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Review

Evolutionary Insights into the Tick Hologenome

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Recently, our knowledge of the composition and complexity of tick microbial communities has increased and supports microbial impact on tick biology. Results support a phylogenetic association between ticks and their microbiota across evolution; this is known as phylosymbiosis. Herein, using published datasets, we confirm the existence of phylosymbiosis between *Ixodes* ticks and their microbial communities. The strong phylosymbiotic signal and the phylogenetic structure of microbial communities associated with Ixodid ticks revealed that phylosymbiosis may be a widespread phenomenon in tick–microbiota evolution. This finding supports the existence of a species-specific tick hologenome with a largely unexplored influence on tick biology and pathogen transmission. These results may provide potential targets for the construction of paratransgenic ticks to control tick infestations and tick-borne diseases.

The Tick Hologenome and Phylosymbiosis

Microbes, their associations, and biochemical processes are important components of life [1,2]. In all biological systems the microbial and host entities that make up the **holobiont** (see Glossary) comprise the genetic information that makes up the **hologenome**, a biological entity that can function as a unit of selection [3–5]. The study of the hologenome composition and its variations using animal and human models has shown that the microbial community within the gut, body cavities, and cells is key to understanding host biology [6], behavior [7,8], immunological development [9,10], and ecological plasticity [11]. As a consequence, host–symbiont cooperative and competitive interactions impact host phenotypes, leading to new adaptations, reproductive isolation, and even speciation [11–14].

Ecoevolutionary dynamics between hosts and microbes involves strong bidirectional adaptations leading to **phylosymbiosis** when evolutionary events in the host occur and in parallel to ecological changes in the microbiota [15]. The hypothesis of phylosymbiosis is a conceptual framework useful for understanding host–microbe associations within an ecoevolutionary perspective and not necessarily implying coevolution, cospeciation, or **cocladogenesis**, that in turn provides methodological tools for evaluating whether microbial assemblages track host phylogenetics [15]. Theoretical and empirical analysis suggests that microbes have driven evolution and speciation across the tree of life [11,16]. A growing body of research also demonstrates the presence of a phylosymbiotic signal in microbes associated with different organisms, including plants, sponges, insects, and mammals [15,17–24].

The study of tick microbiota has revealed a complex and diverse microbial community associated with these arthropods [25–27]. In a recent study, Cabezas-Cruz *et al.* [28] showed the presence of genus-specific microorganisms associated with the main tick genera. Remarkably, a large number of bacterial genera were exclusively associated with *Ixodes* spp. From these observations, we can hypothesize an intimate evolutionary association between ticks and bacterial genera present in their microbiota [27,28]. While researchers have sought to capture solely the systematics of tick–symbiont interactions [26,27], the positive association between microbial community ecological relationships and tick species evolution might explain much of their evolutionary success.

Highlights

Microbes and ticks form a functional unity known as the tick hologenome.

Coevolutionary events between ticks and symbionts have benefited tick fitness and adaptation.

Phylosymbiosis occurs in ticks across their phylogeny.

Phylosymbiosis between microbial communities and the tick genome provides the basis for future research on the role of microbes on tick biology and speciation.

A better understanding of tick phylosymbiosis and hologenome, together with tick–microbiota and pathogen–microbiota interactions, would help in identifying the best targets for paratransgenesis in ticks for the control of tick-borne pathogens.

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Evolution of Tick–Symbiont Associations

Ticks are among the earliest lineages of terrestrial arthropods, which evolved a blood-feeding behavior about 250 million years ago [29,30]. This fact makes them an excellent model organism to study evolutionary adaptations to a blood-feeding lifestyle, development of vector abilities, and ecological plasticity [31]. Consequently, a large array of tick–symbiont interactions (i.e., mutualism, parasitism, commensalism) evolved across millions of years of tick evolution [32–34].

Mutualistic coevolution with obligate symbionts leads to an evident cocladogenesis, showing congruency of the phylogenetic trees [34]. In contrast, infections by facultative symbionts can be more variable in space and time, and horizontal transmission (HT), a common way in which facultative symbionts are transmitted, prevents the congruency between host and symbiont phylogenies [33,35]. Among ticks, a diversified community of endosymbiotic bacteria (obligate primary symbionts, facultative secondary symbionts) has been recognized across several bacterial genera *Rickettsia, Coxiella, Francisella, Midichloria, Cardinium, Spiroplasma, Arsenophonus, Wolbachia, Rickettsiella*, and *Lariskella* [26,27,33,36]. A good example of **codivergence** was found in the genus *Rhipicephalus* where a perfect match between the phylogenies of *Coxiella*-like endosymbiont (LE) and mtDNA genes of *Rhipicephalus* was found [33].

Nutritional Tick–Symbiont Associations

Tick–symbiont coevolution has contributed to developing beneficial effects in tick fitness [26,27]. Most of the investigations on tick–symbiont interactions have addressed the effect of symbionts on tick nutrition. Blood feeding in ticks is related to coevolution with different symbiotic bacteria that complement nutritional deficiencies in their restrictive diet. For example, the obligate mutualist *Francisella* F-Om has the ability to synthesize seven B vitamins and has kept pathways for the synthesis of biotin, riboflavin, folic acid, and the cofactor coenzyme A, which complement the deficiency of B vitamins in the diet of the soft tick *Ornithodoros moubata* [37,38]. Further examples are *Rickettsia* endosymbionts (e.g., *Rickettsia buchneri*) which are maternally transmitted in *Ixodes pacificus* and *Ixodes scapularis* and harbor all the gene machinery necessary for *de novo* biosynthesis of folate [39,40]. *Coxiella*-like symbionts isolated from *Rhipicephalus turicanus* and *Amblyomma americanum* share many genes implicated in B vitamin synthesis and participate in various biosynthetic pathways providing many cofactors, including biotin and riboflavin, to the ticks [41,42].

