A crucial nexus: Phylogenetic *versus* ecological support of the life-cycle of *Ixodes ricinus* (Ixodoidea: Ixodidae) and *Borrelia* spp. amplification

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ARTICLE INFO

ABSTRACT

The tick *Ixodes ricinus* parasitizes a wide range of vertebrates. These hosts vary in the relative contribution to the feeding of the different tick life stages, and their interplay is pivotal in the transmission dynamics of tick-borne pathogens. We aimed to know if there is a phylogenetic signal in the feeding and propagation hosts of *I. ricinus*, independently of other traits, as well as in the amplification of *Borrelia burgdorferi* (*s.l.*) in feeding larvae. We used a compilation of 1127 published field surveys in Europe, providing data for 96,586 hosts, resulting in 265,124 larvae, 72,080 nymphs and 37,726 adults. The load of immature ticks on hosts showed a significant phylogenetic signal towards the genera *Psammodromus*, *Podarcis,* and *Lacerta* (nymphs only). We hypothesize that such signal is the background hallmark of the primitive hosts associations of *I. ricinus*, probably in the glaciation refugia. A secondary phylogenetic signal for tick immatures appeared for some genera of Rodentia and Eulipotyphla. Results suggest the notion that the tick gained these hosts after spread from glaciation refugia. Analyses support a phylogenetic signal in the tick adults, firmly linked to Cetartiodactyla, but not to Carnivora or Aves. This study provides the first demonstration of host preferences in the generalist tick *I. ricinus*. We further demonstrate that combinations of vertebrates contribute in different proportions supporting the tick life-cycle in biogeographical regions of the Western Palaearctic as each region has unique combinations of dominant hosts. Analysis of the amplification of *B. burgdorferi* (*s.l.*) demonstrated that each genospecies is better amplified by competent reservoirs with which a strong phylogenetic signal exists. These vertebrates are the same along the spatial range: environmental traits do not change the reservoirs along the large territory studied. The transmission of *B. burgdorferi* (*s.l.*) is amplified by a few species of vertebrates, that share biogeographical regions with the tick vector in variable proportions.

1. Introduction

Ticks are ectoparasites that have earned notoriety in recent years due to their pivotal role in the transmission of many pathogens to humans and animals. These blood-feeding vectors are intricately linked to the ecosystems they inhabit, forming complex relationships with their hosts. The tick-host interactions serve as critical determinants in the circulation of pathogens, impacting disease epidemiology, ecology, and public health (Estrada-Peña et al., 2015, 2016; Estrada-Peña and de la Fuente, 2016; Sprong et al., 2018).

Ticks are ubiquitous, distributed across diverse geographical regions. This global occurrence places humans and animals in frequent contact with these parasites, making understanding their role in disease transmission paramount. Ticks have coevolved with vertebrates, but most probably not co-speciated, except perhaps a few species (see e.g. Mccoy et al., 2001; Gómez-Díaz et al., 2010) with a wide range of vertebrate hosts, including mammals, birds, and reptiles. Each tick species may exhibit host specificity to varying degrees, often displaying a preference for host species or higher taxa, that in most cases seems to derive from an ecological common background (Hoogstraal and Aeschlimann, 1982; Cumming, 1998; Wells and Clark, 2019). Studies interpreted these findings under an ecological perspective in which ticks could track their environmental niche associating to vertebrates that would maximize tick survival under a range of abiotic variables, i.e. by environmental

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filtering; therefore, generalist ticks would associate with hosts by environmental sharing (Estrada-Peña et al., 2020).

Also, the evolutionary relationships of tick-borne pathogens with ticks and vertebrates are difficult to unravel since they may result from either co-speciation events or from "jumps" or switching among vertebrates; moreover, pathogens could co-evolve along the phylogenetic gradient of tick species probably exploiting several hosts, resulting in a misty phylogenetic picture that obscures the true evolutive relationships. For example, previous studies involving *Rickettsia* spp. in the Neotropics have demonstrated that these phylogenetic associations have been most probably driven by the co-speciation of ticks and the pathogen (Estrada-Peña et al., 2021), considering the symbiotic features of these microorganisms. *Ixodes ricinus* is an interesting example of a generalist tick that is able to circulate several groups of pathogens, probably as the result of the tick-host relationships, with a further adaptation of "strains" or genospecies of pathogens to lineages of the tick. A generalist tick may feed on a large variety of vertebrates, and the carried pathogens could make associations with competent reservoirs based on their geographical or environmental overlap; however, such new relationships could result from co-speciation events between the pathogen and the reservoirs. Pathogens such as *Borrelia burgdorferi sensu lato* (hereinafter abbreviated as *Bb*), utilize the complex of species *I. ricinus* as vectors in their life-cycles. The intricate interactions between this tick, the hosts, competent reservoirs, and pathogens allow for the persistence and circulation of these disease agents in nature. It is therefore important to capture the nature of relationships between vertebrates and *I. ricinus*, and the degree to which hosts contribute to the tick propagation as well as pathogen amplification. The evolutionary ecology of *Bb* and the *I. ricinus* group of ticks is a particularly interesting example of such a complex chain of interactions (see e.g. O'Keeffe et al., 2020). Previous studies on the network of ticks and vertebrates that support the life-cycle of *Borrelia* spp. (Estrada-Peña et al., 2016) found a core of generalist ticks and vertebrates, from which peripheral branches supported by specialist ticks reach secondary reservoirs. Such a structure would expand the network of pathogen relationships, supporting a redundant and resilient circulation. Whether such a pattern is based on associations of the pathogen with the phylogenetic background of the tick(s) or the reservoir(s) has been only partially addressed (Estrada-Peña et al., 2018a). The tick vectors could be environmentally associated with the vertebrates, while the microorganism could have true phylogenetic associations with the vertebrates. Similar associations were recognized for the genus *Ixodes* in the Neotropics (Estrada-Peña et al., 2023). A deeper approach to the evolutionary background of *Borrelia* (O'Keeffe et al., 2020) suggested that the distribution of *Borrelia* and its genetic structure is shaped by the contact with key vertebrates.

The phylogenetic signal (Revell et al., 2008) is the inheritance of traits passed between consecutive generations of organisms that may drive their structured separation in a way related to their phylogeny (Keck et al., 2016). Such phylogenetic signal refers to the "tendency for related species to resemble each other more than they resemble species drawn at random from the [phylogenetic] tree" (Keck et al., 2016). The concept of phylogenetic signal can be applied to the specificity of parasites and vectors with their hosts (Heath, 1981; Wolinska and King, 2009; Wells and Clark, 2019) and therefore to the parasitic burden, which is nothing else than a continuous (i.e. non-categorical) trait. Regarding tick-borne microorganisms, it is necessary to demonstrate that the connections of pathogens to groups of vertebrates are the result of either an ecological connection or a true co-evolutive event. Phylogenetic signal can help unravel the congruence of common phylogenetic histories, looking for long-term coevolutionary relationships (Krasnov et al., 2022).

Regarding the epidemiology of tick-borne pathogens, data reveal that changes in the balance of the abundance of some vertebrates in a community alter infection rates by some pathogens in ticks (Levi et al., 2016; Occhibove et al., 2022). It is however puzzling that many vertebrates contribute to sustaining the life-cycle of generalist ticks but some

of them may not contribute to amplifying the pathogen transmission (e. g. Gandy et al., 2022). It is thus crucial to understand if the tick burden follows a pattern of phylogenetic associations, and if tick-borne pathogens associate with competent reservoirs because of the phylogeny (and not only ecologically shared biotic niche).

The available reports on *I. ricinus* in its entire distribution range make this tick species suitable for the search of a hypothetical phylogenetic signal between any of the feeding stages of the tick and its hosts. These reports may help capture the details of the relationships between vertebrates, the tick, and the circulation of pathogens, providing a background to examine these hypothetical phylogenetic relationships. This study analyzed published data on the *I. ricinus* tick burden in vertebrates and the circulation of *Borrelia* spp., considering climate and landscape variables, as well as the phylogenetic relationships of 201 species of vertebrates. The purpose is to test the presence of a phylogenetic signal driving the tick burden on vertebrates and the associations of *Borrelia* spp. in the Western Palaearctic.

2. Materials and methods

2.1. The tick burden on hosts

Data on the burden of larvae, nymphs, and adults of *I. ricinus* on hosts were obtained from the meta-analysis described in Fabri et al. (2024). This led to an updated database from the previous data by Hofmeester et al. (2016) on tick burden of hosts and infection prevalence of *Bb*. The date of collection introduced a bias in further statistical analyses: the wide variety of intervals and periods of study reported by authors precluded a solid integration into a time framework. Some surveys extended only for one day, while others extended for years in variable intervals of time. Many publications included different dates, but the results were not associated with the dates, rendering it impossible to correlate prevalence and incidence with a period of the year. With all these considerations, the compilation resulted in 1127 different surveys, with a total of 96,586 hosts collected and examined, giving information on 265,124 feeding larvae, 72,080 feeding nymphs and 37,726 feeding adults.

Raw data about tick burden on hosts cannot be used directly since larger animals could accumulate more ticks because of a simple law related to their size. We corrected data on tick burden according to traits of the host(s). Several datasets compiled morphological traits of vertebrates (e.g. PanTHERIA, Therion, COMBINE, AnAge). However, although these datasets are useful for comparing traits *among* classes of vertebrates, a harmonized dataset including the same morphological traits of vertebrates (mammals, birds, lizards, all of them hosts of *I. ricinus*) is missing. One of the few comparable features available in these datasets and with ecological meaning regarding the tick load is the weight and the size of the animal; we considered that the inclusion of the size may bias the results (e.g. a large tail in mammals, or a wide wingspan in small birds, distorting the actual body of the animal). Therefore, the weight of adults of each species of host was used for these calculations as available in the dataset AnAge (De Magalhaes and Costa, 2009). Weight and size are correlated in most vertebrates and should not introduce bias in further calculations; body mass, too, is related to some immunological and behavioral responses (Brace et al., 2017). The weight of species of the genera *Psammodromus* and *Podarcis* (Lacertidae) were taken from general literature because they were not included in AnAge at the time this study was carried out.

