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Short communication

Babesia genotypes in *Haemaphysalis concinna* collected from birds in Hungary reflect phylogeographic connections with Siberia and the Far East

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ABSTRACT

Haemaphysalis concinna is the second most common tick species attaching to birds in Hungary. Recently, *Babesia* genotypes, found in Siberia and the Far East, have been detected in this tick species collected from the vegetation in Hungary and Slovakia. The aim of this study was to molecularly investigate if these piroplasms also occur in *H. concinna* carried by migratory birds, which might explain their occurrence in the western Palaearctic. During a 2-year period, 321 *H. concinna* larvae and nymphs were collected from 121 passerine birds (of 19 species) in Hungary. These were molecularly investigated for the presence of piroplasm DNA with PCR and sequencing. The prevalence of PCR positive ticks was 15.9% (51 out of 321). Piroplasm PCR positivity of *H. concinna* ticks was significantly more frequent during the summer and autumn compared to spring, suggesting that migratory birds arriving in Hungary from the north or north east are the most important in the dispersal of *H. concinna*-associated piroplasms. Three genotypes, i.e. *Babesia* sp. "Irk-Hc133", "Irk-Hc130" (originally found in Irkutsk, Siberia) and "Kh-Hc222" (originally found in Khabarovsk, Far East) were detected. Phylogenetically all these belonged to the group formed by *Babesia* spp. of ruminants. Four bird species, which had 14–60% prevalence of PCR positive ticks, are known to be associated with northeast to southwest autumn migration. In conclusion, the presence of Central and East Asian *Babesia* genotypes in Central Europe are most likely related to bird species with known eastern migratory habit and/or phylogenetically substantiated connections between their eastern and western Eurasian populations.

1. Introduction

Birds play an important role in the dispersal of ticks and tick-borne pathogens into new geographical regions, contributing to risks associated with relevant infections of humans and wild or domestic animals (Reed et al., 2003). The most studied direction of bird migration in this context is between the north and the south, particularly when northern Europe is considered (Hasle, 2013). However, tick transportation via birds has also been reported in eastern and western directions (in Central Europe: Hornok et al., 2016a). Similarly, the geographical distribution of tick-borne viral pathogens reflect latitudinal connection between Europe and Asia (Siberia or Far East) in either eastward or westward direction (Subbotina and Loktev, 2012; Ponomareva et al., 2015).

The most common tick species collected from songbirds in Central-Europe are *Ixodes ricinus* and *Haemaphysalis concinna* (Taragelová et al., 2008; Dubska et al., 2009; Špitalská et al., 2011; Lommano et al., 2014; Hornok et al., 2016a). In Eurasia, the geographical distribution of *I. ricinus* is restricted to the western Palaearctic, whereas *H. concinna* can also be found in central and eastern Asia (Lebedeva and Korenberg, 1981).

The prevalence of infestation with these tick species among birds may depend on several factors. These include the feeding habit of bird species, if it takes place preferentially on ground level or above the ground. In Hungary, *H. concinna* predominantly occurs on birds that feed above the ground, related to the host-seeking behaviour of the larvae and nymphs (differing from that of *I. ricinus*: Hornok et al., 2014). Another important factor in the tick-infestation is the migration strategy characteristic of each bird species, in terms of direction and distance. The latter will also influence the time of arrival in the activity

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period of certain tick species.

In Central Europe *H. concinna* adults are most active in May (Hornok, 2009). Larvae, however, appear questing on the vegetation from June, whereas overwintering nymphs are active from April (Nosek, 1971). The questing activity of larvae and nymphs in this region concur with the arrival of long and short distance migratory birds, respectively. The activity of *H. concinna* larvae also overlap with the nesting of passerines, thus predisposing larvae to feed on these birds. The above factors increase the significance of migratory and non-migratory birds in the local and distant transportation of this tick species. Consequently, birds are among the most important hosts of *H. concinna* (Nosek, 1971; Hornok et al., 2014). Therefore, it is also reasonable to suppose the role of birds in the dispersal of pathogens associated with this tick species.

In the epidemiology of babesioses affecting mammals, birds are not important as reservoirs, but rather as suspected disseminators of *Babesia*-carrier ticks (Hornok et al., 2015). Recently, a diversity of new *Babesia* genotypes has been reported from Asia (Rar et al., 2014). In the last 2 years, some of these newly recognized *Babesia* genotypes, found in Siberia and the Far East, have also been detected in *H. concinna* from Central Europe; in particular, in questing ticks from Hungary (Hornok et al., 2015) and in questing and rodent-attached ticks from Slovakia (Hamšíková et al., 2016). The aim of this study was to molecularly investigate if these piroplasms also occur in *H. concinna* carried by birds, which might explain their occurrence in the western Palaearctic.