Tick–Symbiont Associations and Cospeciation

Host–symbiont coevolution might result in reproductive isolation or chromosomal rearrangements that finally lead to host speciation [12,43]. These features are well studied in the model symbiont *Wolbachia*, a maternally inherited bacterium that persists within arthropod populations favoring the fitness of infected host daughters (via transmitting sex) or manipulating host reproduction [44,45]. *Wolbachia* can manipulate the host, leaving a host sex-ratio distortion (towards a female infection-biased population), or cause cytoplasmic incompatibility (limiting reproductive success of uninfected females) [46,47]. This peculiar behavior makes *Wolbachia* an interesting symbiotic bacterium when screening vector–pathogen systems for disease control strategies [48,49]. *Wolbachia* is frequently found in a wide range of tick species from different genera, including *Amblyomma, Ixodes*, and *Rhipicephalus* [27,50]. For example, in the species *Ixodes ricinus*, 20% of ticks were positive for *Wolbachia* [51]. It is well known that most of these associations are the result of tick tissue contamination by *Wolbachia*-infected parasitoid wasps [27,50]. However, in some species (i.e., *Amblyomma maculatum*) the prevalence of *Wolbachia* was not strictly explained by nematode infection [50]. Altogether, the role of *Wolbachia* in ticks should be viewed with care; however, it remains of interest to fully elucidate the reproductive phenotype in ticks [27,50].

Glossary

Cocladogenesis: cladogenesis that spans two or more lineages and the parallel process of speciation between host and symbiont in a way that phylogenetic trees of each partner are equivalent. However, there are characteristic features associated with cocladogenesis: (i) maintenance within the host in a special host organ, likely bacteriome, (ii) existence of a long evolutionary history of diversification within host lineages, (iii) vertical transmission, (iv) nutritional suppliers to host, (v) extreme genome reduction (final genome size lower than 1 Mb), and (vi) lack of gene uptake, phage, mobile elements, or genome rearrangements.
Codivergence: divergence in parallel of host and microbes which speciate or diverge in parallel as illustrated by topological congruency of phylogenetic trees. Codivergence may not prove cocladogenesis/coevolution in the strict sense.
Holobiont: host organism and its associated microbial community that form an ecological unit.
Hologenome: the collective genomes of the holobiont.
Overdispersion patterns: when microbial taxa patterns observed are less clustered and therefore less related by chance.
Paratransgenesis: genetic manipulation of symbiotic or commensal microorganisms of arthropod vectors.
Phylogenetic clustering: microbial taxa patterns that are phylogenetically clustered because they are closely related bacteria with small genomic diversity differences and therefore more related by chance.
Phylosymbiosis: ecoevolutionary pattern in which evolutionary changes in the host associate with ecological changes in the microbiota.

Another example of male-killing strategy has been evidenced in the interactions of the symbiont *Spiroplasma* sp. with arthropods [43,52]. The male-killing behavior of the symbiont *Spiroplasma ixodetis* was explored in *Ixodes arboricola* and *Rhipicephalus decoloratus* but no evidence of sex-biases between males and females was observed [53,54]. Nevertheless, *S. ixodetis* has an elevated rate of HT and cocladogenesis in ticks, and is closely related to *S. ixodetis* in insects, that is, the ladybird (*Adalia bipunctata*), in which these bacteria were associated with male-killing behavior [54].

Tick–Symbiont Associations and Vector Competence

Vector competence is a component of vectorial capacity that depends on genetic factors affecting the ability of a vector to transmit a pathogen. Symbiotic bacteria have coevolved with ticks, resulting in modulation of tick vector competence [36,55–58]. For example, the *Rickettsia peacockii* endosymbiont modifies *Dermacentor andersonii* physiology by limiting the transmission of rickettsial pathogens [36]. How this process occurs has not been well elucidated, but it is hypothesized that *R. peacockii* infection in *D. andersonii* ovaries activates a molecular response that blocks transovarian invasion, multiplication, and transmission of *R. rickettsii* [59]. In *A. americanum*, the endosymbionts *Coxiella* and *Arsenophonus* seem to impair the transmission of *Ehrlichia chaffeensis* and *Rickettsia* spp., respectively [55,56]. The molecular mechanisms underlying these interactions are not fully elucidated.