We transformed the weight of each animal to its logarithm to avoid the overdispersion of disparate values (e.g. comparing mice and large ungulates). The final "index of larval (or nymph, or adult) burden" was calculated separately for each stage using the simple equation "total number of ticks of a stage" times "total hosts infested" divided by "total hosts examined". The result was divided by the logarithm of the weight of the host to give the "tick burden" for each stage. We could not calculate the aggregation of ticks on vertebrates, because individual

data for each host are necessary to compute the clumping factor and the index of aggregation from the negative binomial distribution (Poulin, 1993). Individual data for each host are commonly unavailable, and even absent in the supplementary materials of published reports.

All the data reported for species of vertebrates were summarized into genera for this part of the study, aiming to simplify its presentation and to have a coherent phylogenetic view. The phylogenetic tree of the vertebrates was obtained from the Open Tree of Life (OTL) initiative (http://opentreeoflife.org) accessed using the package rotl v 3.1.0 (Michonneau et al., 2016) for R (R Core Team, 2023). To note, OTL is not based on some few genes but is a coherent view of the "tree of life" synthetically obtained from published results or available data in genetic repositories (see https://tree.opentreeoflife.org/for an overview). Synonymies in vertebrates were resolved using data available in OTL and adopting the current name for the organism.

Our aim was to check if a phylogenetic signal of the tick burden exists for specific branches of the tree, separately for larvae, nymphs, or adults, uncorrelated with the size or weight of the vertebrate and related only to the position of the host in the phylogenetic tree. We used the package Phylosignal v 1.3.1 (Keck et al., 2016) for R to evaluate the presence of such a phylogenetic signal in the tick burden. This was done separately for larvae, nymphs, and adults, using their respective indices. A phylogenetic signal is the tendency for closely related species to display similar traits because of their phylogenetic proximity. In our context, the trait is the tick burden, that we track across the branches of the phylogenetic tree of vertebrates. Most algorithms are mainly based on the concept of autocorrelation (Keck et al., 2016). Thus, the presence of a phylogenetic signal (as defined by Blomberg and Garland Jr, 2002) can be tested by rejecting the null hypothesis that trait values are distributed independently from their phylogenetic distance in the tree.

Global measures of autocorrelation, commonly performed by Moran's I, a universal measure of autocorrelation, give only generalist information (Gittleman and Kot, 1990). Such an approach assumes that traits evolve similarly across the phylogeny (which is rarely the case) and that phylogenetic signal is scale-dependent and varies among clades. Therefore, it is better to use local statistics ("local" in terms of branches of the phylogeny) to describe a trait pattern as happening separately in phylogenetic clades. Several statistical tools to analyze local patterns exist under the name of Local Indicators of Spatial Association (LISA). The local Moran's I (Anselin, 1995) is a well-described LISA that can be used to detect hotspots of positive and negative phylogenetic signals, associated with the phylogenetic clade(s). We thus performed a local Moran's I on the tick burden for each stage to find the local hotspot(s) of association(s) between a phylogenetic clade and the tick burden index. This was done separately for larvae, nymphs, and adults. The phylogenetic tree of the species of hosts and the Nexus-like file of phylogeny are included in Supplementary file S1.

We aimed to associate the tick burden with other ecological explanatory variables, looking for a better combination of variables explaining the tick burden index other than the purely phylogenetic one. General Linear Models (GLMs) were used to explore other variables affecting the phylogenetic signal of tick burden. In preliminary tests, either the geographical latitude or longitude produced a slightly positive association with the larval and adult tick burden, that we wanted to further explore. We reasoned that an effect related to the coordinates could be derived from the temperature, coordinates being the proxies for the actual variable(s) explaining such an effect on tick burden. We thus obtained the monthly temperature for each collection point and averaged them for the period 1980–2010. Data were obtained from Terra-Climate, available at https://www.climatologylab.org/terraclimate.html (Abatzoglou et al., 2018). TerraClimate is a global gridded dataset at 4 × 4 km resolution of meteorological and water balance variables for the period 1958-present, available on a monthly time step. Using the

coordinates of surveys, we obtained the values of the monthly averaged temperature for each collection point. The latitude and longitude were used in the GLMs together with the values of average temperature of each collection point, looking for significant associations.

We aimed to determine the best feeding hosts (for immature ticks) and propagation hosts (for adult ticks) in the Western Palaearctic: these are the animals that could best increase the population of ticks because of their role in supporting the three stages of the tick (in our context, a concept like "super-spreaders" of the tick). Tick burden as explained above was further weighted by the contact rates of each vertebrate with the tick, using the so-called predicted niche overlap (PNO) as available in the package Phyloclim v 0.9.5 (Heibl and Calenge, 2018) for R. For example, a vertebrate species that shows high prevalence (infested/total) of the tick AND high tick burden AND a high PNO with the tick, will be ranked high as tick feeding/propagation host. Lower values in one of the rules will produce a relative decrease in its capacity of propagation. Most important, these results can be used to produce maps of distribution of the most contributing hosts in the wide known range of the tick.

Since the data of this compilation were scattered (and sometimes aggregated) across Europe, we summarized and referred findings to the bioclimatic regions of the continent, known as European Landscape Classification (LANMAP2) and developed by Mücher and Wascher (2007). LANMAP2 is a hierarchical division of the territory into large climate categories, that are further subdivided according to features of soil, vegetation, or elevation, to produce more than 20,000 combinations of the above features. For this study we used the smallest divisions but referred only to categories of climate, that include 13 categories plus the Anatolian region (see Fig. 1). The Anatolian region was removed from further studies because *I. ricinus* is absent there. The data regarding the feeding/propagation hosts of *I. ricinus* are included in Supplementary file S2.

2.2. The phylogenetic signal and the amplification of Borrelia burgdorferi (s.l.)

We asked if a phylogenetic signal exists between the prevalence of different species of *Bb* in ticks and the amplifying abilities of the vertebrates in the Western Palaearctic. Field and laboratory studies have addressed this issue and demonstrated that, at least for Europe, some species of *Bb* are associated with different groups of vertebrates (e.g. mammals, lizards, or birds; see Wolcott et al., 2021, for a review). We used only data of larvae collected feeding on hosts and processed individually, as per published reports. It is known that other than *Borrelia miyamotoi* (a member of the recurrent fevers group) the *Bb* group is not (or very rarely) transmitted to the next generation of ticks. Calculations on larvae are thus a correct estimation of the amplification of the microorganism after feeding of the vector on hosts. The inclusion of nymphs in these calculations could detect double infections (because of the double bloodmeal of larvae and nymphs) that could obscure such phylogenetic signal. In the analyses, we could not simultaneously include the number of hosts with feeding larvae and the tick load since these data were missing in 774 of 1037 analyzed papers (more than 74%).

To evaluate the phylogenetic signal for each *Borrelia* spp. we calculated their prevalence in the feeding larvae. Spatial maps of amplification were produced using the values of amplification obtained for each species of vertebrate, their expected distribution in the target territory, and their niche overlap with *I. ricinus*, summarized at the level of the bioclimatic regions in Europe, as explained above for the feeding/propagation hosts of the tick vector. We also calculated which species of vertebrate are the best amplifiers of *Bb* in each bioclimatic region. All the data regarding amplification hosts of *Bb* and *I. ricinus* are available in Supplementary file S3.
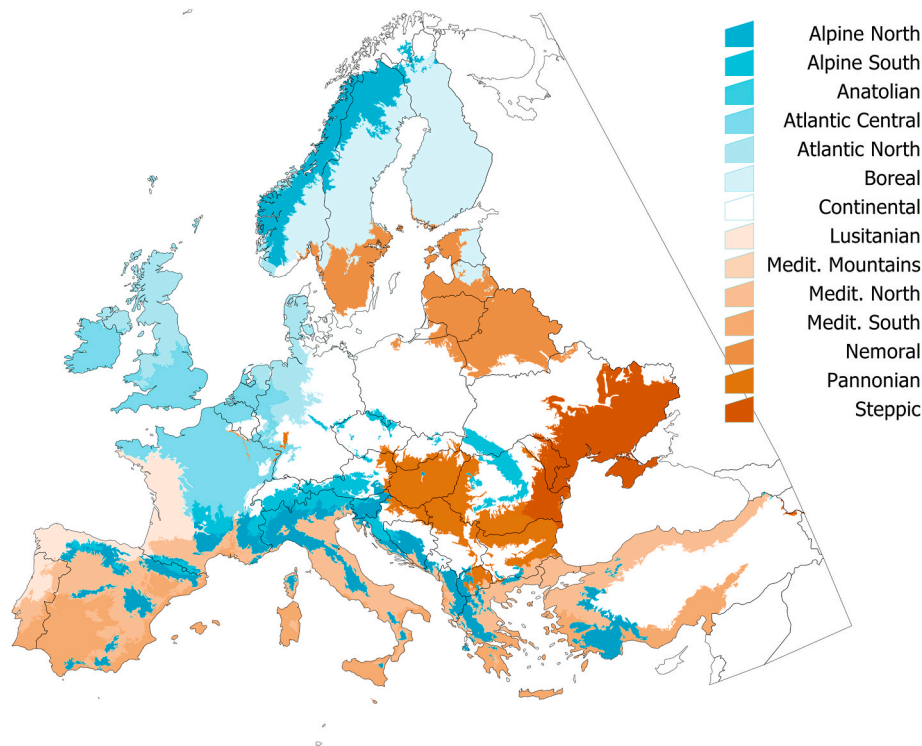
Fig. 1. An overview of the bioclimatic regions in Europe, according to LANMAP2. The legend indicates the official names of the regions.
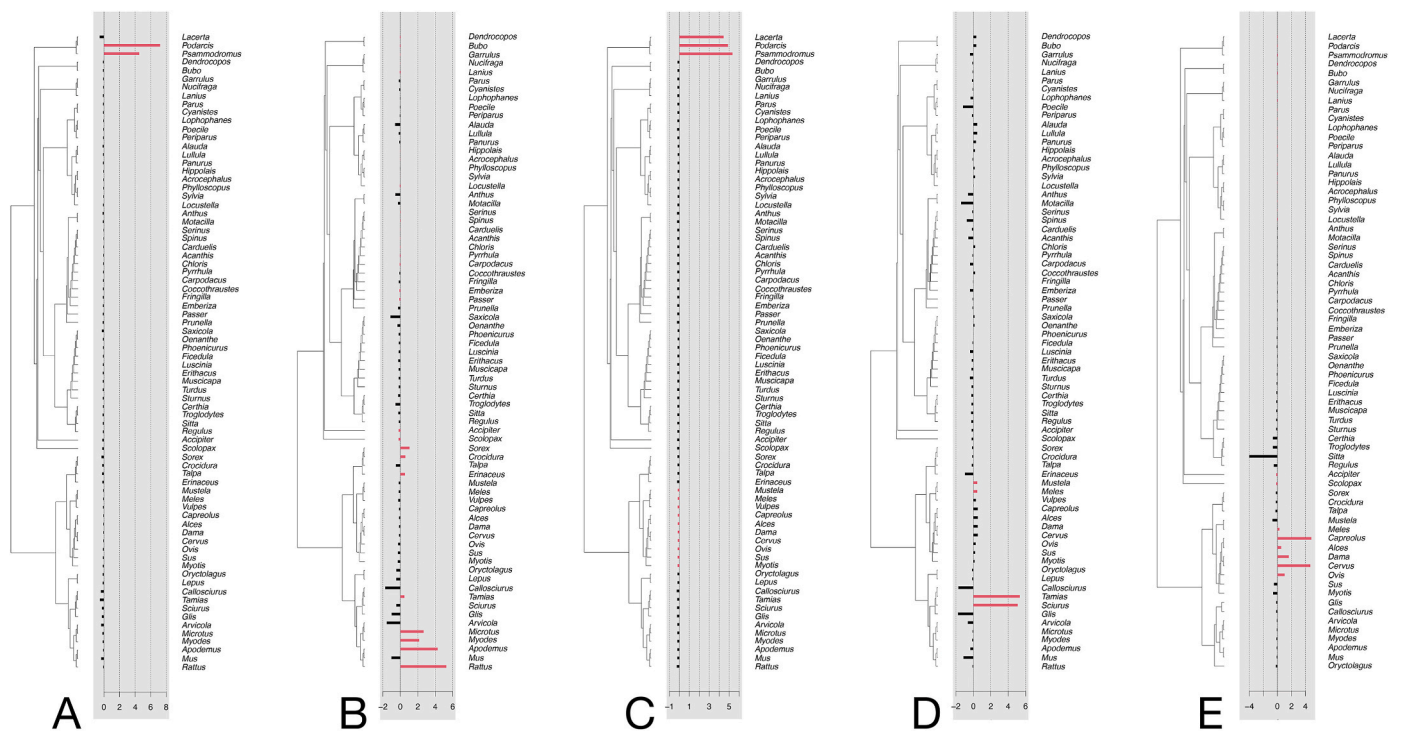

