An Introduction to Phylosymbiosis

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ABSTRACT

Phylosymbiosis was recently formulated to support a hypothesis-driven framework for the characterization of a new, cross-system 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 analyses. Quantitative support for phylosymbiosis is provided by statistical methods evaluating higher microbiome variation between host species than within host species, topological similarities 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, but not universal, in microbiomes of plants and animals from terrestrial and aquatic habitats. Consistent with natural selection shaping phylosymbiosis, microbiome transplant experiments demonstrate reduced host performance and/or fitness upon host-microbiome mismatches. Hybridization can also disrupt phylosymbiotic microbiomes and cause hybrid pathologies. The pervasiveness of phylosymbiosis carries several important implications for advancing knowledge of eco-evolutionary processes that impact host-microbiome interactions and future applications of precision microbiology. Important future steps will be to examine phylosymbiosis beyond bacterial communities, apply evolutionary modeling for an increasingly sophisticated understanding of phylosymbiosis, and 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.

Keywords: symbiosis; phylosymbiosis; microbiome; host-microbe interactions

1. 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 (1,2). Any discussion on the study of host-microbiome interactions must begin with clear definitions. Here, we use the term 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 the 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 research and technological advances have placed microbial symbiosis as a nexus of many subdisciplines within and beyond biology. Scholars now have a suite of tools and increased awareness of the major questions to be answered. These include holistic approaches for the identification of ecological (3) and host (4-7) drivers of microbial taxonomic and functional diversity, as well as reductionist approaches that provide evolutionary and mechanistic insights into transmission processes (8) and phenotypic outcomes of symbiosis (1). 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 rapidly growing research area across diverse systems is the recently defined pattern of phylosymbiosis (9). This review aims to synthesize the topic to provide: (a) a long-lasting definition of the term; (b) a practical guide to test phylosymbiosis; (c) an overview of the prevalence of phylosymbiosis; (d) a

discourse on the biological significance of phylosymbiosis; and (e) future directions in phylosymbiosis research.

2. 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" (9). Phylosymbiosis is first and foremost a significant association between host phylogenetic relationships and host-associated microbial community relationships wherein "phylo" refers to host clade and "symbiosis" refers to the microbial community in or on the host.

Prior to the introduction of the term phylosymbiosis in a study of *Nasonia* parasitoid wasp species (9), early investigations specified relationships between host phylogenies or genetic distances with microbial beta diversity in maize (10), insects (5,11), and mammals (4,12). These studies utilized bacterial 16S rRNA gene sequencing across multiple host species to demonstrate that closely-related species harbor more similar microbiomes than distantly-related species. For example, the sister species *N. giraulti* and *N. longicornis* diverged ~0.4 million years ago and harbor more similar 2nd instar larval, pupal, and adult microbiomes compared to the microbiome in their outgroup species *N. vitripennis* (9,11), which diverged ~1.0 million years ago from the two sister species (13).

Phylosymbiosis may arise from stochastic and/or deterministic evolutionary and ecological forces. For example, stochastic effects include dispersal fluctuations in microbial communities (ecological drift) or shifts in host geographic ranges (14). Phylosymbiosis can also be shaped by ecological (15-17) and dietary (4) niche variation across host lineages. Deterministic effects include microbial

colonization preferences for certain host backgrounds or host regulation in which microbial community composition is influenced by host trait(s) (18). The first study linking phylosymbiotic patterns to the function of specific host genes found that knockdown of the *Hydra* armenin antimicrobial peptide disrupted phylosymbiosis (6) commonly observed in several freshwater and laboratory *Hydra* species (19). Although phylosymbiosis can potentially arise from long-term, intimate host-microbe associations over evolutionary time, such as through host-microbe co-evolution, co-diversification (20), and co-speciation (21), importantly it may also be driven by relatively short-term changes in microbiome composition. Indeed, a recent *Drosophila melanogaster* study revealed the effects of gut microbiome changes on host genomic divergence in as little as five generations (22).