2. Materials and methods

During a 2-year period (from April 2012 until October 2014), 321 *H. concinna* larvae and nymphs were collected from 121 passerine birds at a ringing station in Hungary (Ócsa: 47.2967°, 19.2101°). Birds were captured by standard Ecotone mist-nets (Gdynia, Poland), 12 m in length, 2.5 m in height and with 16 mm mesh as described (Hornok et al., 2014). Ticks were removed with fine forceps and put into 70% ethanol in separate tubes according to their hosts. Data (bird species, date of collection, ring number) were recorded. Migratory habits were assigned to bird species based on ringing and recapture records from 100 years (Csörgő et al., 2009). Morphological identification of ticks was done with a stereo-microscope (SMZ-2 T, Nikon Instruments, Japan, illuminated with model 5000–1, Intralux, Urdorf-Zürich, Switzerland) according to standard keys (Babos, 1965). Only specimens identified as *H. concinna* were included in this study.

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 (Hornok et al., 2014). An extraction control was also processed in each set of samples. All tick DNA samples were screened for the presence of piroplasms by conventional PCR modified 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 of a 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× Coral Load 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 (Pacific 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).

The MEGA model selection method was applied to choose the appropriate 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.

Confidence interval (CI) for the overall prevalence was calculated at the 95% level. The prevalence of PCR positivity was calculated from the number of PCR positive ticks, expressed as the percentage of all evaluated ticks. Prevalence data were compared by Fisher's exact test, and differences were considered significant when $P < 0.05$.

3. Results and discussion

In this study 321 *H. concinna* larvae and nymphs were collected from 121 passeriform birds belonging to 19 species (Table 1). The prevalence of ticks PCR positive for piroplasms was 15.9% (51 out of 321, CI: 12.1–20.4%). PCR positivity was detected in ticks of 11 bird species (Table 1). Two bird species (*Locustella luscinioides*, *Emberiza citrinella*) found here to be infested with *Babesia*-carrier *H. concinna* ticks, were reported to be significantly more important hosts for this tick species than for *I. ricinus* (Hornok et al., 2016a).

These piroplasms (with one exception of unsuccessful sequencing)

Table 1
Data of evaluated bird species and the piroplasm-carrier status of their *Haemaphysalis concinna* ticks. The names of bird species with documented migratory habit from the east are written with bold characters.

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×)

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).