Fig. 2. The phylogenetic signal of *I. ricinus* on genera of vertebrates, as reported for the target territory (Europe and Turkey). Bars indicate the strength of the phylogenetic signal. Values higher than zero (bars to the right in the figure) are associated with a positive phylogenetic signal; values lower than zero (bars to the left in the figure) are associated with a negative signal. Bars in red display statistically significant phylogenetic signals (either positive or negative). Calculations of phylogenetic signal included all the genera of mammals included in this study. **A** Data for the index of the larvae of *I. ricinus* calculated as explained in *Section 2.1*. Positive and significant values were found only for the genera *Podarcis* and *Psammodromus*. **B** Data on Squamata were removed and calculations were redone, therefore obtaining a secondary phylogenetic signal for the genera *Microtus*, *Myodes*, *Apodemus*, and *Rattus* (all of them highly significant) and with a smaller value, but still significant, for the genera *Sorex*, *Crocidura*, and *Erinaceus*. **C** The phylogenetic signal of the index of nymphs on genera of vertebrates. **D** Results of the same calculations obtained after removal of the data on Squamata. **E** The phylogenetic signal of the index of adults of *I. ricinus* on genera of vertebrates.

3. Results

3.1. There is a phylogenetic signal for the hosts of I. ricinus, but best feeding hosts exhibit a spatial overlap with the tick

For the larvae of *I. ricinus*, data from a total of 59,956 vertebrates of 79 genera in 848 published surveys resulted in 12,551 positive animals (prevalence of 20.93%) totaling 62,639 larvae (2.32 larvae/host). However, the distribution of ticks on hosts was highly skewed: for some species, many individuals carried no ticks. The complete raw data regarding feeding hosts for *I. ricinus* is available in Supplementary file S2. The phylogenetic signal of the larval tick burden was clear and focused on lizards (Fig. 2A) including species of the genera *Podarcis* and *Psammodromus*. It must be noted that different species of the same genus yielded different results probably because of the date of collection (thus affected by the tick seasonal activity), the conditions of the survey, or other uncontrolled factors. We considered that the association between larval *I. ricinus* and species of Squamata could obscure other relationships. After the removal of data related to lizards, we conducted a second phylogenetic test (Fig. 2B). This test uncovered a signal related to some species of Eulipotyphla (genera *Sorex*, *Crocidura*, and *Erinaceus*) and Rodentia (genera *Microtus*, *Myodes*, *Apodemus*, and *Rattus*). The phylogenetic signal of the larval tick burden was not significant for birds and was in some cases negatively correlated (Fig. 2A and B). The mean larval burden on Squamata, Soricomorpha, and Rodentia was 9.01, 36.05, and 25.6, respectively; indices of larvae (after correction by the weight of the hosts) for these three groups were 7.78, 7.31 and 7.67, respectively. It was remarkable that the corrected larval load for each group was very similar, supporting the notion of the correction of tick burden by the weight of the host. Whether this is another clue of phylogenetic relationships cannot be deduced from the data. In other words, Rodentia and Eulipotyphla feed more larvae per host, but the higher weight of these animals seemed to be behind such relations.

We obtained data for a total of 76,790 vertebrates in 76 genera (1009 surveys) for feeding nymphs of *I. ricinus*, resulting in 12,724 positive hosts (prevalence of 16.56%) and 71,185 nymphs (1.32 nymphs/host). The phylogenetic signal of the nymphal tick burden was focused on lizards (Fig. 2C) including the genera *Podarcis*, *Psammodromus*, and *Lacerta*. It was interesting that most Carnivora and Cetartiodactyla had a significant negative phylogenetic signal with the nymphal *I. ricinus* burden. We removed data for lizards and reperformed the phylogenetic tests to pinpoint a secondary (and smaller) phylogenetic relationship related to some Rodentia (genera *Tamias* and *Sciurus*, Fig. 2D). The mean nymphal load on Squamata was 1.84; the nymphal index for Squamata (after correction by the weight of the host) was 6.76, like the indices obtained for larvae.

The available information for adults of *I. ricinus*, resulted in 61,271 hosts of 100 genera in 887 surveys, with 12,561 positive hosts to adults of *I. ricinus* (prevalence of 19%; tick load of 0.26 adults/host). The highest burden was recorded for *Cervus*, *Dama*, *Capreolus*, and *Ovis*. Of note, about 80% of records of the ticks on the genus *Ovis* corresponded to domestic sheep. The management of livestock is not the same as for wildlife and may have distorted the phylogenetic signal. After the removal of tick burden from *Ovis* we obtained an average tick load higher than 10 ticks per animal, with a prevalence in these genera of vertebrates of, or near, 100%. Values of tick load dropped quickly but remained higher than 10 for Carnivora (e.g. *Meles*). Adults of *I. ricinus* had a negative (not significant) phylogenetic signal related to some birds and were focused on a clade that included *Certhia*, *Troglodytes*, *Sitta*, and *Regulus*. Phylogenetic signal for adults of *I. ricinus* was absent for all clades of genera in Squamata, Rodentia, Eulipotyphla, and Aves, but the four genera mentioned above (Fig. 2E).

Results obtained by GLMs included in Supplementary file S3 were aimed to capture the combination of variables that could explain the larval, nymphal, and adult indices, other than the purely phylogenetic. The GLMs were done separately for the three stages of the tick. Although

the discriminatory power was low, all of them did show that both prevalence and tick load were better explained by the genus of the host, supporting previous results on the phylogenetic signal. The contribution of other variables differed. Interestingly, the number of surveyed hosts was not a prominent variable in any of the models. Latitude had a slight effect (for larvae), as well as longitude (for adults). The GLMs for larvae showed that the average temperature in February (the end of winter and beginning of spring), May to July (late spring-summer), and the beginning and late autumn (September) had a significant contribution, in an effect that we interpret as the seasonality of the tick, extracted from many heterogeneous reports that may have contributed to blurring the effect. Similar effects of temperature were found for adults (February–May and July–September) but were absent in the model for nymphal data.

We aimed to detect the vertebrates that most could feed/propagate a population of *I. ricinus* in the target territory, using values of larval, nymphal, and adult indices, the overlap of their environmental preferences among them, and overlap with *I. ricinus*. The environmental overlap of vertebrates was considered because the simultaneous presence of several vertebrates that sustain the tick's life-cycle would better increase its populations. We found several genera of vertebrates (Table 1) that most feed/propagate immature/adult stages of the tick. Of note, some of these hosts were those that demonstrated a positive and significant phylogenetic signal for the tick burden.

Of interest in Table 1 was that some of the best feeding hosts had a relatively low phylogenetic signal, but they shared a large portion of the environmental niche with the tick, resulting in a large overlap over the

Table 1

The best feeding/propagation vertebrates (taxonomic genera) for *I. ricinus*, in alphabetical order. The classification is based on a set of rules that included the prevalence, the tick burden and the habitat overlap among the involved vertebrates, and with the tick.