Certain species of tick symbionts are closely related to pathogens such as *Coxiella burnetti*, and *Francisella tularensis* [36,60]. This is the result of the symbiont–pathogen continuum that occurs repeatedly during evolution. Bacteria can abandon their symbiotic lifestyle, adopting pathogenic
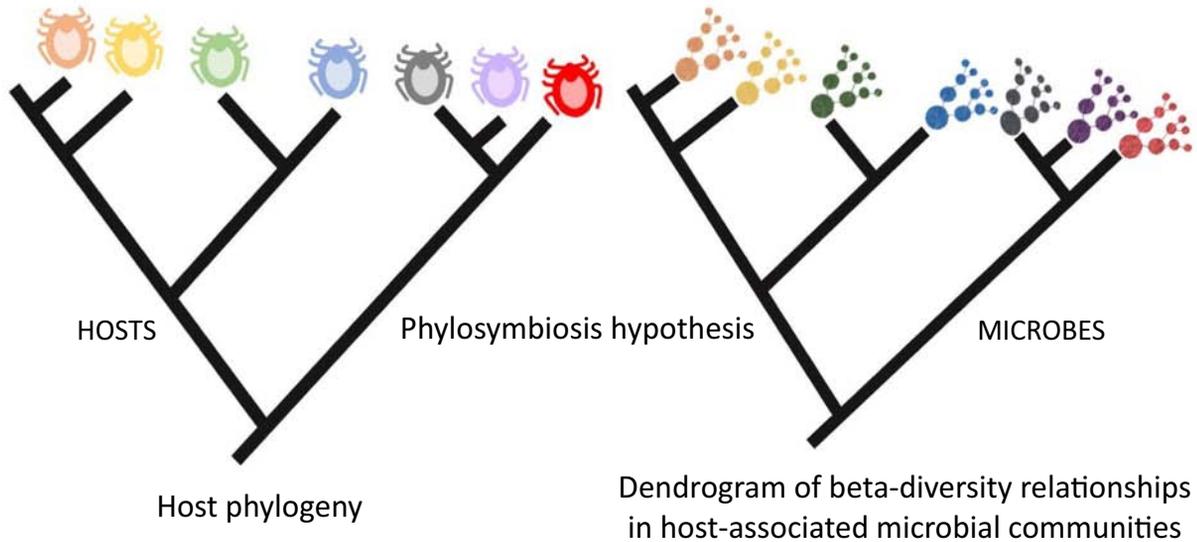
Table 1. Metagenomics Studies of *Ixodes* Ticks

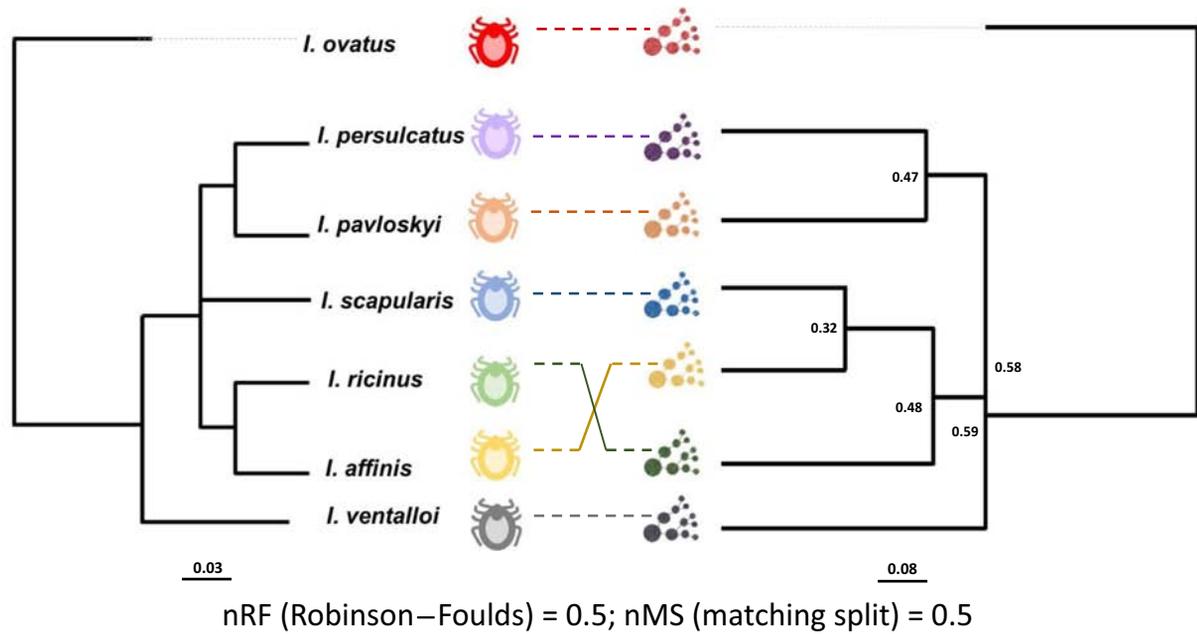| *Ixodes* spp.	No. of ticks	Bioinfo	Location	Coordinates (if applicable)	Sequencing technology	Library construction	Sequences/reads	Refs
I. affinis	11	Adult ticks Wild-caught flagging	North Carolina (USA)	N/A	Illumina MiSeq	16sRNA V1-V3	110 000 reads	[104]
I. persulcatus	414	Adult ticks Wild-caught flagging	Novosibirsk (Russia)	N55°01′07″, E84°06′41″	Illumina MiSeq	16 sRNA V3-V5	36 901 reads	[102]
I. pavloskyi	436	Adult ticks Wild-caught flagging	Novosibirsk (Russia)	N54°49′21″, E83°06′41″	Illumina MiSeq	16 sRNA V3-V5	44 956 reads	[102]
I. scapularis	81	Adult ticks Wild-caught flagging	North Carolina South Carolina Connecticut Virginia New York (US)	N/A	Illumina MiSeq	16 sRNA V1-V3	110 000 reads	[104]
I. ricinus	20	Adult ticks Wild-caught flagging	Trento (Italy)	N/A	454 GS 20	16S rRNA V6	299 510 spots	[101]
I. ventalloi	125	Adult ticks Wild-caught flagging	Sicily (Italy)	Long 13.35141° Lat 38.14946° Long 13.35469° Lat 38.15640°	Illumina HiSeq2000	Whole-shotgun metagenomics	73 976 098 reads	[103]
I. ovatus	10	Adult ticks Wild-caught flagging	Shizuoka (Japan)	N/A	454 GS-Junior Roche	16S rDNA V1-V3	140 584 reads	[64]

N/A, not applicable.

