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The role of birds in the epidemiology of tick-borne pathogens

Ph.D. thesis

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Abbreviations

16S rDNA gene	16S ribosomal deoxyribonucleic acid gene
18S rRNA gene	18S ribosomal ribonucleic acid gene
A.	*Anaplasma* genus
B.	*Borrelia* genus
bp	base pair
CE	collision energy
CI	confidence interval
COI	cytochrome oxidase subunit I
DNA	deoxyribonucleic acid
LC-MS	liquid chromatography–mass spectrometry
MRM	multiple reaction monitoring
MS	mass spectrometry
PCR	polymerase chain reaction
sp.	species (singular)
spp.	species (plural)
TBEV	tick-borne encephalitis virus

Summary

Birds play an important role in short- and long-distance transportation of ixodid ticks and tick-borne pathogens. Passeriforms constitute the majority of all extant bird species. Many of them tend to forage on the ground, consequently, frequently infested with ticks. Apart from this they represent the majority of species inspected during ringing. The most common tick species collected from songbirds in Central Europe are *Ixodes ricinus* and *Haemaphysalis concinna*.

The number of the observed newfound bird species in Hungary has grown over the last decades. As a consequence, new vectors (non-endemic ticks) and/or pathogens may arrive with new avian hosts. From a collection of 3339 ixodid ticks of six species from Hungary *I. ricinus* and *Ha. concinna* were the most abundant species with 2296 and 989 specimens, respectively. Apart from these, 48 specimens of *I. frontalis*, three *Hyalomma rufipes,* two *I. festai* females and one *I. lividus* female were collected. In host-parasite relationship, ticks may affect birds in several ways (e.g. inoculate pathogens, modify immune responses, influence body condition), and birds will influence ticks in return. The prevalence of infestation with ticks among birds may depend on several factors. These include the feeding habit of bird species. In Hungary *Ha. concinna* predominantly occurs on birds that tend to feed above the ground, related to the host seeking behaviour of the larvae and nymphs. On the contrary, the presence of *I. ricinus* and *I. frontalis* larvae/nymphs showed a significant association with ground feeding bird species.

Moulting hormones (ecdysteroids) have a similar role in ticks, as in other arthropod groups. Apolysis of ticks is induced by highly elevated ecdysteroid titers. Our examinations indicate that naturally aquired (food-derived) arthropod moulting hormones reach high levels in the blood of insectivorous passerines. Based on our examinations naturally aquired (food-derived) arthropod moulting hormones are present in the blood of insectivorous passerine birds, reaching high levels. These exogenous ecdysteroids affect bird ticks by inducing on-host apolysis and consequently they have a great biological importance, as they can shorten the average duration of feeding.

Most molecular studies focus on the detection of pathogens associated with bird ticks or on avian hosts as potential pathogen reservoirs. We investigated bird ticks with molecular biological methods in order to make a taxonomic comparison in a geographical context. We provide molecular evidence for the first time on the transportation of immature stages of *Hy. rufipes* by birds into Central Europe. Importantly, even under the continental climate nymphs of *Hy. rufipes* are able to moult to adults, previously reported to infest cattle in Hungary.

Ixodes festai is reported for the first time in Hungary, while *I*. *frontalis* adults had been reported more than half a century ago in our country. Our data suggest that *I. frontalis* and *I. festai* is mainly transported from South West Europe to Central Europe. Based on the analysis of two genetic markers the present data clearly indicate two distinct genetic lineages of *I. frontalis* that are transported by birds in Central Europe. *Haemaphysalis concinna* immature stages carried by songbirds in Central Europe have a high degree of 16S rDNA gene identity with conspecific ticks from East Siberia and the Far East, Japan. In the present study, *Ha. concinna* genotypes highly similar to East Asian isolates were collected during spring and autumn migration. These findings highlight the importance of western and eastern migratory connections by birds (in addition to the southern direction) which is also relevant to the epidemiology of tick-borne diseases.

Birds are among the most important hosts of *Ha. concinna.* Recently new *Babesia* genotypes have been reported from southern Siberia and Far East of Russia. Some of these newly detected genotypes have been isolated from *Ha. concinna* ticks from Central Europe. In the current work three of these *Babesia* genotypes were identified from *Ha. concinna* ticks collected from birds. One out of three genotypes has not been found before in Europe, and none of them have been reported from bird ticks. *Babesia*-carrier ticks were collected significantly more frequently from four bird species with known eastern migratory connections. This finding is in harmony with hitherto reported cases of tick transportation via birds in east-western directions. Relevant sequences align to the phylogenetic group of piroplasms infecting ruminants, in which host they have not yet been reported. Therefore, future investigations are required to find the vertebrate host species of these *Babesia* genotypes.

Összefoglalás

A madarak jelentős szerepet töltenek be a kullancsok és a kullancs közvetítette kórokozók terjesztésében. A Földön élő madárfajok több mint felét az énekesmadarak (más néven verébalakúak; Aves: Passeriformes) teszik ki, melyek között sok faj a talajközeli táplálékkereső életmód következtében gyakrabban hordoz kullancsokat. Ebbe a rendbe tartozik a madárgyűrűző állomásokon kézbe fogott, gyűrűzött, ill. megvizsgált példányok többsége is. Közép-Európa leggyakoribb, énekesmadarakon előforduló kullancsfajai az *Ixodes ricinus* és a *Haemaphysalis concinna*.

Az utóbbi évtizedekben a hazánkban megfigyelt új madárfajok száma folyamatos növekedést mutat, melynek következtében új kórokozó-, ill. vektorfajok (pl. egzotikus kullancsok) is érkezhetnek a madarak közvetítésével. A kutatás során Magyarországon gyűjtött 3339 kullancs hat fajba tartozott, melyek közül az *I. ricinus* és a *Ha. concinna* fordult elő leggyakrabban, 2296 illetve 989 egyedszámmal. Emellett 48 *I. frontalis*-t, három *Hyalomma rufipes*-t, két *I. festai* nőstényt és egy *I. lividus* nőstény kullancsot találtunk. A kullancsok sokrétűen képesek befolyásolni a madarakat a parazita-gazda kölcsönhatás során (pl. kórokozók bejuttatása a gazdába, immunreakciók módosítása, kondíció befolyásolása), ugyanakkor a madarak is hatással vannak a rajtuk vért szívó kullancsokra. A madarak kullancsfertőzöttségét több tényező befolyásolja. Ezek közé tartozik a táplálékkereső életmód jellege is. Magyarországon a *Ha. concinna* kullancsok főleg azokon a madarakon fordulnak elő, amelyek magasan a föld felett táplálkoznak, mivel a lárvák és nimfák keresőmagassága itt található. Ezzel szemben az *I. ricinus* és az *I. frontalis* lárvák/nimfák szignifikánsan gyakrabban fordultak elő talaj közelében táplálkozó madárfajokon.

A vedlési hormonok (ún. ecdysteroidok) azonos szereppel bírnak a kullancsok fejlődésében, mint más ízeltlábú csoportok esetén. A kullancsok apolíziséhez emelkedett ecdysteroid szintek szükségesek. A kutatás során megfigyeltük, hogy a természetes úton szerzett (táplálékból felvett) ízeltlábú vedlési hormonok nagy mennyiségebn jelennek meg a rovarevő madarak vérében. Ezek az exogén ecdysteroidok apolízist idézhetnek elő a kullancsokban már a gazdán való tartózkodás ideje alatt. Ezáltal nagy biológiai jelentőséggel bírnak, mivel lerövidíthetik a vérszívás átlagos időtartamát.

A molekuláris biológiai kutatások többsége a madárkullancsokban fellelhető kórokozókkal vagy a madarakkal, mint kórokozó hordozókkal foglalkozik. Molekuláris biológiai kutatásunk során mi a madarakról gyűjtött kullancsok földrajzi szempontok szerinti taxonómiai összehasonlítását végeztük el. Sikerült molekuláris úton elsőként

igazolnunk, hogy a *Hy. rufipes* fiatal fejlődési alakjai madarak közvetítésével eljuthatnak Közép-Európába. Egy korábban leírt hazai eset is bizonyítja, hogy ennek a fajnak a nimfái még a kontinentális éghajlati viszonyok között is képesek lehetnek a vedlésre és háziállatok (leírt kutatásban szarvasmarhák) fertőzésére. Az *I. festai* jelenlétét elsőként sikerült igazolnunk Magyarországon, ugyanakkor *I. frontalis* adultokat már gyűjtöttek hazánkban több mint fél évszázaddal ezelőtt. Adataink alapján valószínűsíthető, hogy az *I. frontalis* és az *I. festai* főleg Délnyugat-Európa felől érkezik Közép-Európába. A két genetikai marker vizsgálata során két elkülönült *I. frontalis* genetikai vonal jelenlétét bizonyítottuk, melyek madarak közvetítésével Közép-Európába érkezhetnek. A *Ha. concinna* énekesmadarak által hordozott fiatal alakjai Közép-Európában a 16S rDNS gén szempontjából magas szintű azonosságot mutatnak kelet-szibériai ill. japán fajtársaikkal. Jelen kutatásunk során a kelet-ázsiai izolátumokhoz nagyban hasonlító *Ha. concinna* genotípusokat gyűjtöttünk a tavaszi és őszi madárvonulási időszakban vörösbegyekről (*Erithacus rubecula*), feketerigóról (*Turdus merula*) és szürkebegyeről (*Prunella modularis*). Ezek a madárfajok keleti vonulási összeköttetésekkel is rendelkeznek. Az eredmények alapján kijelenthetjük, hogy a nyugat-keleti irányú vonulási kapcsolatokkal (a déli irányokhoz hozzáadódva) rendelkező madárfajok szerepe is jelentős a kullancs-közvetítette kórokozók járványtanában.

A madarak a *Ha. concinna* leggyakoribb gazdái közé tartoznak. Nemrég új *Babesia* genotípusokról számoltak be Szibéria déli területeiről, illetve Oroszország keleti régióiból. Ezek közül néhányat Közép-Európában is kimutattak *Ha. concinna* kullancsokból. Kutatásunk során ezek közül a genotípusok közül hármat azonosítottunk madarakról gyűjtött *Ha. concinna* kullancsokban. Ezek közül az egyiket eddig még nem írták le Európában, illetve egyiket sem találták meg madarakról gyűjtött kullancsokban. A *Babesia*-hordozó kullancsok többsége olyan madárfajokról származott melyek rendelkeznek keleti vonulási kapcsolatokkal. Ez a felfedezés egyezik a korábbi megfigyelésekkel, melyek során madarak közvetítésével jutottak el kullancsok keletről nyugatra. A talált szekvenciák a kérődzőket fertőző piroplasma fajokkal állnak filogenetikai kapcsolatban, azonban gazdafajaikat még nem sikerült leírni.

2. Introduction

2.1 Hard tick infestation of birds, and their role in tick dispersal

Birds are vertebrates with a worldwide distribution and occupying a wide range of habitats. Their epidemiological role has been increasingly recognised, because birds contribute to the transmission of ticks and tick-borne pathogens into new geographical regions (Reed et al. 2003). In a recent study, researchers suggest that evolutionary associations between ticks, pathogens and birds are older than associations between ticks, pathogens and mammals. Therefore, birds had a significant role in the ecological network of ticks and tick-borne pathogens during evolutionary processes (de la Fuente et al. 2015).

Members of the Passeriformes order include more than a half of all bird species (Bankovics 2004). Many of them belong to the ground foraging species, consequently and frequently infested with ticks. Apart from this they represent the majority of inspected species during ringing. In Hungary 179 of 227 breeding resident bird species (Passeriformes and others) are migrants. Winter visitors and rare vagrants merged to the previous number. Their nestling sites are farther north, and they are passing through our country in the fall and/or spring (Bankovics 2004).

Bird migration is the regular seasonal movement between breeding and wintering grounds, with a determinate direction and annual cycle (Bankovics 2004). In Europe birds migrate between northeastern and southwestern regions. Birds fly from North European nesting places towards the Mediterranean in autumn, and backwards during spring. Long distance migrant passerine birds usually fly at night, and search for food after landing by dawn. In case they find favourable habitat, they land more times, to replenish their fat reserves (Bankovics 2004; Csörgő et al. 2009).

The number of observed bird species new to the European fauna has grown over the last decades. Eastern origin of numerous specimens among these new birds (for example from Turcestan, mongol-tibetian), may reflects the influence of climate-change (Csörgő et al. 2009; Bozó et al. 2016). Consequently, new vectors (non-endemic ticks) and/or pathogens may arrive. It was reported over half a century ago that even small local tick populations may establish from nymphs dispersed by migrating birds (Hoogstraal 1956).

Invasion potential of ticks is supported by former cases reported from Hungary. In 2011, adult *Hyalomma rufipes* specimens were found on grazing cows (Hornok and Horváth

2012). *Hyalomma rufipes* subadults collected from migratory birds were reported several times throughout Europe (Hasle et al. 2009). Locally warmer climate may allow the establishment of these ticks transported from larger distances (Hoogstraal et al. 1961), however *Hyalomma* nymphs need much more time to moult in such conditions, with a consequent increase in mortality. Adult ticks are cold-hardy and can survive continental winters, on the other hand the eggs and larvae that have not completed development would not survive the cold season in Central Europe (Gray et al. 2009). Therefore the survival of exotic ticks in northern latitudes depend on factors that affect moulting, rather than low temperatures which can be well tolerated by the adults (Hornok and Horváth, 2012).

Information of migratory movements is derived from bird ringing databases. Banding is one of the most effective methods to study the biology, ecology, behavior, movement, breeding and population demographics of birds (USGS 2016). Ringing activities also allow us to study how many and what kind of parasites infest them. Blood sucking parasites of birds include hard ticks, mites, fleas, louse flies, and some other Dipterans. Members of the first four groups stay longer on their hosts, compared to other Dipteran parasites (for example mosquitoes). In most cases ringers find hard ticks around the bird's eyes, ears and the beak, in regions that are inaccessible for preening (Figure 1). Inspection of these parasites provide information on the species diversity of ticks transported by migratory and non-migratory birds. If these carriers arrive from other parts of Europe, or distant lands, additional ornithological data may be obtained from the results.

Figure 1. Tick infested Robin (*Erithacus rubecula*)

Avian hosts may contribute to the transmission of ticks and tick-borne pathogens to urban habitats. Numerous tick species of medical and veterinary importance may infest livestock or humans by means of birds tick-dispersion. The most common tick species collected from passerine birds in Central Europe are *Ixodes ricinus* (Taragel'ová et al. 2008; Dubska et al. 2009; Lommano et al. 2014) and *Haemaphysalis concinna* (Špitalská et al. 2011) (Figure 2).

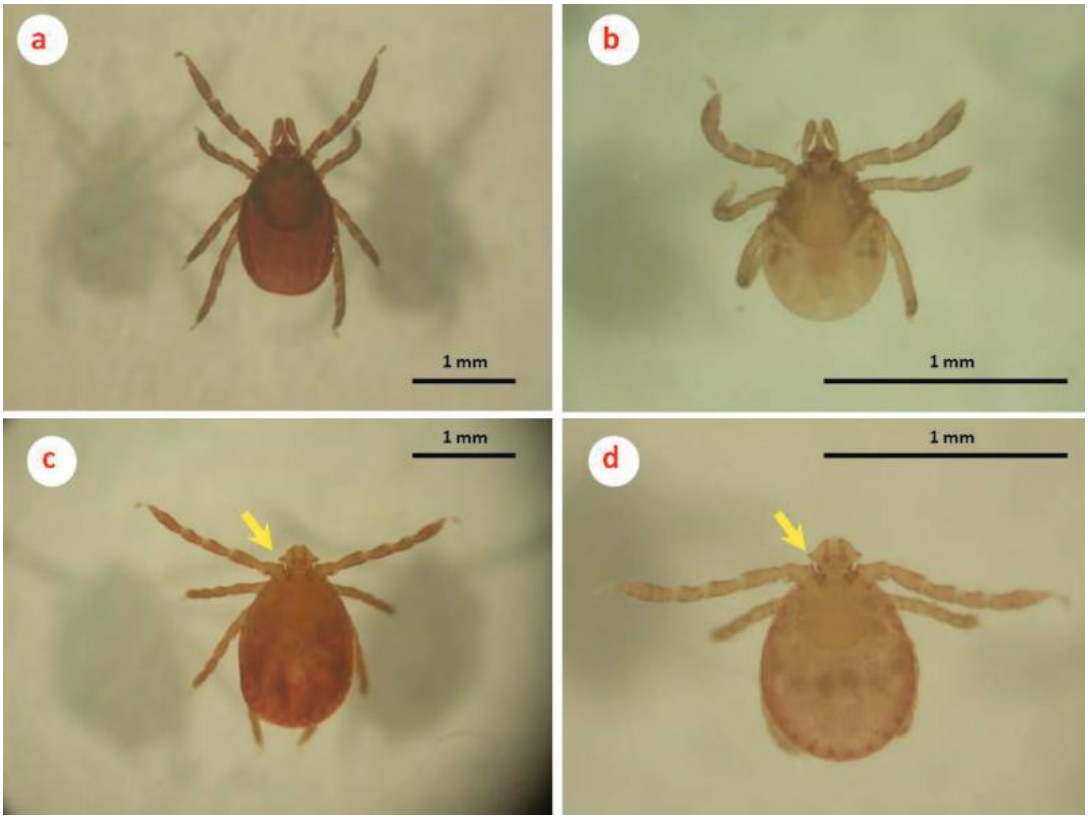


Figure 2. The two tick species most frequently collected from birds in Hungary: *Ixodes ricinus* (a: nymph, b: larva) and *Haemaphysalis concinna* (c: nymph, d: larva). The latter species has laterally pointed second palpal segment (arrows).

Ornithophilic tick species that are usually considered strictly specific to birds, include *I. frontalis* (Lommano et al. 2014), *I. arboricola* (Dubska et al. 2011; Špitalská et al. 2011) and *I. lividus* (Jaenson et al. 1994). Occurance of these species is relatively rare both in Central Europe and in Hungary, substantiated by *I. arboricola*, that had been reported from Hungary more than 50 years ago (Babos 1965). Non-ornithophilic ticks parasitize birds mainly in the immature stages (larvas, nymphs), however ornithophilic ticks may be found on hosts in adult forms.

Apart from indigenous tick species a *Hyalomma* tick was also found in Hungary on a migratory bird half a century ago (Janisch 1959), but it was identified only on the genus level. Molecularly confirmed occurrence of *Hy. marginatum* in our country was reported in 2013 (Hornok et al. 2013a). Immature phases of this tick species were collected from a European Robin (*Erithacus rubecula*), a partly urbanized bird species. This finding highlighted the epidemiological significance of migratory birds importing exotic tick species to urban gardens and parks. Tick dissemination by synanthropic birds may increase local prevalance of tick-borne pathogens and sometimes may introduce non-endemic agents (Hornok et al. 2013a). There are several publications about the exotic tick importations (e.g. *Rhipicephalus sanguieus*) by companion animals into non-endemic countries and emphasizing the importance of tick-control (Jameson et al. 2010). This kind of transportation (on humans or on companion/zoo animals) is called unnatural transfer and depends on human activity (Siuda et al. 2005). Natural transfer by migratory mammals and birds observed frequently, but tick control is much more difficult in these cases. Consequently, the epidemiological risk may be higher, particularly when the tick hosts arrive from another continent.

2.2 Characteristics of birds which influence their tick-infestation

Tick infestation of birds is influenced by multiple factors. Ground-foraging birds are prone to carry much more ticks than other bird species (Elfving et al. 2010). Blackbirds (*Turdus merula*), European Robins (*E. rubecula*) and Dunnocks (*Prunella modularis*) are infested with ticks (most of the time with *I. ricinus*) frequently, as they are considered ground feeders. Significant tick infestation of Blackbirds and European Robins is a well known phenomenon in Europe (Germany, Slovakia, Portugal, Czech Republic) (Taragel'ová et al. 2008; Dubska et al. 2009; Norte et al. 2015; Klaus et al. 2016).