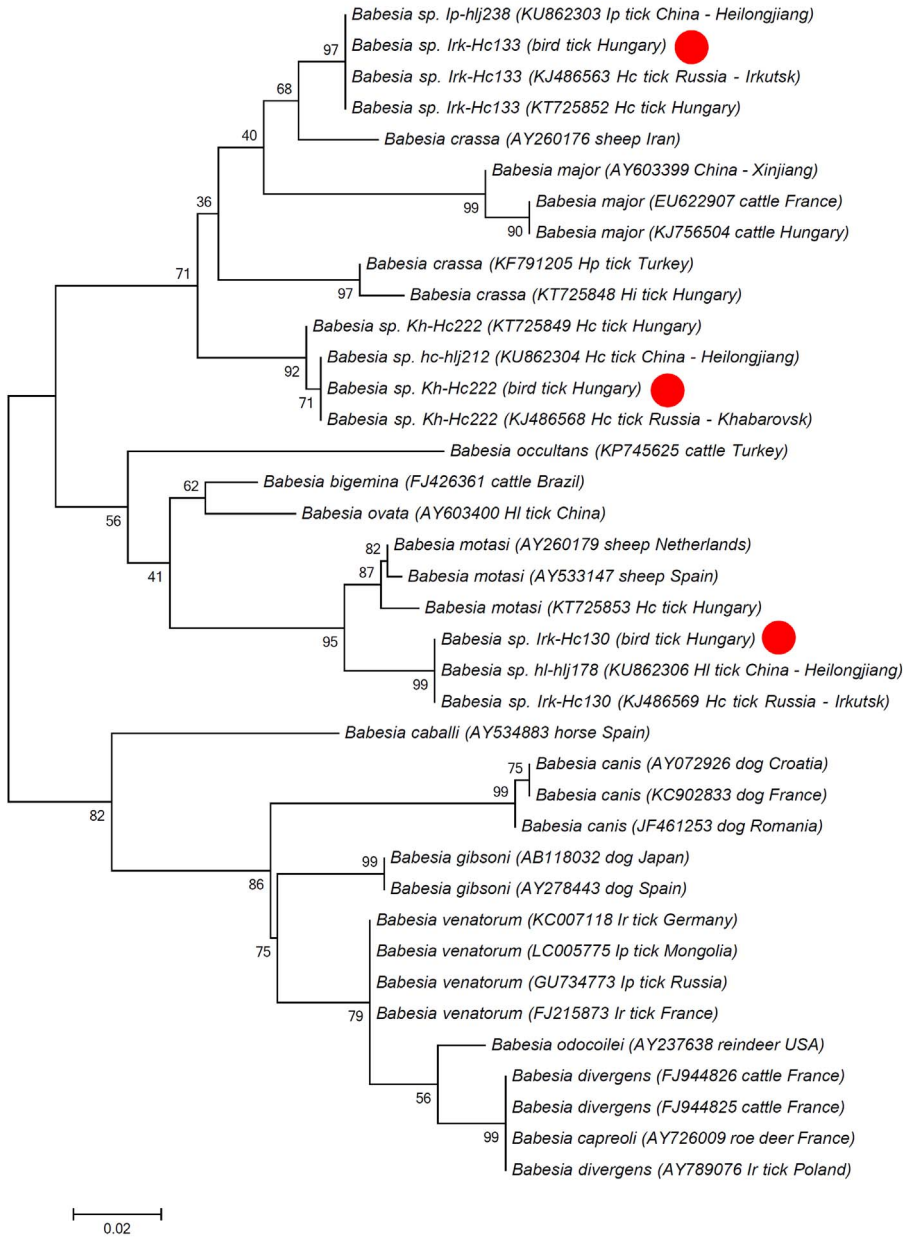

Fig. 1. Maximum Likelihood phylogenetic tree of *Babesia* spp./genotypes based on 18S rRNA gene. Sequences from this study are indicated with red dots. Branch lengths represent the number of substitutions per site inferred according to the scale shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were molecularly identified as 100% (405/405 or 409/409 bp) identical to three *Babesia* genotypes that have been recently reported from southern Siberia (Baikal region) and Far East of Russia (Rar et al., 2014). Genotypes "Irk-Hc133" (originally found in Irkutsk, Siberia) and "Kh-Hc222" (originally found in Khabarovsk, Far East) were present in the majority of ticks analyzed here (designated "A" and "B", respectively, in Table 1). These two *Babesia* genotypes have recently been detected in questing *H. concinna* ticks in Hungary (Hornok et al., 2015), and in questing or rodent-attached *H. concinna* ticks in Slovakia (Hamšíková et al., 2016). In the present study one additional genotype ("Irk-Hc130": described also from Irkutsk: Rar et al., 2014) was shown to be present in three *H. concinna* specimens (Table 1). To the best of our knowledge, this latter genotype has not been found before in Europe, and none of the three genotypes have been reported from ticks of birds.

In the phylogenetic analysis (Fig. 1), 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*, whereas the clade containing *Babesia* sp. Irk-Hc130 was a sister group to *B. motasi*. Taken together, all three above genotypes belonged to the phylogenetic group formed by *Babesia* spp. of ruminants. These three genotypes have unknown pathogenicity and (to the best of our knowledge) have not been reported from ruminants. Nevertheless, it is likely that *H. concinna* could have access to piroplasms from wild ruminants (particularly from roe deer), because these are the preferred hosts of its larvae and nymphs (Hornok et al., 2012).

There was no significant difference between the prevalence of PCR positive ticks among larvae (17.2%: 27 out of 157) and nymphs (14.6%: 24 out of 164). Considering that birds are unlikely reservoirs of piroplasms infecting mammals, PCR positivity of larvae suggests that the above Siberian, Far Eastern *Babesia* genotypes (similarly to other members of *Babesia* sensu stricto) are transovarially transmitted and thus maintained by *H. concinna*. This factor may also help their gradual dispersal over large geographical distances.

Piroplasm PCR positivity of *H. concinna* ticks was significantly less frequent during the spring (2.5%: one out of 40) than in the summer (17.6%: 45 out of 255) or autumn (19.2%: five out of 26) ($P = 0.009$ and $P = 0.003$, respectively). The latter finding suggests that migratory birds arriving in Hungary from the south during the spring are the least important in the dispersal of *H. concinna*-associated piroplasms, as contrasted to those arriving from the north or northeast to Hungary during late summer and autumn.