(A)

Phylosymbiotic approach based on topological congruence

HOSTS   Phylosymbiosis hypothesis   MICROBES

Host phylogeny   Dendrogram of beta-diversity relationships in host-associated microbial communities

(B)

Topological congruence

I. ovatus

I. persulcatus

I. pavloskyi

I. scapularis

I. ricinus

I. affinis

I. ventalloi

0.47

0.32

0.48

0.59

0.58

0.03

0.08

nRF (Robinson−Foulds) = 0.5; nMS (matching split) = 0.5

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activity as in the case of the pathogen *C. burnetti*, to which is attributed a nonpathogenic ancestor [36,60]. In contrast, a *Francisella*-like endosymbiont was found to share a common ancestor with the mammalian pathogen *F. tularensis* [38,61].

Phylosymbiosis in *Ixodes* spp. Ticks

Despite evidence showing coevolution of ticks and bacteria, one of the outstanding questions in the study of the tick–microbe associations that remains to be answered is whether phylosymbiosis has occurred during the evolution of tick–microbe associations. Various methods have been used to study the phylosymbiotic signal and the structure of microbial communities [62]. Given evidence of the existence of bacterial genera-specific associations within *Ixodes* spp. [28], we compiled metagenomic data from different studies (Table 1) and used a dendrogram-based approach for testing the hypothesis of phylosymbiosis between *Ixodes* spp. and their microbial communities (Figure 1A, Box 1). Our estimation of the phylosymbiotic signal showed evidence of differences in the composition of microbial communities within phylogenetically related *Ixodes* spp. ticks (Figure 1B). Phylosymbiosis can be qualitatively explained by the topological similarity between the *Ixodes* spp. phylogenetic tree and the hierarchical clustering of the microbiota (Figure 1B). This analysis showed evidence of species-specific associations in tick-associated microbiota and reflected a paired congruency within the *Ixodes* spp. phylogeny and microbial dendrograms. However, a discrepancy was found in the microbial communities of *I. ricinus* and *Ixodes affinis*. These results give rise to the hypothesis that factors intervening and shaping the microbial communities might be very similar between these two tick species.

Taxonomic composition, based on detrended correspondence analysis (DCA) ordination, was performed to illustrate community dissimilarities throughout *Ixodes* spp. ticks based on the microbial composition (Figure 2A). The microbial structure in the DCA evidenced a dispersion of *Ixodes ventalloi* and *Ixodes ovatus* from the members of the *I. ricinus* species complex, *Ixodes pavloskyi*, *Ixodes persulcatus*, *I. affinis*, *I. scapularis*, and *I. ricinus* [63] (Figure 2B). This result can be attributed to a mixed effect of the local environment, as *I. pavloskyi*, *I. persulcatus*, *I. affinis*, *I. scapularis*, and *I. ricinus* share a similar ecological niche, whereas *I. ventalloi* and *I. ovatus* occupy different niche ecosystems [64,65], and tick-derived factors such as *I. pavloskyi*, *I. persulcatus*, *I. affinis*, and *I. ricinus* are closely related members within the *I. ricinus* complex, in contrast to *I. ventalloi* and *I. ovatus* that are more distantly related. Therefore, tick genomes might have evolved similar or unique genomic features that participate in microbial filtering and acquisition.

The concordance observed between these results, and the phylogenetic reconstruction of *Ixodes* spp., can be a good indicator of the importance of specific *Ixodes*–microbe associations on the evolutionary time scale. For instance, whether the observed phylosymbiotic signal corresponds to an ecological filtering process and its functional significance needs to be further assessed under controlled conditions. In addition, more *Ixodes* spp. should be included in this model so that the reconstruction of the phylogeny accounts for other factors such as abundance and seasonality of ticks. Indeed, more microbiome data from ecologically diverse tick species are clearly

Figure 1. Phylosymbiosis Signal of Microbial Communities in *Ixodes* spp. Ticks. We compiled metadata and the microbial profile of seven different *Ixodes* species, including *I. affinis*, *I. ovatus*, *I. ricinus*, *I. persulcatus*, *I. pavloskyi*, *I. scapularis*, and *I. ventalloi*, with metagenomic datasets publicly available [64,101–104] (Table 1). (A) A dendrogram-based approach was used to test the hypothesis of phylosymbiosis in the microbiota associated with *Ixodes* spp. ticks. (B) *Ixodes* spp. tick phylogeny was constructed based on the *cytochrome oxidase 1* (*COI*) gene sequences. The tree was built in RaxML and the hierarchical clusterization of *Ixodes* spp. based on microbial composition was performed by using the Vegan package in R (R Studio) using Jaccard distance matrices and the Waird method [105]. The strength of phylosymbiotic signal in *Ixodes* spp. ticks was quantitatively measured by normalized Robinson-Foulds (nRF)/Matching-split (nMS) metrics (complete congruence = 0, complete incongruence = 1). Qualitative measures were determined by the topological congruence of the trees [15,106].

