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INVITED REVIEW

The Microbiome as a Maternal Effect: A Systematic Review on Vertical Transmission of Microbiota

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Synopsis The microbiome is an interactive and fluctuating community of microbes that colonize and develop across surfaces, including those associated with organismal hosts. A growing number of studies exploring how microbiomes vary in ecologically relevant contexts have recognized the importance of microbiomes in affecting organismal evolution. Thus, identifying the source and mechanism for microbial colonization in a host will provide insight into adaptation and other evolutionary processes. Vertical transmission of microbiota is hypothesized to be a source of variation in offspring phenotypes with important ecological and evolutionary implications. However, the life-history traits that govern vertical transmission are largely unexplored in the ecological literature. To increase research attention to this knowledge gap, we conducted a systematic review to address the following questions: (1) How often is vertical transmission assessed as a contributor to offspring microbiome colonization and development? (2) Do studies have the capacity to address how maternal transmission of microbes affects the offspring phenotype? (3) How do studies vary based on taxonomy and life history of the study organism, as well as the experimental, molecular, and statistical methods employed? Extensive literature searches reveal that many studies examining vertical transmission of microbiomes fail to collect whole microbiome samples from both maternal and offspring sources, particularly for oviparous vertebrates. Additionally, studies should sample functional diversity of microbes to provide a better understanding of mechanisms that influence host phenotypes rather than solely taxonomic variation. An ideal microbiome study incorporates host factors, microbe–microbe interactions, and environmental factors. As evolutionary biologists continue to merge microbiome science and ecology, examining vertical transmission of microbes across taxa can provide inferences on causal links between microbiome variation and phenotypic evolution.

Introduction

Most surfaces, including the internal and external membranes of vertebrates, are colonized by microorganisms that form interactive and fluctuating communities, commonly referred to as the "microbiome". The microbiome is a network of bacteria, archaea, fungi, algae, and protists as well as associated structural elements (e.g., DNA/RNA) and metabolite products that create an environment with distinct bio-physiochemical properties (Berg et al. 2020). Microorganisms are important to the health and function of organ systems in many species (McFall-Ngai et al. 2013) and often exhibit symbiotic relationships with their host (Douglas and Werren 2016). Indeed, deviations away from microbiome community structures that promote health (i.e., dysbiosis) have been shown to reduce host survival (Shreiner et al. 2015). Thus, characterizing microbiomes across organismal regions and lifespans, as well as identifying the source of different microorganisms that make up a microbiome, is critical for understanding how variation in microbiota may influence host phenotypes.

Identifying life-history traits, such as reproductive mode or presence of maternal care, that have the potential to influence vertical transmission (i.e., the passing of symbionts or pathogens from mother to offspring) of

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microbiota may help in guiding research that addresses the process of microbiome colonization and development in offspring. In viviparous species, for example, neonates obtain critical microbial symbionts via vertical transmission from mothers (Funkhouser and Bordenstein 2013; Pelzer et al. 2017; Grieneisen et al. 2021). Vertical transmission of microbes aids in the development of neonates by stimulating metabolic processes (Mueller et al. 2015) and can influence survival through the acquisition of critical bacterial groups (Korpela et al. 2018). In many oviparous organisms (e.g., many reptiles, including birds), eggshells may present a barrier to vertical transmission to the neonate. Nevertheless, eggshells are readily colonized by bacteria from maternal sources (Martínez-García et al. 2016; Li et al. 2022) as well as the nest environment (van Veelen et al. 2018) in ways that have the potential to affect developing embryos and the microbiome of hatchlings. For example, the microbiome associated with eggshells prevents fungal growth and pathogenic bacteria from negatively impacting embryo survival (Bunker et al. 2021). Thus, sampling at this early stage is critical for understanding how microbiomes may be passed from mothers to offspring and how microbial communities change throughout host growth.

The timing and order of colonization by different microbial taxa on their hosts can have important long-term effects on host physiology and fitness (Wallace et al. 2016). Because neonates are at an early ontogenetic stage with little, if any, prior exposure to microbes, the initial microbes that they receive (potentially transmitted from the mother) could have important impacts on the arrival order and relative abundance of bacteria at subsequent periods of life. This is often referred to as a priority effect (Debray et al. 2022), where the first microbes to which an organism is exposed will colonize some internal or external surface (e.g., gastrointestinal tract) and then influence subsequent colonization of other microbial species (Kapourchali and Cresci 2020). Consequently, early microbes transmitted by mothers might have direct effects on offspring or indirect effects at other ontogenetic stages of the host. Hence the ontogenetic patterns of co-evolution between host and microbes could have been critical in organismal adaptation to different environments, which potentially originate from vertical transmission. These long-term influences of the maternal microbiome on offspring may resemble the effects that have been documented for many other maternal factors (Mousseau and Fox 1998).

Maternal influence on offspring microbiomes

Our understanding of how interactions among host genomes, the environment, and associated microbiome(s) determine the phenotype of an organism is still developing and has been an important focus of recent research (Oyserman et al. 2021). The relationship between a host and its microbiome is defined in two ways: first, microbiome community structure, or the taxonomic and functional diversity represented in a microbiome that interact with the host genotype and the environment, is a phenotype of the host (Oyserman et al. 2021). This definition implies that microbiomes differ by taxonomic structure and/or function (e.g., metabolite production), and this phenotype is unique to an individual host. That is, the microbiome is an extended phenotype that is an integral part of the host's phenotype. Second, the microbiome may be defined by its impact on the host phenotype (or defined as "microbiome-associated phenotype") rather than a host-specific characterization of the microbiome; this metric is quantified by physiological changes in the host generated by the microbiome (Oyserman et al. 2021). In this context, the origin and initial colonization of the microbiome can also influence host phenotypes. This variety in definitions of host microbe-interactions can influence our understanding of the role that vertical transmission plays in shaping host phenotypes, which has implications for organismal evolution (Osmanovic et al. 2018).