The degree of tick infestation depends on the occurrence of ticks in a geographical region and their current seasonal activity. Weather conditions and local geographical characteristics may influence the activity and survival of these parasites (Hasle et al. 2009). Dubska et al. (2011) found less *Ixodes* ticks on birds living at higher altitudes. Higher mean temperature in urban areas also influences tick activity during winter, thus birds living here (especially in parks) have a higher chance to become infested by ticks. Seasonal differences in the distribution of ticks on birds were reported from Hungary too. The majority of bird ticks were *Ixodes* subadults in the spring and in the autumn. On the contrary, highest abundance of *Ha. concinna* larvae and nymphs was observed in the summer (Hornok et al. 2013a).

The prevalence of tick infestation may depend also on the height of the bird's territory. This phenomenon may be related to the difference in the host seeking behaviour of those ticks. *Ixodes* larvae and nymphs feed on small mammals (primary hosts) and ground feeding birds (Rigó et al. 2011), whereas the preferred host of *Ha. concinna* immature stages in Hungary is roe deer (*Capreolus capreolus*) (Hornok et al. 2012a). To the best of our knowledge there is no significant difference in the level of infestation between birds of different ages and sexes (Dubska et al. 2009; Sándor et al. 2014). Former authors showed no correlation between body weight by species and number of larvae/nymphs they carried (Newman et al. 2015).

Vector-transmitted pathogens may affect birds in several ways. Heylen et al. (2014a) found that *Borrelia* bacteria indirectly increase the body condition, by elevating the bird's energetic needs, which may result in an increase of bacteria-transmission events. They found that the increase of body condition correlated with the *Borrelia* infection rate. Bacteria could proliferate more successfully in these birds by increasing foraging efforts which entails the growth of tick infestation risk. As ticks attach to birds, they enhance the *Borrelia* proliferation as a consequence of immune response suppression (Heylen et al. 2014a). Infection and its implications facilitate the transmission and spatial distribution of the bacteria and its vector.

A Czech study in 1995 revealed that ecdysteroids (hormones that effect cell proliferation and growth in insects) may have anabolic effects on vertebrate animals (Japanese quails). Oral administration of ecdysteroid–containing plants significantly increased the living mass of birds in comparison to the control group. Furthermore, 20-hydroxyecdisone (the ecdysteroid originated from the diet) was present in remarkably high amount in the blood of the quails (Koudela et al. 1995).

Apolysis, the separation of cuticule from the epidermis, is the initiative act of moulting in arthropods. On-host apolysis of ixodid ticks (due to phytoecdysteroids) was observed in Hungary on goats that were kept in a meadow rich in ecdysteroid containing plants (Hornok et al. 2012a). This phenomenon has recently been reported also in ticks feeding on bats (Hornok et al. 2014a). A plausible explanation for this observation is that bats feed on insects that may contain ecdysteroids.

2.3 Vector-borne pathogens in bird ticks

Avian hosts can act as important dispersers not only of ticks but also of the pathogens transmitted by them (Maturano et al. 2015). Since adult ticks rarely infest passeriform birds, the majority of bird ticks in studies are larvae and nymphs (Scott et al.

2010; Molin et al. 2011; Berthová et al. 2016). Ticks (three-host ixodid ticks) moult to the next stage after finishing their blood meal and return to the off-host environment. After that, they acquire another host (larger animal or human) to feed on them. During blood sucking they will be able to infect the new host with the pathogens they received during their previous developmental stage (transstadial pathogen transmission) or from their mother (with transovarial transmission). Naive vectors may also acquire vector-borne pathogens via co-feeding, a special transmission when they feed close to each other on the same host at the same time. Co-feeding transmission has been reported for a various number of vector-borne pathogens including viruses and bacteria like TBEV or *B. burgdorferi sensu lato* (Belli et al. 2017). However, significance of this type of transmission has not yet been proved for *Borrelia* in bird ticks (Heylen et al. 2017).

Bird ticks can be infected by several zoonotic pathogens (Table 1). In Central Europe *Anaplasma phagocytophilum* (Špitalská et al. 2011), *Rickettsia* species (Hildebrandt et al. 2010; Lommano et al. 2014), *Borrelia* species (Taragel'ová et al. 2008; Dubska et al. 2009; Špitalská et al. 2011) and *Francisella tularensis* (Franke et al. 2010a) are the most common bacteria in ticks of birds. Viruses, like tick-borne encephalitis (Lommano et al. 2014) and protozoans of the genus *Babesia* (Franke et al. 2010a; Hildebrandt et al. 2010), have been also detected in bird-fed ticks. Apart from monospecific infections multiple infections may also occur in these ectoparasites (Hildebrandt et al. 2010). Blackbirds (*T. merula*) were more often infested with coinfected ticks than other bird species in Germany (Franke et al. 2010a), but coinfected ticks could be found on other bird species too (Franke et al. 2010b).

The assumed role of specialised bird ticks (i.e. *I. arboricola, I. frontalis*) as biological vectors for *B. burgdorferi* sensu lato was controverted by a Belgian experiment (Heylen et al. 2014a). Although, these spirochetes have been detected in European ornithophilic tick species, they were not capable of transmitting bacteria to pathogen-free bird hosts. Heylen et al. (2014a) assumed that the presence of *Borrelia* in bird-fed ticks (collected by previous studies) is the result of their ability to carry the spirochetes. The haematocytes of non-competent vectors, destroy the spirochetes, inhibiting them to reach the salivary glands (Soares et al. 2006). Moreover, these tick's saliva might not contain modulators that inhibit the host's immune response (pro-inflammatory citokine response), which could be advantageous both to the arthropod vector and the spirochete (Hovius et al. 2007).

Hyalomma ticks (*Hy. aegyptum, Hy. marginatum*) collected from passerine birds in Europe were negative for *Borrelia* species while some of them were positive for rickettsiae (Hornok et al. 2014b; Diakou et al. 2016). These bacteria belong to rickettsiae of the spotted fever group (except for *R. felis* and *R. acari*). Hard ticks serve both as vectors and reservoirs of these pathogens (Rizzoli et al. 2014), furthermore songbirds play a major role in the epidemiology of rickettsiae. *Rickettsia aeschlimannii* was identified for the first time in

Hungary in *Hy. marginatum*, collected from European Robin (*E. rubecula*) (Hornok et al. 2013a). In La Rioja (Spain) a new *Candidatus* Rickettsia sp. (*Candidatus* Rickettsia vini) was found in *I. ricinus* and *I. arboricola* ticks collected from birds (Palomar et al. 2012). Elfving found several different *Rickettsia* species (including *R. helvetica* and *R. monacensis*) in *Ixodes* spp. ticks of migratory passerine birds (Elfving et al. 2010). Birds take a prominent part in the circulation (dissemination) of *Anaplasma phagocytophilum* in nature (de la Fuente et al. 2015). However, according to some authors intensity of pathogen infection in bird-feeding ticks may be lower than in ticks collected from mammals (Franke et al. 2010b).

Among tick-transmitted apicomplexan parasites, *Babesia* and *Theileria* species have significant importance in public and animal health. Norwegian scientists published the first report of *Babesia* in bird ticks. *Babesia venatorum* was found in *I. ricinus* ticks collected from migratory birds. The authors concluded that the infected ticks were brought to Norway by the birds (northward migrants) they were feeding on (Hasle et al. 2011). In a previous German study Franke et al. (2010a) detected the majority of *Babesia* infections (*B. divergens*, *B. microti*) in bird-feeding *I. ricinus* ticks. Migratory birds may also harbour ticks infected by *Candidatus* Neoehrlichia mikurensis, a newly described zoonotic intracellular bacteria, that was detected by Swiss scientists in nymphs from common chaffinches (Lommano et al. 2014). In Hungary it was found in ticks from vegetation (Hornok et al. 2013b), however, it was not detected in the blood of tick-infested birds (Hornok et al. 2014b).

Table 1. Common tick-infested bird species, ticks and tick-borne pathogens in Europe. (Abbreviations: *A.*- *Anaplasma*, *B.*- *Borrelia*)

Bird species	**Tick species**	**Tick-borne pathogen**	**Country**	**Reference**
Blackbird (*Turdus merula*)	*Ixodes ricinus*	*B. garinii*	Czech Republic	Dubska et al. (2009)
		B. garinii	Slovakia	Taragel'ová et al.(2008)
		B. valaisiana	Czech Republic	Dubska et al. (2009)
		B. valaisiana	Slovakia	Taragel'ová et al.(2008)
		Rickettsia spp.	Germany	Hildebrandt et al. (2010)
		Rickettsia spp.	Hungary	Hornok et al. (2013a)
		Babesia spp.	Germany	Franke et al. (2010a)
	Ixodes frontalis	*B. turdi*	Belgium/Portugal	Heylen et al. (2014a)
	Hyalomma spp.	*Rickettsia* spp.	Greece	Diakou et al. (2016)
Song Thrush (*Turdus philomelos*)	*Ixodes ricinus*	*B. garinii*	Czech Republic	Dubska et al. (2009)
		B. garinii	Slovakia	Taragel'ová et al.(2008)
		B. valaisiana	Czech Republic	Dubska et al. (2009)
		B. valaisiana	Slovakia	Taragel'ová et al.(2008)
Great Tit (*Parus major*)	*Ixodes ricinus*	*B. garinii*	Czech Republic	Dubska et al. (2009)
		A. phagocytophilum	Czech Republic	Špitalská et al. (2011)
		Rickettsia spp.	Germany	Hildebrandt et al. (2010)
	Ixodes arboricola	*Rickettsia* spp.	Czech Republic	Špitalská et al. (2011)
Marsh Tit (*Poecile palustris*)	*Ixodes arboricola*	*B. garinii*	Czech Republic	Špitalská et al. (2011)
European Robin (*Erithacus rubecula*)	*Ixodes ricinus*	*A. phagocytophilum*	Czech Republic	Špitalská et al. (2011)
		Rickettsia spp.	Germany	Hildebrandt et al. (2010)
		Rickettsia spp.	Switzerland	Lommano et al.(2014)
		Rickettsia spp.	Hungary	Hornok et al. (2013a)
		A. phagocytophilum	Hungary	Hornok et al. (2013a)
		Francisella sp.	Hungary	Hornok et al. (2013a)
		TBEV	Switzerland	Lommano et al. (2014)
		Babesia spp.	Germany	Hildebrandt et al. (2010)
	Haemaphysalis concinna	*Rickettsia* spp.	Hungary	Hornok et al. (2013a)
	Hyalomma marginatum	*Rickettsia* spp.	Hungary	Hornok et al. (2013a)

2.4 The reservoir role of birds in the epidemiology of tick-borne infections

Birds in the thrush family Turdidae are frequently infested with *Borrelia* infected ticks. Blackbirds (*T. merula*), and Song thrushes (*T. philomelos*) have been shown to be important reservoirs of *B. garinii* and *B. valaisiana* (Dubska et al., 2009). Franke et al. (2010a) states that birds may also serve as reservoir hosts for *B. afzelii*. Since transovarial transmission of *Borrelia* spp. from female tick to larvae is very rare, *Borrelia*-prevalence in larvae suggest that they became infected during feeding on these birds. The main tick vector of *B. burgdorferi sensu lato* in Europe is *I. ricinus* (Heylen et al. 2014b), which is one of the most common ectoparasite of passerine birds. Consequently, birds (as tick-hosts and reservoir competent hosts of *Borrelia* bacteria) have a significant role in the maintenance of these pathogens.

Birds can become infected with other tick-borne pathogens. Zoonotic *R. helvetica* and *A. phagocytophilum* were detected in blood samples collected from synanthropic birds in Hungary. Samples from European Robins (*E. rubecula*) and a Dunnock (*P. modularis*) were found to be PCR positive for *R. helvetica*, and another sample of a Redwing (*T. iliacus*) was PCR positive for *A. phagocytophilum*. Additionally, further birds carried an unidentified member(s) of the Anaplasmataceae family, and also rickettsiae, other than *R. helvetica* (Hornok et al. 2014b).

In a Slovakian study 336 blood samples of tick-infested and tick-free birds were investigated. The most heavily infested bird species was the great tit (*Parus major*) with early developmental stages of *I. ricinus* carrying *R. helvetica* and *Coxiella burnetii* and was found to be rickettsaemic. However, xenodiagnostic studies are needed to prove that birds may act as reservoirs of Rickettsiae, because these pathogens can be transmitted transovarially. *Coxiella burnetii* was present in three blood samples, but none of the *Coxiella* positive birds carried infected ticks (Berthová et al. 2016).

3. Aims of the study

The aims of the study were:

1. to investigate species and genetic diversity of ixodid ticks transported by migratory and non-migratory bird species in Hungary. In particular to analyse molecular taxonomic characteristics of bird ticks in a geographical context.

2. to determine species specific characteristics of birds that influence their tick-infestation.

3. to investigate the presence of food-borne hormones in host blood and their effect on tick infestations and to compare the findings with population density data of lepidopterans, in order to analyse the influence of exogenous moulting hormones on apolysis.

4. to investigate the occurrence and prevalence of piroplasms in *Ha. concinna* ticks, carried by birds.

4. Materials and methods

4.1 Collection of ticks from birds

During a three-year period (from January 2012 until December 2014) ixodid ticks were collected from passerine birds. Collection took place at ringing stations in Hungary (Ócsa, Fenékpuszta, Bódva-völgy). Birds were captured by standard Ecotone mist-nets (Gydnia Poland), 12 m in length, 2.5 m in height and with 16 mm mesh (Hornok et al. 2014b). All captured birds were examined for the presence of ticks. Ectoparasites were removed with fine tweezers and put into 70% ethanol in separate tubes according to their hosts. Morphological examination of ticks was carried out under a stereomicroscope (SMZ-2 T, Nikon Instruments, Japan, illuminated with model 5000-1, Intralux, Urdorf-Zürich Switzerland) according to standard keys (Babos 1965), and were subsequently stored at room temperature.

4.2 Collection of blood samples from birds

Blood samples were collected from passerine birds in order to detect the presence of food-borne hormones (ecdysteroids). Blood samples were taken from the brachial vein (*vena ulnaris cutanea*) using a fine (28G) needle and a 0.5 ml syringe (Kendall Monoject: Tyco Healthcare Group Lp., Mansfield, MA, USA). Samples were collected into EDTA-containing microtubes and stored frozen at -20 ^{o}C until analyses.

4.2.1 Preparation of blood samples for LC-MS/MS studies

Ecdysteroid detection was performed by LC-MS/MS assays. Physiological saline solution (100 µl or 250 µl, according to the sample) was added to the blood samples. After homogenization, each sample was transferred to Eppendorf tubes with a Hamilton syringe. Following this step as much volume of methanol as the original volume of blood was added, and after homogenization the solution was left at room temperature for at least half an hour. After centrifugation at 10.000 rpm for 10 min at 8 ^{o}C, the clear supernatant was utilized for LC-MS/MS studies.

4.3 Calibration for LC-MS/MS studies

The seven standard ecdysteroids (purity > 95%) that were obtained from previous phytochemical studies (Hunyadi et al. 2007; Tóth et al. 2008) were 20-hydroxyecdysone (20E), polypodine B (pB), poststerone (pS), ecdysone (E), 2-deoxy-20-hydroxyecdysone (2d20E), ajugasterone C (ajC) and dacryhainansterone (Ds). Standard stock solutions were prepared in methanol at 1.0 mg/ml and stored at 4 ^{o}C. Equal volumes of the stock solutions were mixed and the obtained mixture (142.8 µg/ml for each analyte) was diluted first 100-fold and then 4-fold with methanol to obtain 8 concentration levels for calibration (1428.60; 357.14; 89.29; 22.32; 5.58; 1.40; 0.35 and 0.09 ng/ml, respectively). Calibration curves were constructed from at least six appropriate concentrations in triplicate. The limit of detection (LOD) and the limit of quantification (LOQ) were determined at the signal-to-noise ratio of about 3 and 10, respectively (Table 2).

Table 2. Calibration data for each standard ecdysteroid. Abbreviations: 20-hydroxyecdysone (20E), polypodine B (pB), poststerone (pS), ecdysone (E), 2-deoxy-20-hydroxyecdysone (2d20E), ajugasterone C (ajC), dacryhainansterone (Ds). [a]LOD: limit of detection. [b]LOQ: limit of quantification

Compound	Regression equation	R^2	Linear range (ng/mL)	LOD[a] (ng/mL)	LOQ[b] (ng/mL)
20E	y = 59.27x + 44.0	0.9988	1.40–1428.6	0.24	0.79
pB	y = 36.61x + 19.1	0.9998	1.40–1428.6	0.20	0.66
pS	y = 78.44x + 28.3	0.9998	1.40–1428.6	0.34	1.12
E	y = 67.86x + 22.0	0.9995	5.58–1428.6	0.77	2.56
2d20E	y = 98.14x + 66.2	0.9996	1.40–1428.6	0.27	0.89
ajC	y = 32.42x–47.7	0.9999	1.40–1428.6	0.38	1.27
Ds	y = 134.57x + 26.8	0.9995	0.35–1428.6	0.14	0.47

4.4 LC-MS/MS analysis

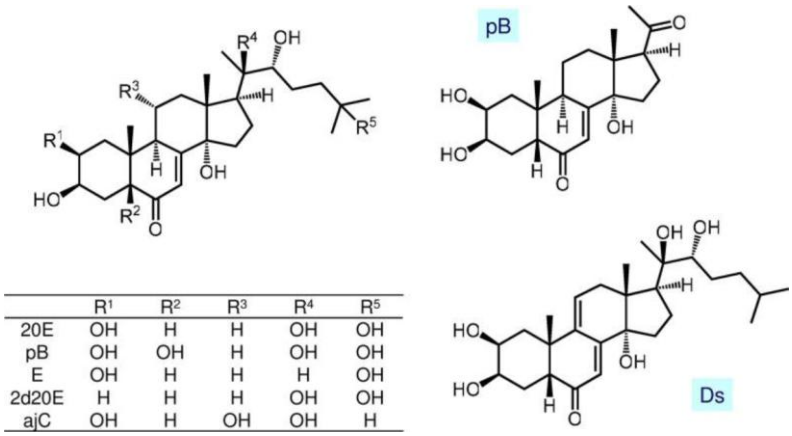
Equipements of the experiments were an Agilent 1200 liquid chromatography system equipped with a vacuum degasser, a binary pump, an autosampler, a column temperature controller and a diode array detector. Chromatographic analysis was carried out using a Kinetex XB-C18 column at 40 ^{o}C (100 x 2.1 mm, 2.6 µm) (Phenomenex, Torrance, CA, USA), with a mobile phase flow rate of 0.5 ml/min. The optimum separation was obtained under gradient elution with two isocratic time segments using 0.1% (v/v) formic acid in water as solvent A and 0.1% (v/v) formic acid in pure acetonitrile as solvent B. The linear gradient profile was: 0-0.5 min, 12% B; 0.5-2.0 min, 12-20% B; 2-3 min, 20% B; 3-9 min, 20-90% B.

Post time was 6.0 min. The volume of injection was set to 25 µL and the needle was rinsed and washed with methanol three times between the injections in order to minimize carryover.

Mass spectrometry detection was carried out on a 6410A triple quadrupole MS (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray ionization (ESI) source used in positive ionization mode. The source settings were as follows: drying gas temperature, 350 ^{o}C; gas flow rate, 11 L/min; nebulizer, 40 psig; capillary voltage, 4000 V. Analyte detection was performed by multiple reaction monitoring (MRM) using an electron multiplier voltage (EMV) of 700 volts. Fragmentor voltage and collision energy (CE) were optimized individually for each target compound and are listed in Table 3. Data acquisition and qualitative analysis was carried out with MassHunter B.04.01.

Table 3. Optimized LC-MS/MS conditions for each standard ecdysteroid. Abbreviations of compounds can be found in the legend of Table 2.