Box 1. Predicting Phylosymbiotic Signal and Microbial Communities

Phylosymbiotic Signal

There are two common methods for predicting phylosymbiosis: (i) the dendrogram-based approach, which measures congruence between tree representations of host phylogenies and the tree of microbial community similarities, using a metric that calculates the dissimilarity of tree branching structures, and (ii) the Mantel approach, which measures the correlation between two distance matrices (i.e., host phylogenetic divergences vs. microbial community dissimilarities) [62]. In this review, we used a dendrogram-based approach. First, we constructed the *Ixodes* phylogeny based on the marker cytochrome oxidase 1 (COI) GenBank sequences and aligned with CLUSTALw. The *Ixodes* phylogenetic tree was built in RaxML using the T-REX software platform and the GTR substitution model. The dendrogram of *Ixodes*-associated microbial communities was constructed using the *Ixodes*-associated microbiota matrix and analyzed using the Vegan package in R (R Studio) [105] to calculate the dendrogram of beta-diversity relationships in *Ixodes*-associated microbiota using the Jaccard distance index and the Ward clustering method. Finally, the identification of topological congruence between *Ixodes* phylogeny and the *Ixodes*-associated microbiota dendrogram was calculated using the Matching Split and Robinson-Foulds metrics for unrooted trees (software TreeCmp) [106] with normalized distances ranging from 0.0 (complete congruence) to 1.0 (complete incongruence).

Structure of Microbial Communities

Common methods for estimating microbial community structure include measures of phylogenetic beta-diversity, such as mean pairwise phylogenetic distance (MPD); they reflect phylogenetic structuring across the whole phylogeny dendrogram, while mean nearest taxon distance (MNTD) reflects phylogenetic structure closer to the tips [66]. Likewise, the life history features of microbes describe processes related to microbial community structure. Thus, the phylogeny of the bacterial species representing each bacterial genus within each *Ixodes* sp. was constructed using the bacterial 16S rDNA nucleotide sequences downloaded from GenBank (a total of 327 bacterial species, one representing each bacterial genus). This approach assumes that bacterial species within a given genus are more related to each other than they are to bacteria of other genera [69]. Further, sequences were aligned using MAFFT, and the evolutionary history was inferred using the neighbor-joining method. Finally, MPD was calculated to predict the microbial community structure of *Ixodes* spp. (mpd.obs.z = standardized MPD; mpd.bos.p = P value vs. null communities). Negative values of mpd.obs.z and low P values (P <0.05) imply phylogenetic clustering observed in microbial communities in which taxa are closely related [32,68]. Positive values of mpd.obs.z and high P values (P >0.95) illustrate phylogenetic evenness that reflects over dispersed patterns observed when taxa are less clustered and less related by chance [32].

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needed in order to establish strong species-specific patterns and a core microbiome separated by tick geographical isolation.

Structure of the Tick Microbial Communities

How are tick microbial communities structured? To answer this question we examined the phylogenetic structure of *Ixodes* spp. microbial communities using the phylogenetic analysis of diversity, mean pairwise distance (MPD) that tests the phylogenetic relatedness of individuals in a community, and whether patterns of clustering or overdispersion across a phylogenetic tree occur [66–68] (Figure 3A,B; Box 1).

The microbial communities of *I. affinis*, *I. scapularis*, *I. ricinus*, and *I. ovatus* showed **overdispersion patterns** (Figure 3B). The interpretation of this result is that phylogenetic evenness and a greater phylogenetic distance among co-occurring individuals might exist.

Previous observations by Estrada-Peña *et al.* [69] correlated with these results, given that the structure of the microbiome in ticks in that study revealed a high phylogenetic diversity but lacked co-occurring genera. Phylogenetically overdispersed microbial communities are the result of species-specific interactions between closely related taxa when they compete for similar resources [70], and bacterial traits that have converged throughout evolution to prevail in the environment, resulting in redundant capacity of their microbiomes [67,71]. In nature, overdispersed taxa across a phylogeny might be considered the rule rather than the exception [67].

Microbial communities of *I. persulcatus*, *I. pavloskyi*, and *I. ventalloi* revealed a pattern of **phylogenetic clustering**, being significantly strong in *I. ventalloi* in correspondence to small phylogenetic distances among co-occurring bacteria (Figure 3B). A clustering or co-occurrence pattern is explained by phylogenetic aggregation of closely related taxa shaped by a strong selection of important traits that facilitate adaptation to a particular environment [66]. Microbes tend to have similar ecological requirements due to an increased effect of environmental filters, such as when the habitat is unfavorable to facilitate the adaptation of species to that environment [68]. Consequently, the *I. ventalloi*, *I. pavloskyi*, and *I. persulcatus* clustering phylogenetic signal could be partially explained under a niche-based scenario due to the small area of distribution that these tick species tend to occupy. The interpretation of these results showed evidence that *Ixodes* spp. ticks likely harbor phylogenetically clustered microbial communities, which in turn are prone to occur under strong selection forces related to environmental filtering processes, differential dispersal, colonization abilities, and adaptive radiation events [68]. Environmental filtering and microbial competition are forces that might act together, shaping the bacterial community [62].