The second definition described above is relevant to the growing area of research on microbiome-mediated plasticity, which refers to how variation in the structure and composition of microbiota shapes the phenotypes of the host organism (Fischbach 2018; Henry et al. 2021). For example, the immune system of many organisms is shaped by their gut microbiome and must maintain mutualistic homeostasis with microbial communities (Hooper et al. 2012). Examples of microbiome-mediated plasticity are most studied within specific life stages (Blaser et al. 2013; Cresci and Bawden 2015), thus we have a poor understanding of the impacts of ontogenetic changes in the microbiome (Caporaso et al. 2011) as well as the potential for maternal microbiota to directly or indirectly impact offspring phenotypes independent of any vertically transmitted microbes. This area of microbiome research warrants attention (Mueller et al. 2015; Rowe et al. 2020; Comizzoli et al. 2021).

Maternal effects are a form of developmental plasticity in which offspring phenotypes are influenced by the mother at embryonic or neonatal stages (Uller 2008). Maternal effects tend to arise when a mother's environment or phenotype, rather than only her own genetic material, influences the phenotypes of her offspring (Wade 1998; Wolf and Wade 2009). Under this definition, vertical transmission of microbes from mothers to offspring is a clear example of a maternal effect. Yet, the

microbiome is poorly studied in the context of a maternal effect in ecological and evolutionary research. Indeed, only a few studies demonstrate a causal link between maternal microbiomes and offspring phenotypic variation. For example, mice delivered via cesarean section that are colonized with microbiota from the vaginal fluid of mothers had a different microbiome (taxonomic and functional) than those that were not exposed to vaginal fluid (Jašarević et al. 2018). The researchers also exposed mothers and offspring to various stressors and demonstrate that the microbiota that colonized offspring (i.e., maternal origin) decreased body weight and growth of offspring when taxonomic and functional diversity was reduced in the sourced maternal microbiome (Jašarević et al. 2018).

The influence of the maternal microbiome on offspring may vary considerably among taxa, particularly those with different modes of reproduction. Exploration of relationships between host genotype, the environment, and associated microbiome can lead to new hypotheses on how these interactions drive evolutionary change (Abdul-Aziz et al. 2016; Osmanovic et al. 2018; Henry et al. 2021; Oyserman et al. 2021). Variation in reproductive strategies and mode (i.e., time point of vertical transmission) is suggested to critically influence microbial colonization in neonates (Comizzoli et al. 2021). For example, microbiota transfer to offspring in viviparous species is more direct (e.g., there is no eggshell barrier) than in oviparous species. Indeed, the eggshell and yolk might hinder maternal transfer of microbiota to the embryo or neonate. In addition, the time gap between oviposition and hatching in oviparous species means that mothers can be absent at time of hatching. In these cases, much of the maternally transferred microbes might later get swamped by microbes in the nest, and maternal signature could decline with time. However, to describe these causal mechanisms, studies need to measure the taxonomic and functional diversity of microbes as well as the phenotypic traits of hosts under different environmental conditions.

Mode of vertical transmission

Studies that examine vertical transmission of the microbiome from parents to offspring typically assess the timing and mechanisms of microbial transfer. Microbial transfer can occur before fertilization, which is likely common in species with external fertilization where microbes originating from the mother colonize the external oocyte. Eggs obtained from female Sydney rock oysters (*Saccostrea glomerata*), for example, share over 20% of bacteria with their day-old larvae, and these colonizing microbes may have shaped the development of the larval oyster microbiome through colonization from maternal sources (Unzueta-Martínez et al. 2022). Importantly, ontogenetic stages had distinct microbiomes but harbored a core microbiome, overlapping with maternal sources, that persisted throughout development and were distinguishable from the water column (Unzueta-Martínez et al. 2022).

A second time point when maternal microbes may be acquired by offspring is during embryonic development (i.e., following fertilization). Bacterial phyla have been shown to be present inside eggs from four different bird species (*Poecile atricapillus*, *Sialia sialis*, *Passer domesticus*, and *Tachycineta bicolor*) and eastern fence lizards (*Sceloporus undulatus*) shortly after oviposition (Trevelline et al. 2018), and this pattern likely occurs in other taxa. Importantly, comparisons of bacterial communities inside eggs with substrate from lizard nests reveal that microbes present within the egg differ in abundance and taxonomic diversity than those from the surrounding environment (Trevelline et al. 2018). These findings strongly suggest that maternally derived microbes contribute to the diversity within eggs. However, whether microbes are present within the yolk and/or developing embryos across oviparous taxa and when they are transmitted (i.e., prior to vitellogeneis; Nyholm 2020) remains largely understudied, particularly for animals with no agricultural importance. Moreover, some studies characterize the microbial content in yolk of poultry eggs (Lee et al. 2019; Ding et al. 2022; Jin et al. 2022), but little is explored in free-ranging wild animals. Notably, embryonic development occurs externally to mothers in oviparous species and continues following oviposition. This distinction may guide future questions into how and when vertically transmitted microbes colonize offspring in species with varying reproductive modes.