Compound	Retention time (min)	Quantitative MRM transition	CE (eV)	Qualitative MRM transition	CE (eV)	Fragmentor voltage (V)
20E	2.43	481 > 445	16	481 > 165	24	135
pB	2.53	497 > 443	20	497 > 369	24	135
pS	3.7	363 > 345	12	363 > 215	22	100
E	4.82	447 > 429	20	447 > 109	28	135
2d20E	5.52	465 > 429	16	465 > 355	20	135
ajC	5.65	481 > 427	16	481 > 299	22	135
Ds	6.77	463 > 299	20	463 > 209	26	135



	R¹	R²	R³	R⁴	R⁵
20E	OH	H	H	OH	OH
pB	OH	OH	H	OH	OH
E	OH	H	H	H	OH
2d20E	H	H	H	OH	OH
ajC	OH	H	OH	OH	H

Figure 3. Structures of the ecdysteroids tested in the blood samples.

4.5 Molecular methods

4.5.1 Molecular taxonomic analysis

4.5.1.1 DNA extraction from ticks for molecular analysis

DNA was extracted from individual ticks with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction, including an overnight digestion in tissue lysis buffer with Proteinase-K at 56 °C, as reported by Hornok et al. (2014b). An extraction control was also processed in each set of samples. For the investigation of genetic diversity, DNA was extracted from 46 larvae/nymphs of *I. frontalis* and 12 larvae/nymphs of *Ha. concinna*. One *Hyalomma* nymph and one hind leg of an *I. festai* were investigated out of the three and two collected specimens, respectively. The DNA was extracted also from the only specimen of *I. lividus*.

4.5.1.2 Tick specific COI (cytochrome oxidase subunit I) gene based conventional PCR

The PCR was modified from Folmer et al. (1994) and amplifies a 710 bp fragment of the gene. The primers HCO2198 (forward: 5' -TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (reverse: 5' -GGT CAA CAA ATC ATA AAG ATA TTG G-3') were used in a reaction volume of 25 µl, containing 1 U (0.2 µl) HotStarTaq Plus DNA polymerase, 2.5 µl 10x CoralLoad Reaction buffer (including 15 mM $MgCl_2$), 0.5 µl PCR nucleotide Mix (0.2 mM each), 0.5 µl (1 µM final concentration) of each primer, 15.8 µl ddH_2O and 5 µl template DNA. During the amplification, the initial denaturation step at 95 ^oC for 5 min was followed by 40 cycles of denaturation at 94 ^oC for 40 s, annealing at 48 ^oC for 1 min and extension at 72 ^oC for 1 min. Final extension was performed at 72 ^oC for 10 min.

4.5.1.3 Tick specific 16S rDNA based conventional PCR

A PCR method, described by Black and Piesman (1994) was used to amplify a 460 bp fragment of the 16S rDNA gene from one sample among those that yielded the same COI genotype. Primers were 16S+1 (forward: 5'-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3') and 16S-1 (reverse: 5' -CCG GTC TGA ACT CAG ATC AAG T-3'). Other components of the reaction and tha cycling conditions were the same as at the COI gene based conventional PCR, except for annealing at 51 ^oC.

PCR products for the tick specific PCR were electrophoresed in a 1.5 % agarose gel (100 V, 60 min), stained with ethidium-bromide and visualised under ultra-violet light. Purification and sequencing was done by Biomi Inc. (Gödöllő, Hungary) and the sequences were submitted to GenBank (Table 4).

Table 4. Tick species, genotypes and GenBank accession numbers of sequences

Tick species	Accession number for part of the:	
	COI gene (corresponding genotypes)	16S rDNA gene (corresponding genotypes)
Ixodes frontalis	KU170492-500 (A-Hu1 to A-Hu9)	KU170518 (A-Hu16S)
	KU170501-9 (B-Hu1 to B-Hu9)	KU170519 (B-Hu16S)
Ixodes festai	-	KU170521-2 (Hu165, Hu166)
Ixodes lividus	KU170510	KU170520
Hyalomma rufipes	KU170491	KU170517
Haemaphysalis concinna	KU170511-6 (Hu1 to Hu6)	KU170523-5 (Hu167 to Hu169)

4.5.2 Piroplasm detection in bird ticks

4.5.2.1 DNA extraction from ticks for piroplasm detection

DNA was extracted from individual ticks with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction, including an overnight digestion in tissue lysis buffer with Proteinase-K at 56 °C, as reported by Hornok et al. (2014b). An extraction control was also processed in each set of samples.

4.5.2.2 Piroplasm specific 18S rRNA based conventional PCR

Samples were screened for the presence of piroplasms by conventional PCR modifed from Casati et al. (2006). The primers BJ1 (forward: 5′-GTC TTG TAA TTG GAA TGA TGG-3′) and BN2 (reverse: 5′-TAG TTT ATG GTT AGG ACT ACG-3′) were used to amplify an approximately 500 bp portion of the 18S rRNA gene of *Babesia*/*Theileria* spp. The reaction volume was 25 µl, i.e. 5 µl of extracted DNA was added to 20 µl reaction mixture containing 0.5 U HotStarTaq DNA Plus polymerase (5 U/µl), 200 µM of PCR nucleotide mix, 1 µM of each primer and 2.5 µl of 10× CoralLoad PCR buffer (15 mM $MgCl_2$ included). Cycling conditions included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 40 s. The final extension was performed at 72 °C for 5 min. All PCRs were run with positive control (DNA extracted from canine blood, and the presence of *B. canis* confirmed with sequencing) and negative control (non-template reaction mixture). PCR products were subjected to electrophoresis in 1% standard agarose gel (SeaKem LE Agarose, Lonza Inc.) and were visualized with ECO Safe (Pacifc Image Electronics Inc.) nucleic acid staining solution. Extraction controls and negative controls were PCR negative. Purification and sequencing of PCR products was performed by Macrogen Europe (Amsterdam, The Netherlands). Obtained sequences were manually edited, then aligned with GenBank sequences by nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). Representative sequences were submitted to GenBank (accession numbers: KY471448–50).

4.6 Ethical approval

The investigations were carried out according to the national animal welfare regulations of Hungary (28/1998). Bird ringing was approved by the National Inspectorate for Environment and Nature (under license number 14/3858-9/2012). Sampling of birds (ticks and blood) was approved by the regulation of Conservancy of Environmental and Protection Areas of Central Danube valley (number of the regulation: 27251-1/2014).

4.7 Statistical analysis

Confidence intervals (CI) for the prevalence rates were calculated at the 95% level according to Sterne's method (Reiczigel, J. 2003). Prevalence data were analyzed by Fisher's exact test. Mean values for the intensity of tick infestation (number of all ticks collected from a bird species, divided by the number of all tick-infested individuals of the same bird species) were compared between bird categories by Mann-Whitney *U*-Test. Differences were considered significant when $P < 0.05$.

Activity of caterpillars was deduced from the population density data of moths (Insecta: Lepidopterans). These data are based on the Hungarian Plant Protection and Forestry Light Trap Network records, that were collected between 1974 and 2006 (Gimesi et al. 2012). The monthly regional population density of lepidopterans was expressed as a percentage of the total yearly number. The data were obtained from the mean monthly number of lepidopterans.

To test the association between the monthly proportion of apolytic ticks and the population density of lepidopterans Spearman rank correlation was used. The association of blood ecdysteroids with season and tick apolysis (Table 7) was compared by using Fisher's exact test. The differences were considered significant if $P < 0.05$.

Fisher's exact test was used to compare the proportion of piroplasm positive *H. concinna* ticks. Confidence interval (CI) for the overall prevalence was calculated at the 95% level according to Sterne's method (Reiczigel, J. 2003). The prevalence of PCR positivity was calculated from the number of PCR positive ticks, expressed as the percentage of all evaluated ticks. Differences were considered significant when $P < 0.05$.

4.8 Phylogenetic analyses of bird ticks

Phylogenetic analyses were conducted with the Tamura-Nei model and Maximum Composite Likelihood method by using MEGA version 5.2 as reported previously (Hornok et al. 2015a).

4.9 Phylogenetic analyses of piroplasms in *Ha. concinna* ticks

The MEGA model selection method (Tamura et al. 2013) was applied to choose the suitable model for phylogenetic analyses. Sequences were trimmed from the same starting point to the same end (405-409 bp length). The dataset was resampled 1000 times to generate bootstrap values. Phylogenetic analyses were conducted with the Maximum Likelihood method (Jukes Cantor model) by using MEGA version 6.0 (Tamura et al. 2013).

5. Results

5.1.1 Species diversity of ixodid ticks carried by migratory and non-migratory bird species

A total of 3339 ixodid ticks were collected from 1167 infested passerines (representing 47 species) in the period between 2012-2014. Most of the tick specimens were early developmental stages belonging to four species. *Ixodes* ricinus and *Ha. concinna* were the most common species with 2296 (68.8% of all collected ticks, CI: 67.2-70.3%) and 989 (29.6%, CI: 28.1-31.2%) specimens (only larvae and nymphs), respectively. There were 48 specimens of *I. frontalis* (including three adults) (Figure 5a), and three nymphs of *Hy. rufipes* (Figure 5b). Moreover, two *I. festai* females (Figure 5c,d) and one *I.lividus* female were collected (Figure 4).

Ixodes ricinus ticks occurred on birds between March and November, and *Ha. concinna* was noted from March to October. However, *I. frontalis* specimens occurred during all seasons (August to November and January to April), the majority were collected in springtime (Table 6). Thirty-eight *I. frontalis* specimens were collected from European Robins (*E. rubecula*) (79.2%, CI: 65-89.5%) (Table 5). *Hyalomma rufipes* nymphs and *I. festai* females were found also in the spring (in May and March, respectively). *Hyalomma* ticks were collected from a Common Whitethroat (*Sylvia communis*), the two *I. festai* females were found on a Greenfinch (*Carduelis chloris*) and a Dunnock (*P. modularis*). The *I. lividus* female was collected in July from a Sand Martin (*Riparia riparia*).

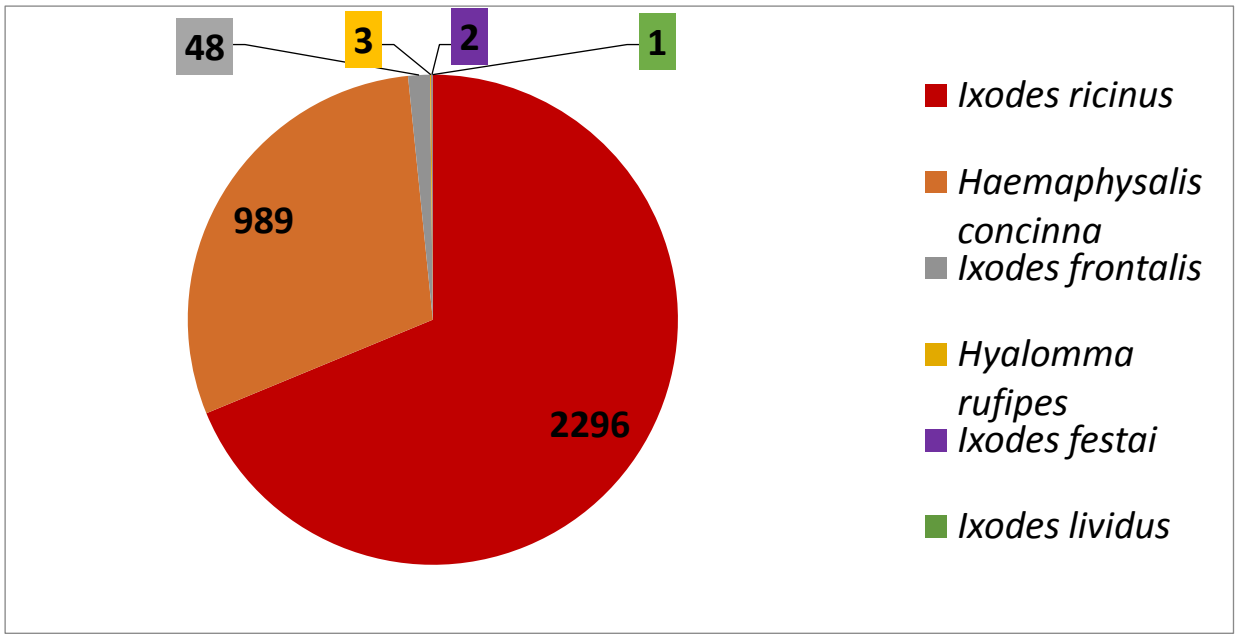


Figure 4. Proportion of the collected bird ticks between 2012-2014

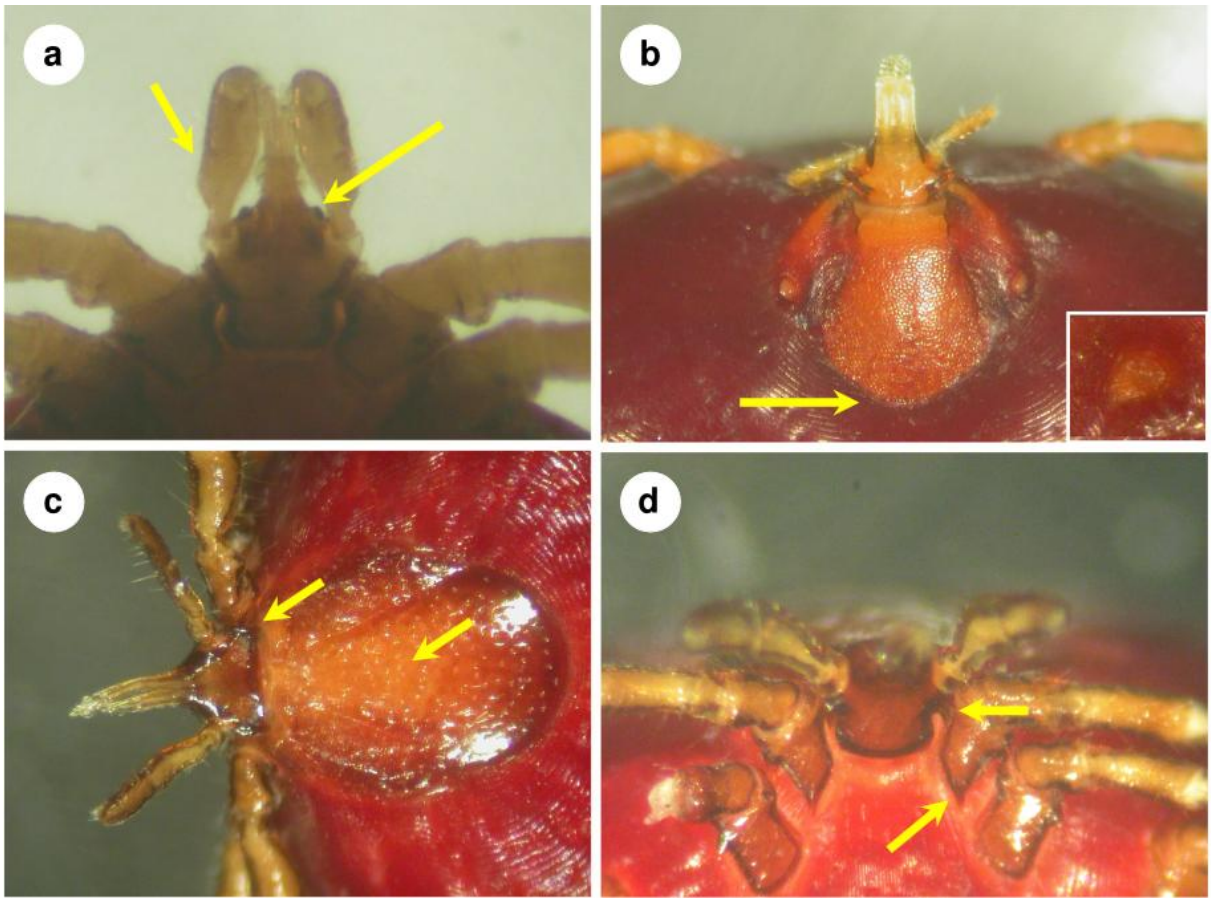


Figure 5. Morphology of tick species identified in the relevant stage for the first time in Hungary. **a**: *Ixodes frontalis* nymph showing parallel sides of palps and “frons” (arrows); **b**: *Hyalomma rufipes* nymph with broadly rounded posterior margin of the scutum (arrow) and elongated spiracular plate (insert); **c**: *Ixodes festai* female, dorsal view the scutum with deep punctuations and few long hairs, distinct cornuae on the basis capituli (arrows); **d**: *Ixodes festai* female, ventral view – broad auriculae curved backwards, long internal spur on coxa I (arrows)

Table 5. Host traits and tick-infestation of most important bird species in this study (of which at least eight tick-infested individuals were captured or at least 10 ticks were collected between March 2012 and November 2014)

Bird species characteristics					t/n	Cumulative number of tick specimens						
						I. ricinus		*H. concinna*		*I. fr.*	*I. fe.*	*H. r.*
Species[a]	Feeding	Migration	Weight (g)	n		L	N	L	N	L/N/F	F	N
ACR PAL	ABOVE GROUND LEVEL	long	10–17	53	2.1	39	41	18	14	-	-	-
ACR SCH			10–13	30	2.6	3	10	10	56	-	-	-
ACR SCI			9–12	70	1.8	29	45	18	36	-	-	-
LOC LUS			14–17	92	4.9	1	7	206	236	-	-	-
LOC NAE			13–16	2	11	-	-	18	4	-	-	-
PHY COL		middle	6–11	8	1.4	5	6	-	-	-	-	-
SYL ATR			16–25	69	1.7	45	39	7	22	-/1/-	-	-
CAR CHL		short	25–35	30	1.7	1	47	-	1	-/-/1	1	-
COC COC			**46–80**	11	2.4	-	25	1	1	-	-	-
EMB CIT		local	27–30	2	20	-	-	38	2	-	-	-
EMB SCH			27–30	2	8.5	3	-	2	12	-	-	-
PAR MAJ			16–22	81	1.8	49	88	2	1	1/-/1	-	-
LUS LUS	GROUND	long	24–38	10	4.2	40	2	-	-	-	-	-
LUS MEG			17–28	24	4	61	17	18	1	-	-	-
SYL COM			13–20	12	1.1	5	8	-	-	-	-	3
TUR ILI			**55–75**	3	6.5	2	12	-	-	-	-	-
ERI RUB		short	16–22	318	2.3	469	195	27	5	22/15/1	-	-
PRU MOD			16–25	67	3.1	24	173	3	4	-	1	-
TRO TRO			7–12	13	1.8	15	8	-	-	-	-	-
TUR MER			**80–140**	149	4.6	137	421	47	73	-	-	-
TUR PHI			**65–95**	56	4.5	49	118	31	50	5/1/-	-	-

Bold numbers indicate weight of bird species in the larger body weight category. The number of tick specimen refers to the number of larvae, nymphs or female ticks collected from all individuals of the relevant bird species during the study period. Abbreviations: *n* number of tick-infested individuals, *t/n* mean intensity of tick infestation (number of all ticks divided by the number of all tick-infested birds), *L* larva, *N* nymph, *F* female, *I. fr. - Ixodes frontalis*; *I. fe. - Ixodes festai*; *H. r. - Hyalomma rufipes* [a]ACR PAL= *Acrocephalus palustris*, ACR SCH = *A. schoenobaenus*, ACR SCI = *A. scirpaceus*, LOC LUS = *Locustella luscinioides*, LOC NAE = *L. naevia*, PHY COL = *Phylloscopus collibita*, SYL ATR = *Sylvia atricapilla*, CAR CHL = *Carduelis chloris*, COC COC = *Coccothraustes coccothraustes*, EMB CIT = *Emberiza citrinella*, EMB SCH = *E. schoeniclus*, PAR MAJ = *Parus major*, LUS LUS = *Luscinia luscinia*, LUS MEG = *L. megarhynchos*, SYL COM= *Sylvia communis*, TUR ILI = *Turdus iliacus*, ERI RUB = *Erithacus rubecula*, PRU MOD= *Prunella modularis*, TRO TRO = *Troglodytes troglodytes*, TUR MER = *T. merula*, TUR PHI = *T. philomelos*

5.1.2 Genetic diversity of less frequent ixodid ticks in a geographical context

The COI gene was chosen as the first target for molecular analysis, on account of its suitability as a DNA-barcode sequence for tick species identification. Among the 46 *I. frontalis* specimen for which part of the COI gene was sequenced, two genetic lineages (each containing nine genotypes) were discovered ("A": KU170492-500, and "B": KU170501-9). The separation of the two lineages had a high bootstrap support on the phylogenetic tree (Figure 6), with 56 nucleotide difference (598/654 bp, means only 91.4% identity). Genotypes within lineage "A" had one to two nucleotide differences, whereas within lineage "B" this amounted to one to four nucleotide differences. The following 16S rDNA gene analysis included DNA samples of each different COI genotype, but revealed only two distinct genetic variants (KU170518: genotype A-Hu16S, KU170519: genotype B-Hu16S), which showed a four bp difference (402/406 bp, i.e. 99% identity). These two 16S rDNA genotypes had 100% sequence identity to South-Western European isolates from the Azores (KP769863 and KP769862). The phylogenetic analysis of 16S rDNA sequences confirmed the separation of the two *I. frontalis* lineages (Figure 7). The isolation sources of *I. frontalis* genotypes are represented in Table 6.