While phylosymbiosis distinguishes itself from non-phylosymbiosis by a significant degree of association between host phylogenetic and microbiome community relationships, it is not universal (**Section 5**) and therefore provides a testable hypothesis. Determining the presence of phylosymbiosis is a first step preceding further investigations into eco-evolutionary mechanisms, such as the nature of species-species associations, selective or neutral forces driving phylosymbiosis, and the (in)consequences of the pattern on host and microbial phenotypes. If phylosymbiosis results from an evolutionary selective pressure, then decreases in host or microbial fitness are expected upon host exposure to microbiomes from different host lineages in an evolutionary-informed manner. Evolutionary selective pressures for phylosymbiosis could drive the spread of host traits that regulate microbiome composition or microbial traits that enhance host colonization. In this general light, we refer to “functional phylosymbiosis” when host and/or microbial phenotypes impact or are impacted by phylosymbiotic associations.

Interspecific microbiome transplant experiments are useful in elucidating functional phylosymbiosis. A large-scale phylosymbiosis investigation spanning 24 species across four laboratory-reared host clades (*Nasonia* wasps, *Drosophila* flies, mosquitoes, and *Peromyscus* deer mice) demonstrated that interspecific transplants of gut microbial communities between *Peromyscus* species decreased dry matter digestibility and increased food intake, while transplants between *Nasonia* species markedly lowered survival to adulthood by nearly half (23). In addition, interspecific microbiomes are more costly to *Nasonia* larval growth and pupation than intraspecific microbiomes (24). Similarly, reciprocal maternal symbiont transplants between two wild, sympatric *Ontophagus* dung beetle species caused developmental delay and elevated mortality in non-native hosts that persisted to the next generation (25). Collectively, phylosymbiotic associations that impact host fitness support the premise that hosts are adapted to their native microbiomes rather than non-native microbiomes, although more studies are needed to confirm these associations and effects in captive and wild host populations.

Hybridization between host species causes host-microbiome mismatches since combining independently-evolved host genotypes in a hybrid may cause a breakdown in either microbial colonization preferences for certain hosts or host control of the microbiome. As demonstrated in *Nasonia* (9), house mice (26), and whitefish (27), hybrids have an altered microbiome relative to the parental microbiome, suggesting a reduced capacity for hosts to regulate their microbiomes and an increased capacity for pathogenic microbes to bloom. These breakdowns in host-microbiome interactions can associate with maladaptive phenotypes in hybrids including immune dysfunction, pathology, inviability, and sterility (9,26) that can reduce interbreeding between

142 species or populations. In *Nasonia*, lethality of hybrids between the older species pair was rescued
143 by germ-free rearing and restored by feeding an inoculum of select, resident gut bacterial species
144 from parents to germ-free hybrids (9). In contrast, hybrids between a younger *Nasonia* species pair
145 did not have an altered microbiome nor suffer functional costs. Collectively, the results from
146 interspecific microbiome transplant experiments and host hybridization studies illustrate that host-
147 microbiome interactions across host species can have important functional consequences that
148 impact evolutionary events within and between species, including wedging host populations into
149 species.
150

151 **3. WHAT IS NOT PHYLOSYMBIOSIS**

152 Having now summarized phylosymbiosis, we briefly accentuate what phylosymbiosis is not for
153 clarity. Early misconceptions associated the term with strictly narrow presumptions such as
154 vertical transmission, mutualistic interactions, or evolutionary splitting from a common ancestor
155 via co-evolution, co-speciation, co-diversification, or co-cladogenesis. Although these processes
156 may lead to phylosymbiosis, the pattern may alternatively arise by antagonistic interactions and/or
157 horizontal microbial transmission whereby interactions between hosts and environmental
158 microbes establish phylosymbiosis anew each generation. As such, phylosymbiosis has varied
159 underpinnings subject to empirical investigation, and it may appear at certain points of time and
160 space rather than be stable throughout a host's entire lifespan.
161

162 **4. A PRACTICAL GUIDE TO STUDYING PHYLOSYMBIOSIS**

163 Investigations of phylosymbiosis vary in approach (qualitative vs quantitative), methodology, and
164 statistical power (18). Thus, a clear, consistent, and robust workflow to detect phylosymbiosis is

desirable for newcomers and experts alike. Here, we suggest a comprehensive workflow for examining phylosymbiosis (**Figure 1**).