Forces Shaping the Structure of Tick Microbial Communities

What processes shape the composition and assembly of the tick microbiome and phylosymbiosis? In general, the colonization and assemblage of microbes in invertebrates and vertebrates depend on four factors: (i) local environment, (ii) host-derived factors, (iii) microbe-derived factors, and (iv) interactions between bacteria [72,73]. Previous studies have already discussed the gradient in which environmental filtering intervenes in shaping the host microbiome [74,75]. Mazel *et al.* [62] demonstrated, through a simulated data set, that host-related ecological filtering can generate phylosymbiosis. This is of importance as host traits might conditionate assembly of adapted microbes from the environment [62].
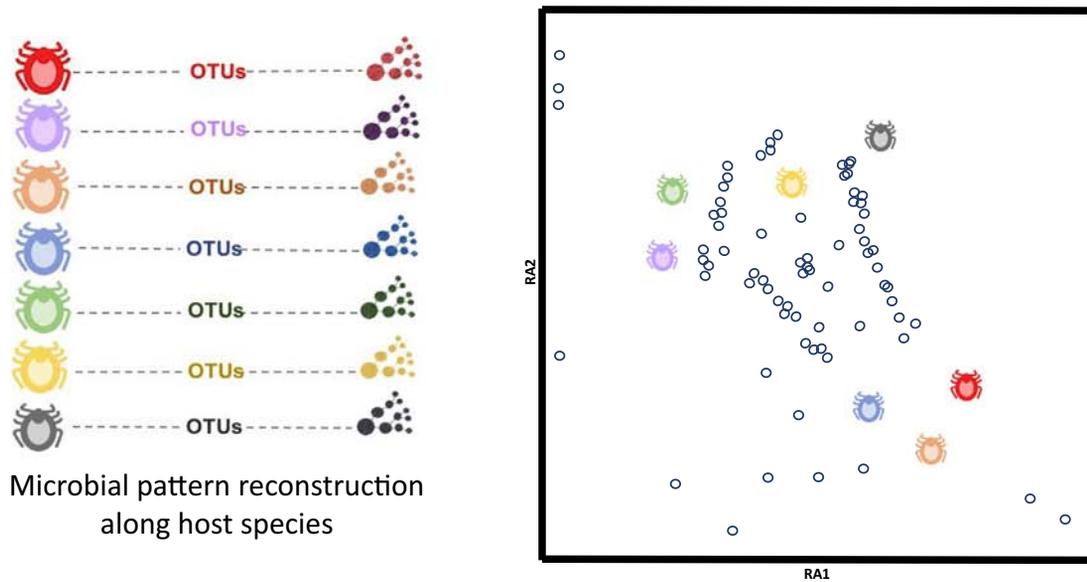
In a tick–microbe system the presence of unique features is very appealing for addressing these questions. However, knowledge about the mechanisms of microbial assembly is practically non-existent. Common observations in ticks are limited to the acquisition of bacteria that resulted from the tick's lifestyle, transmitted vertically or horizontally, which in turn is mainly attributed to obligate or facultative endosymbiotic bacteria [27,28]. Beyond endosymbiotic bacteria, other genera, such as *Pseudomonas, Sphingobacterium, Acinetobacter, Enterobacter,* and *Stenotrophomonas*, are common colonizers of the midgut in hard ticks [26].

Recent insights showed the importance of the environment in the internal shape and assemblage of tick microbial communities due to bacteria that can be eventually acquired from soil and plants [69,76,77]. Indeed, an obvious part of the microbial community can be acquired from the environment through the spiracles, the mouth, and the anal pore, or even during copulation (paternal transmission route) and on each blood-feeding event (skin microbes and pathogens) [26].

Another important component impacting microbial acquisition and assembly would result from the tick's immune system. Ticks are armed with a great variety of immunological strategies that condition microbial invasions [78]. It is well known that, after microbial acquisition, the process of microbe colonization activates a cascade of host-derived pathways associated with the immune system, for example by increasing the expression of genes coding for antimicrobial peptides (AMPs) among other immune response proteins [15,72,79,80]. In soft and hard ticks, the AMP family of defensins might play a crucial role in structuring tick microbial communities [81–83]. Hence, recent studies have shown evidence that the wide antimicrobial activity of defensins can limit or favor the assembly of various Gram-negative and Gram-positive bacteria even if they are distantly related bacteria [81,82]. Nevertheless, other unexplored tick pathways, such as the biochemical mechanisms related to the development of the gut lining [84,85],
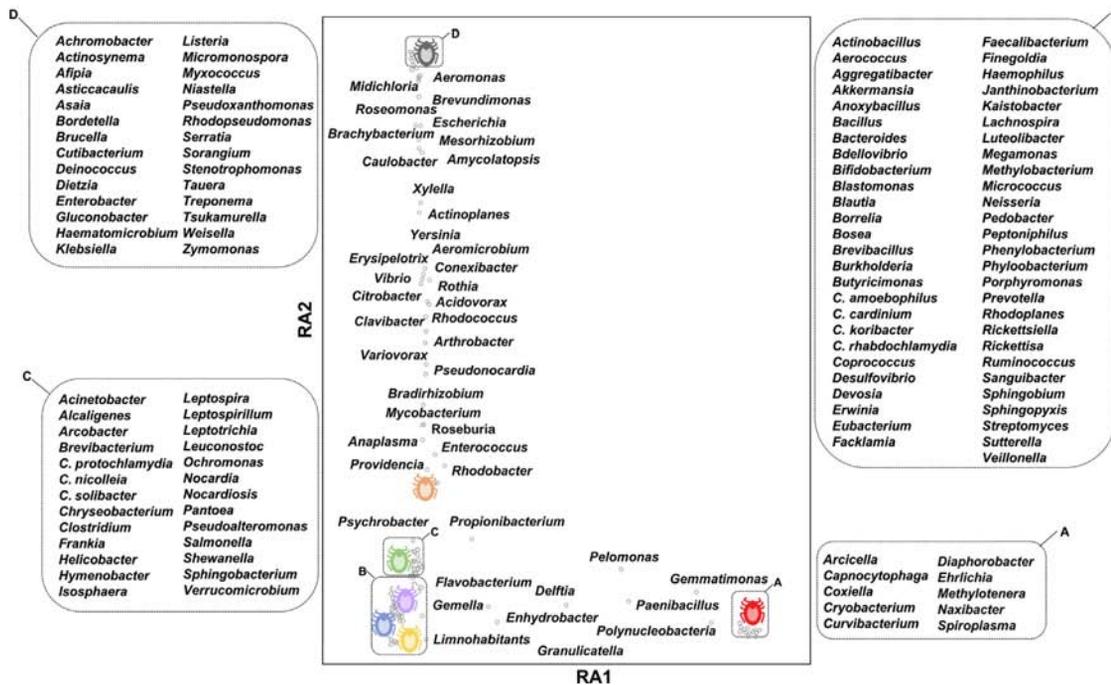
(A)