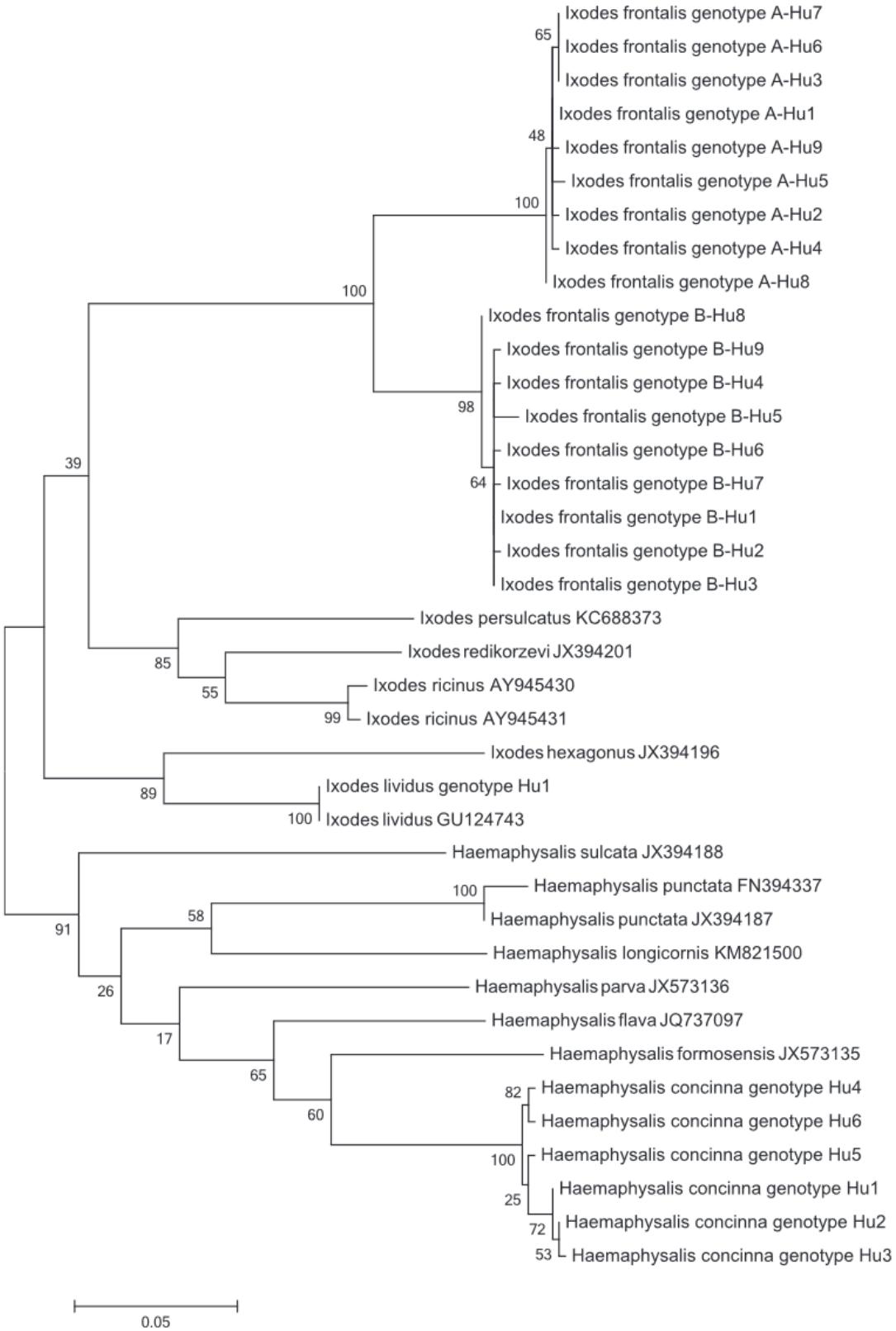


Figure 6. Phylogenetic relationships of *Ixodes* and *Haemaphysalis* sp. ticks based on COI gene. Specimens collected in this study (genotypes with “Hu”) and related data from GenBank are included. Branch lengths correlate to the number of substitutions inferred according to the scale shown.

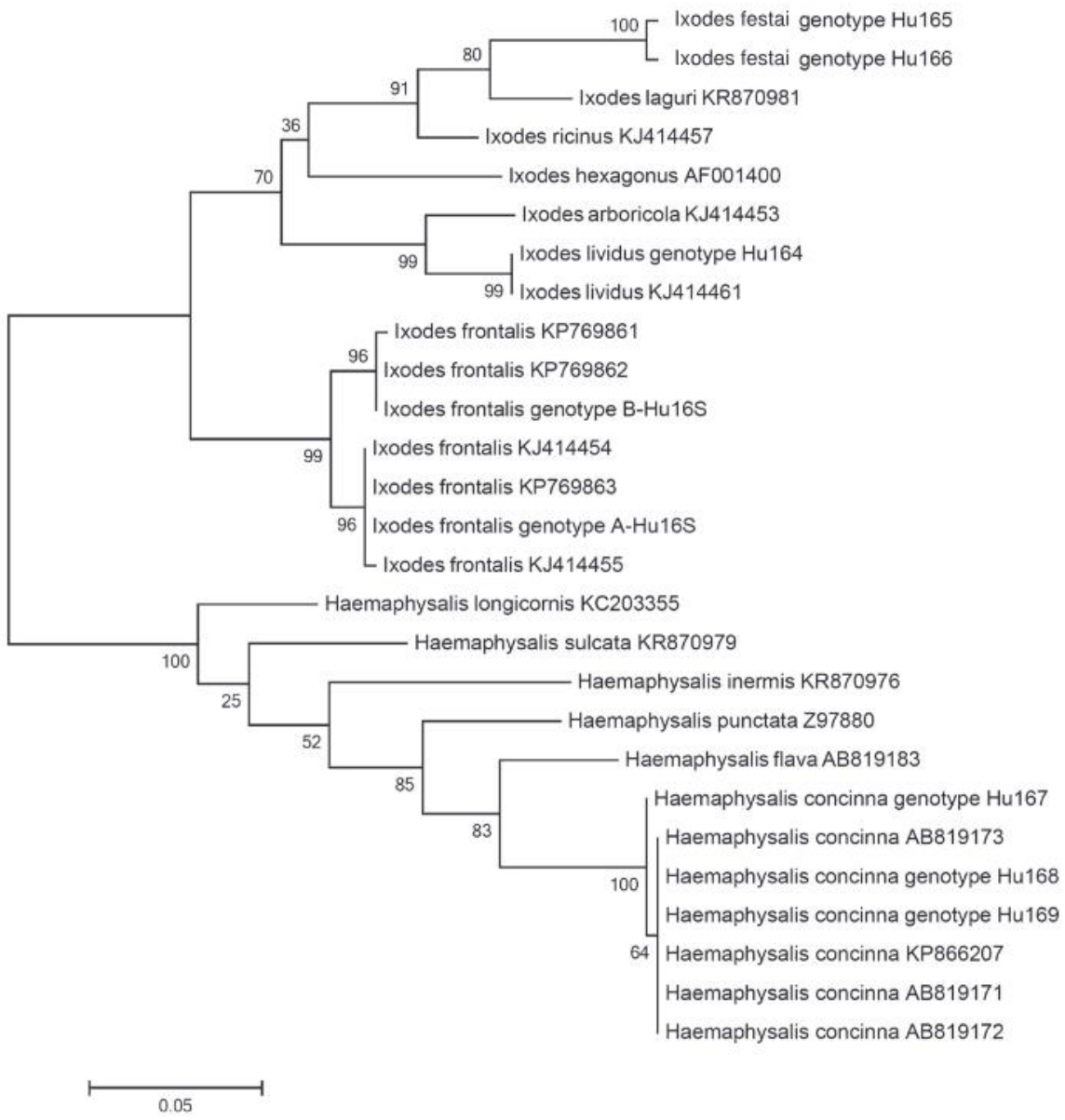


Figure 7. Phylogenetic comparison of 16S rDNA sequences of *Ixodes* and *Haemaphysalis* sp. ticks. Specimens identified in the present study (genotypes including Hu) and other sequences from GenBank are included. Branch lengths correlate to the number of substitutions inferred according to the scale shown.

Table 6. Genotypes of *Ixodes frontalis* and *Haemaphysalis concinna* identified in this study, according to bird species and season.

	Genotype		Bird species							
	COI	16S rDNA	ERI RUB			TUR PHI	PAR MAJ	SYL ATR	CAR CHL	
I. frontalis	A-Hu1	A-Hu16S	S^1 S^1 S^1 S^2 S^2 S^2 S^8 S S S S S A			S^5	S			
	A-Hu2		S^2							
	A-Hu3		S							
	A-Hu4		S S							
	A-Hu5		S^4 S^4							
	A-Hu6		S							
	A-Hu7								w	
	A-Hu8					A^7				
	A-Hu9		S^8							
	B-Hu1	B-Hu16S	S^1 S^1 S S			M				
	B-Hu2		S^1							
	B-Hu3		S^2 S S S				A			
	B-Hu4							S		
	B-Hu5		S							
	B-Hu6					S^5				
	B-Hu7		S^6 S^6 S^6 S^6							
	B-Hu8					A^7 A^7				
	B-Hu9		A							
	COI	16S rDNA	ERI RUB	ACR SCH	TUR MER	PRU MOD	SYL NIS	SYL ATR	LOC LUS	EMB CIT
H. concinna	Hc-Hu1	Hu167		M						
	Hc-Hu2						M		M	
	Hc-Hu3				MA			S		M
	Hc-Hu4	Hu168			S					
	Hc-Hu5		S A			S				
	Hc-Hu6	Hu169	A							

The number of letters of a season below one bird species in the given row indicates the number of ticks belonging to the relevant genotype. The same upper index on these letters indicate the number of ticks that were found simultaneously on the same bird. Abbreviations: *S* spring, *M* summer, *A* autumn, *W* winter. Abbreviations of bird names can be found in the legend of Table 5.

The 16S rDNA sequences of the two *I. festai* specimens (KU170521-2) differed in three nucleotides (373/376 bp, i.e. 99.2% identity). However, the two genotypes clustered together on the phylogenetic tree (Figure 7). Sequencing of the amplified part of the COI gene was not successful in the case of *I. festai*.

The COI sequence of *I. lividus* (KU170510) had 100% identity with an isolate of the same tick species from the UK (GU124743). Furthermore, the partial 16S rDNA sequence of the collected specimen had 99.7% (398/399 bp) identity with an isolate from Belgium (KJ414461).

The three *Hyalomma* nymphs were collected from a Common Whitethroat (*S. communis*) in 2014. The partial COI sequence of one specimen (KU170491) showed the highest degree of identity (645/649 bp, i.e. 99.4%) to a *Hy. rufipes* x *Hy. dromedarii* hybrid from Africa, Ethiopia (AJ437079), whereas a 99.2% (644/649 bp) identity to *Hy. marginatum*

(AJ437091) from the same geographical region. However, this nymph showed the highest degree of identity to *Hy. rufipes* (405/406 bp, i.e. 99.8% identity to L34307 and only 403/407 bp: 99% identity to KP776645, *Hy. marginatum*) based on the partial sequence of its 16S rDNA gene (KU170517).

Twelve specimens of *Ha. concinna* ticks were analysed with molecular methods. Among them six different COI genotypes were found (KU170511-6) with a difference in up to eight nucleotides (622/630 bp, i.e. 98.7% identity), and clustered in two lineages on the phylogenetic tree (Figure 6). These COI genotypes represented three 16S rDNA genotypes (KU170523-5). The difference between them was one to two nucleotides. The phylogenetic analysis of these genotypes confirmed the separation of Hu167 (encompassing COI genotypes Hu1-3) from Hu168-9. The latter (e.g. KU170524) showed high degree of identity (meaning 387/388 bp, i.e. 99.7%) to isolates of *Ha. concinna* from Japan (e.g. AB819171) and another from East Siberia (KP866207: 384/387 bp, i.e. 99.2% identity). The Hungarian genotypes clustered together with the Far Eastern isolates on the phylogenetic tree (Figure 7). The host species and seasonality of COI and 16S rDNA genotypes of *Ha. concinna* are shown in Table 6.

5.2 Species specific characteristics of birds that influence their tick-infestation

Characteristics (feeding site preference, migration distance and body weight) were assigned to bird species based on ornothological observations (Csörgő et al. 2009). Among the most important tick-infested bird species in this survey (Table 5), the majority of *I. ricinus* and *I. frontalis* early developmental stages (1756 of 2239: 78.4%, CI: 76.7-80.1% and 44 of 48: 91.7%, CI: 80-97.7%, respectively) occurred on birds that preferentially feed from the ground. However, most of *Ha. concinna* larvae and nymphs (73.1%, 705 of 964, CI: 70.2-75.9%) were found on birds that feed above the ground level. The difference of the host seeking habit between the two tick genus was significant (Fisher's exact test: $P < 0.0001$). The mean intensity of tick infestation (Table 5) had no significant association with the lower (6-38 g) or higher (39-140 g) body weight of host species (arbitrary threshold: 39 g), or with long vs. short distance (or no) migration route of hosts (Mann-Whitney *U*-Test: $P > 0.05$).

5.3 Food-borne hormone in host blood influencing tick infestations

During the three year period, 3330 ixodid ticks were collected from 1164 passerine birds (representatives of 46 mainly or partly insectivorous species). Early developmental stages of *Ixodes* spp. predominated, followed by *Ha. concinna* larvae and nymphs, accounting for 70.3% (2341 out of 3330, CI: 68.7-71.9%) and 29.7% (989 out of 3330, CI: 28.2-31.3%) of all collected ticks, respectively.

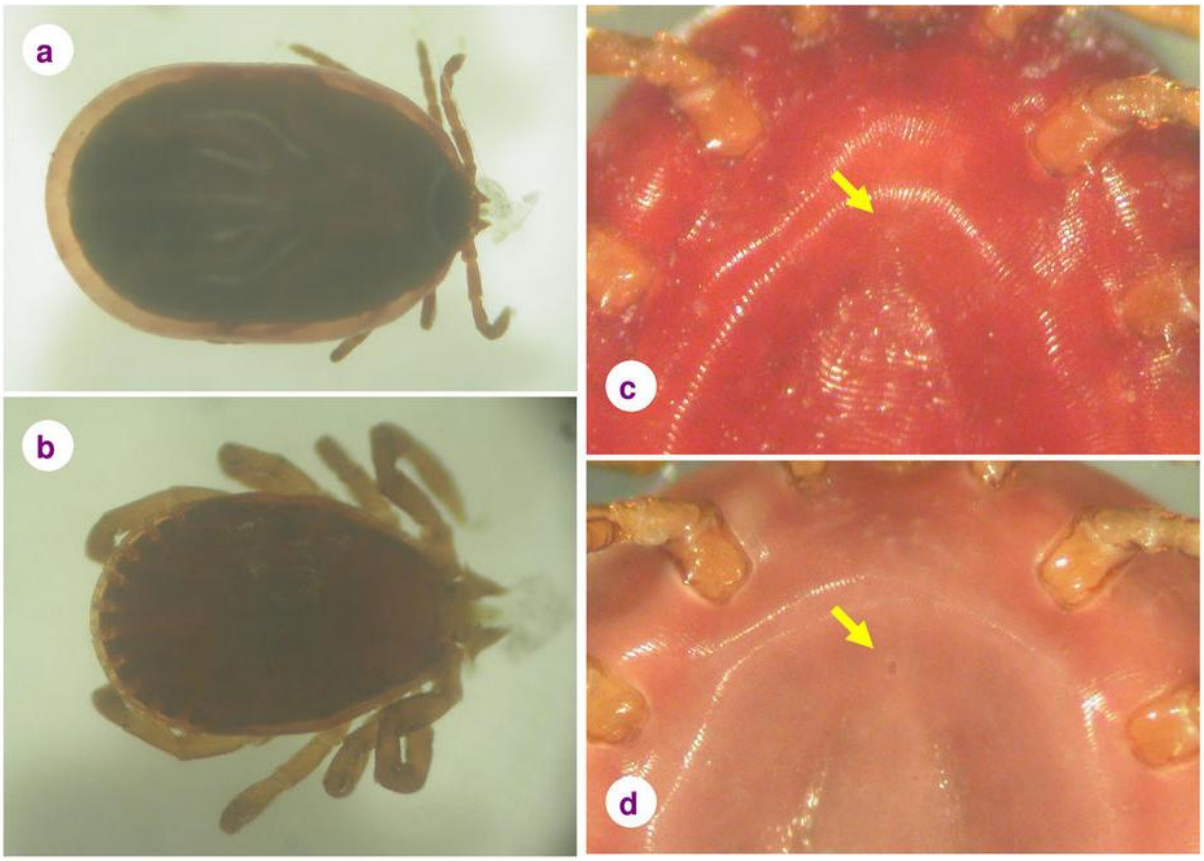


Figure 8. *Haemaphysalis concinna* nymphs showing apolysis: (**a**) when close to full engorgement, (**b**) at the beginning of engorgement. Compared to nymphs that did not show the signs of apolysis (**c**), the place of the genital pore (arrow) is more apparent on apolytic nymphs (**d**).

A noteworthy proportion, 20.5% (683 out of 3330, CI: 19.2-21.9%) of immature ticks collected from birds showed apolysis (Figure 8a). In the case of engorged apolytic nymphs, the place of the genital pore is frequently darker and more visible (Figure 8c,d). In this study the signs of apolysis were also observed in the case of unengorged ticks, i.e. at the beginning of their blood meal (Figure 8b). We found the greatest proportion of apolytic ticks on birds in July (250/750=35.5%, CI: 31.9-39.1%). There was a significant association between the monthly proportion of apolytic immature stages and the reported monthly regional population density of lepidopterans (Spearman's rank correlation: r=0.93, P=0.00001) (Figure 9).

Figure 9. The monthly regional population density of lepidopterans and the percentage of ticks showing apolysis. The latter indicates the number of apolytic ticks expressed as the percentage of all ticks removed from birds, calculated for each month.

Ecdysteroids were found in eight out of 18 blood samples of tick-infested birds. In these samples up to seven ecdysteroids or their derivatives were present in detectable quantities (Figure 10; Table 7). There were significantly more positive samples in the summer (61.5%=8/13) than in spring (0.0%=0/5) (Fisher's exact test: P=0.036). Among the birds that carried apolitic ticks, the proportion of ecdysteroid-positive samples (87.5%=7/8) was almost nine times higher than in birds with no apolytic ticks (10.0%=1/10) and this difference was statistically significant (Fisher's exact test: P=0.003).