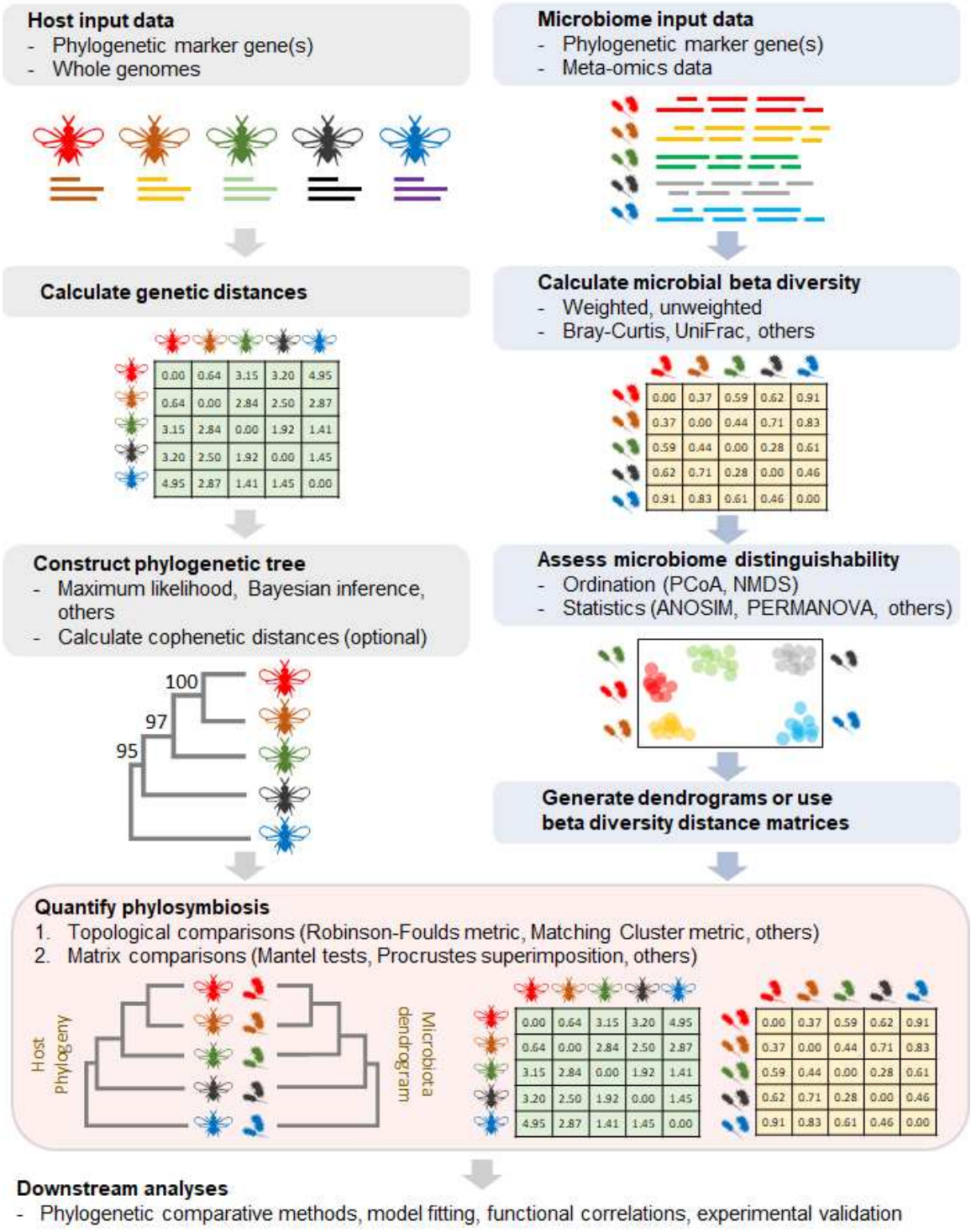

Figure 1. Sequential overview of bioinformatic methods commonly used for phylosymbiosis analyses.