Genera[a]	Larval index	Nymphal index	Adult index	Overlap with *I. ricinus* (0–1)
Alauda	190.59			0.80
Alces			85.38	0.45
Anthus	125.86			0.78
Apodemus	266.30			0.72
Capreolus		73.17	214.34	0.79
Cervus			100.29	0.67
Crocidura	68.64			0.46
Dama			71.42	0.55
Erinaceus[b]	2167.04	449.98		0.78
Lacerta	94.59	169.09		0.87
Microtus	434.57			0.93
Myodes	178.49			0.93
Ovis	50.18	110.40	100.37	0.83
Podarcis	241.94	75.37		0.75
Prunella		68.59		0.97
Psammodromus	2621.27	179.95		0.56
Rattus	2254.82	264.30		0.85
Saxicola	141.79			0.96
Sciurus	527.60	839.07		0.94
Sorex	100.40			0.94
Talpa	114.43			0.96
Tamias	1321.32	100.34		0.88
Troglodytes	65.93			0.96
Turdus[c]	55.39	71.73		0.96

Notes: Data are summarized in three columns including the propagation effectivity for each tick stage, plus a fourth one that includes the niche overlap between the vertebrate(s) and *I. ricinus*.

[a] Values of prevalence/burden for the same species of vertebrate may be different according to different sites of the wide area considered: included is the mean of all the observations.

[b] Only six studies were available for *Erinaceus roumanicus*, therefore the bias of the results should be considered.

[c] Several species of the genus were included, namely *Turdus iliacus*, *Turdus merula*, *Turdus philomelos*, and *Turdus pilaris*. The mean was calculated for these species.

territory. This is an important finding. Hosts with high phylogenetic signal may be quite restricted in space, and therefore sustain the tick only locally. However, hosts with large environmental overlap with the tick sustain the life-cycle almost everywhere, even with low tick loads; these are regarded as gained by "environmental sharing".

The expected geographical range of the indices of tick burden for the three stages of *I. ricinus* is summarized in Fig. 3. Data were obtained by the sum of the larval (or nymphal, or adult) indices for every host involved, weighted by the environmental overlap of the tick with these hosts, and averaged according to the biogeographical regions of Europe. Areas of maximum support of the tick's life-cycle were similar for the three stages, with small differences across the wide territory examined. Highest support extended in areas of central-western Europe, Ireland, United Kingdom, and northern Iberian Peninsula, overlapping with the biogeographical regions Atlantic North and Atlantic Central, with portions of the Lusitanian region. Ranked second were areas of the Baltic and northern countries. In these domains, the tick finds the largest number of hosts that most contributed to the feeding of immatures and propagate adults. We aimed to capture the composition of the communities of vertebrates that support the life-cycle of the tick in the different biogeographical regions (Fig. 4). It was of interest that the genera of vertebrates sustaining the life-cycle change according to regions. For example, the importance of Lacertidae for larvae decreases along the gradient from Atlantic Central to Continental regions (Fig. 4A). Similar results could be observed for adult ticks and their propagation hosts, that increase notably in the Lusitanian and Atlantic Central ranges (Fig. 4C). In any case, the results supported the hypothesis that a few hosts take different relative importance according to the biogeographical domain. The community of vertebrates supporting the *I. ricinus* life-cycle changes spatially and each host has a relative importance that changes spatially.

3.2. The phylogenetic signal and the amplification of Borrelia burgdorferi (s.l.)

Calculations were carried out on reports including 61,446 larvae of which 7664 (12%) were further individually analyzed for identification of the genospecies of *Bb*; this resulted in a total of 882 host specimens for which the prevalence data of *Bb* in feeding larval *I. ricinus* were available. Hereinafter, we include the results for the species of *Bb* for which enough data were available in the compilation. The raw data regarding the amplification of *B. burgdorferi* (*s.l.*) by larvae of *I. ricinus* feeding on hosts in the Western Palaearctic are available in Supplementary file S4.

The phylogenetic signal of four species of the genus *Borrelia* (*Borrelia afzelii*, *Borrelia garinii*, *Borrelia lusitaniae*, and *Borrelia valaisiana*) was clear (Figs. 5 and 6). We could not carry out the analysis of the data for *Borrelia bavariensis* because of the low number of available records. The

reliability of the identification of these *Bb* was not re-assessed and was included as published. The larvae of *I. ricinus* carrying *Borrelia afzelii* had a strong phylogenetic link with *Microtus arvalis*, *Myodes glareolus*, *Apodemus flavicollis*, and *Apodemus sylvaticus* (Fig. 5A). It is noteworthy that many species of birds showed a significant but very small signal with larvae infected by *B. afzelii*. On the other hand, larvae infected with *B. garinii* were phylogenetically associated with three species of the genus *Turdus* (Fig. 5B). The main phylogenetic signal of *B. lusitaniae* was associated with lizards of the genera *Lacerta*, *Podarcis*, and *Psammodromus* (Fig. 6A), with a small and significant signal for *Erinaceus* and *Meles* (Fig. 6B) that we could not ignore. The largest positive and significant phylogenetic signal of *B. valaisiana* was related to birds (*Turdus merula* and *Turdus philomelos*) with a small but significant signal associated with a large cluster of vertebrates, including *Capreolus*, *Alces*, *Dama*, *Meles*, *Erinaceus*, *Apodemus*, and *Myodes*.

Table 2 includes the genera of hosts found to be involved in the amplification of every species of *Borrelia* analyzed. It showed the involvement of *Apodemus* spp. in the amplification of both *B. afzelli* and *B. garinii* (but only seven positive larvae out of 480 for the latter), or the supposed high contribution of *Motacilla* spp. for *B. afzelii* (for which only one larva was analyzed and found positive). However, both genera of vertebrates had a clear negative phylogenetic signal with these *Bb*, which supported the comments about these probably serendipitous results.

We succeeded to associate the results of the amplification of four species of *Borrelia* (*B. afzelii*, *B. garinii*, *B. lusitaniae*, and *B. valaisiana*, Fig. 7A–D, respectively) according to the bioclimatic regions of Europe. It must be considered that a heterogeneous number of records for each species and competent reservoirs was available for each bioclimatic region; results may be biased because of an unbalance of surveys. All the vertebrates reported in each biogeographical region have been included, even if they resulted in zero percent of infection in feeding larvae. The different spatial amplification of each species was well notable and overlapped the territories in which main competent reservoirs are prevalent. In the maps in Fig. 7, different amplification was observed in the four genospecies of *Bb*. *Borrelia lusitaniae* was the genospecies with the highest amplification (with a few vertebrates contributing to its amplification and the local-regional patterns of contact with the vector). Interestingly, the minimum (averaged) value of amplification of the species was higher than maximum values for *B. afzelii* and well over the range of values of either *B. garinii* or *B. valaisiana*.

We further addressed the distribution of the competent reservoirs across the bioclimatic regions of Europe to know if different vertebrates are associated with the amplification of *Borrelia* spp. according to the region (Fig. 8). All the available reports were included in these figures, even if this resulted in an amplification of zero percent. *Borrelia afzelii*
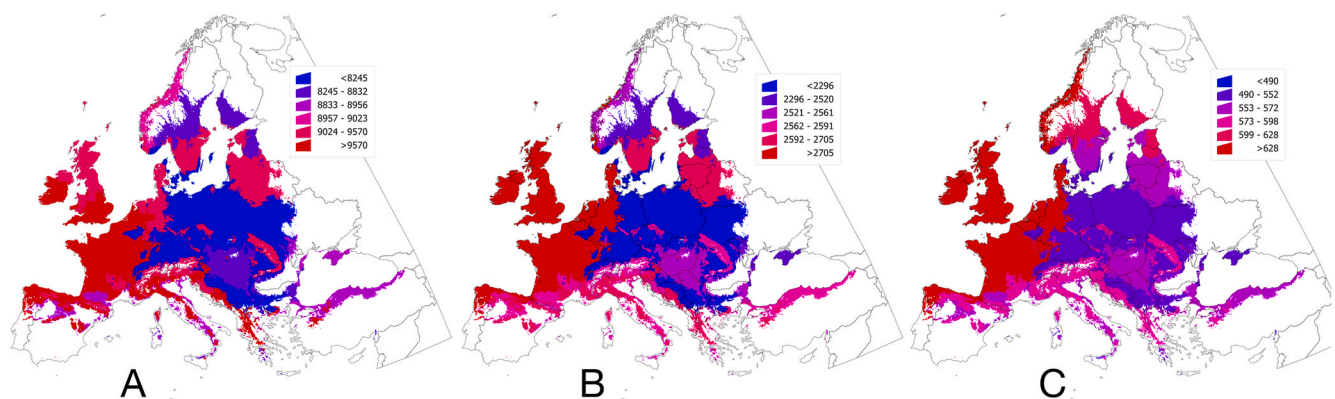