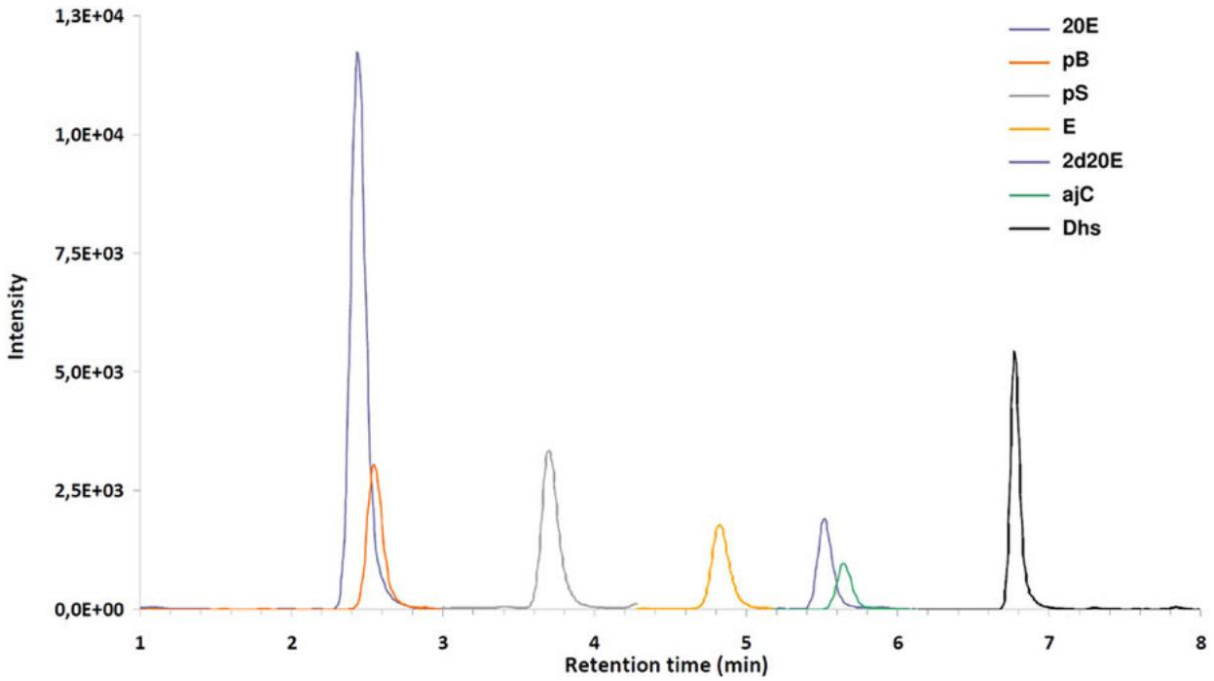


Figure 10. MRM chromatogram of sample No. 6. Abbreviations can be found in the legend of Table 2, sample data are shown in Table 7.

Table 7. Data of eighteen tick-infested birds: the presence/absence of ticks showing apolysis and ecdysteroid concentrations in corresponding blood samples. Abbreviations of compounds can be found in the legend of Table 2; "<" symbol denotes detectable ecdysteroid content below the limit of quantification.

Bird number	Bird species*	Sampling date (2014)	Presence of ticks with apolysis	Fold dilution at sample preparation	Ecdysteroid concentrations in the blood (ng/ml)						
					20E	pB	pS	E	2d20E	ajC	Ds
1.	LOC LUS	August 22	**yes**	100	440 ± 80	230 ± 20	990 ± 40	–	650 ± 10	–	390 ± 120
2.	LOC LUS	July 7	**yes**	20	–	–	100	–	–	–	–
3.	LOC LUS	August 5	**yes**	100	180	–	900	190	160	–	560
4.	TUR MER	August 5	**yes**	100	4480 ± 320	1850 ± 110	3100 ± 220	1950 ± 180	1540 ± 220	2060 ± 170	7640 ± 380
5.	LOC LUS	August 5	**yes**	100	220	–	290	200	250	–	80
6.	ACR SCH	August 7	**yes**	5	7920 ± 670	3330 ± 230	1760 ± 130	1130 ± 60	660 ± 30	1100 ± 20	980 ± 10
7.	LUS MEG	August 8	**yes**	100	310	–	650	–	–	–	100
8.	ERI RUB	August 8	no	8.3	30	–	40	–	–	–	< 5
9.	TUR MER	August 5	**yes**	20	no detectable ecdysteroid contents						
10.	TUR MER	March 29	no	2.5							
11.	ERI RUB	March 29	no	5							
12.	PRU MOD	March 29	no	50							
13.	PRU MOD	April 7	no	100							
14.	LUS MEG	August 4	no	50							
15.	ERI RUB	April 8	no	5							
16.	LUS MEG	August 6	no	100							
17.	LOC LUS	August 6	no	20							
18.	ACR SCI	August 7	no	20							

*Abbreviations: LOC LUS= Locustella luscinioides, TUR MER= Turdus merula, ACR SCH= Acrocephalus schoenobaenus, LUS MEG= Luscinia megarhynchos, ERI RUB= Erithacus rubecula, PRU MOD= Prunella modularis, ACR SCI= Acrocephalus scirpaceus.

5.4 Prevalence and molecular investigation of piroplasms in *Ha. concinna* ticks, carried by birds

A total of 321 *Ha. concinna* larvae and nymphs were tested for the presence of piroplasms, collected from April 2012 until October 2014. Ticks were collected from 121 passeriform birds belonging to 19 species. Fifty-one ticks were positive for piroplasms (15.9%, CI: 12.1-20.4%), and belonged to 11 bird species (Table 8). The piroplasms were molecularly identified identified (with the exception of one failed sample) and proven to be 100% (405/405 or 409/409 bp) identical to three *Babesia* genotypes that have been reported previously from southern Siberia (Baikal region) and Far East of Russia (Rar et al. 2014). In the majority of ticks two genotypes were present: “Irk-Hc133” (previously found in Irkutsk, Siberia) and “Kh-Hc222” (recently reported from Khabarovsk, Far East) (designated “A” and “B”, respectively, in Table 8). An additional genotype (“Irk-Hc130”: described also from Irkutsk: Rar et al., 2014) was isolated from three *Ha. concinna* specimens (designated “C” in Table 8). Phylogenetic analysis showed (Figure 11) that bird tick-associated Kh-Hc222 clustered separately from the phylogenetic group formed by bird tick-associated *Babesia* sp. Irk-Hc133, *B. crassa* and *B. major*. The clade containing *Babesia* sp. Irk-Hc130 was a sister group to *B. motasi.* All three genotypes belonged to the phylogenetic group formed by *Babesia* spp. of ruminants.

The proportions of PCR positive *Ha. concinna* larvae (17.2%: 27 out of 157) and nymphs (14.6%: 24 out of 164) were similar. Piroplasm PCR-positive ticks were collected more frequently during summer (17.6%: 45 out of 255) and autumn (19.2%: 5 out of 26) than in the spring (2.5%: 1 out of 40) and this difference was statistically significant (Fisher’s exact test: P = 0.003, and P = 0.009, respectively).

Haemaphysalis concinna immature ticks PCR positive for piroplasms were collected significantly more frequently from five bird species with known eastern migratory connections (24 out of 92 ticks) than from other bird species (27 out of 229 ticks) (Fisher’s exact test: *P* = 0.002, Table 8). These five host species had 14-60% prevalence of PCR positive ticks (Table 8). Bird species with current eastern migratory habit are Yellowhammer (*Emberiza citrinella*), Song Thrush (*T. philomelos*) and River Warbler (*Locustella fluviatilis*) (Csörgő et al. 2009; Scebba and Olivieri Del Castillo 2017; Dove et al. 2017). Additionally, Yellowhammer, Savi’s Warbler (*Locustella luscinioides*) and Nightingale (*Luscinia megarhynchos*) have phylogenetic connections with eastern groups of their species (Irwin et al. 2009; Drovetski et al. 2004; Ács and Kováts 2013).

Table 8. Data of evaluated bird species and the piroplasm-carrier status of their *Haemaphysalis concinna* ticks. The names of bird species with documented current migratory habit from the east are written with bold characters.

Abbreviations: A – GenBank: KY471448, identical with *Babesia* sp. Irk-Hc133 (KJ486563 from Irkutsk, Siberia); B – GenBank: KY471449, identical with *Babesia* sp. Kh-Hc222 (KJ486568 from Khabarovsk, Far East); C – GenBank: KY471450, identical with *Babesia* sp. Irk-Hc130 (KJ486569 from Irkutsk, Siberia).

No.	Bird species (n = tick infested)	PCR positive/all ticks (percentage)	Identified piroplasm (number of sequences)
1.	*Acrocephalus arundinaceus* (1)	0/1	–
2.	*Acrocephalus schoenobaenus* (9)	2/32 (6%)	A (+ not successful)
3.	*Acrocephalus scirpaceus* (6)	3/11 (27%)	A, B, C
4.	*Acrocephalus palustris* (6)	1/6 (17%)	A
5.	*Cardeulis chloris* (1)	0/1	–
6.	*Coccothraustes coccothraustes* (1)	0/2	–
7.	***Emberiza citrinella*** (2)	11/40 (28%)	A (10×), B
8.	*Erithacus rubecula* (17)	2/18 (11%)	A, B
9.	*Lanius collurio* (1)	0/1	–
10.	***Locustella fluviatilis*** (5)	1/7 (14%)	A
11.	*Locustella luscinioides* (33)	15/99 (15%)	A (13×), B, C
12.	*Luscinia megarhynchos* (1)	3/5 (60%)	B (3×)
13.	*Parus major* (1)	0/1	–
14.	*Prunella modularis* (6)	0/7	–
15.	*Sylvia atricapilla* (5)	2/22 (9%)	A (2×)
16.	*Sylvia curruca* (1)	0/1	–
17.	*Sylvia nisoria* (1)	0/1	–
18.	*Turdus merula* (12)	2/26 (8%)	A, C
19.	***Turdus philomelos*** (12)	9/40 (23%)	A (9×)

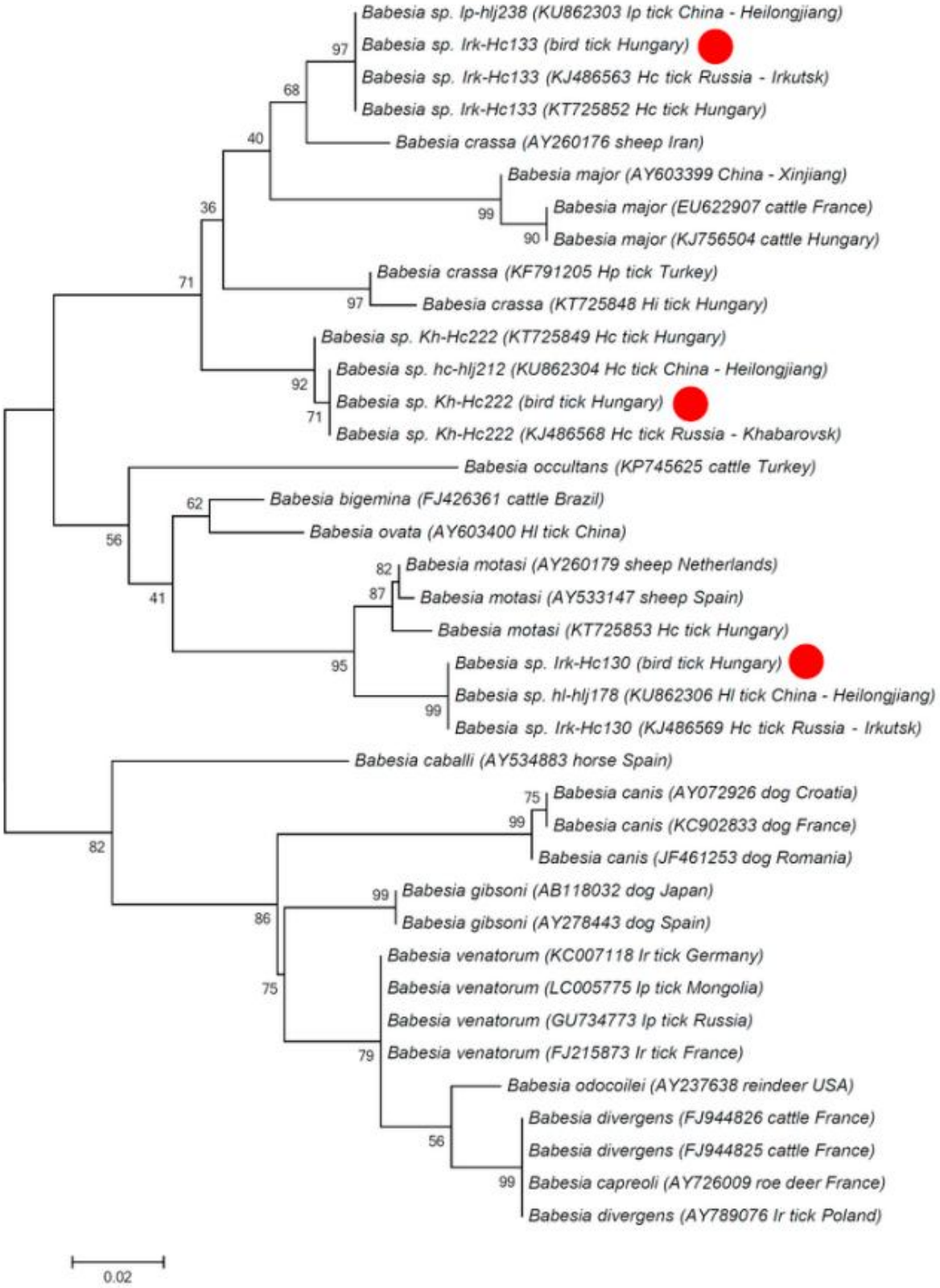


Figure 11. Maximum Likelihood phylogenetic tree of *Babesia* spp./genotypes based on 18S rRNA gene. Sequences from this study are indicated with red dots. Branch lengths represent the number of substitutions per site inferred according to the scale shown.

6. Discussion

We investigated the epidemiological significance of tick infestations of birds. Birds take part in the transportation of ticks and tick-borne pathogens, especially by travelling great distances during migration. On the other hand, inflying birds may promote shorter term changes in urban and periurban habitats than local reservoirs i.e. urban-dwelling mammals. Non-ornithophilic ticks species exhibited the highest mean intensity on birds. Ornithophilc and non-endemic ticks are collected less frequently during bird-tick studies. In Hungary exotic tick species i.e. *Hyalomma* spp. were collected from long distance migrant hosts (Hornok et al. 2013a, 2014b), in line with reports from other European countries (Hasle et al. 2009; Jameson et al. 2012). The prevalence of tick infestation among birds may depend on several factors. The strategy of migration (short or long distance migrants), the level of feeding (on the ground or above), climatic effects and pathogens living in ticks may also contribute to the level of tick infestation (Olsén et al. 1995; Hornok et al. 2014b; Heylen et al. 2014a). The migration strategy in terms of direction and distance influence the time of arrival in the activity period of different tick species. Therefore, the activity *of Ha. concinna* larvae overlap with the nesting of passerine birds, thus predisposing larvae to feed on birds. Consequently, birds are among the most important hosts of *Ha. concinna* (Nosek 1971; Hornok et al. 2014b). The niche birds occupy in an ecosystem will influence the risk they may pose as a source of parasites and vector-borne diseases towards humans and their domestic animals. Species specific characteristic of birds will also influence their mean intensity of tick infestation. Feeding preference of avian hosts has a great influence on their tick burden. Ground feeding birds, i.e. Blackbirds (*T. merula*) and Robins (*E. rubecula*) are infested with ticks more frequently, according to European results (Taragel'ová et al. 2008; Dubska et al. 2009). However, this correlation depends on the tick species, as larvae and nymphs of *Ha. concinna* occurred exclusively on birds that feed above ground level in previous Hungarian studies (Hornok et al. 2014b). *Ixodes ricinus* immature stages that have lower questing heights are associated with rodents as primary hosts (Rigó et al. 2011). In contrast, *Ha. concinna* subadults prefer larger (medium-sized) mammalian hosts i.e. roe deer (*C. capreolus*) in Hungary, i.e. their questing height on the vegetation will be different (Hornok et al. 2014b) similarly to other *Haemaphysalis* ticks (Tsunoda and Tatsuzawa 2004). Climatic effects may change population density and host seeking activity of parasites which entails the changes in bird's tick infestation. Sharply rising/descending ambient temperatures can be significant in triggering the end/beginning of behavioural diapause of hard ticks (Hornok 2009). Late spring and summer dominance of thermophilic *Ha. concinna* ticks was already

observed among collected ticks from birds and from vegetation in Hungary (Hornok 2009; Hornok et al. 2013a).

Ixodes ricinus is one of the most common tick species associated with avian hosts in Europe (Franke et al. 2010a; Hornok et al., 2013a). Compared to other tick species of the genus it is a competent vector of the highest number of important zoonotic bacteria (Estrada-Peña and Jongejan 1999). Bird ticks may carry viruses, bacteria and protozoan parasites which could represent potential threat to humans. Among them well-known zoonotic agents of medical importance are *B. burgdorferi* sensu lato that causes Lyme disease (Gylfe et al. 2000), *A. phagocytophylum* responsible for granulocytic anaplasmosis (Keesing et al. 2012), and tick borne encephalitis virus (Waldenström et al. 2007). *Borrelia* bacteria indirectly elevate the tick-host bird's energetic needs, which may result in an increase of bacteria- and vector-transmission events. These bacteria could proliferate more successfully, by increasing foraging efforts which entails the growth of tick infestation risk. Ticks enhance the *Borrelia* proliferation as a consequence of immune response suppression (Heylen et al. 2014a). At the same time birds may have a defensive response against blood-sucking ectoparasites. It was proved that food-borne ecdysteroids, can reach high levels in the blood of avian hosts. Prior to our study, phytoecdysteroids (structure and effects similar to insect hormones) were shown to promote on-host apolysis of three-host ticks on goats in Hungary (Hornok et al. 2012a). In this work we assumed that the similar phenomenon (on-host apolysis) in case of birds and their ticks, have the same background.

Babesia and *Theileria* species are frequent protozoan parasites of bird ticks. In the epidemiology of babesioses affecting mammals, birds act as disseminators of *Babesia*-carrier ticks, rather than reservoirs (Hornok et al. 2015b). Recently, new *Babesia* genotypes has been reported from Asia (Rar et al. 2014), and some of them have been also detected in *Ha. concinna* ticks in Central Europe (Hornok et al. 2015b; Hamšíková et al. 2016). The geographical distribution of this tick species expands from the western Palearctic to central and eastern Asia (Lebedeva and Korenberg 1981). Tick exchange via migratory birds between Central Europe and East Asia may contribute to transportation of certain piroplasms, similarly to tick-borne viral pathogens (TBEV), that reflect this latitudal connection (Ponomareva et al. 2015). Subbotina and Loktev (2012) suggest that population spread of different birds, rodents, and accompanying ticks facilitated TBEV expansion to new territories. The fact that the virus strains of the European genotype were found in South Korea indicates that TBEV can also spread eastward across Eurasia (Subbotina and Loktev 2012). We assume a similar pattern in the case of *Babesia* genotypes, carried by *Ha. concinna* ticks.

In 2016 an unprecedent influx, more than 200 records of Siberian Accentors (*Prunella montanella*) were recorded throughout Europe. This bird species breeds on both sides of the

Ural and beyond in Siberia, and there were few (circa 32) records of it up to 2015 in Europe (Ławicki et al. 2016). These events may contribute to tick transportation from long distances, but we assume that gradual disposal by birds and the usage of same wintering grounds probably have larger significance.