A third time point when maternal microbes may be acquired by offspring is during oviposition or birth. For example, the microbiome of newborn humans differs between babies from vaginal births versus those from a cesarean sections due to bacteria in vaginal fluid (Tamburini et al. 2016; Mueller et al. 2017). This pattern likely occurs in many other mammals (Kimura et al. 2020; Hummel et al. 2021; Owens et al. 2021), but studies that examine these patterns in wildlife are limited. Nevertheless, in populations of Antarctic fur seals (*Arctocephalus gazella*), mother-offspring pairs shared a greater overlap in bacterial groups or operational taxonomic units (OTUs) than their non-related counterparts and those from different regions (Grosser et al. 2019). In oviparous vertebrates (e.g., reptiles including birds), eggs may be inoculated with bacteria as they pass through the cloaca at oviposition (Lee et al. 2019;

Trudeau et al. 2020). The degree to which the eggshell microbes later colonize embryos or newly hatched offspring is poorly studied but is likely to occur most frequently in viviparous species (e.g., many squamates) where the eggshell does not present a barrier to direct transmission to the neonate.

A fourth time point when maternal microbes may be acquired by offspring is following oviposition or birth, and offspring are actively influenced by mothers (e.g., parental care). Female burying beetles (*Nicrophorus vespilloides*) regulate carcass microbiota by applying anal and oral secretions to carcasses that larvae migrate to and feed on, meaning the carcass functions as both larval nutrition and transmission of microbial communities from mother to offspring (Shukla et al. 2018). Many examples exist in mammals, which often exhibit parental care and can mechanically transfer microbiota through lactation or touch (Yeoman et al. 2018; Klein-Jöbstl et al. 2019; Owens et al. 2021). Additionally, eggshells and nestlings of various bird species have been shown to share microbiota with parental counterparts through nest attendance (Martínez-García et al. 2016; Martin-Vivaldi et al. 2018; van Veelen et al. 2018; Chen et al. 2020).

Lastly, vertical transmission may also occur from adults to offspring during early neonatal stages but may not involve direct transfer from mothers to their own offspring. This is illustrated by hatchling green iguanas (*Iguana iguana*), where juveniles behaviorally acquire microbes that are necessary for hindgut fermentation of plant matter they consume (Troyer 1982, 1984). Upon hatching, young iguanas will not only feed on nest site material but will also actively feed on adult feces (which may or may not be solely from maternal sources; Troyer 1982, 1984). This behavior is critical to the development of gut microbiota that facilitate digestive efficiency in these herbivorous lizards (Troyer 1982, 1984). Similar types of adult-to-juvenile transfer of microbes may also be present in highly social species (e.g., many mammals and hymenopteran insects [Ezenwa et al. 2012; Tung et al. 2015; Sarkar et al. 2020]). The diversity of potential mechanisms for vertical transmission that are described above highlight the complexity of this field and challenges that researchers face in better understanding variation in life history traits that are shaped by interactions between hosts, their associated microbiomes, and the environment.

Overall, maternal transfer of microbiota to offspring is well accepted, but the mechanisms and timing of transmission, as well as their implications for evolutionary adaptation in taxa with different life histories are poorly understood. Moreover, in addition to its potential role in ecology and evolutionary biology, vertical transmission of microbes is important for informing efforts in wildlife conservation (Trevelline et al. 2019). One factor that contributes to our poor understanding of the microbiome as a maternal effect is the lack of a comprehensive synthesis on this topic, which would bring attention to knowledge gaps and help guide future research. Thus, the goal of this review is to identify trends in the literature on vertical transmission of microbes, review key findings, identify knowledge gaps, provide suggestions for future research, and discuss the ecological and evolutionary implications of vertically transmitted microbiomes as a maternal effect. We argue that microbiota acquired from mothers should be considered a maternal effect. Examining the relationship between microbiota shared between maternal and offspring sources through a multi-faceted approach will provide a robust foundation for future microbiome studies seeking to address knowledge gaps in evolutionary biology. Here, we provide a systematic review, and where possible, conduct analyses to address the following questions:

1) How often is vertical transmission assessed as a contributor to offspring microbiome colonization and development?
2) Do studies have the capacity to address how maternal transmission of microbes affects the offspring phenotype?
3) How do studies vary based on taxonomy and life history of the study organism, as well as the experimental, molecular, and statistical methods employed?

By addressing these three broad questions with a systematic review and focusing on non-human studies, we hope this paper provides a useful guide that stimulates research on vertical transmission of the microbiome in a wide range of taxa.

Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (Page et al. 2021).

Study identification

An extensive literature search was performed using three databases, including Web of Science (Core Collection), BIOSIS Citation Index, and Zoological Record, on 22 November 2022, using the comprehensive keywords: ("microbiome*" OR "microbe*" OR "microbial") AND ("maternal*" OR "mother" OR "vertical transmission") AND ("offspring" OR "egg*" OR "neonat*") NOT ("human" or "child*" OR "infant"). In each of these databases, these keywords were all searched under "Topic". This setting searches titles, abstracts, author keywords, and KeyWord Plus. We lim-