Fig. 3. The propagation of the three life stages of *I. ricinus* in the target territory. It is an estimation of the "force of support" of *I. ricinus* disaggregated according to the biogeographical regions in the Western Palaearctic. Units in the legend represent the index of larvae (nymphs or adults) multiplied by the environmental overlap with hosts. Maps were produced with the larval, nymphal, or adult indices (see *Section* 2.1) multiplied by the sum of habitat overlap between each host and *I. ricinus*, separately for each stage. **A** Larvae. **B** Nymphs. **C** Adults.
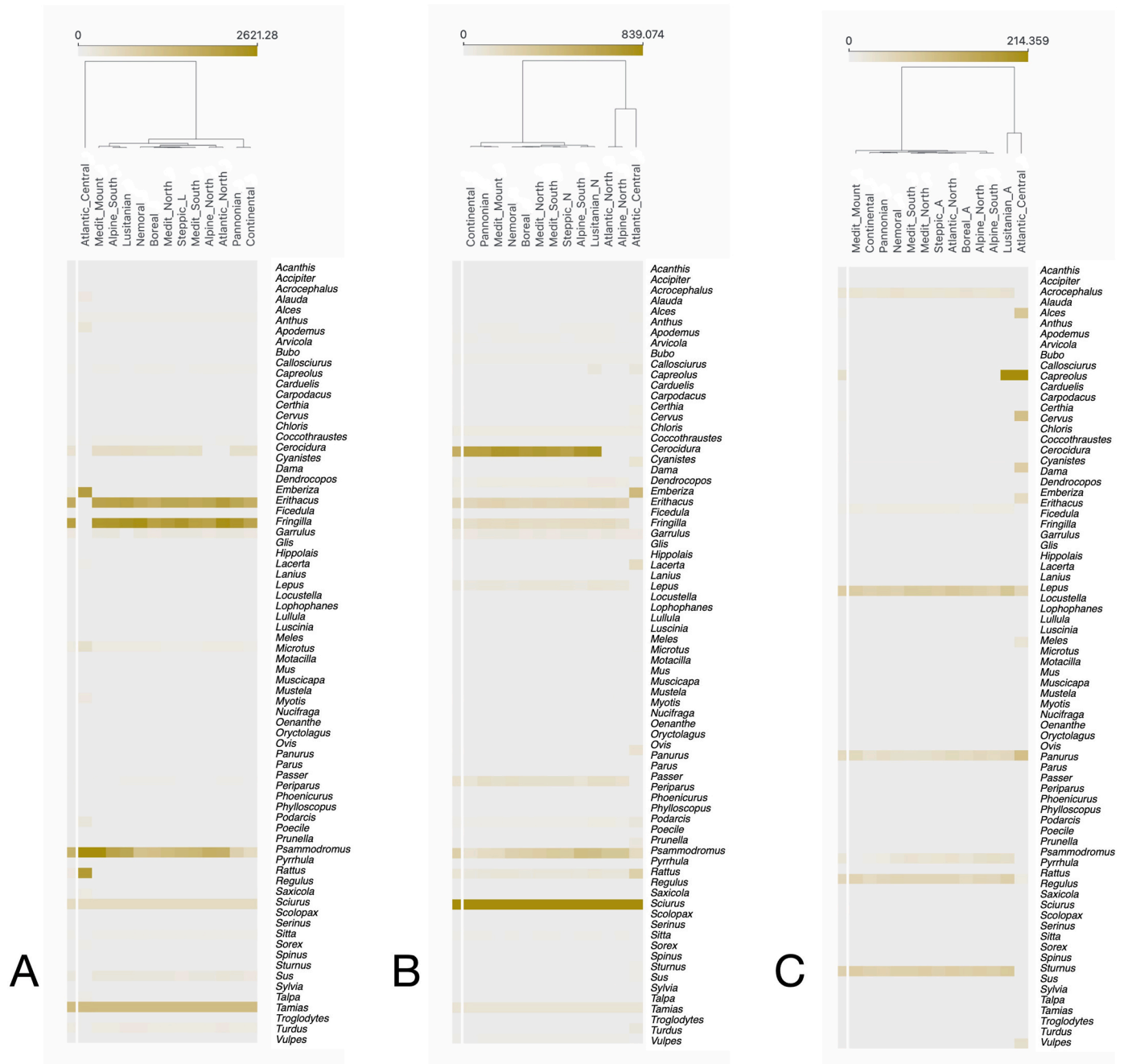
Fig. 4. The relative importance of each species of vertebrate in the feeding/propagation of *I.* ricinus. The importance of each host in different biogeographical regions was evaluated according to the environmental overlap with *I. ricinus* and its ability to feed/propagate each stage of the tick's life-cycle. Each panel represents a stage of the tick's life-cycle, with the relative contribution of the vertebrates supporting such life stage in each bioclimatic region. The results indicate which vertebrate(s) are better supporters of the tick life-cycle in each biogeographical region. The left colored band in each panel indicates the average for such a stage. The colors are proportional to the larval, nymphal, or adult indices, weighted by the environmental overlap in each region with the available hosts. Columns represent bioclimatic regions clustered with a k-means algorithm to display their similarity according to results. Rows represent vertebrate genera. Units in the legend represent the index of larvae, nymphs, or adults, multiplied by the environmental overlap with hosts. **A** Larvae. **B** Nymphs. **C** Adults.

(Fig. 8A) had medium amplification rates, observed mainly in larvae feeding on *Myodes glareolus*, *Apodemus flavicollis*, and *Apodemus sylvaticus* mostly in the Atlantic regions. Therefore, *B. afzelii* used at least three vertebrates (which are also good propagation hosts for larvae) as amplification reservoirs in most of the target territory. In the case of *B. garinii*, the data showed a high link with *Turdus* spp., as already observed for the phylogenetic signal (Fig. 8B). However, it is necessary to mention the high plasticity of this microorganism, since it could be transmitted to larvae of the vector by a variety of other reservoirs, such as species of *Anthus*, *Carduelis* or *Parus*, present in most biogeographical regions of the target region. We could not find a coherent argument to explain the reports on *Rattus* or *Apodemus*. Two genera of lizards have different amplification abilities according to the territory: *Psammodroums algirus* is replaced by *Podarcis muralis* in other regions. *Borrelia valaisiana* resulted deeply linked to *Turdus* spp., in similar proportions at every bioclimatic region, with some other species of birds participating in its circulation; no mention of mammals infecting larval ticks of *I. ricinus* with *B. valaisiana* has been found in the published literature
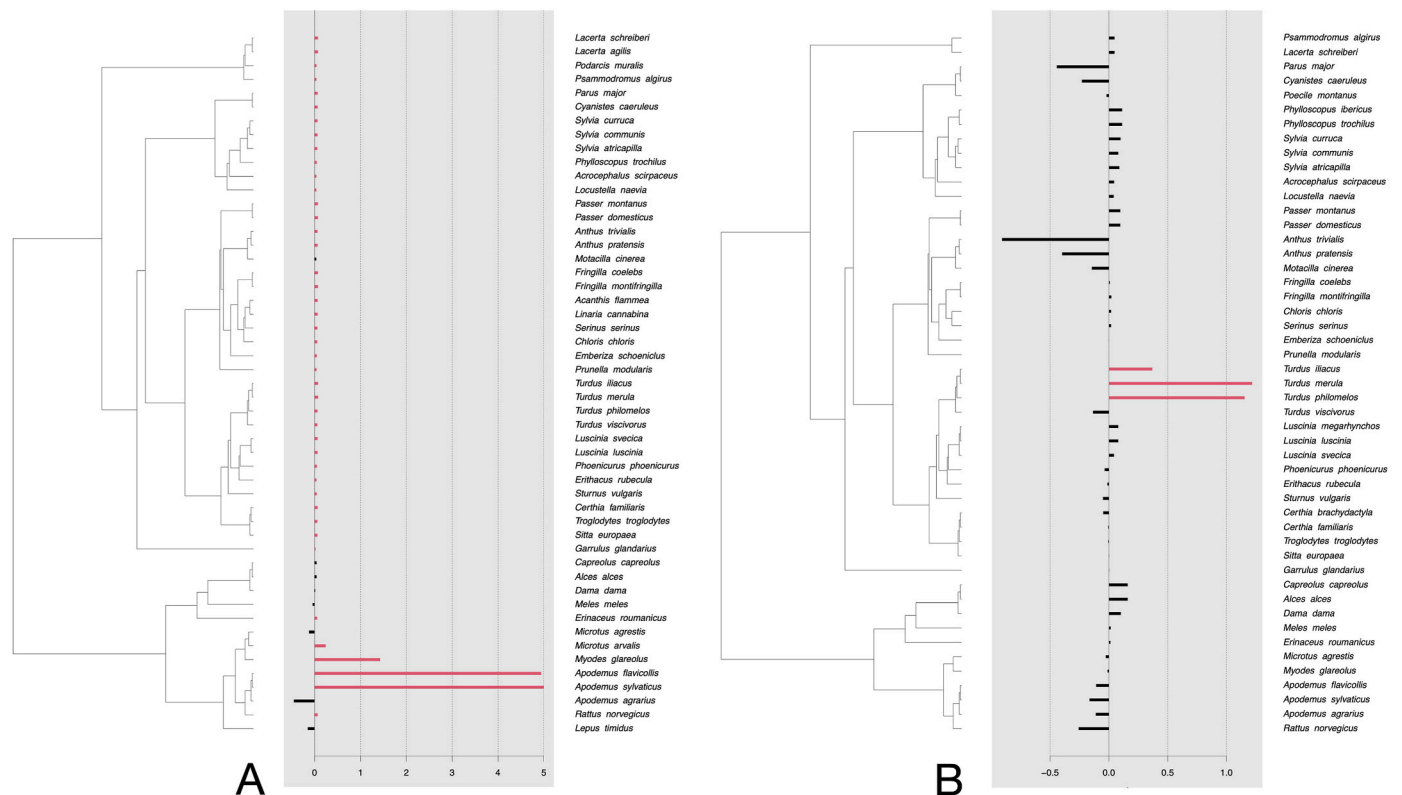
Fig. 5. The phylogenetic signal for the prevalence of *B. afzelii* and *B. garinii* in feeding larvae of *I. ricinus*, as reported for the target territory. Bars indicate the strength of the phylogenetic signal. Values higher than zero (bars to the right in the figure) are associated with a positive phylogenetic signal; values lower than zero (bars to the left in the figure) are associated with a negative signal. Bars in red display statistically significant phylogenetic signals (either positive or negative). Calculations of phylogenetic signal included all the genera of mammals included in this study. **A** *B. afzelii.* **B** *B. garinii.*

that fulfill all the requirements to be included in the meta-analysis. Each genospecies of *Bb* circulates by the same and few species of vertebrates, for which phylogenetic signal was significantly high. There were no "replacement" reservoirs that could assume the role of amplifying hosts in the absence of those mentioned herein. Interestingly, the main amplification hosts are present in every bioclimatic region where the tick exists. Moreover, as far as our data demonstrated, the community of vertebrates circulating *Borrelia* spp. is similar in every part of the continent.