6.1.1 Species diversity of ixodid ticks carried by migratory and non-migratory bird species

The species of ticks attaching to bird hosts and the intensity of infestation depends on several factors. Three host-generalist tick species, *I. ricinus* and *Ha. concinna* occur more often on birds in Central Europe (consequently collected more frequently), than ornithophilic ticks (Dubska et al. 2009; Špitalská et al. 2011). Additionally, *Ha. concinna* is geographically widespread in Eurasia (Lebedeva and Korenberg 1981) presumably because some of it's main hosts are birds, icluding migratory species. Investigation of bird ticks revealed the same pattern, as the most abundant tick species found were *I. ricinus* and *Ha. concinna* immature stages. The presence of *I. ricinus* on birds from March to November coincided with previous Hungarian results reporting the activity of this tick species (Hornok 2009). *Ha. concinna* ticks were found during a wider period on avian hosts (one or two months earlier) than their known seasonal activity in Central Europe (Nosek 1971). Ornithophilic ticks occurred less frequently on the birds studied by us, with adult forms also collected besides early developmental stages. *Ixodes frontalis* has been previously reported from Hungary more than half a century ago (Janisch 1959). In that study only two female *I. frontalis* have been collected from birds. Our present results attest that this may be a quite common tick species in our country. The observed spring predominance of *I. frontalis* larvae and nymphs on birds followed the late winter seasonal peak reported in Portugal (Norte et al. 2015). In the present study the great majority were collected from European Robins (*E. rubecula*), a bird species that known to have predominantly south-west to north-east spring migration from the Mediterranean region to Hungary (Hornok et al. 2012b). It is remarkable that *I. arboricola*, another common ornithophilic tick species in Central Europe (Mihalca et al. 2012; Novakova et al. 2015), was not found in this survey, despite the fact that its preferred hosts (i.e. cavity-nesting birds) were included in the study, and it has been reported earlier from Hungary (Babos 1965). Exotic ticks i.e. *Hyalomma* spp. subadults were not identified on the species level in our country (Janisch, 1959) until 2013, when it was evidenced by molecular biologic methods that *Hy. marginatum* larvae and nymph were found on a European Robin (*E. rubecula*) (Hornok et al. 2013a). In the present work the transportation of *Hy. rufipes* immature stages by birds in Central Europe has been proved for the first time with molecular methods. In the same way, the presence of *I. festai* in Hungary is reported as a new observation.

6.1.2 Genetic diversity of less frequent ixodid ticks in a geographical context

Migratory birds take a significant role in short- and long-distance transportation of ixodid ticks and tick-borne pathogens. From a molecular taxonomic point of view it may be important to investigate bird ticks, to find out the connections within the species between

separated geographical regions. In this work the majority of *I. frontalis* ticks were derived from European Robins (*E. rubecula*), a bird species that migrate predominantly from south-west to north-east. The spring predominance of larvae and nymphs on birds in Hungary during this study, followed the late winter seasonal peak in Portugal, as Norte et al. reported (2015). Molecular studies also supported this migratory connection. The two 16S rDNA genotypes (KU170518: genotype A-Hu16S, KU170519: genotype B-Hu16S) had 100% sequence identity to the corresponding isolates from the Azores. This observation suggests that *I. frontalis* is mainly transported between Central European fields and South-Western Europe. The two 16S rDNA genotypes represented two distinct genetic lineages of *I. frontalis*, similarly to the results of COI gene sequencing, where the two genetic lineages were recognizable. The separation is supported by high bootstrap values on the COI and 16S rDNA phylogenetic trees (Figures 6,7). Additionally, the degree of COI sequence divergence between the two lineages (9%) exceeds the proposed approximated sequence difference as species boundary for ticks (6.1% of COI gene) (Lv et al., 2014). Nevertheless, detailed morphological examination of *I. frontalis* nymphs collected later did not reveal morphological differences between these two genetic lineages (data not shown).

It is remarkable that *I. festai* was reported from Hungary for the first time. It was found on a Greenfinch (*C. chloris*) and a Dunnock (*P. modularis*), which species migrate to the Mediterranean Basin in autumn. *I. festai* is known to occur in Italy (Contini et al. 2011), from where these birds may arrive to Hungary during their spring migration. *I. lividus* that we collected from a Sand Martin (*R. riparia*) is host specific tick species. We identified the same genotype as Graham et al. (2010) did in the UK, despite the fact that Sand Martins have no direct migration routes between the two geographical regions (Csörgő et al. 2009). Therefore, this result indicates that the same genotype is present in separated European populations of *I. lividus*.

In Hungary *Hyalomma* spp. ticks were collected previously from middle distance migrants (Hornok et al. 2013a, 2014b), including Wood Warbler (*Phylloscopus sibilatrix*) and European Robin (*E. rubecula*). In the present study all three *Hyalomma* nymphs morphologically resembled *Hy. rufipes*. One specimen showed close identity in its partial COI gene to an Ethiopian *Hy. rufipes* hybrid, which was also reliably identified according to taxonomic keys (Rees et al. 2003). The Common Whitethroat (*S. communis*) that hosted the *Hyalomma* ticks is known to overwinter in sub-Saharan Africa (Csörgő et al. 2009) and breeds in Central Europe. *Hy. rufipes* larvae and nymphs occur frequently on birds in the Middle East (Hoogstraal and Kaiser 1958; Fain et al., 1995), where the migrating birds stop as they travel towards Europe, even carrying *Hy. rufipes* to northern territories like Norway (Hasle et al. 2009). Infestation of cattle with *Hy. rufipes* adults was reported in 2011 from Hungary (Hornok and Horváth 2012). Infestation occured presumably with the assistance of

migratory birds who taking part in the transportation of immature stages of this tick species. This result means that early developmental stages of primarily exotic ticks may be able to moult to adults under the continental climatic conditions. *Haemaphysalis concinna* ticks are broadly distributed in Eurasia, and typically attaching to birds, whereas other *Haemaphysalis* ticks in the genus were shown to be phylogeographically different in parts of Eurasia. In the present study six different COI genotypes, clustered in two lineages, were found among *Ha. concinna* larvae and nymphs collected from songbirds. The COI genotypes represented three 16S rDNA genotypes. Two of them have a high degree of 16S rDNA gene identity with conspecific ticks from East Siberia (Khasnatinov et al. 2016) and Japan (Takano et al. 2014). The reason for this close identity for some specimens can be the ectoparasite exchange via migratory birds between Europe and East Asia. The isolates that were highly similar to East Asian ones were collected during spring and autumn bird migration from bird species - i.e. Robins (*E. rubecula*), a Blackbird (*T. merula*) and a Dunnock (*P. modularis*) - that have eastern migratory connections towards Eastern Europe and Asia (Csörgő et al. 2009; Collar 2014).

6.2 Species specific characteristics of birds that influence their tick-infestation

The prevalence of tick infestation among birds depends on several factors. These factors may influence the epidemiological role of avian hosts from the point of view of tick-borne diseases. Feeding location preference is one of these factors. In previous Hungarian and international results the prevalence of tick infestation was significantly associated with the habit of ground feeding in birds (Ishiguro et al. 2000; Dubska et al. 2009; Hornok et al. 2014b). Similarly to previous observations there were significantly ($P < 0.0001$) more tick-infested birds among the ground-feeding bird species i.e. *Turdus* spp., Thrush Nightingale (*Luscinia luscinia*), Dunnock (*P. modularis*) and Robin (*E. rubecula*) (Taragel'ová et al. 2008; Dubska et al. 2009; Hornok et al. 2014b). *Ixodes ricinus* and *I. frontalis* immature stages were found mostly on these birds. Interestingly, in case of *I. frontalis* association with ground-feeding bird species was demonstrated here for the first time. In contrast to this, *Ha. concinna* larvae and nymphs were found significantly more frequently on birds that feed higher above the ground level i.e. Sedge warbler (*Acrocephalus schoenobaenus*), Savi's warbler (*Locustella lusciniodides*), Common grasshopper warbler (*L. naevia*) and Yellowhammer (*Emberiza citrinella*). The difference may be related to the different questing height of the two groups of ticks. *Haemaphysalis concinna* immature stages hold a relatively high questing position on the vegetation, as a result of an adaptation to the body size of their

preferred host species (roe deer, *C. capreolus*) in Hungary (Hornok et al. 2012a). The larvae and nymphs of *I. ricinus* had lower questing heights in association with small foraging mammals as primary hosts (Rigó et al. 2011). In the present study the body weight and length of migration route of birds had no significant association with intensity of tick infestation. However, in a previous Hungarian study it was demonstrated that the distance of migration significantly influences the tick burden of avian hosts. Hornok et al. (2014b) found that the prevalence of tick infestation was higher among local birds and short migrants, which could be explained their presence during the main tick season, while middle to long distance migrants are known to arrive at the end of the decisive period (in May and June). On the other hand, we collected *Hyalomma* early developmental stages from a long distance migrant, Common Whitethroat (*S. communis*). This finding is in harmony with previous results, and emphasize the importance of migratory birds as potential carriers of exotic tick species (Hornok et al. 2013a, 2014b).

The lack of association between body weight and mean intensity of tick infestation is in agreement with previous data published by Newman et al. (2015). They hypothesized that body weight may serve as an adequate null model of tick infestation and abundance and *Borrelia burgdorferi* infection prevalence, either because of larger surface area of skin, or correlation with life history traits (e.g. home range size, ground nesting and foraging behaviors). However, they found that average body weight of birds by species did not predict the number of subadults carried per bird.

6.3 Food-borne hormone in host blood influencing tick infestations

Interactions between parasites and their hosts depend on the prevalence and abundance of the parasite, effects on the fecundity and mortality of hosts, the degree of anti-parasite defence (immune response) by host and the effects of transmitted pathogens (Møller et al. 2013; Heylen et al. 2014b). In the present study we revealed another interesting factor that influences host-parasite relationship.

Birds usually carry immature stages of three-host ixodid ticks in temperate climate zone. Fully engorged ticks fall from their host and moult to the next stage in the environment, controlled by ecdysteroids (moulting hormones). High ecdysteroid concentration is required in tick's haemolymph in order to start apolysis (the preliminary phase of moulting)(Diehl et al. 1982). Few days after the end of feeding ecdysteroid levels are elevating progressively in nymphs until apolysis and stay high during epicuticle deposition and production of preecdysial cuticle. As the nymphs accomplice moulting ecdysteroid concentration relapse to

initial levels. Former laboratory studies reviewed by Rees (2004) proved that exogenous source of moulting hormones accelerate the moulting of ticks, by inducing apolysis. Investigation of the natural occurrence of this phenomenon, to the best of our knowledge, has never been reported. In the current study the percentage of apolytic ticks was the highest in July, but a second lower peak in November has been also observed. Population density of lepidopterans (data obtained from the Forestry Light Trap Network) refer to the activity of caterpillars in the same region. Moreover, these data may reflect seasonal activity of other arthropods. The summer peak of apolytic ticks followed the regional top activity of caterpillars, furthermore significant seasonal correlation was demonstrated between the population density of lepidopterans by month and the ratio of apolytic ticks. Caterpillars, that form the major part of diet of forest-dwelling passerine birds (Cholewa and Wesołowski 2011; Matrková and Remeš 2014), may contain high titres of ecdysteroids, even up to 780 ng/g (Sehnal et al. 1981). Caterpillars may even appear in the diet, if some passerine species mostly feed on other classes of arthropods (Haraszthy, L. 1998). Ecdysteroids influence the development of lepidopterans (metamorphosis), and have a similar physiological effect on other arthropods including ticks (Palmer et al. 1999). The high ratio of apolytic ticks feeding on insectivorous birds and the peak activity of caterpillars at the same time can be regarded as a consequence of ecdysteroids in caterpillars (and probably other arthropods). Nevertheless, further studies are required to investigate the interrelation between insect-derived ecdysteroids in the avian diet and on-host apolysis of hard ticks.

The previously mentioned late peak of apolytic ticks in November can be explained by phytoecdysteroid containing fruits and grains that are accessible for partly frugivorous/granivorous birds. Phytoecdysteroids are analogues of ecdysteroids, that occur in several plant species (Dinan et al. 2001). Some bird species switch from fully insectivorous diet to berry/grain eating in the late fall, if they cannot find enough insects (Jordano 1989). Some fruits that usually ripen from late summer in Central-Eastern Europe (e.g. Malus spp.) were reported as ecdysteroid-positive during the investigations with radioimmunoassay. However, scientists suggest that almost all plant species retain the genetic capacity to produce phytoecdysteroids (Dinan et al. 2001). If these organic compounds influence the function of ecdysteroid receptors in arthropods, their endocrine balance will be disrupted. This mechanism could be also utilised for insect control (Muema et al. 2017). Ecdysteroids affect not only moulting processes, but induce salivary gland degeneration, therefore shorten the time of blood sucking (Kaufman 1991; Rees 2004). As the duration of feeding gets shorter, the risk of pathogen transmission is decreasing. Wilhelmsson et al (2013) examined the effects of feeding duration on the *Borrelia* load of ticks. After 36 hours of feeding, adult female ticks infected with *Borrelia* bacteria contained a significantly lower *Borrelia* load compared to ticks with a shorter duration of feeding. This study suggests, that the lower

Borrelia loads of long-feeding ticks are caused by the pathogen transmission to the hosts (Wilhelmsson et al. 2013).

A Czech study that investigated the anabolic effects of ecdysteroids on birds found that levels of 20E (20-hydroxyecdysone) in the serum of birds were proportional to the amount of seeds (rich in ecdysteroid) they consumed (Koudela et al. 1995). During their experiment, 20E reached as much as 80 ng/ml concentration in the serum, measured by radioimmunoassay. Our results on insectivorous passerine birds showed high levels of 20-hydroxyecdysone (ca 8 µg/ml) and ecdysone (2 µg/ml). The ecdysteroid values in our work showed greater accumulation in several individuals confirming that accumulation of ecdysteroids in birds is possible.

In our study, five additional compounds were detected in several blood samples in relatively high concentrations. These ecdysteroids occur in different plant families as Asteraceae (e.g. Serratula, Leuzea), Lamiaceae (e.g. Ajuga), and Caryophyllaceae (e.g. Silene) in Europe (Lafont et al. 2002), but the concentration of these compounds is typically an order of magnitude lower than that of 20E. A possible explanation of this phenomenon is that poststerone and 2-deoxy-20-hydroxyecdysone identified as an in vivo metabolite of 20E in mice and in humans, respectively (Tsitsimpikou et al. 2001; Kumpun et al. 2011). In birds, dietary 20E may have similar metabolites as in mammals. However, the other three ecdysteroids that were detected in bird's blood have a specific structure that makes it unlikely that they are the metabolic products of dietary 20E in birds. We assume that polypodine B, ajugasterone C and particularly dacryhainansterone made their way from plant sources through caterpillars to the birds. These compounds were detected in higher or equal concentration as 20E in blood samples, which strongly suggest that their metabolism and/or elimination is much slower. Based on the few available studies on the metabolism of ecdysone in mice (Girault et al. 1988; Lafont et al. 1988), and of 20E in rodents (Ramazanov et al. 1996; Kumpun et al. 2011) and in humans (Tsitsimpikou et al. 2001; Brandt 2003), reduction at the B-ring is among the major metabolic routes of ecdysteroids. Moieties like a 5α -OH forming intramolecular H-bond with the 6-oxo group (polypodine B) or a conjugated 7 (9,11)-dien-6-one (dacryhainansterone) might interfere with this process, and the lack of OH-25 (ajugasterone C and dacryhainansterone) can possibly decrease phase II metabolism i.e. sulphate or glucuronide conjugation (Figure 3). It should also be noted, that all these compounds, and mainly dacryhainansterone, are more lipophilic than 20E, based on which other pharmacokinetic properties (absorption, plasma protein binding etc.) can also significantly contribute to a relatively higher accumulation rate. To the best of our knowledge no related studies are available with these ecdysteroids, apart from 20E and ecdysone. Nevertheless, these results emphasize the biological importance of the minor phytoecdysteroids.

6.4 Prevalence and molecular investigation of piroplasms in *Ha. concinna* ticks, carried by birds

Haemaphysalis concinna is one of the most common tick species collected from passerine birds in Central Europe (Hornok et al. 2016a). This tick species is present throughout Europe and Asia (Lebedeva and Korenberg 1981), while *I. ricinus* is restricted to the western Palaearctic. Considering that birds are preferred hosts of *Ha. concinna*, transportation of this parasite by migratory birds is also likely to occur frequently. On the contrary, *Haemaphysalis erinacei* which prefers hedgehog, carnivore and rodent hosts, exhibits a considerable spatial genetic heterogenity. This phenomenon is due to the separation during ice age(s), when southern peninsulas of Europe acted as major refugia for animals, from where distinct clades of animal species emerged, and glacial surfaces confluent with the Caspian sea inhibited genetic mixing for parasites whose typical hosts do not migrate (Hornok et al. 2016b).

Piroplasms (Apicomplexa: Piroplasmida) are geographically widespread, unicellular, tick-borne parasites that infect blood cells of vertebrates (Homer et al. 2000). Members of the genus *Babesia*, may affect both domesticated and game animal species, and even humans (Homer et al. 2000). Among them, increasing number of new species and genotypes (Rar et al. 2014; Baneth et al. 2015) are being molecularly characterized, however pathogenicity of the latter is frequently unknown. In the epidemiology of mammal babesioses, *Haemaphysalis* spp. are important in the transmission of piroplasms to ruminants (Alani and Herbert 1988; Yin et al. 1996), and birds are the suspected disseminators of *Babesia*-carrier ticks. An earlier Hungarian study confirmed the role of *Haemaphysalis* ticks in the epidemiology of piroplasms. Hornok et al. (2015b) reported the presence of *B. crassa* from *Ha. inermis* and *B. motasi*, along with four other different piroplasms (genotypes) form *Ha. concinna*. These pathogens were newly detected in Central-Eastern Europe, and none of them were reported from these vectors before. Additionally, among the *Babesia* genotypes found in *Ha. concinna* in that study, two were formerly reported from Far Eastern Russia and in East Siberia. These results were in accordance with the broad geographical range of *Ha. concinna* and its longitudinal transportation via migratory birds.

In the current study 321 *Ha. concinna* immature stages were collected from passeriform birds and tested for the presence of piroplasms. Piroplasms detected in these ticks were molecularly identical to three *Babesia* genotypes that have been reported previously from southern Siberia (Baikal region) and Far East of Russia (Rar et al. 2014). In the majority of ticks two genotypes were present: “Irk-Hc133” (found in Irkutsk, Siberia) and “Kh-Hc222” (reported from Khabarovsk, Far East), and only three *Ha. concinna* specimens harboured the third genotype, “Irk-Hc130” (described also from Irkutsk: Rar et al. 2014).

Genotypes “Irk-Hc133” and “Kh-Hc222” have been detected before in questing *Ha. concinna* ticks in Hungary (Hornok et al. 2015b) and in questing or rodent-attached *Ha. concinna* ticks in Slovakia (Hamšíková et al. 2016). The third *Babesia* genotype, that was formerly described also from Irkutsk, has not been found in Europe until now. None of these three *Babesia* genotypes have been detected in ticks of birds previously. All three genotypes belonged to the phylogenetic group formed by *Babesia* spp. of ruminants, but they have unknown pathogenicity. It is likely that *Ha. concinna* could have access piroplasms from wild ruminants, because these are the preferred hosts of its larvae and nymphs, but *Ha. concinna* can also infect domestic small ruminants in Central-Eastern Europe (Hornok et al. 2012a). However, to the best of our knowledge these *Babesia* genotypes have not been reported from ruminant hosts.

There were no significant differences between the prevalence of PCR positive ticks among early developmental stages. PCR positivity of larvae suggests that the Siberian, Far Eastern *Babesia* genotypes are transovarially transmitted (similarly to other members of *Babesia* sensu stricto) and thus maintained and dispersed over large geographical distances by *Ha. concinna*. Piroplasm PCR positivity of *Ha. concinna* ticks was significantly less frequent during the spring. This finding suggest that migratory birds arriving from the south during the spring are the least important in the dispersal of *Ha. concinna*-associated piroplasms, as contrasted to those arriving from the north or northeast to Hungary during late summer and autumn. Larvae and nymphs of *Ha. concinna* are active only during summer and autumn (until October) (Nosek 1971), this confirms the importance of autumn migration in the long distance transportation of this tick species via birds. The immature stages of *Ha. concinna* suck blood for up to six days (Meng et al. 2014), during which their avian host may fly even a few hundred kilometres (Newton et al. 2008).