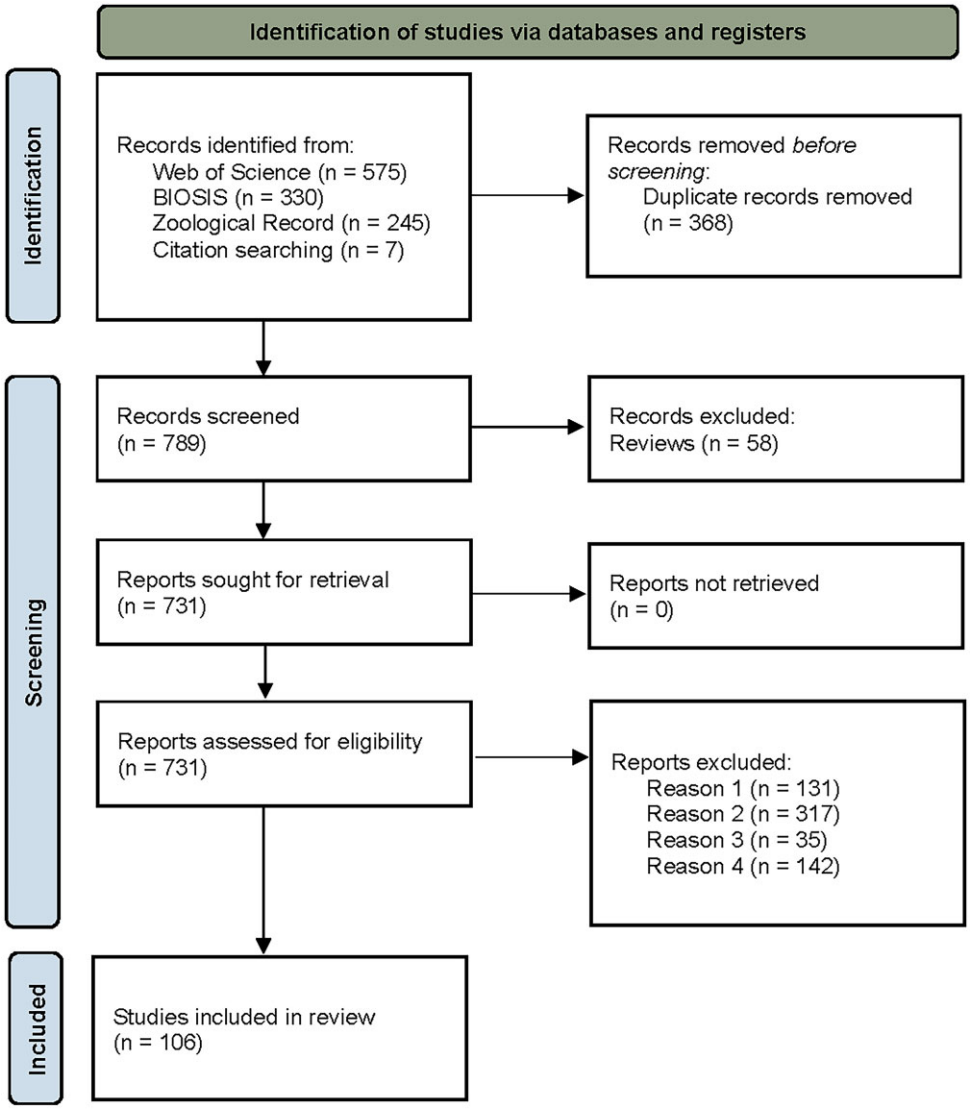

Fig. 1 Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) flow diagram of the study selection process for this systematic review on vertical transmission of microbes across taxa. Studies were excluded from analysis if they did not meet the following criteria: (1) utilized only plants, single-celled organisms, or humans to address research question(s), (2) did not sample whole microbiome, (3) utilized only metatranscriptomic and/or metaproteomic sequencing and/or did not sequence microbiota (i.e., culture-dependent methods), or (4) did not sample microbiota from both maternal and offspring sources.

ited our search to non-human animals to address microbiome sampling among wildlife populations.

Documents were filtered to only scientific articles written in English and published between 2010 and 2022, which is when high-throughput sequencing methods were standardized (Rocca et al. 2019). This initial search resulted in 575 studies on the Web of Science (Core Collection), 330 studies on BIOSIS Citation Index, and 245 studies on Zoological Record for a total of 1150 studies. Of these, 368 were duplicates and were thus removed from the dataset. Seven additional articles were identified through citation searching for a total of 789 articles screened. However, 58 of these were reviews or papers that focused on developing statistical models only rather than conducting an empirical study. If a study conducted a meta-analysis and met the following inclusion criteria, then it was included in downstream analyses. Thus, a total of 731 articles were filtered using the criteria below (Fig. 1).

Inclusion and exclusion criteria

Articles were screened from November 2022 to February 2023 and were included in the systematic review if they: (1) examined invertebrates and/or vertebrates that reproduce sexually and/or asexually (except for humans), (2) collected whole microbiome samples (i.e., all or most microbiota present), (3) identified micro-

biota present (i.e., using 16S rRNA gene-sequencing, shotgun-metagenomic sequencing), and (4) sampled and reported findings, at a minimum, of both microbial communities derived from mothers and their offspring to assess vertical transmission of microbiota (as defined by transfer or acquisition of microbiota from maternal, rather than environmental, sources).

The exclusion criteria used for filtering studies were (1) utilized only plants, single-celled organisms or humans to address research question(s), (2) did not sample the microbiome (i.e., only examined a single species or set of microbes), (3) utilized only metatranscriptomic and/or metaproteomic sequencing and/or did not sequence microbiota (i.e., culture-dependent methods), or (4) did not sample microbiota from both maternal and offspring sources and/or only assessed horizontal transmission and/or environmental input (e.g., soil, diet other than maternal milk) on microbial colonization. If a study only sampled from the amniotic fluid of a developing fetus, this was not considered as maternal sampling in viviparous organisms because the amniotic fluid at gestation is mostly comprised of fetal urine (Gilbert and Brace 1993). Additionally, studies that only collected from the placenta (in terms of an "offspring" sample) were excluded. However, in oviparous reptiles, the yolk and surrounding egg compartments besides the developing embryo could be considered as "maternal" sources as these are deposited by the mother during vitellogenesis (Schwabl 1993).

Study selection

Articles were initially screened based on titles and abstracts to determine fit within our inclusion criteria. A second screening of the full scientific article was conducted following this to determine fit within our inclusion criteria. If the article did not meet the inclusion criteria, it was given a number correlating to which exclusion criteria it fit with first (e.g., examined microbiota transfer in plants, thus was excluded due to exclusion criteria 1).