Host taxa and input data. Because phylosymbiosis detection involves the collection of replicated samples across multiple taxa, both optimization of statistical sensitivity (28) and specificity (18) as well as minimization of sequencing batch effects are crucial for differentiating between noise and signal. Although our 2016 study showed that rooted trees with four *Nasonia* species are sufficient to detect phylosymbiosis within the clade (23), we suggest the use of appropriate power and effect size analyses (reviewed in (29) for microbiome data) to determine sufficient replicates and taxa for the optimization of statistical power (28). Sampling multiple individuals per species will help resolve noise from signal in microbial community relationships, but further study is required on how replicates of inter- and intraspecies samples are best utilized in studying phylosymbiosis across host clades that can vary in divergence times. If available, experimental designs of successful phylosymbiosis studies with similar sample types can also be adapted accordingly (30). Previous studies have successfully detected phylosymbiosis in host taxa spanning ~0.3-100 million years of evolutionary history (21,23), and whether longer times since a last common ancestor impacts phylosymbiosis detection requires further study. Nucleotide or amino acid sequence(s) from host species can be used to generate a phylogenetic or phylogenomic tree that is confidently supported at branching nodes with bootstrap (31) or other measures (32) and across several phylogenetic inference methods (e.g., maximum likelihood (33) and Bayesian inference (34)). Because an accurate host phylogenetic topology is essential for evaluating phylosymbiosis, the tree should be free from systematic artifacts such as long branch attraction; polytomies should be resolved in the host phylogeny when possible. As methods used to

reconstruct a host phylogeny from a sequence alignment have been extensively reviewed (35), we will not discuss them further here. With a host evolutionary 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 (36).

Microbiome input data. Phylosymbiosis analysis requires microbial diversity data from each host lineage. Short-read sequencing of microbial phylogenetic marker genes (e.g 16S rRNA gene) is common and economical 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 (37,38). OTU clustering cutoffs reflect genetic distances between taxa over evolutionary time and may affect phylosymbiosis detection (39); such variability has also been observed in practice (reviewed in (18)). Second, reads can be resolved into amplicon sequence variants (ASVs) without clustering, which may offer single-nucleotide resolution, though sequencing error rates should be accounted for (40). For the greatest sensitivity in phylosymbiosis assessment, meta-omics datasets are advantageous because finer-scale taxonomic and functional profiling can be achieved (41). Metagenomic sequence data were used to demonstrate viral phylosymbiosis in *Nasonia* (42) as well as the varying effects of host phylogeny and ecology on the composition and functions of non-human, primate gut microbiomes (43,44).

Microbial beta diversity measures. Microbial beta diversity, which measures dissimilarities in microbial composition and structure across host samples, is conventionally used to measure phylosymbiosis. Binary measures, such as Jaccard distance and Sørensen-Dice distance (45,46),

214 are calculated with OTU presence/absence data. Quantitative descriptors of OTU abundances can
215 also compute beta diversity, including the Bray-Curtis dissimilarity (47) derived from Motyka *et*
216 *al.*'s coefficient (48). It simplifies as 1-[2w/(a+b)], in which w is the sum of the minimum
217 abundances of common species across two host samples, a is the sum of the abundance of all
218 OTUs/species in one sample, and b is the sum of the abundance of all OTUs/species in the other.
219 Phylogeny-based metrics, such as weighted and unweighted unique fraction (UniFrac), use
220 phylogenetic distances between communities (samples) to calculate microbial community
221 differences, necessitating the use of a phylogenetic tree as input (49).
222
223 Because beta diversity metrics reflect different aspects of dissimilarity, the choice of metric is
224 study specific and depends partly on the microbial composition and evolutionary history of the
225 lineages studied. Binary metrics based on presence/absence are more sensitive to variations in rare
226 taxa and were implemented to study host specificity of sponge microbiomes, where rare taxa
227 comprised more than 90% of distinct OTUs (50). Binary metrics may also be sensitive to recent
228 microbial diversification because recently diverged OTUs/ASVs will exert the same effect as
229 OTUs/ASVs with a longer divergence history (39). In contrast, quantitative metrics are more
230 sensitive to variations in abundant taxa. Besides taxonomy-based phylosymbiosis studies (23,51-
231 53), quantitative metrics have also been applied to metagenomics data (42,43). Metrics that
232 consider phylogenetic relationships between OTUs, such as UniFrac distances, (54) are applied in
233 many other phylosymbiosis studies, including bats (55), corals (20), and mammals (4,43).
234
235 Microbiome distinguishability, representative of microbial beta diversity differences between host
236 lineages under evaluation, is a prerequisite for phylosymbiosis (20,23,51-53). Microbiome