Multivariate approach based on detrended correspondence analysis (DCA)

Microbial pattern reconstruction
along host species

(B)

Bacterial genera distribution across *Ixodes* ticks

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circadian clock genes [86], miRNAs [87], and digestive enzymes [88], can participate in the formation of host-microbe alliances.

Microbes can actively participate in the host selection process. For instance, microbe-associated molecular signaling patterns (MAMPs) activate the communication between the commensal bacteria and the host which, in turn, permits the maintenance of cooperative alliances [89,90]. Host exposure to microbes across evolution can initiate the mechanisms of immune priming with a demonstrated impact on the structure of the microbial communities in arthropods [91]. In addition, trained immunity or the capacity of the innate immunity to adapt and confer immunological memory, is observed in ticks in response to infection with the pathogenic bacteria *Anaplasma phagocytophilum* and *Borrelia burgdorferi* [91,92].

Microbe–microbe synergies underlying competitive or cooperative processes have direct effects on a successful colonization [27]. It has been demonstrated that pathogens can modulate the tick's microbiota to facilitate the pathogen's own colonization [93–95]. A recent study testing microbe–microbe interactions in different biotypes observed a minor effect in shaping phylosymbiosis [62]. However, the coevolutionary interactions among members of the tick's microbial communities resulting in specific microbiome composition are largely unexplored.

Development of Interventions for the Control of Tick-Borne Diseases

Paratransgenesis was proposed for the genetic manipulation of symbiotic or commensal microorganisms as an effective and environmentally sound approach for the control of vector-borne diseases [96]. Paratransgenic ticks with symbiotic bacteria producing antimicrobial compounds have been produced and could offer a safe and effective way to reduce pathogen transmission by ticks [97–100]. Future advances in this area have been directed to strategies aimed at developing a shuttle vector system capable of transforming rickettsial symbionts (i.e., *R. buchneri* and *R. peacockii*) and producing antipathogen factors [98]. In this review we want to emphasize the need to understand the tick hologenome in terms of tick–microbiota and pathogen–microbiota interactions, and the existence of phylosymbiotic patterns, to enhance efficiency when designing paratransgenic strategies for tick-borne disease control. For example, symbiotic bacteria identified by phylosymbiosis as specific to a certain tick species may be an interesting target for the control of pathogen infection and transmission by this vector. Similarly, symbionts that are commonly found in various tick vector species may be used for the control of pathogens transmitted by multiple tick species.

Concluding Remarks

Despite recent advances in the study of tick microbiota, most of the mechanisms implicated in the recruitment, assemblage, and transmission of specific microbes across the tick tree of life are in the spotlight of ongoing research (see Outstanding Questions). Future directions should describe the long-term relationships between each component in the phylogeny, including other factors such as the abundance and seasonality of ticks. Additionally, more microbiome data from ecologically diverse species are clearly needed in order to explore the principles and the central hypothesis related to tick microbial ecology.

Little is known about the prevalence in nature and the strength of phylosymbiosis along tick phylogeny. In these studies, some limitations derive from the use of different metagenomic datasets