Data extraction and analysis

A total of 106 studies were used for downstream analyses (see below). Data were manually extracted from text when reported and checked for accuracy. Information on study taxa, such as taxonomic classification and reproductive mode (e.g., oviparous, viviparous), was extracted from articles. Study design metrics, such as sequencing method (e.g., 16S region, metagenomic), experimental vs. observational designs, whether functional diversity of microbes was explored (e.g., through metatranscriptomics), whether phenotypic variables of hosts were measured (e.g., mass, growth rates, immune parameters), and sample region (e.g., cloacal, egg, fecal) from maternal and offspring sources, were also recorded. If a study did not use 16S rRNA gene sequencing and/or metagenomic sequencing, then they were labeled as not meeting inclusion criteria 3 and removed from analyses. For functional predictions, if the authors used metagenomic data (e.g., shotgun metagenomic sequencing of microbiota), metatranscriptomic data (e.g., RNA-seq), or compared targeted sequencing to known databases (e.g., National Center for Biotechnology Information [NCBI], BugBase [Ward et al. 2017]), they were recorded as exploring microbial functional diversity in the article. If a study collected cecal samples without specifying if samples were tissue or cecal contents, then the sample region was labeled as "gut".

Denotation of statistical methods used in articles was examined to quantify variation in analyses across microbiome studies. We recorded whether a study analyzed amplicon sequence variants (ASVs) or OTUs, used Shannon's diversity index (Shannon 1948), Bray–Curtis dissimilarity index (Bray and Curtis 1957), or weighted Unifrac distances (Lozupone et al. 2011), and reported results using a principal coordinate analysis (PCoA; Lozupone et al. 2011) and permutational multivariate analysis of variance (PERMANOVA; Anderson 2017). These metrics were selected because they are commonly used as alpha and beta diversity metrics in microbiome studies (Allali et al. 2017; Kim et al. 2017; Berg et al. 2020). Lastly, if the authors reported any overlap between maternal and offspring sources, such as relative abundances of OTUS/ASVs between mother and offspring pairs (most often reported as bar graphs), then they were considered to have reported "overlap". A study that did not report "overlap" is one that (1) did not analyze or visually display abundances of microbial taxa (i.e., absolute or relative abundance) and/or (2) did not describe variations in maternal/offspring taxa (most commonly in studies that did not solely focus on vertical transmission).

Using these data, we quantified publication rates over time as well as variation in study factors between and within articles using RStudio software version 2022.12.0 (R Development Core Team 2018). Figures were generated using ggplot2 (Wickham 2011).

Results

From 2010 to 2022, 782 articles were identified from a literature search of the Web of Science (Core Collection, BIOSIS, and Zoological Record). An additional seven articles were identified from citations in other articles resulting in a total of 789 papers screened. The number of publications over this period increased by 7.3% on average each year (Fig. 2A).

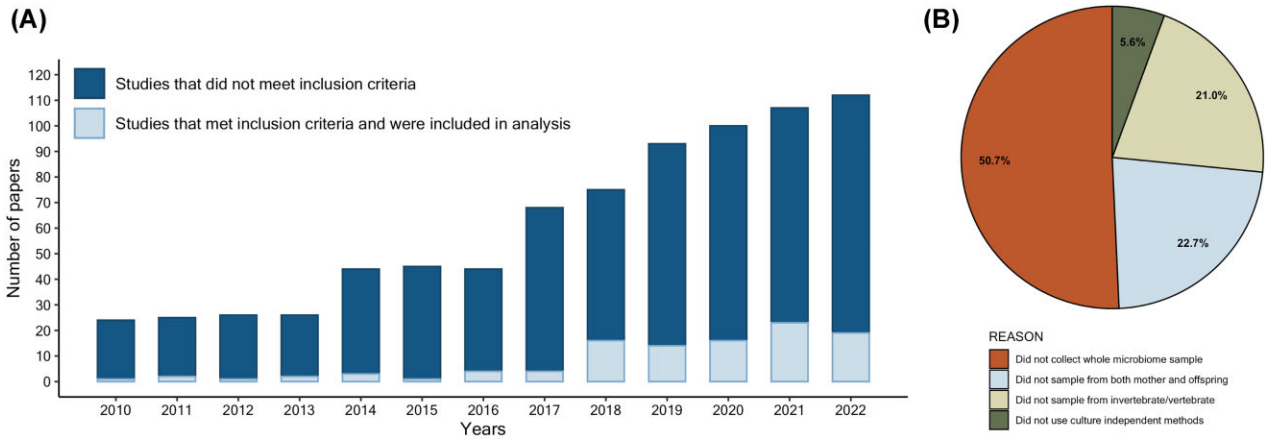

Fig. 2 (A) Increase in the number of publications over time. Dark blue bars denote publications that did not fit inclusion criteria, while light blue bars are studies that met all inclusion criteria and were included in downstream analyses. (B) Percentages of publications that failed to meet inclusion criteria based on the listed reasons. For studies that did not collect whole microbiome samples (orange, 50.7%), $n = 317$. For studies that did not sample from both mother and offspring (light blue, 22.7%), $n = 142$. For studies that did not sample from invertebrate/vertebrate (tan, 21.0%), $n = 131$. For studies that did not use culture-independent methods or only used metabolomics/transcriptomics (green, 5.6%), $n = 35$.