237 distinguishability can be visualized from beta diversity data and categorical sample grouping data
238 using ordination plots, such as principle coordinate analysis (PCoA) and non-metric
239 multidimensional scaling (NMDS) plots (56). In addition, microbiome distinguishability can be
240 further evaluated using typically non-parametric multivariable analyses, such as analysis of
241 similarities (ANOSIM) (57) and variants of permutational multivariate analysis of variance
242 (PERMANOVA) (58). Specific pairwise comparisons of intra- and interspecific microbial beta
243 diversity distances can also be performed with an appropriate non-parametric two-sample test (23).
244
245 *Quantifying phylosymbiosis*. The determination of phylosymbiosis relies on evaluating a
246 significant association between host phylogenetic relationships and host-associated microbial
247 community distances. To this end, topological congruency tests directly compare topologies of a
248 host phylogenetic tree and a microbiome dendrogram (23,42,51-53,59). To generate a hierarchical
249 dendrogram, several agglomerative hierarchical clustering methods (reviewed in (56)) can cluster
250 microbial beta diversity distances. The most commonly used method, unweighted pair group
251 method with arithmetic mean (UPGMA), performs pairwise sample clustering from their average
252 dissimilarity values and gives all samples equal weights (60). Compared to linkage clustering
253 approaches, UPGMA prioritizes relationships among groups over individual samples (56). By
254 assigning equal weights to all samples, UPGMA assumes that samples in each group are
255 representative of groups in the larger reference population (56). As such, it may be sensitive to
256 sample sizes and may generate unstable topologies with imbalanced data where some groups are
257 oversampled while some are undersampled. Newer clustering methods, such as the
258 phylogenetically-aware squash clustering method, directly compute distances between samples
259 (rather than differences between beta diversity distances) based on their positions on a

phylogenetic tree (61). In general, the effects of clustering methods on phylosymbiosis detection require further study.

Topological comparison metrics, such as the Robinson-Foulds metric and the more robust and sensitive Matching Cluster metric, are frequently used to detect phylosymbiosis (23,42,51,52,59,62). Robinson-Foulds analyzes the distance between two trees as the smallest number of operations required to convert one topology to the other (63), while Matching Cluster considers congruency at the subtree level and is therefore a more refined evaluation of small topological changes that affect incongruence (64). We strongly recommend the use of both metrics. Statistical significance (p-values) is typically evaluated by determining the probability of 100,000 randomized bifurcating dendrogram topologies yielding equivalent or more congruent phylosymbiotic patterns than the microbiome dendrogram (23); normalized Robinson–Foulds and Matching Cluster scores can be calculated as the number of differences between the two topologies divided by the total possible congruency scores for the two trees, with normalized distances ranging from 0 (complete congruence) to 1 (complete incongruence) (23).

Matrix correlation methods identify phylosymbiosis by comparing the similarities between host-derived and microbial-derived distance matrices. Methods implemented in phylosymbiosis studies (20,21,39,50,65-71) include the Mantel test, which statistically evaluates the linear correlation between all corresponding elements from two independent matrices by permutation (72), and the more powerful Procrustean superimposition approach, which rotates and fits two matrices to minimize their differences association. Partial Mantel tests (73) measuring correlations between two matrices while controlling for the effects of a third variable described in another matrix are

283 also used to evaluate associations between microbial communities and multiple aspects of host
284 characteristics, such as phylogeny, identity, genetic distances, and geographic distances
285 (39,66,67,69).
286
287 Although both topology-based and matrix-based tests are specific and sensitive enough to detect
288 phylosymbiosis in a variety of empirical cases, there are several differences between them.
289 Topological comparison metrics do not use branch length information as there is no *a priori* reason
290 to assume rates of host evolution in each lineage should equal rates of ecological community
291 change in the microbiome. Indeed, rates of microbiome change may be expected to be far more
292 rapid than gradual evolution of host genetic changes. As such, tests of topology without relative
293 branch lengths are conservative relative to matrix correlation methods that directly rely on
294 comparisons of host genetic divergence with microbial community dissimilarity. A simulation
295 analysis suggested that the Mantel test has higher sensitivity and power than the Robinson-Foulds
296 metric when phylosymbiosis is based on the assumption of microbial preferences for a host trait
297 (19). The practical relevance of this conclusion is not clear because phylosymbiosis will arise from
298 reasons other than microbial colonization preferences, such as host preferences, neutral processes,
299 and microbe-microbe interactions. Moreover, the performance between Mantel test and the more
300 sensitive topology-based Matching Cluster distance was not evaluated in this simulation, and such
301 comparisons are likely to yield different insights. Systematic benchmarking of type I and II error
302 rates of phylosymbiosis measurement methods across various possible scenarios will aid
303 experimental design and result interpretation. As such, research opportunities for the development
304 and implementation of improved phylosymbiosis detection methods are ample.
305