Haemaphysalis concinna larvae and nymphs PCR positive for piroplasms were collected significantly more frequently from five bird species with known eastern migratory connections, supporting their eco-epidemiological role in the above context. Eastern connections of concerning bird species with key role in dispersing *Babesia*-carrier *Ha. concinna* ticks are well documented.

Bird species with current migratory habit showing eastern connections are Yellowhammer (*Emberiza citrinella*), Song Thrush (*T. philomelos*) and River Warbler (*Locustella fluviatilis*) (Csörgő et al. 2009; Scebba and Olivieri Del Castillo 2017; Dove et al. 2016).

Yellowhammers (*E. citrinella*) ringed in Hungary were recaptured as far as Russia 2800 km to the east (Csörgő et al. 2009). The geographical range of this bird species extends to the Irkutsk region of Siberia (Irwin et al. 2009), the place of origin for two *Babesia* genotypes (“Irk-Hc133” and “Irk-Hc130”) detected here.

Nightingales (*Luscinia megarhynchos*) in Hungary derived from eastern European or Asian populations, as demonstrated with phylogenetic methods (Ács and Kováts 2013).

Savi's warbler's (*L. luscinioides*) western palearctic populations are phylogenetically more closely related to Asian warblers (*Bradypterus* spp.) than to certain *Locustella* spp. (Drovetski et al. 2004). Phylogenetic comparison of current populations of Savi's warblers and several other long distance migratory species reflected that the direction during post-glacial recolonisation followed eastward or westward directions (Irwin and Irwin 2005). Moreover, in the last decades some of the newly observed emerging bird species in Hungary were of eastern origin, from the region of Turkestan and Mongolia (Csörgő et al. 2009).

Transportation of *Babesia*-carrier ticks more likely happened towards West, because the peak activity of *H. concinna* larvae and nymphs is during the late summer and autumn migration, when birds are heading westward. Moreover, an other pathogen (TBEV) shows a similar pattern in westward distribution (Subbotina and Loktev 2012). Nevertheless, spreading in the opposite direction is also possible.

7. Overview of the new scientific results

1. In the present work the transportation of *Hy. rufipes* immature stages by birds in Central Europe has been proved by molecular methods, and *I. festai* was collected for the first time in Hungary. Our present results attest that *I. frontalis* ticks are transported by avian hosts frequently in Hungary. Two genetic lineages of *I. frontalis* and *Ha. concinna* are transported by birds in Central Europe, which reflect a high degree of sequence identity to South-Western European and East Asian isolates of the same tick species, respectively. These findings highlight the importance of western and eastern migratory connections by birds, which are also relevant to the epidemiology of tick-borne diseases.

2. In case of *I. frontalis*, association of immature stages with ground-feeding bird species was demonstrated in Hungary for the first time. *Haemaphysalis concinna* larvae and nymphs occured significantly more frequently on vegetation-foraging birds of higher altitude.

3. The presence of naturally acquired ecdysteroids in the blood of passerine birds, which induce on-host apolysis in ticks (not normal in three-host ticks) was reported here for the first time. Investigation of the natural occurrence of this phenomenon, to the best of our knowledge, has never been reported. Exogenous ecdysteroids may reach high levels in the blood of insectivorous passerine birds, and might affect ticks by shortening their parasitism.

4. *Babesia* genotype “Irk-Hc130” has been found in Europe for the first time. This is the first report of “Irk-Hc130”, “Irk-Hc133” and “Kh-Hc222” genotypes in *Ha. concinna* ticks of birds. Findings of the present study indicate that birds may play a significant role in the long distance geographical dispersal of *Babesia* genotypes within *Ha. concinna* ticks. *Babesia*-carrier ticks collected from resident bird species might reflect Central European establishment of Siberian or Far Eastern *Babesia* genotypes.

8. References

Ács, Z., Kováts, D.: **Phylogenetic patterns of a nightingale population in a contact zone of *Luscinia megarhynchos* and *L. luscinia* in Hungary**. North. West. J. Zool., 9. 365–373, 2013.

Alani, A.J., Herbert, I.V.: **The morphometrics of *Babesia motasi* (Wales) and its transmission by *Haemaphysalis punctata* (Canestrini and Fanzago 1877) to sheep.** Vet. Parasitol., 30. 87–95, 1988.

Babos, S.: **Kullancsok-Ixodidea.** Fauna Hungarie 18. 1–38, 1965.

Baneth, G., Florin-Christensen, M., Cardoso, L., Schnittger, L.: **Reclassification of *Theileria annae* as *Babesia vulpes* sp. nov.** Parasit. Vectors, 8. 207, 2015.

Bankovics, A.: **A madarak.** Budapest: Press Publica, 2004.

Belli, A., Sarr, A., Rais, O., Rego, R.O.M., Voordouw, M.J.: **Ticks infected via co-feeding transmission can transmit Lyme borreliosis to vertebrate hosts.** Sci. Rep., 7. 5006, 2017.

Berthová, L., Slobodník, V., Slobodník, R., Olekšák, M., Sekeyová, Z., Svitálková, Z., Kazimírová, M., Špitalská, E.: **The natural infection of birds and ticks feeding on birds with *Rickettsia* spp. and *Coxiella burnetii* in Slovakia.** Exp. Appl. Acarol., 68. 299–314, 2016.

Black, W.C., Piesman, J.: **Phylogeny of hard and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences.** Proc Natl Acad Sci U S A., 91. 10034–10038, 1994.

Bozó, L., Heim, W., Harnos, A., Csörgő, T.: **Can we explain vagrancy in Europe with the autumn migration phenology of Siberian warbler species in East Russia?** Ornis Hungarica.,24. 150-171, 2016.

Brandt, F.: **Pharmakokinetik und Metabolismus des 20-Hydroxyecdysons im Menschen.** University of Marburg (Germany), 2003.

Casati, S., Sager, H., Gern, L., Piffaretti, J.C.: **Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland,** Ann. Agric. Environ. Med., 13, 65-70, 2006.

Cholewa, M., Wesołowski, T.: **Nestling food of European hole-nesting passerines: do we know enough to test the adaptive hypotheses on breeding seasons?** Acta Ornithol., 46. 105–138, 2011.

Collar, N.: **Handbook of the Birds of the World Alive.** Barcelona: Lynx Edicions, 2014.

Contini, C., Palmas, C., Seu, V., Stancampiano, L., Usai, F.: **Redescription of the male of *Ixodes festai* Rondelli, 1926 (Ixodida: Ixodidae) on specimens from Sardinia (Italy)**. Parasite., 18. 235–240, 2011.

Csörgő, T. et al. (szerk): **Magyar madárvonulási atlasz.** Budapest: Kossuth Kiadó, 2009.

de la Fuente, J., Estrada-Peña, A., Cabezas-Cruz, A., Brey, R.: **Flying ticks: anciently evolved associations that constitute a risk of infectious disease spread.** Parasit. Vectors., 8. 538, 2015.

Diakou, A., Norte, A.C., Lopes de Carvalho, I., Núncio, S., Nováková, M., Kautman, M., Alivizatos, H., Kazantzidis, S., Sychra, O., Literák, I.: **Ticks and tick-borne pathogens in wild birds in Greece.** Parasitol. Res., 115. 2011–2016, 2016.

Diehl, P.A., Germond, J.E., Morici, M.: **Correlations between ecdysteroid titers and integument structure in nymphs of the tick, *Amblyomma hebraeum* Koch (Acarina: Ixodidae).** Rev. Suisse Zool., 89. 859–868, 1982.

Dinan, L., Savchenko, T., Whiting, P.: **On the distribution of phytoecdysteroids in plants.** Cell. Mol. Life Sci., 58. 1121–1132, 2011.

Dove, C.J., Saucier, J., Whatton, J.F., Schmidt, B., Roble, H.R.: **First record of River Warbler Locustella fluviatilis and additional records for Plain Nightjar Caprimulgus inornatus and Lesser Masked Weaver Ploceus intermedius in Djibouti.** Bull. B.O.C., 137. 67-70, 2016.

Drovetski, S.V., Zink, R.M., Fadeev, I.V., Nesterov, E.V., Koblik, E.A., Red'kin, Y.A., Rohwer, S.: **Mitochondrial phylogeny of *Locustella* and related genera.** J. Avian Biol., 35. 105–110, 2004.

Dubska, L., Literak, I., Kocianova, E., Taragelova, V., Sverakova, V., Sychra, O., Hromadko, M.: **Synanthropic birds influence the distribution of *Borrelia* species: analysis of *Ixodes ricinus* ticks feeding on passerine birds.** Appl. Environ. Microbiol., 77. 1115–1117, 2011.

Dubska, L., Literak, I., Kocianova, E., Taragelova, V., Sychra, O.: **Differential role of passerine birds in distribution of *Borrelia* spirochetes, based on data from ticks collected from birds during the postbreeding migration period in Central Europe.** Appl. Environ. Microbiol., 75. 596–602, 2009.

Elfving, K., Olsen, B., Bergström, S., Waldenström, J., Lundkvist, Å., Sjöstedt, A., Mejlon, H., Nilsson K.: **Dissemination of spotted fever rickettsia agents in Europe by migrating birds.** PLoS One., 5. e8572, 2010.

Estrada-Peña, A., Jongejan, F.: **Ticks feeding on humans: A review of records on human-biting Ixodoidea with special reference to pathogen transmission.** Exp. Appl. Acarol., 23. 685–715, 1999.

Fain, A., Roggeman, W., Symens, P., Suhaibani, A.: **Notes on a small collection of immature *Hyalomma* ticks from migrating birds during their spring migration in Karan Island, Persian Gulf, Saudi Arabia [Hyalomma marginatum rufipes].** Bull. l'Institut R. Des Sci. Nat. Belgique Entomol., 65. 101–103, 1995.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R.: **DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates.** Mol Mar Biol Biotechnol., 3. 294-299, 1994.

Franke, J., Fritzsch, J., Tomaso, H., Straube, E., Dorn, W., Hildebrandt, A.: **Coexistence of pathogens in host-seeking and feeding ticks within a single natural habitat in Central Germany.** Appl. Environ. Microbiol., 76. 6829–6836, 2010a.

Franke, J., Meier, F., Moldenhauer, A., Straube, E., Dorn, W., Hildebrandt, A.: **Established and emerging pathogens in *Ixodes ricinus* ticks collected from birds on a conservation island in the Baltic Sea.** Med. Vet. Entomol., 24. 425–432, 2010b.

Gimesi, L., Homoródi, R., Hirka, A., Szabóki, C., Hufnagel, L.: **The effect of climate change on the phenology of moth abundance and diversity.** Appl. Ecol. Environ. Res., 10. 349–363, 2012.

Girault, J.P., Lafont, R., Kerb, U.: **Ecdysone catabolism in the white mouse.** Drug Metab. Dispos., 16. 716–720, 1988.

Graham, R.I., Mainwaring, M.C., Du Feu, R.: **Detection of spotted fever group *Rickettsia* spp. from bird ticks in the U.K.** Med. Vet. Entomol., 24. 340–343, 2010.

Gray, J.S., Dautel, H., Estrada-Pena, A., Kahl, O., Lindgren, E.: **Effects of climate change on ticks and tick-borne diseases in Europe.** Interdiscip. Perspect. Infect. Dis., 2009. e593232, 2009.

Gylfe, A., Bergström, S., Lundstróm, J., Olsen, B.: **Epidemiology: Reactivation of *Borrelia* infection in birds.** Nature., 403. 724–725, 2000.

Hamšíková, Z., Kazimírová, M., Haruštiaková, D., Mahríková, L., Slovák, M., Berthová, L., Kocianová, E., Schnittger, L.: ***Babesia* spp. in ticks and wildlife in different habitat types of Slovakia.** Parasit. Vectors., 9. 292, 2016.

Hasle, G., Bjune, G., Edvardsen, E., Jakobsen, C., Linnehol, B., Røer, J.E., Mehl, R., Røed, K.H., Pedersen, J., Leinaas, H.P.: **Transport of ticks by migratory passerine birds to Norway.** J. Parasitol., 95. 1342–1351, 2009.

Hasle, G., Leinaas, H.P., Røed, K.H., Øines, Ø.: **Transport of *Babesia venatorum*-infected *Ixodes ricinus* to Norway by northward migrating passerine birds.** Acta Vet. Scand., 53. 41, 2011.

Haraszthy, L.: **Magyarország madarai.** Budapest: Mezőgazda Kiadó, 1998.

Heylen, D.J.A., Sprong, H., Oers, K. van, Fonville, M., Leirs, H., Matthysen, E.: **Are the specialized bird ticks, *Ixodes arboricola* and *I. frontalis*, competent vectors for *Borrelia burgdorferi* sensu lato?** Environ. Microbiol., 16. 1081–1089, 2014a.

Heylen, D.J.A., Müller, W., Vermeulen, A., Sprong, H., Matthysen, E.: **Virulence of recurrent infestations with Borrelia-infected ticks in a *Borrelia*-amplifying bird.** Sci. Rep., 5. 16150, 2014b.

Heylen, D.J.A., Sprong, H., Krawczyk, A., Van Houtte, N., Genné, D., Gomez-Chamorro, A., van Oers, K., Voordouw, M. J.: **Inefficient co-feeding transmission of *Borrelia afzelii* in two common European songbirds.** Sci. Rep., 7. 39596, 2017.

Hildebrandt, A., Franke, J., Meier, F., Sachse, S., Dorn, W., Straube, E.: **The potential role of migratory birds in transmission cycles of *Babesia* spp., *Anaplasma phagocytophilum*, and *Rickettsia* spp.** Ticks Tick. Borne. Dis., 1. 105–107, 2010.

Homer, M.J., Aguilar-Delfin, I., Telford, S.R., Krause, P.J., Persing, D.H.: **Babesiosis.** Clin. Microbiol. Rev., 13. 451–469, 2000.

Hoogstraal, H.: **African Ixodoidea- I. Ticks of the Sudan.** Washington DC: US Department of the Navy, Bureau of Medicine and Surgery, 1956.

Hoogstraal, H., Kaiser, M.N.: **Observations on Egyptian *Hyalomma* ticks (Ixodoidea, Ixodidae). 2. Parasitism of migrating birds by immature *H. rufipes* Koch.** Ann. Entomol. Soc. Am., 51. 12-16, 1958.

Hoogstraal, H., Kaiser, M.N., Traylor, M.A., Gaber, S., Guindy, E.: **Ticks (Ixodidea) on birds migrating from Africa to Europe and Asia.** Bull. World Health Organ., 24. 197–212, 1961.

Hornok, S.: **Allochronic seasonal peak activities of *Dermacentor* and *Haemaphysalis* spp. under continental climate in Hungary.** Vet. Parasitol., 16. 366–369, 2009.

Hornok, S., Csörgő, T., de la Fuente, J. , Gyuranecz, M., Privigyei, C., Meli, M.L., Kreizinger, Z., Gönczi, E., de Mera, I.G.F., Hofmann-Lehmann, R.: **Synanthropic birds associated with high prevalence of tick-borne rickettsiae and with the first detection of *Rickettsia aeschlimannii* in Hungary**. Vector Borne Zoonotic Dis., 13. 77–83, 2013a.

Hornok, S., Estók, P., Kováts, D., Flaisz, B., Takács, N., Szőke, K., Krawczyk, A., Kontschán, J., Gyuranecz, M., Fedák, A., Farkas, R., Haarsma, A.J., Sprong, H.: **Screening of bat faeces for arthropod-borne apicomplexan protozoa: *Babesia canis* and *Besnoitia***

***besnoiti*-like sequences from Chiroptera**. Parasit. Vectors., 8. 441, 2015a.

Hornok, S., Flaisz, B., Takács, N., Kontschán, J., Csörgő, T., Csipak, Á., Jaksa, B.R., Kováts, D.: **Bird ticks in Hungary reflect western , southern , eastern flyway connections and two genetic lineages of *Ixodes frontalis* and *Haemaphysalis concinna*.** Parasit. Vectors., 9. 101, 2016a.

Hornok, S., Horváth, G.: **First report of adult *Hyalomma marginatum rufipes* (vector of Crimean-Congo haemorrhagic fever virus) on cattle under a continental climate in Hungary.** Parasit. Vectors., 5. 170, 2012.

Hornok, S., Horváth, G., Jongejan, F., Farkas, R.: **Ixodid ticks on ruminants, with on-host initiated moulting (apolysis) of *Ixodes*, *Haemaphysalis* and *Dermacentor* larvae.** Vet. Parasitol., 187. 350–353, 2012a.

Hornok, S., Karcza, Z., Csörgő, T.: **Birds as disseminators of ixodid ticks and tick-borne pathogens: note on the relevance to migratory routes.** Ornis Hungarica., 20. 86–89, 2012b.

Hornok, S., Kontschán, J., Kováts, D., Kovács, R., Angyal, D., Görföl, T., Polacsek, Z., Kalmár, Z., Mihalca, A.D.: **Bat ticks revisited: *Ixodes ariadnae* sp. nov. and allopatric genotypes of *I. vespertilionis* in caves of Hungary.** Parasit. Vectors., 7. 202, 2014a.

Hornok, S., Kováts, D., Csörgő, T., Meli, M.L., Gönczi, E., Hadnagy, Z., Takács, N., Farkas, R., Hofmann-Lehmann, R.: **Birds as potential reservoirs of tick-borne pathogens: first evidence of bacteraemia with *Rickettsia helvetica*.** Parasit. Vectors., 7. 128, 2014b.

Hornok, S., Meli, M.L., Gönczi, E., Hofmann-Lehmann, R.: **First evidence of Candidatus *Neoehrlichia mikurensis* in Hungary.** Parasit. Vectors., 6. 267, 2013b.

Hornok, S., Takács, N., Kontschán, J., György, Z., Micsutka, A., Iceton, S., Flaisz, B., Farkas, R., Hofmann-Lehmann, R.: **Diversity of *Haemaphysalis*-associated piroplasms of ruminants in Central-Eastern Europe, Hungary.** Parasit. Vectors., 8. 627, 2015b.

Hornok, S., Wang, Y., Otranto, D., Keskin, A., Lia, R.P., Kontschán, J., Takács, N., Farkas, R., Sándor, A. D.: **Phylogenetic analysis of *Haemaphysalis erinacei* Pavesi, 1884 (Acari: Ixodidae) from China, Turkey, Italy and Romania.** Parasit. Vectors., 9. 643, 2016b.

Hovius, J.W., van Dam, A..P., Fikrig, E.: **Tick-host-pathogen interactions in Lyme borreliosis.** Trends Parasitol., 23. 434–438, 2007.

Hunyadi, A., Gergely, A., Simon, A., Tóth, G., Veress, G., Báthori, M.: **Preparative-scale chromatography of ecdysteroids of *Serratula wolffli* Andrae.** J. Chromatogr. Sci., 45. 76–86, 2007.

Irwin, D.E., Irwin, J.H.: **Siberian Migratory Divides. The Role of Seasonal Migration in**

Speciation. In: *Birds of two worlds: The ecology and evolution of migration,* Editors: Greenberg, R.,Marra, P.P., Baltimore: John Hopkins University Press, 2005.

Irwin, D.E., Rubtsov, A.S., Panov, E.N.: **Mitochondrial introgression and replacement between yellowhammers (*Emberiza citrinella*) and pine buntings (*Emberiza leucocephalos*) (Aves: Passeriformes)**. Biol. J. Linnean Society., 98. 422–438, 2009.

Ishiguro, F., Takada, N., Masuzawa, T., Fukui, T.: **Prevalence of Lyme disease *Borrelia* spp. in ticks from migratory birds on the Japanese mainland.** Appl. Environ. Microbiol., 66. 982–986, 2000.

