. *Microbiome*, 7:79-019-0694-6 DOI:10.1186/s40168-019-0694-6.

Sanders JG, Powell S, Kronauer DJC, Vasconcelos HL, Frederickson ME, Pierce NE. 2014. Stability and phylogenetic correlation in gut microbiota: lessons from ants and apes. *Molecular ecology*, 23:1268-1283 DOI:10.1111/mec.12611.

Sarkar SF, Gordon JS, Martin GB, Guttman DS. 2006. Comparative genomics of host-specific virulence in *Pseudomonas syringae*. *Genetics*, 174:1041-1056 DOI:10.1534/genetics.106.060996.

Schlaeppi K, Dombrowski N, Oter RG, Ver Loren van Themaat E, Schulze-Lefert P. 2014. Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives, *Proceedings of the National Academy of Sciences of the United States of America*, 111:585-592 DOI:10.1073/pnas.1321597111.

Schmitt S, Tsai P, Bell J, Fromont J, Ilan M, Lindquist N, Perez T, Rodrigo A, Schupp PJ, Vacelet J, Webster N, Hentschel U, Taylor MW. 2012. Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *The ISME journal*, 6:564-576 DOI:10.1038/ismej.2011.116.

Schottner S, Hoffmann F, Cardenas P, Rapp HT, Boetius A, Ramette A. 2013. Relationships between host phylogeny, host type and bacterial community diversity in cold-water coral reef sponges. *PloS one*, 8:e55505 DOI:10.1371/journal.pone.0055505.

Smouse PE, Long JC, Sokal RR. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Biology*, 35:627-632 DOI:10.2307/241312.

Sokal RR, Rohlf FJ. 1962. The comparison of dendrograms by objective methods. *Taxon*, 11:33-40 DOI:10.2307/1217208.

Tedersoo L, Mett M, Ishida TA, Bahram M. 2013. Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. *New Phytologist*, 199:822-831 DOI:10.1111/nph.12328.

Theis KR, Dheilly NM, Klassen JL, Brucker RM, Baines JF, Bosch TCG, Cryan JF, Gilbert SF, Goodnight CJ, Lloyd EA, Sapp J, Vandenkoornhuyse P, Zilber-Rosenberg I, Rosenberg E, Bordenstein SR. 2016. Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. *mSystems*, 1:e00028-16 DOI:10.1128/mSystems.00028-16.

Thomas T, Moitinho-Silva L, Lurgi M, Bjork JR, Easson C, Astudillo-Garcia C, Olson JB, Erwin PM, Lopez-Legentil S, Luter H, Chaves-Fonnegra A, Costa R, Schupp PJ, Steindler L, Erpenbeck D, Gilbert J, Knight R, Ackermann G, Victor Lopez J, Taylor MW, Thacker RW, Montoya JM, Hentschel U, Webster NS. 2016. Diversity, structure and convergent evolution of the global sponge microbiome. *Nature communications*, 7:11870 DOI:10.1038/ncomms11870.

Vincent JB, Weiblen GD, May G. 2016. Host associations and beta diversity of fungal endophyte communities in New Guinea rainforest trees. *Molecular ecology*, 25:825-841 DOI:10.1111/mec.13510.

Wehner J, Powell JR, Muller LAH, Caruso T, Veresoglou SD, Hempel S, Rillig MC. 2014. Determinants of root-associated fungal communities within *Asteraceae* in a semi-arid grassland. *Journal of Ecology*, 102:425-436 DOI:10.1111/1365-2745.12197.

Werren JH, Richards S, Desjardins CA, Niehuis O, Gadau J, Colbourne JK, *Nasonia* Genome Working Group, Werren JH, Richards S, Desjardins CA, Niehuis O, Gadau J, Colbourne JK, Beukeboom LW, Desplan C, Elsik CG, Grimmelikhuijzen CJ, Kitts P, Lynch JA, Murphy T, Oliveira DC, Smith CD, van de Zande L, Worley KC, Zdobnov EM, Aerts M, Albert S, Anaya VH, Anzola JM, Barchuk AR, Behura SK, Bera AN, Berenbaum MR, Bertossa RC, Bitondi MM, Bordenstein SR, Bork P, Bornberg-Bauer E, Brunain M, Cazzamali G, Chaboub L, Chacko J, Chavez D, Childers CP, Choi JH, Clark ME, Claudianos C, Clinton RA, Cree AG, Cristino AS, Dang PM, Darby AC, de Graaf DC, Devreese B, Dinh HH, Edwards R, Elango N, Elhaik E, Ermolaeva O, Evans JD, Foret S, Fowler GR, Gerlach D, Gibson JD, Gilbert DG, Graur D, Grunder S, Hagen DE, Han Y, Hauser F, Hultmark D, Hunter HC,4th, Hurst GD, Jhangian SN, Jiang H, Johnson RM, Jones AK, Junier T, Kadowaki T, Kamping A, Kapustin Y, Kechavarzi B, Kim J, Kim J, Kiryutin B, Koevoets T, Kovar CL, Kriventseva EV, Kucharski R, Lee H, Lee SL, Lees K, Lewis LR, Loehlin DW, Logsdon JM,Jr, Lopez JA, Lozado RJ, Maglott D, Maleszka R, Mayampurath A, Mazur DJ, McClure MA, Moore AD, Morgan MB, Muller J, Munoz-Torres MC, Muzny DM, Nazareth LV, Neupert S, Nguyen NB, Nunes FM, Oakeshott JG, Okwuonu GO, Pannebakker BA, Pejaver VR, Peng Z, Pratt SC, Predel R, Pu LL, Ranson H, Raychoudhury R, Rechtsteiner A, Reese JT, Reid JG, Riddle M, Robertson HM, Romero-Severson J, Rosenberg

M, Sackton TB, Sattelle DB, Schluns H, Schmitt T, Schneider M, Schuler A, Schurko AM, Shuker DM, Simoes ZL, Sinha S, Smith Z, Solovyev V, Souvorov A, Springauf A, Stafflinger E, Stage DE, Stanke M, Tanaka Y, Telschow A, Trent C, Vattathil S, Verhulst EC, Viljakainen L, Wanner KW, Waterhouse RM, Whitfield JB, Wilkes TE, Williamson M, Willis JH, Wolschin F, Wyder S, Yamada T, Yi SV, Zecher CN, Zhang L, Gibbs RA. 2010. Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science*, 327:343-348 DOI:10.1126/science.1178028.

Wiley EO, Lieberman BS. 2012. *Phylogenetics: the theory and practice of phylogenetics, second edition*. Hoboken, New Jersey: John Wiley & Sons, Inc.

Wong AC, Chaston JM, Douglas AE. 2013. The inconstant gut microbiota of *Drosophila* species revealed by 16S rRNA gene analysis. *The ISME journal*, 7:1922-1932 DOI:10.1038/ismej.2013.86.

Yeoh YK, Dennis PG, Paungfoo-Lonhienne C, Weber L, Brackin R, Ragan MA, Schmidt S, Hugenholtz P. 2017. Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. *Nature communications*, 8:215-017-00262-8 DOI:10.1038/s41467-017-00262-8.