306 *Parameter selection*. Phylosymbiosis detection involves selection of various parameters, such as
307 OTU identity cutoff, beta diversity metric, clustering method, and congruency test, each with their
308 strengths and limitations that will vary with study design and questions. Although various
309 parameter combinations can be tested and compared simultaneously (39), in the case when only a
310 few of all possible parameter combinations detect phylosymbiosis, we recommend cautious
311 interpretation of results with respect to the chosen parameters. If available, results should also be
312 compared to those from previous phylosymbiosis studies with similar sample types using the same
313 parameter combinations. Experimental replication is also necessary to confirm phylosymbiosis,
314 especially when it is not consistently detected.
315
316 *Phylogenetic comparative methods.* The effects of phylogenetic signal, defined as "a tendency for
317 related species to resemble each other more than they resemble species drawn at random from the
318 tree" (74), on univariate traits (e.g., microbial alpha diversity) have been examined in parallel with
319 phylosymbiosis studies (66,67). Phylogenetic signal indices like Pagel's λ (75), and Blomberg's
320 *K* (76) are based on a random Brownian model of trait evolution (77), but can also be used with
321 and compared to more complex models that take into account natural selection. Although these
322 methods are less commonly used on multivariable data and have not yet been applied to evaluate
323 phylosymbiosis explicitly, they are promising alternatives for not only examining host
324 phylogenetic signal on microbial beta diversity, but also testing evolutionary models relevant to
325 phylosymbiosis.
326
327 Phylogenetic comparative methods, such as phylogenetic independent contrasts (77) and
328 phylogenetic generalized linear mixed models (pGLMMs) (78), predict the evolutionary

correlation between two or more discrete or continuous traits given a known phylogeny and an evolutionary model. These can also be integrated into phylosymbiosis studies. pGLMMs were recently implemented in coral microbiome (20) and passerine feather microbiome studies (71) 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 predicting ecological interactions, such as predator-prey relationships, mutualism, competition, and habitat filtering, as well as environmental interactions, all of which can affect microbial community structure and therefore 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 resolve linkages between host phylogeny, host functions, microbial diversity, microbial functions, selective forces, and environmental factors.

5. THE PREVALENCE OF PHYLOSYMBIOSIS

A major goal of microbiome science is to find general paradigms and rules, if any, that are comparable across varied systems. In this light, phylosymbiosis is emerging as a bona fide trend because of its frequent recurrence across insect, animal, and plant systems. (**Figure 2**). Phylosymbiosis in insects include viromes of *Nasonia* parasitoid jewel wasps (42) and gut microbiomes of cockroaches, termites (79), lab-reared (23) and wild mosquitoes (59), *Cephalotes* turtle ants (39), and *Apis* social corbiculate bees (69). In *Drosophila* flies, phylosymbiosis patterns are either weakly supported (23) or not detected (80) in lab strains and wild populations.