Figure 2. Microbial Pattern Reconstruction along *Ixodes* spp. Ticks. (A) A detrended correspondence analysis (DCA) approach was used to reconstruct the microbial pattern along *Ixodes* spp. ticks. (B) Bacterial genera distribution across *Ixodes* spp. ticks (DCA plot) was obtained using the Vegan package in R [103]. The genus *Candidatus* is abbreviated as *C*.
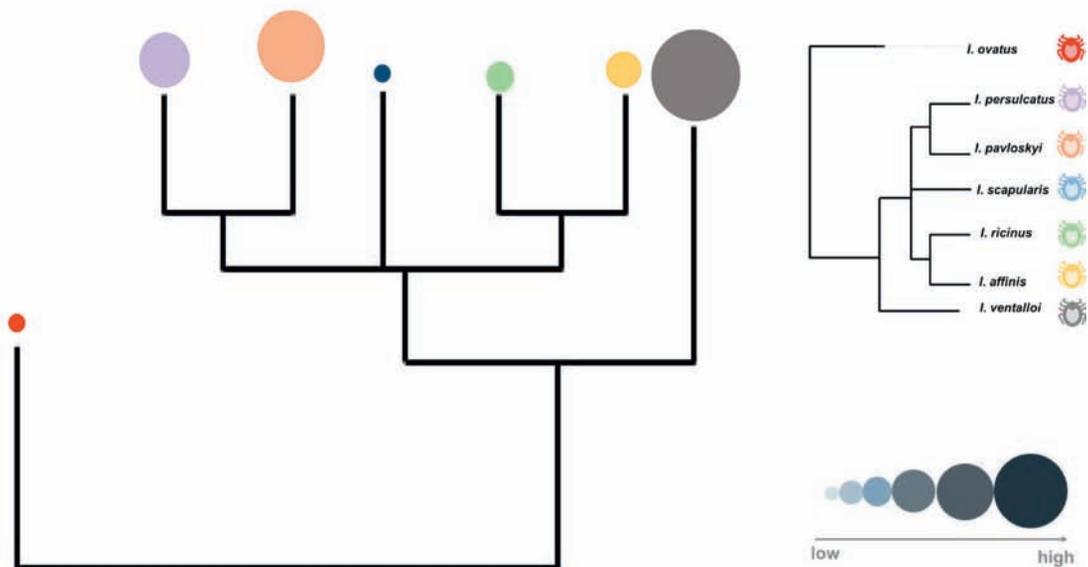
(A)

Phylogenetic clustering approach based on mean pairwise phylogenetic distance

Microbial phylogenetic tree → Phylogenetic signal

(B)

Phylogenetic signal

I. ovatus
I. persulcatus
I. pavloskyi
I. scapularis
I. ricinus
I. affinis
I. ventalloi

low — high
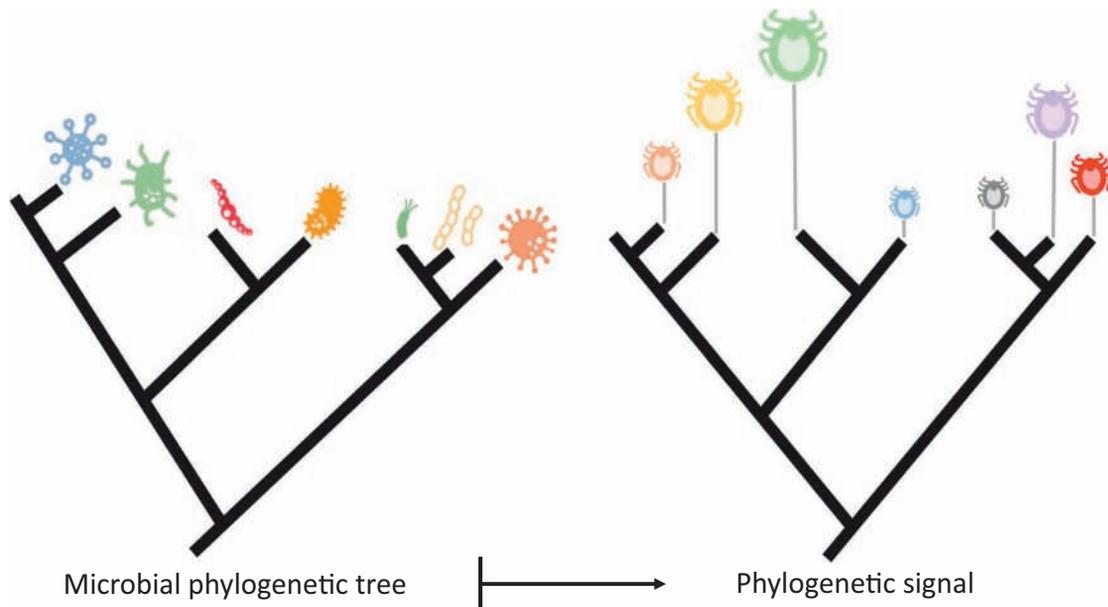
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Figure 3. Structure of Microbial Communities in *Ixodes* spp. Ticks. (A) The mean pairwise distance (MPD) approach was used to assess the structure of the microbial communities in *Ixodes* spp. ticks based on the microbial phylogenetic tree. The 16S rDNA nucleotide sequences of 327 bacteria species were obtained from GenBank, aligned using MAFFT and inferred evolutionary history was determined using the NJ method. The phylogenetic relatedness was obtained using Picante package in R [107]. (B) MPD values near zero indicate a strong phylogenetic signal, those near one indicate a relatively low phylogenetic signal.

obtained in different studies. One of the most important limitations is the methodological differences in data collection and metagenomic workflow between different studies, which may affect the conclusions of the tick phylosymbiosis and hologenome (experimental and sequencing artifacts, host-related variability, stochastic microbial community changes). Additionally, future studies need to consider microbial intraspecific variability that may not be reflected when using pooled metagenomic data.

Despite these limitations, and the small number of metagenomes included in our meta-analysis, we have revealed a consistent emergent phylosymbiotic and microbial pattern in *Ixodes* ticks that is worth further exploration. Finally, these results may guide the identification of potential targets for the control of tick infestations and pathogen infection and transmission through the production of paratransgenic ticks using different components of the tick microbiota.

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