Of the 789 articles, 58 were removed because they did not collect empirical data (i.e., were reviews or focused on developing statistical models). Thus, a total of 731 were filtered using the inclusion criteria. A total of 625 studies failed to meet the inclusion criteria (85.5% of total studies; see Methods above). Of these, 131 papers (21.0% of total) utilized only plants, single-celled organisms, or humans to address research questions, thus failing to meet inclusion criteria 1 (Fig. 2B). If a study used an invertebrate or vertebrate group but did not sample the microbiome (i.e., only examined a single species or set of microbes), then they were excluded based on inclusion criteria 2. Of these, 317 papers were excluded (50.7% of total) based on inclusion criteria 2 (Fig. 2B). If an article met the first two criteria, but did not sequence the whole microbiome (e.g., utilized only qPCR), then they were excluded based on inclusion criteria 3. Of these, 35 papers were excluded (5.6% of total) based on inclusion criteria 3 (Fig. 2B). Lastly, if an article met all three of these original criteria but did not sample microbiota from both maternal and offspring sources, then they were excluded based on inclusion criteria 4. Of these, 142 papers were excluded (22.7% of total) based on inclusion criteria 4 (Fig. 2B).

Of the original 731 articles filtered, 106 studies met all inclusion criteria and were included in the systematic review (14.5% of total studies). Of these 106, about a quarter (27.3%, $n = 29$) used invertebrates for their study. The vertebrates used in studies spanned across major classes, including 62.3% mammals ($n = 66$), 2.8% fish ($n = 3$), 5.7% birds ($n = 6$), and 1.9% non-avian reptiles ($n = 2$; Fig. 3A). No studies on amphibians in the current literature met all the inclusion criteria. Most (64.2%, $n = 68$) of these study organisms are viviparous (i.e., live-bearing, largely because most studies were on mammals), with only 23.6% ($n = 25$) of studies using organisms representing oviparous (egg-laying) species and 12.3% ($n = 13$) that used other modes for reproduction such as broadcast spawning, fragmentation, or other forms of asexual reproduction (Fig. 3A).

Less than a half of the 106 studies (46.2%, $n = 49$) measured variables related to offspring phenotype and fewer (40.6%, $n = 43$) measured microbiome functional diversity. Most of the 106 studies (66.0%, $n = 70$), however, used an experimental approach to address research questions (Fig. 3A). The most common anatomical regions that were sampled for microbes from mothers were fecal contents (37.7%, $n = 40$), followed by a combination of tissue types (32.1% of studies, $n = 34$; e.g., feces, gut). Like that of mothers, the most common anatomical regions that were sampled for microbes from offspring were from fecal contents (35.0%, $n = 37$), followed by a combination of tissue types (24.5% of studies, $n = 26$; e.g., feces, gut). Additionally, the majority of studies used 16S rRNA gene-sequencing on the Illumina Miseq (Caporaso et al. 2012) for microbiome analyses (79.2%, $n = 84$).

A substantial amount of variation exists in bioinformatics pipelines for microbiome data, where 61.3% ($n = 65$) of studies used OTUs while the remaining 38.7% ($n = 41$) either used ASVs or did not report their variant method. Additionally, 68.9% ($n = 73$) reported Shannon's diversity index as an alpha diversity metric, while others used Chao1 (Chao 1984) or Faith's phylogenetic index (Faith 1992). Bray–Curtis indices and weighted UniFrac distances for beta-diversity met-

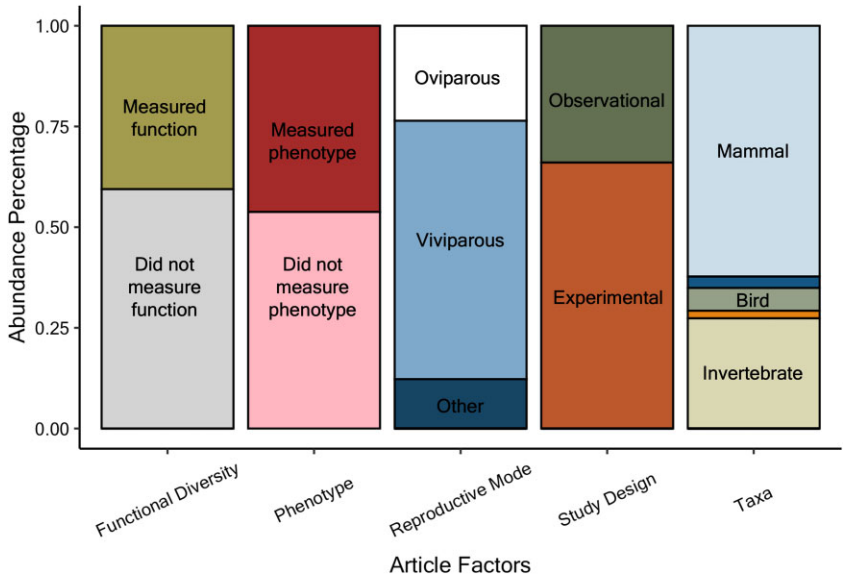

Fig. 3 Percent abundance for article factors (left to right): whether or not a study measured microbiome functional diversity, whether or not a study measured phenotypes of offspring, whether the study organism was oviparous (egg-laying) or viviparous (live-bearing), whether or not the study design was observational or experimental, and taxonomic classification of the organism used in a study (dark blue bar [second from top] = fish, light orange bar [second from bottom] = reptile).

rics were used in 48.1% ($n = 51$) and 37.7% ($n = 40$) of the studies, respectively. To ordinate data for beta-diversity analyses, 56.6% ($n = 60$) of studies used a PCoA while others used a principal component analysis or non-metric multidimensional scaling analysis. To quantify beta-diversity analyses, 49.1% ($n = 52$) of studies used a PERMANOVA while others used an analysis of similarities or an analysis of compositions. Lastly, 85.8% ($n = 91$) of studies reported any sort of overlap between maternal and offspring sources.