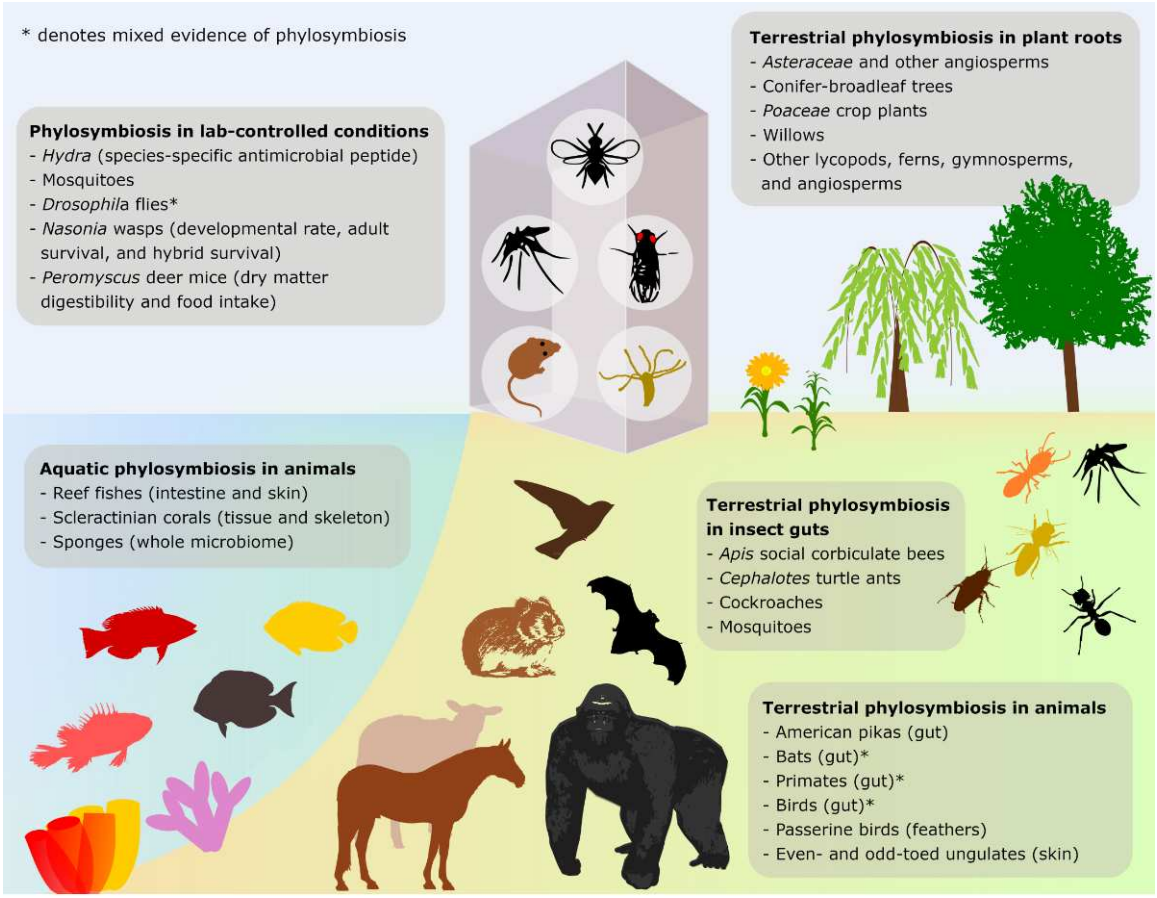

Figure 2. Representative diversity of phylosymbiosis across host species, tissues, habitats, and functions. The * symbol denotes taxa with mixed evidence for phylosymbiosis.

The first phylosymbiosis study on mammalian gut microbiomes (4) demonstrated effects of animal phylogeny and diet on gut microbial community dissimilarity (12,21,23,39,70,81). Studies focusing on gut microbiomes of specific animal groups detected phylosymbiosis in American pikas (51) and *Peromyscus* deer mice (23,52), no phylosymbiosis in western chipmunks (82), and mixed evidence of phylosymbiosis in primates (17,43,44,70), bats (55,83), and birds (62,68,84,85). Besides gut or fecal microbiomes, animal surface microbiomes have also been analyzed for phylosymbiotic associations (86), which for example occur on mammalian skin (53) and passerine feathers (71), but not on amphibian skin (3). A meta-analysis of phylosymbiosis

literature highlighted an increased prevalence of the trend in microbiomes inhabiting internal host compartments in relation to those inhabiting external host compartments (18). 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 is steadily growing (**Figure 2**), spanning global sponge microbiome surveys (67,87,88) and taxon-specific sponge surveys (50,65,66) with mixed results. Two previous studies in sponges showed that the host phylogenetic signal on microbial beta diversity was reduced but still significant when host phylogeny is examined given host identity (66,67). In Australian scleractinian corals, phylosymbiosis was generally observed in tissue and skeleton compartments, but not mucus specimens that are predominantly influenced by the environment (20), suggesting different anatomical impacts on the pattern. Phylosymbiosis and host dietary impacts also occur on the skin microbiomes of 44 fish species from the Western Indian Ocean (89), but do not exist on the surface microbiomes of sympatric kelp species (90).