4. Discussion

We examined whether the tick burden of *I. ricinus* on vertebrates, as revealed by a compilation of published field surveys, had a positive association with branches of the phylogenetic tree of the hosts. This may seem obvious for specialist species of ticks but has never been tested in a generalist tick, like *I. ricinus*. Our findings showed that the three stages of *I. ricinus* have such phylogenetic signal towards some genera of vertebrates. This means that the tick burden of *I. ricinus* is not only passively derived from ecological relationships (environmental sharing) but has a phylogenetic root with a few vertebrates. *Ixodes ricinus* is a plastic species, adapted to feed on a large range of vertebrates, being resilient under varied combinations of the communities of vertebrates. While phylogenetic relationships may be important in this context, the plasticity is clearly manifested in the variable degree of ecological overlap with 222 species of vertebrates, resulting in an intricate network of contact rates, as described (Estrada-Peña and de la Fuente, 2016). *Ixodes ricinus* persists in its range using a combination of hosts that co-evolved with the tick, together with many species of vertebrates sharing the environmental niche. This is a novel finding, demonstrating the echoing primitive associations with some vertebrates before the last glaciation.

The removal of such a main signal uncovered a second group of phylogenetically associated vertebrates (Rodentia and Eulipotyphla). These could be associations among groups of taxa spreading quickly from glaciation refugia and sharing portions of the environmental niche, that remained today as a watermark in the many reported tick-hosts associations.

It is important to remark that neither larvae nor nymphs of *I. ricinus* displayed significant phylogenetic signal to birds, but they were pointed out as prominent reservoirs across the target territory. The most plausible conclusion is that the wide use of birds by the immatures of the tick could result from environmental sharing, resulting in spatial overlap. We postulate that the relationship of *I. ricinus* with birds is a recently gained trait that may have been underrated by de la Fuente et al. (2015) in terms of its geological age.

The phylogenetic signal of immatures to lizards and secondarily to Rodentia and Eulipotyphla overlaps well with the southern refugia of animals in the last glaciation. It could indicate how the tick adapted to available hosts before its widespread European distribution after the Pleistocene glaciations (Røed et al., 2016) acquiring new hosts while spreading northern from the refugia in the south, although refugia were not restricted to the Mediterranean region (Ferrero et al., 2011). This could result in Squamata being the primary hosts found in the Mediterranean region, then acquiring the feeding habit on warm blooded small mammals, as the tick expanded and reached the regions where these vertebrates also spread after the last glaciation (Vollmer et al., 2013; Røed et al., 2016). Interestingly, this is the same situation existing for species of the *I. ricinus* group in the USA, where immatures may parasitize Squamata, even if the most important hosts reported for other parts of the country are present (Tietjen et al., 2020).

We demonstrated that larvae of *I. ricinus* have a strong phylogenetic signal to lizards of the genera *Psammodromus* and *Podarcis*, which have
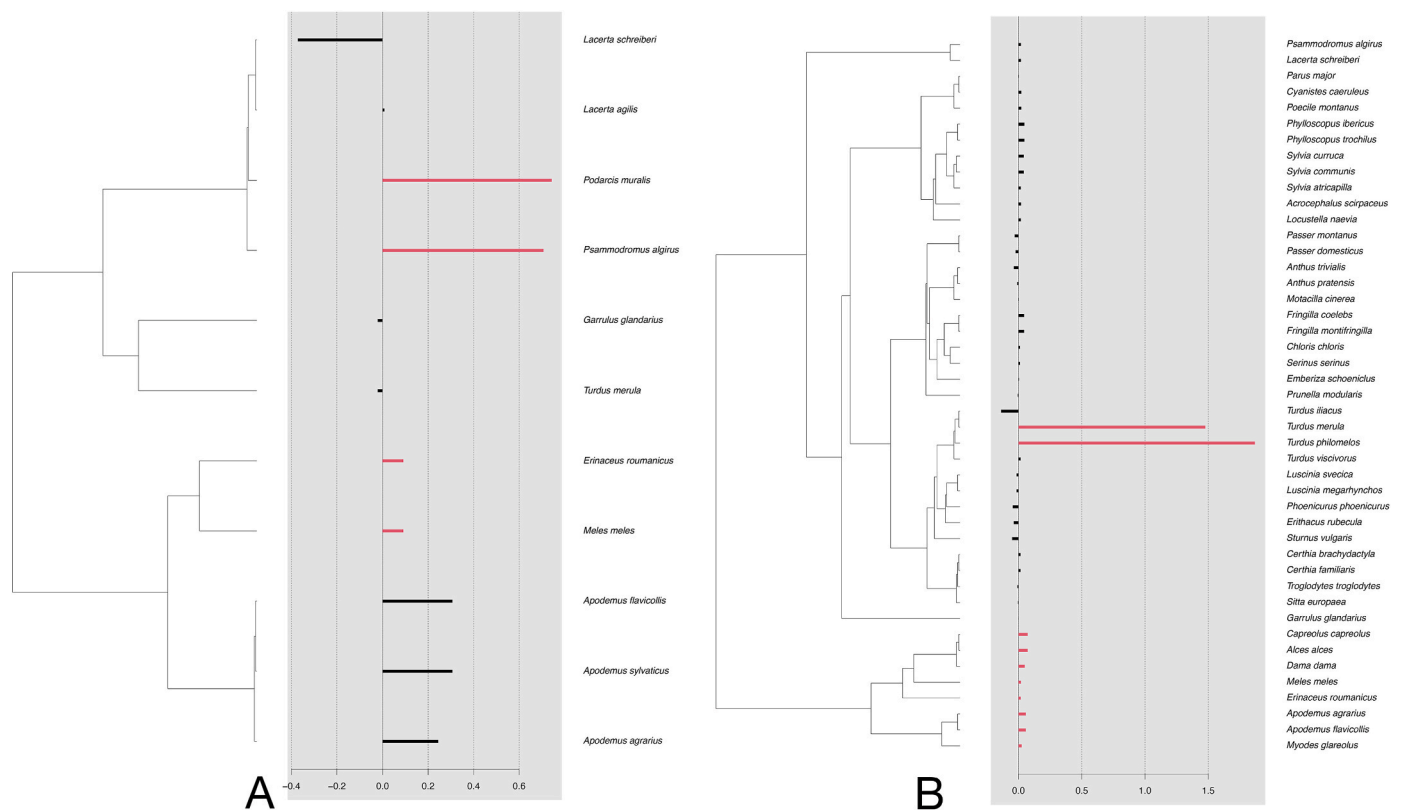
Fig. 6. The phylogenetic signal for the incidence of *B. lusitaniae* or *B. valaisiana* in feeding larvae of *I. ricinus*, as reported for the target territory. Bars indicate the strength of the phylogenetic signal. Values higher than zero (bars to the right in the figure) are associated with a positive phylogenetic signal; values lower than zero (bars to the left in the figure) are associated with a negative signal. Bars in red display statistically significant phylogenetic signals (either positive or negative). Calculations of phylogenetic signal included all the genera of mammals included in this study. **A** *B. lusitaniae.* **B** *B. valaisiana.*

mainly a Mediterranean distribution, as well as parts of Atlantic north and Atlantic central (Mücher and Wascher (2007), Escoriza and Amat, 2021; ecological terminology derived from Mücher and Wascher (2007)). The phylogenetic signal of nymphs is similar, and includes reptiles of the genus *Lacerta*, supporting the tick in central and northern Europe. Interestingly, the current distribution of *Lacerta* makes to consider that this association was gained later, after the spread from glaciation refugia. These are short-lived animals, living in shelters, with a high reproductive potential. Further on this, the tick is environmentally (ecologically) associated with birds (medium lifespan, medium reproductive potential, living in nests) that contribute to support tick's resilience in most of its spatial range. Lacertida, Rodentia, Eulipotyphla and Aves (Passeriformes) are the core of the vertebrates upon which the immatures feed. Such phylogenetic signal of *I. ricinus* could reflect primitive associations between the tick and some vertebrates tracking the spread of the tick after the last glaciation.

Our results support the existence of a previously unknown phylogenetic link between the adults of *I. ricinus* and ungulates. Such signal exists, despite the many reports of adult ticks on Carnivora, and the few records on small mammals and birds. Females of *Ixodes ricinus* need a large bloodmeal to copulate; an association with the large animals that share the environmental niche, and that can support the feeding of many adult ticks, looks coherent. We found a clear phylogenetic signal of adult ticks towards the ungulates of the genera *Capreolus*, *Alces*, *Dama*, and *Cervus*, with a minimal contribution by Carnivora. These findings corroborate previous reports about the suitability of medium-large ungulates for adults of the tick (Mysterud et al., 2021). In any case, this is the first demonstration of host preferences by the adults of *I. ricinus*. Our results also shed light on the reports of large numbers of nymphs on ungulates: adults of *I. ricinus* are phylogenetically associated with animals of medium or large size, long-lived, that have a low number of

descendants (commonly one-two per year).

Some unexpected results were derived from the GLMs, like the slight effect of the coordinates of collection on the general results. This effect was only apparent for larvae and in a lesser degree, for adults. The GLMs demonstrated that the monthly temperature in February or June–July could positively influence the larval index. The data compiled for this study were not designed to demonstrate the effect of temperature on development and/or survival of ticks, and therefore we can only hypothesize. These dates corresponded well with the beginning of the larval activity after the winter, as well as the probable negative effect of the high summer temperature or low humidity on larval questing. It did not escape our attention that these correlations could outline the overlap of activity of the larvae and their hosts, therefore impacting the results on prevalence and tick burden. These results are supported by previous reports about the seasonal variability of the relative importance of each host for *I. ricinus* (e.g. Craine et al., 1995). The point is not only *when* the ticks are questing, but *which hosts* are also active at such period, and how predominant are they in the community of vertebrates. These periods of "concentration" of ticks on hosts could be in fact driven by behavioral traits of the tick, related to the prevailing climate, allowing larvae to be recruited into the cohort of active specimens at the right moment of the year to overlap with hosts. If a genetic basis for such behavior exists, the pattern reported in this study would reflect a deep relationship between *I. ricinus* and vertebrates based on ecological determinants; most probably these are local adaptations aimed to overlap with predominant hosts. On the other hand, it is necessary to remind that ticks were collected when they were actively feeding on hosts. It seems possible that such a pattern (reflected by the prevailing temperature) could be reflected in GLMs giving the wrong impression that a stational pattern drives prevalence and tick load.