Jaenson, T.G.T., Talleklint, L., Lundqvist, L., Olsen, B., Chirico, J., Mejlon, H.: **Geographical distribution, host associations , and vector roles of ticks (Acari : Ixodidae , Argasidae) in Sweden.** J. Med. Entomol., 31. 240–256, 1994.

Jameson, L.J., Morgan, P.J., Medlock, J.M., Watola, G., Vaux, A.G.C.: **Importation of *Hyalomma marginatum*, vector of Crimean-Congo haemorrhagic fever virus, into the United Kingdom by migratory birds.** Ticks Tick. Borne. Dis., 3. 95–99, 2012.

Jameson, L.J., Phipps, L.P., Medlock, J.M.: **Surveillance for exotic ticks on companion animals in the UK.** Vet. Rec., 166. 202–203, 2010.

Janisch, M.: **A hazai kullancsfauna feltérképezése.** Állattani közlemények., 47. 103-110, 1959.

Jordano, P.: **Diet variation among wintering frugivorous Robins, Erithacus rubecula: effects on body condition.** Ardeola., 36. 161–183, 1989.

Kaufman, W.R.: **Furher investigations on the action of ecdysteroids on the salivary glands of the female tick *Amblyomma americanum*.** Exp. Appl. Acarol., 10. 259–265, 1991.

Keesing, F., Hersh, M.H., Tibbetts, M., Mchenry, D.J., Duerr, S., Brunner, J., Killilea, M., Logiudice, K., Schmidt, K.A., Ostfeld, R.S.: **Reservoir competence of vertebrate hosts for *Anaplasma phagocytophilum***. Emerg. Infect. Dis., 18. 10–13, 2012.

Khasnatinov, M.A., Liapunov, A.V., Manzarova, E.L., Kulakova, N.V., Petrova, I.V., Danchinova, G.A.: **The diversity and prevalence of hard ticks attacking human hosts in Eastern Siberia (Russian Federation) with first description of invasion of non-endemic tick species.** Parasitol. Res., 115. 501–510, 2016.

Klaus, C., Gethmann, J., Hoffmann, B., Ziegler, U., Heller, M., Beer, M.: **Tick infestation in birds and prevalence of pathogens in ticks collected from different places in Germany**. Parasitol. Res. 115. 2729–2740, 2016.

Koudela, K., Tenora, J., Bajer, J., Mathová, A., Sláma, K.: **Stimulation of growth and**

development in Japanese quails after oral administration of ecdysteroid-containing diet. Eur. J. Entomol., 92. 349–354, 1995.

Kumpun, S., Girault, J.P., Dinan, L., Blais, C., Maria, A., Dauphin-Villemant, C., Yingyongnarongkul, B., Suksamrarn, A., Lafont, R.: **The metabolism of 20-hydroxyecdysone in mice: relevance to pharmacological effects and gene switch applications of ecdysteroids**. J. Steroid Biochem. Mol. Biol., 126. 1–9, 2011.

Lafont, R., Girault, J.P., Kerb, U.: **Excretion and metabolism of injected ecdysone in the white mouse.** Biochem. Pharmacol., 37. 1174–1177, 1988.

Lafont, R., Harmatha, J., Marion-Poll, F., Dinan, L., Wilson, I.D.: **Ecdybase− The Ecdysone Handbook.** available on-line at: http:// ecdybase.org, 2002. Accessed: 2017.12.05.

Lebedeva, N.N., Korenberg, E.I.: **Distribution of *Haemaphysalis concinna* Koch in the Soviet Union and some general features of its ecology.** Folia Parasitol. (Praha), 249–261, 1981.

Lommano, E., Dvořák, C., Vallotton, L., Jenni, L., Gern, L.: **Tick-borne pathogens in ticks collected from breeding and migratory birds in Switzerland.** Ticks Tick. Borne. Dis., 5. 871–882, 2014.

Ławicki, L., van Egmond, A., Hellström, M., Bruun, M.: **Unprecedented influx of Siberian Accentors in Europe - a monthly summary.** available on-line at: https://www.dutchbirding.nl, 2016. Accessed: 2018.02.07.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L., Lin, X.: **Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida)**. Parasit. Vectors., 7. 93, 2014.

Matrková, J., Remeš, V.: **Vitamin E improves growth of collared flycatcher *Ficedula albicollis* young: A supplementation experiment.** J. Avian Biol., 45. 475–483, 2014.

Maturano, R., Faccini, J.L.H., Daemon, E., Fazza, P.O.C., Bastos, R.R.: **Additional information about tick parasitism in Passeriformes birds in an Atlantic Forest in southeastern Brazil.** Parasitol. Res., 114. 4181–4193, 2015.

Meng, H., Xu, S., Yu, Z., Liu, Z., Liu, J., Yang, X., Liu, J.: **The life cycle and occurrence of *Haemaphysalis concinna* (Acari: Ixodidae) under field conditions.** Ticks Tick. Borne. Dis., 5. 887–891, 2014.

Mihalca, A.D., Dumitrache, M.O., Magdaş, C., Gherman, C.M., Domşa, C., Mircean, V., Ghira, I.V., Pocora, V., Ionescu, D.T., Sikó Barabási, S., Cozma, V., Sándor, A.D.: **Synopsis of the hard ticks (Acari: Ixodidae) of Romania with update on host associations and geographical distribution.** Exp. Appl. Acarol., 58. 183–206, 2012.

Molin, Y., Lindeborg, M., Nyström, F., Madder, M., Hjelm, E., Olsen, B., Jaenson, T.G.T., Ehrenborg, C.: **Migratory birds, ticks, and *Bartonella***. Infect. Ecol. Epidemiol., 1. 5997, 2011.

Møller, A.P., Merino, S., Soler, J.J., Antonov, A., Badás, E.P., Calero-Torralbo, M.A., de Lope, F., Eeva, T., Figuerola, J., Flensted-Jensen, E., Garamszegi, L.Z., González-Braojos, S., Gwinner, H., Hanssen, S.A., Heylen, D., Ilmonen, P., Klarborg, K., Korpimäki, E., Martínez, J., Martínez-de la Puente, J., Marzal, A., Matthysen, E., Matyjasiak, P., Molina-Morales, M., Moreno, J., Mousseau, T.A., Nielsen, J.T., Pap, P.L., Rivero-de Aguilar, J., Shurulinkov, P., Slagsvold, T., Szép, T., Szöllősi, E., Török, J., Vaclav, R., Valera, F., Ziane, N.: **Assessing the effects of climate on host-parasite interactions: a comparative study of European birds and their parasites**. PLoS One., 8. e82886, 2013.

Muema, J.M., Bargul, J.L., Njeru, S.N., Onyango, J.O., Imbahale, S.S.: **Prospects for malaria control through manipulation of mosquito larval habitats and olfactory-mediated behavioural responses using plant-derived compounds.** Parasit. Vectors., 10. 184, 2017.

Newman, E.A, Eisen, L., Eisen, R.J., Fedorova, N.: ***Borrelia burgdorferi* sensu lato spirochetes in wild birds in northwestern California : Associations with ecological factors , bird behavior and tick infestation.** PLoS One., 1–26, 2015.

Newton, I.: **The migration ecology of birds, 1st ed.** London: Academic Press, 2008.

Norte, A. C., da Silva, L.P., Q.Tenreiro, P.J., Felgueiras, M.S., Araújo, P.M., Lopes, P.B., Matos, C., Rosa, A., Ferreira, P.J.S.G., Encarnação, P., Rocha, A., Escudero, R., Anda, P., Núncio, M.S., Lopes de Carvalho, I.: **Patterns of tick infestation and their *Borrelia burgdorferi* s.l. infection in wild birds in Portugal.** Ticks Tick. Borne. Dis., 6. 743–750, 2015.

Nosek, J.: **The ecology, bionomics and behaviour of *Haemaphysalis* (Haemaphysalis) *concinna*** Tick. Parasitol. Res., 36. 233–241, 1971.

Novakova, M., Bulkova, A., Costa, F.B., Kristin, A., Krist, M., Krause, F., Liznarova, E., Labruna, M.B., Literak, I.: **Molecular characterization of '*Candidatus* Rickettsia vini' in *Ixodes arboricola* from the Czech Republic and Slovakia.** Ticks Tick. Borne. Dis., 6. 330–333, 2015.

Olsén, B., Jaenson, T.G.T., Bergström, S.: **Prevalence of Borrelia burgdorferi sensu lato-infected ticks on migrating birds.** Appl. Environ. Microbiol., 61. 3082-3087, 1995.

Palmer, M.J., Harmon, M.A., Laudet, V.: **Characterization of EcR and RXR homologues in the ixodid tick, *Amblyomma americanum* (L.).** Am. Zool., 39. 747–757, 1999.

Palomar, A.M., Portillo, A., Santibáñez, P., Santibáñez, S., García-álvarez, L., Oteo, J.A.: **Genetic characterization of *Candidatus* Rickettsia vini, a new rickettsia amplified in ticks from La Rioja, Spain.** Ticks Tick. Borne. Dis., 3. 319–321, 2012.

Ponomareva, E.P., Mikryukova, T.P., Gori, A.V., Kartashov, M.Y., Protopopova, E. V., Chausov, E.V., Konovalova, S.N., Tupota, N.L., Gheorghita, S.D., Burlacu, V.I., Ternovoi, V.A., Loktev, V.B.: **Detection of Far-Eastern subtype of tick-borne encephali tis viral RNA in ticks collected in the Republic of Moldova**. J. Vector Borne Dis., 52. 334–336, 2015.

Ramazanov, N.S., Saatov, Z., Syrov, B.N.: **Study of ecdysterone metabolites isolated from rat urine.** Chem. Nat. Compd., 32. 545–549, 1996.

Rar, V.A., Epikhina, T.I., Suntsova, O.V., Kozlova, I.V., Lisak, O.V., Pukhovskaya, N.M., Vysochina, N.P., Ivanov, L.I., Tikunova, N.V.: **Genetic variability of *Babesia* parasites in *Haemaphysalis* spp. and *Ixodes persulcatus* ticks in the Baikal region and Far East of Russia.** Infect. Genet. Evol., 28. 270–275, 2014.

Reed, K.D., Meece, J.K., Henkel, J.S., Shukla, S.K.: **Birds, migration and emerging zoonoses: west nile virus, lyme disease, influenza A and enteropathogens.** Clin Med Res., 1. 5–12, 2003.

Rees, H.H.: **Hormonal control of tick development and reproduction.** Parasitology., 129. 127–143, 2004.

Reiczigel, J.: **Confidence intervals for the binomial parameter: some new considerations.** Statist. Med., 22. 611-621, 2003.

Rigó, K., Gyuranecz, M., Tóth, G.Á., Földvári, G.: **Detection of *Borrelia burgdorferi s*ensu lato and *Anaplasma phagocytophilum* in small mammals and ectoparasites in Hungary.** Vector Borne Zoonotic Dis., 11. 1499–1501, 2011.

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Spitalská, E., Kazimírová, M.: ***Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: New hazards and relevance for public health.** Front. Public Heal., *2*. 251, 2014.

Sándor, A.D., Mărcuţan, D.I., D'Amico, G., Gherman, C.M., Dumitrache, M.O., Mihalca, A.D.: **Do the ticks of birds at an important migratory hotspot reflect the seasonal dynamics of *Ixodes ricinus* at the migration initiation site? A case study in the Danube Delta.** PLoS One., 9. e89378, 2014.

Scebba, S., Olivieri Del Castillo, M.: **Timing of Song Thrush *Turdus philomelos* on pre-nuptial migration in southern Italy.** Ornis Hungarica., 25. 109-119, 2017.

Scott, J.D., Lee, M.K., Fernando, K., Durden, L.A, Jorgensen, D.R., Mak, S., Morshed, M.G.: **Detection of Lyme disease spirochete, *Borrelia burgdorferi* sensu lato, including three novel genotypes in ticks (Acari: Ixodidae) collected from songbirds (Passeriformes) across Canada.** J. Vector Ecol., 35. 124–139, 2010.

Sehnal, F., Maróy, P., Malá, J.: **Regulation and significance of ecdysteroid titre fluctuations in lepidopterous larvae and pupae.** J. Insect Physiol., 27. 535–544, 1981.

Siuda, K., Nowak, M., Karbowiak, G., Solarz, K.: **Transfer of exotic tick (Acari : Ixodida) on hosts to Poland.** Vestn. Zool., 19. 336–338, 2005.

Soares, C.A.G., Zeidner, N.S., Beard, C.B., Dolan, M.C., Dietrich, G., Piesman, J.: **Kinetics of *Borrelia burgdorferi* infection in larvae of refractory and competent tick vectors.** J. Med. Entomol., 43. 61–67, 2006.

Špitalská, E., Literák, I., Kocianová, E., Taragel'ová, V.: **The importance of *Ixodes arboricola* in transmission of *Rickettsia* spp., *Anaplasma phagocytophilum* , and *Borrelia burgdorferi* sensu lato in the Czech Republic, Central Europe.** Vector-Borne Zoonotic Dis., 11. 1235–1241, 2011.

Subbotina, E.L., Loktev, V.B.: **Molecular evolution of the tick-borne encephalitis and Powassan viruses.** Mol. Biol., 46. 75–84, 2012.

Takano, A., Fujita, H., Kadosaka, T., Takahashi, M., Yamauchi, T., Ishiguro, F., Takada, N., Yano, Y., Oikawa, Y., Honda, T., Gokuden, M., Tsunoda, T., Tsurumi, M., Ando, S., Andoh, M., Sato, K., Kawabata, H.: **Construction of a DNA database for ticks collected in Japan: application of molecular identiication based on the mitochondrial 16S rDNA gene.** Med. Entomol. Zool., 65. 13–21, 2014.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S.: **MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0.** Mol. Biol. Evol., 30. 2725-2729, 2013.

Taragel'ová, V., Koci, J., Hanincová, K., Kurtenbach, K., Derdáková, M., Ogden, N.H., Literák, I., Kocianová, E., Labuda, M.: **Blackbirds and song thrushes constitute a key reservoir of *Borrelia garinii*, the causative agent of borreliosis in Central Europe.** Appl. Environ. Microbiol., 74. 1289–1293, 2008.

Tóth, N., Simon, A., Tóth, G., Kele, Z., Hunyadi, A., Báthori, M.: **26-hydroxylated ecdysteroids from *Silene viridiflora*.** J. Nat. Prod., 71. 1461–1463, 2008.

Tsitsimpikou, C., Tsamis, G.D., Siskos, P.A., Georgakopoulos, C., Spyridaki, M.E.: **Study of excretion of ecdysterone in human urine.** Rapid Commun. Mass Spectrom., 15. 1796–1801, 2001.

Tsunoda, T., Tatsuzawa, S.: **Questing height of nymphs of the bush tick ,**

Haemaphysalis longicornis **, and its closely related species , *H. mageshimaensis:* correlation with body size of the host.** Parasitology., 128. 503–509, 2004.

USGS (United States Geological Survey) : **The uses of marking and recovery data in migratory bird conservation, research, and management.** avaliable on-line at: https://www.pwrc.usgs.gov/BBL/homepage/gswhy.cfm, 2016. Accessed: 2017.12.05.

Waldenström, J., Lundkvist, Å., Falk, K.I., Garpmo, U., Bergström, S., Lindegren, G., Sjöstedt, A., Mejlon, H., Fransson, T., Haemig, P.D., Olsen, B.: **Migrating birds and tickborne encephalitis virus.** Emerg. Infect. Dis., 13. 1215–1218, 2007.

Wilhelmsson, P., Lindblom, P., Fryland, L., Ernerudh, J., Forsberg, P., Lindgren, P.E.: **Prevalence, diversity, and load of *Borrelia* species in ticks that have fed on humans in regions of Sweden and Åland islands, Finland with different Lyme borreliosis incidences.** PLoS One., 8. e81433, 2013.

Yin, H., Lu, W., Luo, J., Zhang, Q., Lu, W., Dou, H.: E**xperiments on the transmission of *Babesia major* and *Babesia bigemina* by *Haemaphysalis punctata*.** Vet. Parasitol., 67. 89–98, 1996.

9. Scientific publications

In peer-reviewed journals

Flaisz, B., Sulyok, K.M., Kováts, D., Kontschán, J., Csörgő, T., Csipak, Á., Gyuranecz, M., Hornok, S.: ***Babesia* genotypes in *Haemaphysalis concinna* collected from birds in Hungary reflect phylogeographic connections with Siberia and the Far East**, Ticks Tick Borne Dis., 8. 666-670, 2017.

Flaisz, B., Hornok S.: **A madarak szerepe a kullancs közvetítette kórokozók öko-járványtanában**, Magy. Állatorvosok., 139. 489-497, 2017. (in Hungarian with English abstract)

Hornok, S., Flaisz, B., Takács, N., Kontschán, J., Csörgő, T., Csipak, Á., Jaksa, B.R., Kováts, D.: **Bird ticks in Hungary reflect western, southern, eastern flyway connections and two genetic lineages of *Ixodes frontalis* and *Haemaphysalis concinna***, Parasit. Vectors., 9. 101., 2016.

Hornok, S., Kováts, D., Flaisz, B., Csörgő, T., Könczöl, Á., Balogh, G.T., Csorba, A., Hunyadi, A.: **An unexpected advantage of insectivorism: insect moulting hormones ingested by song birds affect their ticks**, Sci. Rep., 6. 23390, 2016.

Conference oral presentation

Hornok, S., Flaisz, B., Csörgő, T., Csipak, Á., Jaksa, B.R., Kováts, D.: **Does insectivorism of birds affect their ticks? Results of a triannual survey**, Second Conference on Neglected Vectors and Vector-Borne Diseases (EurNegVec), Izmir, Turkey, 2015.

Other publications in peer-reviewed journals

Hornok, S., Sugár, L., Horváth, G., Kovács, T., Micsutka, A., Gönczi, E., Flaisz, B., Takács, N., Farkas, R., Meli, M.L., Hofmann-Lehmann, R.: **Evidence for host specificity of *Theileria capreoli* genotypes in cervids,** Parasit. Vectors., 10. 473., 2017.

Hornok, S., Estók, P., Kováts, D., Flaisz, B., Takács, N., Szőke, K., Krawczyk, A., Kontschán, J., Gyuranecz, M., Fedák, A., Farkas, R., Haarsma, A.J., Sprong, H.: **Screening of bat faeces for arthropod-borne apicomplexan protozoa: *Babesia canis* and *Besnoitia besnoiti*-like sequences from Chiroptera**, Parasit. Vectors., 8. 441. 2015.

Hornok, S., Takács, N., Kontschán, J., György, Z., Micsutka, A., Iceton, S., Flaisz, B., Farkas, R., Hofmann-Lehmann, R.: **Diversity of *Haemaphysalis*-associated piroplasms of ruminants in Central-Eastern Europe, Hungary**, Parasit. Vectors., 8. 627., 2015.

Other conference oral presentation

Norte, A.C., Ramos, J.A., Núncio, M.S.,Margos, G., Fingerle, V., Adamik, P., Alivizatos, H., Barba, E., Barrientos, R., Cauchard, L., Csörgő, T., Diakou, A., Dingemanse, N.J., Doligez, B., Dubiec, A., Eeva, T., Flaisz, B., Grim, T., Hau, M., Heylen, D., Hornok, S., Kazantzidis S., Kováts D., Krause F., Literak I., Mänd R., Mentesana L., Morinay J., Mutanen, M., Neto, J., Nováková, M., Sanz, J.J., Silva, L.P., Sprong, H., Tirri, I.S., Török, J.,Trilar, T., Tyller, Z., Visser, M.E., Lopes de Carvalho, I.: **Pandora's flying box - *Borrelia burgdorferi* s.l. prevalence in *Ixodes* species from birds throughout Europe,** International Symposium on Tick-Borne Pathogens and Disease (ITPD), Vienna, Austria, 2017.

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