Phylosymbiosis has been assessed in plants, mainly to distinguish the effects of host phylogeny and soil determinants on microbial beta diversity. A comparative analysis of lycopods, ferns, gymnosperms, and angiosperms across a coastal tropical soil chronosequence indicated host phylogeny is a secondary but statistically significant factor shaping root-associated bacterial community structure, after soil age (15). More taxonomically- and/or spatially-restricted surveys have also revealed phylosymbiosis between rhizobacterial communities and *Poaceae* crop plants (91), endosphere bacterial communities and 30 plant species (92), rhizosphere-associated fungal

388 communities and willows from hydrocarbon-contaminated soils (93), root-associated eumycotan
389 fungal communities and *Asteraceae* flowering plants in a dry grassland (94), ectomycorrhizal
390 fungal communities and conifer-broadleaf forest trees (95), and ectomycorrhizal fungal
391 communities and Estonian Salicaceae willows (96). Contrarily, qualitative incongruency between
392 Brassicaceae host phylogeny and their root microbiomes has been observed (97), whereas non-
393 statistically significant phylosymbiotic correlations have been reported in other plant microbiome
394 studies (16,98).
395
396 **6. SIGNIFICANCE AND FUTURE DIRECTIONS OF PHYLOSYMBIOSIS**
397 Microbiome research will continue to be revolutionized by the multi-omics era, where a deluge of
398 data has enabled unprecedented insights into the extensive taxonomic, genetic, and functional
399 composition of microbial communities and their associated hosts. Such large-scale accumulation
400 of empirical and theoretical findings can potentiate the development of new hypotheses, unifying
401 concepts, and frameworks across diverse host-microbiome systems. Indeed, the recurrence of
402 phylosymbiosis across host systems lends itself to large comparative surveys across kingdoms of
403 life that may uncover taxonomic range restrictions of phylosymbiosis as well as the environmental
404 parameters (e.g., soil and water properties) and ecological interactions (e.g., diet and predator-prey
405 relationships) that determine the boundaries of where and when phylosymbiosis occurs. If the
406 microbiome field will have general trends to test in new systems, phylosymbiosis is well-poised
407 for this circumstance.
408 Phylosymbiosis distinguishes itself from non-phylosymbiosis by characterizing a significant
409 degree of association between host phylogenetic and microbiome community relationships. It is
410 not universal, and thus provides a testable hypothesis, reflects the variation likely to be seen in
411 nature, and is amenable to explanation by mechanisms that require further investigation. The

412 determination of whether phylosymbiosis is present or not is a first step preceding further
413 investigations into mechanistic details, such as the nature of species-species associations and the
414 type(s) of ecological and evolutionary genetic processes underpinning phylosymbiosis. Given the
415 growing evidence for the pattern and increasingly sophisticated tools available to detect
416 phylosymbiosis, phylosymbiosis is relatively clearer and more specific than other terms such as
417 dysbiosis.
418
419 Phylosymbiosis also engenders a holistic view of ecology and evolution in which hosts are
420 communities or holobionts whose microbial members can contribute to genetic and phenotypic
421 variation subject to natural selection. Several questions that have been conventionally overlooked
422 include what are the microbial effects on host allele frequencies? Does host gene flow in natural
423 populations impact microbiome variation and phylosymbiosis? Does phylosymbiosis accelerate or
424 decelerate host speciation? What are the genetic and mechanistic factors that regulate
425 phylosymbiosis, and how do these factors vary across populations or species? Collectively, studies
426 determining the magnitude of ecological, evolutionary, and genetic forces in structuring
427 phylosymbiosis is an important area of future research.
428
429 **CONCLUSIONS**
430 Phylosymbiosis defines a link between host evolutionary relationships and microbial diversity that
431 is quantifiable and applicable across living systems. As research in this area proliferates, a
432 definition, conceptual framework, and workflow for assessing phylosymbiosis will facilitate
433 identification of phylosymbiotic host-microbe interactions. Future cause-and-effect studies of
434 phylosymbiosis will bring a mechanistic understanding of the evolutionary, genetic, and molecular
435 bases. Just as no mature theory of evolutionary genetics was possible until we understood the mode

436 of inheritance, no mature principle of evolutionary ecology for host-associated microbiomes seems
437 possible until we understand the general mechanisms establishing host-microbiome associations.
438

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