Discussion

Vertical transmission of microbes from mothers to offspring likely plays an important role in generating variation in phenotypes and fitness among individuals. While many studies demonstrate convincing evidence for this maternal effect (Mueller et al. 2015; Kapourchali and Cresci 2020), its prevalence and consequences in natural systems are still poorly understood. The main goals of our study are to identify reasons why knowledge in this field is lacking and to provide recommendations for future research that will fill in knowledge gaps. We address these goals with a systematic review of the literature aimed at answering three primary questions. First, how often is vertical transmission assessed as a contributor to offspring microbiome colonization and development across ecological literature? We observed a steady annual increase in the number of publications on vertical transmission over time. This positive trend may reflect reductions in sequencing costs associated with microbiome studies within the last decade (Bharti and Grimm 2021) and will likely keep the field moving in a productive direction. Indeed, even if future studies fall outside of our inclusion criteria, such work will likely stimulate future research that provides a better understanding of vertical transmission. Of the papers that did not meet the inclusion criteria, few (5.6%) were excluded for absence of culture-independent methods. Next-generation sequencing can provide a much more accurate representation of microbiomes compared to culture-dependent methods (Rani et al. 2009). However, 16S rRNA gene-sequencing largely overestimates prokaryotes and is unable to account for eukaryotic microbes present compared to whole genome sequencing (Sun et al. 2013). Many identified papers (21.0%), even with search terms excluding humans, still resulted in articles including human studies and a few that focused on plants. The goal of this study was to assess ecological literature to provide a robust understanding of knowledge gaps related to non-human animals (Trevelline et al. 2019). The second-largest group excluded from the review (22.7%) were articles that did not sample mother/offspring pairs. Maternal microbiota sampling is important for (1) comparison to offspring microbiota, (2) confirming offspring microbiota were not environmentally acquired, and (3) comparative analyses across literature. The largest majority failed the inclusion criteria for not collecting whole microbiome samples; many articles focused on a single, vertically transmitted symbiont. This is useful because individual symbionts can

be easily traced through ontogeny; however, whole microbiome sampling is needed to understand interactions between host genomes, microbe–microbe interactions, and the external environment (Douglas and Werren 2016).

Of the 106 papers that met our criteria, the majority (90%) were from mammalian and invertebrate sources. The mammalian studies included a majority of laboratory (e.g., mice and rats) and agricultural animals (e.g., pigs and cattle), while invertebrate taxa spanned across insects, sponges, and corals. Fish, reptiles, and birds made up very few papers identified, and not one study in amphibians met all the criteria. Of the taxa represented, 64.2% are viviparous, signifying that the mode and consequences of vertical transmission for oviparous vertebrates and those with other modes of reproduction (i.e., asexual) are highly understudied. Because most research focused on mammals, it is not surprising that most studies were on viviparous species. However, given the repeated origins of viviparity in reptiles (Pyron and Burbrink 2014), these organisms might be good models to explore the implications of maternal transfer of microbiota in the evolution of viviparity.

Second, we asked if studies have the capacity to address how maternal transmission of microbes affects the offspring phenotype? We showed that offspring phenotypes were rarely measured in most studies (46.2% of studies). Those studies that did measure phenotypic variables demonstrated that there is an association between host phenotype and microbiota taxonomic and/or functional diversity (a few include: Bansal et al. 2011; Ma et al. 2014; Bunker et al. 2021). Studies must address causation rather than just correlation between microbiome(s) and host phenotypes (Hanage 2014; Klassen 2018). For microbiome studies, this is often examined through up- or down-regulated gene pathways or metabolomics. However, only 40.6% of studies examined functional diversity in microbiomes. One reason behind this may be that many microbiome studies are designed as observational rather than experimental, which would not allow for delimiting the interaction between microbial diversity from mother/offspring sources and offspring phenotypes. Experimental approaches that manipulate maternal microbiomes and examine those treatment effects on offspring will be the most robust for addressing question #2. The current gaps in the literature hinder a comprehensive understanding of the effects of microbiomes on organismal evolution for at least a couple critical reasons. First, development and colonization of microbiomes in offspring are likely influenced by host life-history traits, but many microbiome studies lack phenotypic measurements of hosts (Klassen 2018). Second, methods vary among studies, which in turn, can affect results and interpretations. Therefore, research would benefit from standardizing sampling methods to allow for comparison of vertical transmission of microbiota across taxa (see below).

The structure of the host's microbiome is influenced by numerous factors, including its own genotype and gene expression, as well as general health, age, and life history traits (Abdul-Aziz et al. 2016). Additionally, interactions between microbiota themselves, including any functional hubs, critical groups, and/or metabolites produced by microbes, can influence microbiome community structure (Mueller et al. 2015; Tamburini et al. 2016). Lastly, environmental factors that both influence host factors and microbe–microbe interactions can shape functional and taxonomic diversity of microbes (Bernardo-Cravo et al. 2020; Oyserman et al. 2021). Indeed, microbial effects shaped by environmental variation can favor microbial transmission (Bruijning et al. 2022). These environmental factors incorporate climate, prey availability, and other important host and environmental factors, such as maternal effects. Altogether, a multi-faceted approach to microbiome studies will provide a robust foundation for future microbiome studies seeking to address evolutionary knowledge gaps (Fig. 4; adapted from Dastogeer et al. 2020).