H. concinna is a geographically widespread tick species in Eurasia (Lebedeva and Korenberg, 1981). Because it is only during summer and autumn (until October) when both its larvae and nymphs are active (Nosek, 1971), this confirms that autumn migration is the most important in the long distance transportation of this tick species via birds. The larvae and nymphs of *H. concinna* suck blood for up to 6 days (Meng et al., 2014), during which their avian hosts may fly even a few hundred kilometres (the average speed of migration characteristic of passerine birds is 27–75 km per day, but can be up to 300 km per day) (Newton, 2008).

H. concinna larvae/nymphs PCR positive for piroplasms were collected significantly more frequently from four bird species with known eastern migratory connections (24 out of 92 ticks) than from other bird species (27 out of 229 ticks) ($P = 0.002$, Table 1), supporting their eco-epidemiological role in the above context. These four bird species had 14–60% prevalence of PCR positive ticks (Table 1).

Concerning bird species with key role in dispersing *Babesia*-carrier *H. concinna* ticks as shown here, their eastern connections are well documented. (1) 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 detected here. (2) Nightingales (*Luscinia megarhynchos*) in Hungary derive from eastern European or Asian populations, as demonstrated with phylogenetic methods (Ács and Kováts, 2013). (3) Savi's warbler (*L. luscinioides*) was shown here to carry 15% PCR positive ticks (Table 1). Western Palaearctic populations of this species are phylogenetically more closely related to Asian warblers (*Bradypterus* spp.) than to certain *Locustella* spp. (Drovetski et al., 2004). Savi's warbler and several other long-distance migratory species followed eastward or westward direction during post-glacial recolonization, as reflected by the phylogeographical comparison of their current populations (Irwin and Irwin, 2005).

Moreover, in the last decades a continuous increase was observed in the number of bird species emerging in Hungary, and some of these were of eastern origin, from the region of Turkestan and Mongolia (Csörgő et al., 2009).

On the other hand, PCR positivity of ticks collected from resident bird species (e.g. Blackbird, *Turdus merula*: Table 1) around the peak activity of *H. concinna* support the persistence and natural maintenance of the above Siberian and Far-Eastern *Babesia* genotypes in Central Europe. This is in line with their presence in questing ticks of the region (Hornok et al., 2015).

The host range of *Babesia*-carrier ticks is highly relevant in this context: *Haemaphysalis* ticks that do not typically occur on long-distance migratory species (such as birds) were shown to be phylogeographically different in parts of Eurasia (as exemplified by *Haemaphysalis erinacei*: Hornok et al., 2016b), whereas *H. concinna*, a tick species typically attaching to birds, was reported to have minimal genetic distance between Europe and East Asia (Hornok et al., 2016a).

4. Conclusions

Findings of the present study indicate that birds may play a significant role in the long distance geographical dispersal of *Babesia* genotypes within *H. concinna* ticks, which are broadly distributed in Eurasia. This is supported by the presence of *Babesia* DNA in ticks collected from bird species with known eastern migratory habit and/or phylogenetically substantiated connections between their eastern and western Eurasian populations. At the same time, *Babesia*-carrier ticks collected from resident bird species in the main activity period of *H. concinna* might reflect Central European establishment of Siberian or Far Eastern *Babesia* genotypes. Relevant sequences align to the phylogenetic group of piroplasms infecting ruminants, in which hosts they have not yet been reported. Therefore, it is an important task for the future to find the vertebrate host species of these *Babesia* genotypes.