Ixodes ricinus is a generalist feeder (e.g. Mysterud et al., 2015).

Table 2

The vertebrates that amplify genospecies of *Bb* measured as their prevalence in feeding larvae, in alphabetical order. All the vertebrates for which identification of *Borrelia* spp. genospecies was available are included.

Vertebrate	B. afzelii	B. bavariensis	B. burgdorferi (s.s.)	B. garinii	B. lusitaniae	B. valaisiana
Acanthis flammea	0					
Acrocephalus scirpaceus	0		0	0		0
Alces alces	0		0	0		0
Anthus pratensis	0		0	0		0
Anthus trivialis	0	3.55	0	7.69		0.89
Apodemus agrarius	0.38	0	0	0	0	0
Apodemus flavicollis	25.84	1.51	0.66	2.40	0	0
Apodemus sp.	1.89				0	
Apodemus sylvaticus	28.36	5.84		0	0	
Capreolus capreolus	0		0	0		0
Carduelis cannabina			0	0		0
Carduelis flammea			0	0		0
Certhia brachydactyla				0		0
Certhia familiaris	0		0	1.04		0
Chloris chloris	0		0	0		0
Cyanistes caeruleus	0		0	0		0
Dama dama	0.41		0	0		0
Emberiza schoeniclus	0		0	0		0
Erinaceus roumanicus	6.10	0.34	0	0.17	0	0
Erithacus rubecula	0.42	0	0.15	0.53		0.08
Fringilla coelebs	0.13	0	0	0.82		0
Fringilla montifringilla	0	0	0	0		0
Garrulus glandarius	0		0	0	0	0
Lacerta agilis	0				11.09	
Lacerta schreiberi	0			0	0	0
Lepus timidus	0		3.23			
Linaria cannabina	0					
Locustella naevia	0		0	0		0
Luscinia luscinia	0		0	0		
Luscinia megarhynchos				0		0
Luscinia svecica	0		0	0		0
Meles meles	0		0	0.24	0	0
Microtus agrestis	1.40			0		
Microtus arvalis	4.17					
Motacilla cinerea	0.81		0.81	0		0
Myodes glareolus	44.62	0	0	0.25		0
Parus major	0.13	0	0	3.49		0.43
Passer domesticus	0			0		0
Passer montanus	0			0		0.76
Phoenicurus phoenicurus	0	0	0	0		0
Phylloscopus ibericus				0		0
Phylloscopus trochilus	0	0	0	0		0
Podarcis hispanica	0			0		0
Podarcis muralis	0				50.22	
Podarcis spp.					7.31	
Poecile montanus				0		0
Prunella modularis	0	0	0	0.64		0.91
Psammodromus algirus	0			0	54.81	0
Rattus norvegicus	2.83		2.02	5.25		
Serinus serinus	0	0	0	0		0
Sitta europaea	0			1.15		0
Sturnus vulgaris	0		0	0		0
Sylvia atricapilla	0		0	0		0.25
Sylvia communis	0		0	0.26		0
Sylvia communis				0		0
Sylvia curruca	0		0	0		0
Sylvia melanocephala				0		0
Troglodytes troglodytes	0.26	0	0.36	1.34		0.62
Turdus iliacus	0.14	0	0	1.26		0.37
Turdus merula	0.06	0.41	0	10.42	0	10.42
Turdus philomelos	0	0.29	0	6.05		5.07
Turdus viscivorus	0	0	0	0.48		0.48

Notes: The table is compiled from published data and is based on the ability of a vertebrate to infect feeding larvae feeding on it, without any other transformation of the value. Empty cells are those for which data were lacking. Because the results on incidence can be diluted if several species of the same genus are averaged, species of vertebrates were used in this table instead of genera.

Evidence presented in the present study suggests that (i) the immatures of the tick have primary and secondary preferences towards different groups of hosts, (ii) the wide availability of some hosts ensures the support and resilience of the population of ticks, and (iii) local changes in the community of vertebrates allow the "switch of hosts" to be a cornerstone of tick population resilience. The plasticity of *I. ricinus* does not rely only on its phylogenetic signal with vertebrates, but on its ability to exploit a range of vertebrates that share the environmental niche. This conclusion has been underlined previously using network constructs that demonstrated how the structure and relationships within the tick host's community evolved in such direction (Estrada-Peña et al., 2015, 2017).
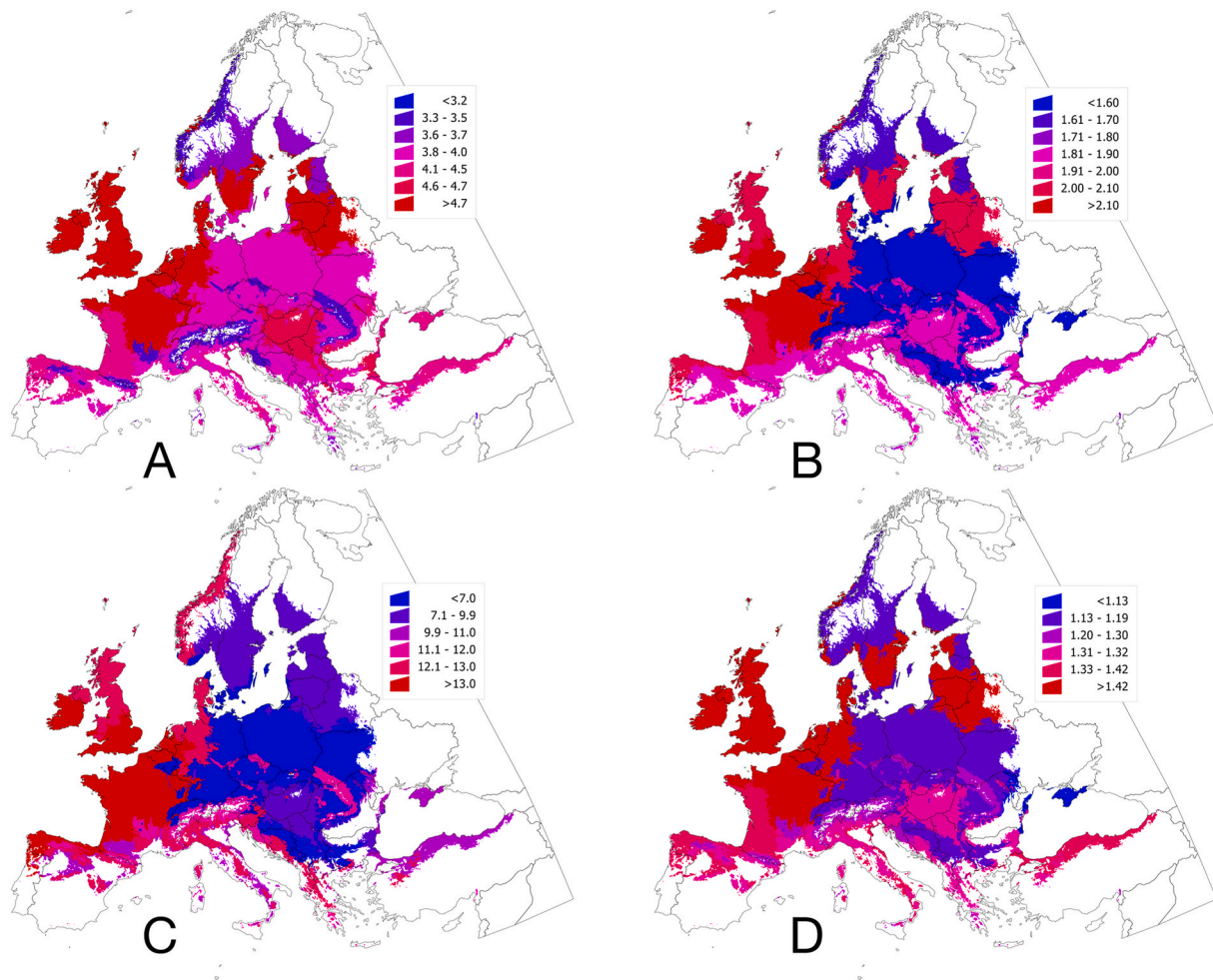
Fig. 7. The expected amplification in the target territory of *B. afzelii* (**A**), *B. garinii* (**B**), *B. lusitaniae* (**C**), and *B. valaisiana* (**D**) by the feeding larvae of *I. ricinus*. It is an estimation of the amplification of *Borrelia* spp. disaggregated according to the biogeographical regions in the Western Palaearctic. Units in the legend represent the amplification of each species of *Borrelia* by each host involved, multiplied by the sum of the environmental overlap of each host with *I. ricinus*. **A** *B. afzelii*. **B** *B. garinii*. **C** *B. lusitaniae*. **D** *B. valaisiana*.

We further used a large dataset of the ability of vertebrates to infect feeding larvae of *I. ricinus* with *Bb*. We considered the larvae of the tick as the best indicator of infection amplification from the infection rates; nymphs may have been infected with two genospecies because they pass through two bloodmeals. Data about the amplification of *Bb* by competent reservoirs feeding larval ticks have been already reported (Steinbrink et al., 2022). We demonstrated that the pathogen's genospecies are differently amplified in the bioclimatic regions of Europe, using *the same reservoirs* for each genospecies in the communities of vertebrates, which change across the gradient of abiotic conditions. This is the first study pinpointing areas of different amplification in a large geographical range because of the spatial restriction of competent reservoirs that follow the environmental niche. Although we detected a "noise" in the background of the reports, the relationships between the genospecies and competent reservoirs was demonstrated to have a phylogenetic signal. For example, *Borrelia afzelii* is not associated with a wide array of Rodentia, but mainly with *Apodemus* and *Myodes*. The signal of *B. garinii* is linked to *Turdus,* even if other small birds carry infected larvae and nymphs of the vector. We hypothesize that these "secondary species of birds" could have an active role in both spread and amplification when the populations of main reservoirs are critically low. We could not find the use of divergent taxa as competent reservoirs (e.g. Steinbrink et al., 2022), but we found small differences in ability to infect tick larvae according to the biogeographical regions. We would like to call the attention on unexpected relationships, like *B. bavariensis*

and birds, that most probably represent *B. garinii* OspA type 4 (Margos et al., 2019).