Conducting a robust and meaningful microbiome meta-analysis

Our third research question sought to address differences across studies based on taxonomy and life history of the study organism, as well as the experimental, molecular, and statistical methods employed. Our literature survey revealed substantial variation in study designs and microbiome bioinformatic pipelines regarding the microbiome as a maternal effect. This variation results in many inconsistencies among studies, and therefore comparison of results from different studies are sometimes difficult to interpret (Debelius et al. 2016). Consequently, this variation may hinder a meaningful meta-analytic approach in identifying overarching patterns of vertical transfer of microbiota. First, some microbiome meta-analyses directly analyze microbial genome sequences themselves. However, this route is very time-consuming and does not incorporate mechanistic or phenotypic variables of hosts. Without re-analyzing all microbial genomic sequences, studies and reviews (including those seeking to perform meta-analyses) would benefit from a streamlined pipeline for microbiome results. Indeed, simply using OTUs vs. ASVs can influence microbiome results (Caruso et al. 2019). This also includes incorporating and report-

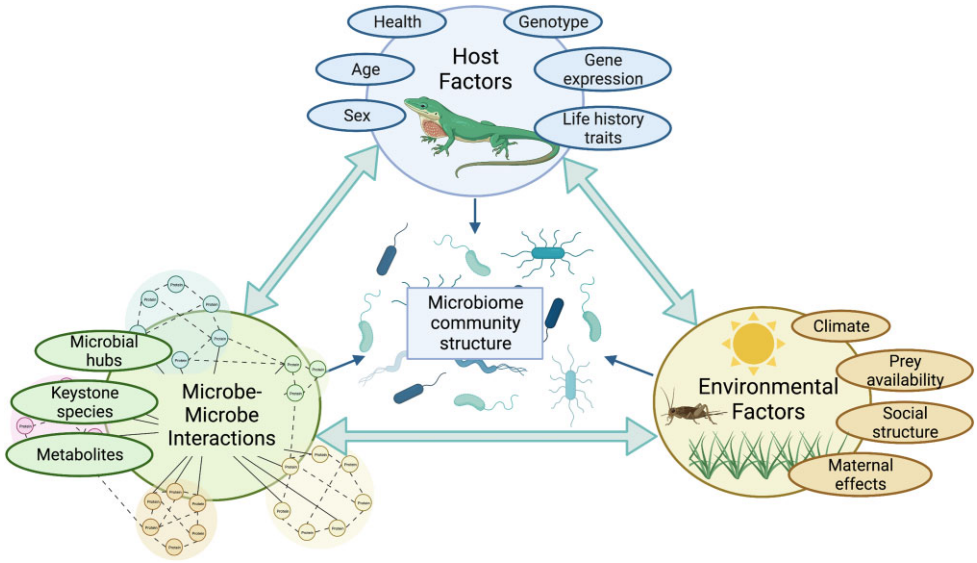

Fig. 4 An ideal microbiome study is one that incorporates host factors, microbe–microbe interactions, and environmental variables. Adapted from Dastogeer et al. (2020). Created with Biorender.com.

ing multiple common alpha and beta diversity metrics (such as Shannon's diversity and/or weighted UniFrac distances) from both mother and offspring sources. Reporting multiple variables for both alpha and beta diversity would allow for comparisons across studies. Of the papers analyzed in this study, there were too few with overlapping analyses (i.e., without having to reanalyze all sequencing data) to conduct a strong and meaningful meta-analysis. Second, sharing bioinformatics pipelines (including all code and scripts) on a public platform for others to access will benefit researchers, facilitate communication among research groups, and minimize redundancy in research. A common repository will help to streamline this process and would be very useful in advancing the field. Third, experimental designs that consistently manipulate experimental variables (i.e., presence/absence of maternal microbes) across studies would allow for comparisons between studies. For vertical transmission research, this could accomplished by removing maternal input or measuring both maternal and offspring sources over time.

Programs and resources exist to streamline microbiome pipelines into a common form. A common tool used for examining shared factors between two sources (e.g., maternal and offspring) is VennDiagram (Chen and Boutros 2011), which gives exact numbers of OTUs or ASVs shared between two sources. Additionally, the tool SourceTracker (Knights et al. 2011) can predict the source of a microbiome; however, this tool relies on sampling from multiple sources including maternal and environmental to predict offspring microbiome origin. FEAST (Shenhav et al. 2019) is also a method used for quantifying source contributions. For additional information on sequencing platforms and bioinformatics pipelines of microbiome data, see Allali et al. (2017), Kim et al. (2017), Bharti et al. (2019) and Berg et al. (2020).

Recommendations for future microbiome studies

Our systematic review revealed several important knowledge gaps in microbiome studies on vertical transmission. First, disproportionate representation exists among animal classes. If more studies focused on taxa such as various invertebrates, fish, amphibians, birds, and non-avian reptiles, then it would enable phylogenetic methods to be applied to transmission modes/microbiota data across large classes. Greater taxonomic representation will also aid in formulating new questions on how microbiota shape host phenotypes and are transmitted from maternal sources. Secondly, more studies should target sampling from both maternal and offspring sources to provide robust evidence for transmission among the two, potentially allowing for identification of the time point when this occurred. Third, many studies lack functional diversity profiles of microbes. If provided for both maternal and offspring sources, then functional diversity profiles could elucidate the specific metabolic pathways and metabolites gained from vertical transmission of microbes. Fourth, many microbiome studies could benefit from experimental manipulation in their research designs. If this is logistically difficult, then a solution could be standardizing or manipulating offspring developmental en-

vironments to remove maternal influence and then documenting of any changes to microbiome community structures. Lastly, as pointed out earlier, many studies do not measure offspring and/or host phenotypes, and therefore the potential consequences of this maternal effect cannot be assessed. This can greatly limit our ability to draw ecologically meaningful conclusions about vertical transmission of microbes and hinder our understanding of the role of this particular maternal effect in evolutionary adaptation. We recommend that more researchers quantify offspring phenotypes (particularly morphological, behavioral, or physiological traits that are likely associated with fitness) and make more effort in examining how these offspring traits are associated with their own microbiome, as well as that of their mother. Such measurements will help us better understand the ecological and evolutionary importance of microbiome as a maternal effect.

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Supplementary data

Supplementary data available at *ICB* online.

Conflict of interest

The authors declare no conflict of interest.

Data availability

All scripts and datasets used in this study are available at GitHub: https://github.com/kmm0155/SystematicReview_Murphyetal_2023.

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