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References

Ács, Z., Kováts, D., 2013. 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.
Babos, S., 1965. Kullancsok Ixodidea. Fauna Hungarie 18, 1–38 (in Hungarian).
Casati, S., Sager, H., Gern, L., Piffaretti, J.C., 2006. Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. Ann. Agric. Environ. Med. 13, 65–70.
Csörgő, T., Karcza, Z., Halmos, G., Gyurácz, J., Magyar, G., Szép, T., Bankovics, A., Schmidt, A., Schmidt, E., 2009. Hungarian Bird Migration Atlas. Kossuth Publishing Zrt, Budapest (in Hungarian with English summary).
Drovetski, S.V., Zink, R.M., Fadeev, I.V., Nesterov, E.V., Koblik, E.A., Red'kin, Y.A., Rohwer, S., 2004. Mitochondrial phylogeny of *Locustella* and related genera. J. Avian Biol. 35, 105–110.
Dubska, L., Literak, I., Kocianova, E., Taragelova, V., Sychra, O., 2009. 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.
Hamšíková, Z., Kazimírová, M., Haruštiaková, D., Mahríková, L., Slovák, M., Berthová, L., Kocianová, E., Schnittger, L., 2016. *Babesia* spp. in ticks and wildlife in different habitat types of Slovakia. Parasit. Vectors 9, 292.
Hasle, G., 2013. Transport of ixodid ticks and tick-borne pathogens by migratory birds. Front. Cell. Infect. Microbiol. 3, 48.
Hornok, S., 2009. Allochronic seasonal peak activities of *Dermacentor* and *Haemaphysalis* spp. under continental climate in Hungary. Vet. Parasitol. 163, 366–369.
Hornok, S., Horváth, G., Jongejan, F., Farkas, R., 2012. Ixodid ticks on ruminants, with on-host initiated moulting (apolysis) of *Ixodes*, *Haemaphysalis* and *Dermacentor* larvae. Vet. Parasitol. 187, 350–353.
Hornok, S., Kováts, D., Csörgő, T., Meli, M.L., Gönczi, E., Hadnagy, Z., Takács, N., Farkas, R., Hofmann-Lehmann, R., 2014. Birds as potential reservoirs of tick-borne pathogens: first evidence of bacteraemia with *Rickettsia helvetica*. Parasit. Vectors 7, 128.
Hornok, S., Takács, N., Kontschán, J., György, Z., Micsutka, A., Iceton, S., Flaisz, B., Farkas, R., Hofmann-Lehmann, R., 2015. Diversity of *Haemaphysalis*-associated piroplasms of ruminants in Central-Eastern Europe, Hungary. Parasit. Vectors 8, 627.
Hornok, S., Flaisz, B., Takács, N., Kontschán, J., Csörgő, T., Csipak, Á., Jaksa, B.R., Kováts, D., 2016a. Bird ticks in Hungary reflect western, southern, eastern flyway connections and two genetic lineages of *Ixodes frontalis* and *Haemaphysalis concinna*. Parasit. Vectors 9, 101.
Hornok, S., Wang, Y., Otranto, D., Keskin, A., Lia, R.P., Kontschán, J., Takács, N., Farkas, R., Sándor, A.D., 2016b. Phylogenetic analysis of *Haemaphysalis erinacei* Pavesi, 1884 (Acari: Ixodidae) from China, Turkey, Italy and Romania. Parasit. Vectors 9, 643.
Irwin, D.E., Irwin, J.H., 2005. Siberian migratory divides: the role of seasonal migration in speciation. In: Greenberg, R., Marra, P.P. (Eds.), Birds of Two Worlds: The Ecology and Evolution of Migratory Birds. Johns Hopkins University Press, Baltimore, MD, pp. 27–40.
Irwin, D.E., Rubtsov, A.S., Panov, E.N., 2009. Mitochondrial introgression and replacement between Yellowhammers (*Emberiza citrinella*) and Pine Buntings (*Emberiza leucocephalos*) (Aves: Passeriformes). Biol. J. Linn. Soc. 98, 422–438.
Lebedeva, N.N., Korenberg, E.I., 1981. Distribution of *Haemaphysalis concinna* Koch in the Soviet Union and some general features of its ecology. Folia Parasitol. 28, 249–261.
Lommano, E., Dvořák, C., Vallotton, L., Jenni, L., Gern, L., 2014. Tick-borne pathogens in ticks collected from breeding and migratory birds in Switzerland. Ticks Tick Borne Dis. 5, 871–882.
Meng, H., Xu, S., Yu, Z., Liu, Z., Liu, J., Yang, X., Liu, J., 2014. The life cycle and occurrence of *Haemaphysalis concinna* (Acari: Ixodidae) under field conditions. Ticks Tick Borne Dis. 5, 887–891.
Newton, I., 2008. The Migration Ecology of Birds, 1st ed. Academic Press, London.
Nosek, J., 1971. The ecology, bionomics and behaviour of *Haemaphysalis* (Haemaphysalis) *concinna* tick. Z. Parasitenkd. 36, 233–241.
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., 2015. Detection of Far-Eastern subtype of tick-borne encephalitis viral RNA in ticks collected in the Republic of Moldova. J. Vector Borne

Dis. 52, 334–336.
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., 2014. 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.
Reed, K.D., Meece, J.K., Henkel, J.S., Shukla, S.K., 2003. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. Clin. Med. Res. 1, 5–12.
Špitalská, E., Literák, I., Kocianová, E., Taragel'ová, V., 2011. 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.
Subbotina, E.L., Loktev, V.B., 2012. Molecular evolution of the tick-borne encephalitis and Powassan viruses. Mol. Biol. 46, 75–84.
Taragelová, V., Koci, J., Hanincová, K., Kurtenbach, K., Derdáková, M., Ogden, N.H., Literák, I., Kocianová, E., Labuda, M., 2008. 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.