We wanted to stress the impact of the combined plasticity of the tick together with the phylogenetic relationships with the vertebrate that contribute to the circulation of microorganisms. The tick overlaps its range with more than two hundred vertebrates at spatially variable rates, circulating the pathogen to potential reservoirs. We consider that the most important conclusion regarding *Borrelia* spp. is that at the studied scale and with the limitations of this compilation, data do not suggest that changes in the community of vertebrates could drive a switch in the reservoirs used by the pathogen. While the network of hosts for *I. ricinus* is redundant and resilient, the opposite stands for *Borrelia* spp., as proposed previously (Estrada-Peña et al., 2017). These results overlap with those obtained in a previous approach reporting the incidence of *Borrelia* spp. in questing nymphs of *I. ricinus* (Estrada-Peña et al., 2018b). However, an explicit numerical comparison cannot be provided, since maps of such previous meta-analysis were developed as six categories of the incidence of *Borrelia* in ticks, and not as a numeric approach. In any case, we include these maps for comparative purposes in Supplementary file S5.

Borrelia lusitaniae is the genospecies with the highest amplification (with a few vertebrates contributing to its amplification). Interestingly, the minimum (averaged) value of amplification of the species is higher than maximum values for *B. afzelii* and well over the range of values of either *B. garinii* or *B. valaisiana*. *Borrelia lusitaniae* is so far linked to small
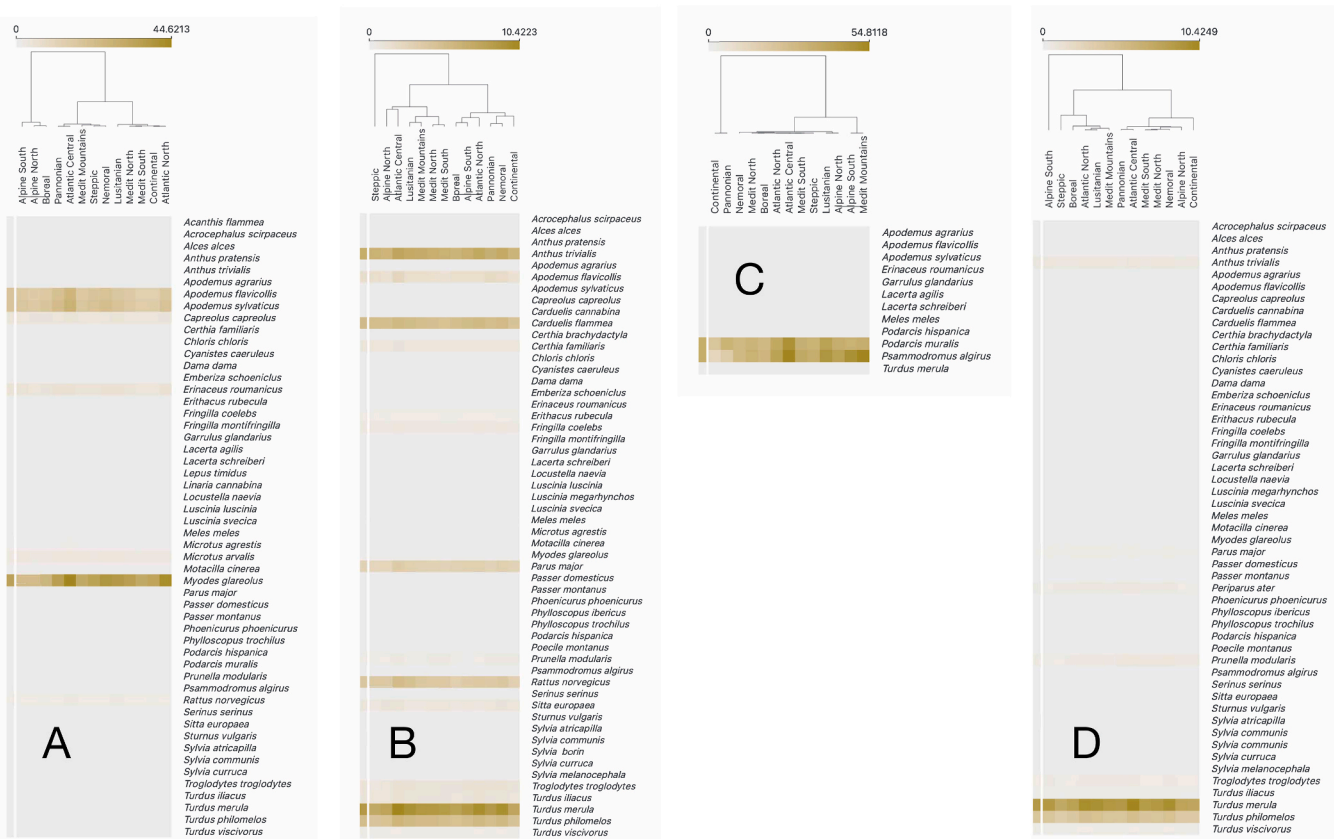
Fig. 8. The relative importance of each species of vertebrate in the amplification of *B. afzelii*, *B. garinii*, *B. lusitaniae*, and *B. valaisiana* by feeding *I. ricinus* larvae, in the different biogeographical regions. The importance of each host has been evaluated according to the environmental overlap with *I. ricinus* and its ability to amplify *Borrelia* spp. Each panel represents a species of *Borrelia*, with the relative contribution of the vertebrates supporting such species in each bioclimatic region. The results indicate which vertebrate(s) are better amplifiers of the bacterium. The colored band at left in each panel indicates the average for such species. The colors and the associated values in the legend are the amplification of each *Borrelia* spp. Columns represent bioclimatic regions clustered with a k-means algorithm to display their similarity according to results. Rows represent vertebrate genera. **A** *B. afzelii*. **B** *B. garinii*. **C** *B. lusitaniae*. **D** *B. valaisiana*.

and medium lacertids, not to every species of Squamata existing in the target region. We propose that this genospecies of *Borrelia* has the highest amplification in feeding larvae because the number of competent reservoirs is low, and its environmental niche is relatively small. Margos et al. (2022) already pointed out that *B. lusitaniae* has a low prevalence in eastern Europe. Here, we demonstrate that known competent reservoirs with high phylogenetic affinities towards the microorganism (and feeding hosts for the vector) are absent in that area and therefore its amplification should be very low.

This study has gaps and limitations. Results and conclusions have been drawn from already published data that may have an obvious bias towards locally abundant vertebrates, easily trappable species, or not protected animals. The geographical distribution of the studies could be related to the spatial pattern of activity of the research teams working on the topic. Moreover, the identification of genospecies of *Borrelia* may be unreliable; in any case, we addressed data "as is" and reported the crude findings, without subjective evaluations. Further, current datasets compiling morphological and/or physiological data for vertebrates tend to be incomplete and heterogeneous. For example, AVONET (Tobias et al., 2022) includes six ecological variables and 11 continuous morphological traits for birds, that are not comparable with those for mammals, like in PanTHERIA (Jones et al., 2009), EltonTraits (Wilman et al., 2014) or COMBINE (Soria et al., 2021). Further, the abundant reproduction data available in COMBINE are unavailable for many (if not all) of the genera in Passeriformes rendering comparisons difficult. EltonTraits (Wilman et al., 2014) includes data on the foraging stratum for both birds and mammals, but mammals reported as hosts are

classified always as "over the ground". The study was designed to understand the probable phylogenetic signal among ticks, pathogens, and vertebrates, behind a large dataset of these associations, restricted to Europe. It is not purposed to address the possible dilution effect emanating from these associations. Analytical studies (e.g. Halsey and Miller, 2020) have focused on the importance of the composition of communities of vertebrates to produce such dilution of the pathogen in the tick populations. We could underline similar conclusions, indirectly obtained from our conclusions on the amplification effect. Changes in the community composition and the behavior of vertebrate species would undoubtedly promote local or regional phenomena compatible with the dilution effect as originally enunciated (Ostfeld and Keesing, 2000).

Our approach to the amplification rates of *Borrelia* spp. or the support of *I. ricinus* life-cycle adhered to the expected spatial distribution of each vertebrate and grading the relative importance of each host feeding the tick from its PNO, a well-known measure of niche overlap in organisms (Broennimann et al., 2012; Vela Diaz et al., 2020). Because of the lack of other indices, the use of prevalence and tick burden corrected by the weight of hosts, and the contact rates among vertebrates and the tick vector, may not be the best input of the phylogenetic signal, but is one of the few that could be applied to the variety of formats of each report. We encourage providing reports about the prevalence of hosts with ticks and the tick load on individual hosts with enough data. This would allow a complete view of the studied system.

5. Conclusions

Our bibliographical study on the prevalence and incidence of the prominent tick *I. ricinus* in its large distribution range, including more than 1100 published reports, concluded that the phylogenetic relationships of their hosts have an influence on the tick burden. Some climate variables also have a general influence on these parameters. Results of this study point to the need for deeper studies on the influence of faunal composition (and hence host diversity and relative abundance) on the circulation of tick-borne pathogens. We succeeded in isolating the phylogenetic signal of the relationships of a tick with many hosts, which is pioneering. These relationships may arise because of ecological overlapping instead of co-evolution and co-speciation of ticks and hosts. In any case, we can foresee an important area of study, disentangling links between vectors and reservoirs behind epidemiological associations and their importance for the circulation of pathogens.

Funding

Ethical approval

Not applicable.

CRediT authorship contribution statement

Agustin Estrada-Peña: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Hein Sprong:** Conceptualization, Methodology, Funding acquisition, Data curation, Writing – review & editing, Supervision. **Sara R. Wijburg:** Validation, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Given their role as Co-Editor, Agustín Estrada-Peña had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Professor Aneta Kostadinova (Editor-in-Chief).

Data availability

The data supporting the conclusions of this article are included within the article and its supplementary files.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2024.100198.

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