AKADÉMIAI KIADÓ

Acta Veterinaria Hungarica

70 (2022) 3, 215–219

DOI:
10.1556/004.2022.00021

SHORT COMMUNICATION

Zoonotic ecotype-I of *Anaplasma phagocytophilum* in sympatric wildcat, pine marten and red squirrel – Short communication

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Received: 13 April 2022 • Accepted: 21 July 2022
Published online: 26 August 2022

ABSTRACT

Anaplasma phagocytophilum is the causative agent of granulocytic anaplasmosis in humans, dogs, cats, horses and tick-borne fever in ruminants. In Europe, its main vector is the tick species *Ixodes ricinus*. In this study, spleen and liver samples, as well as ticks from 18 wild-living mammals (belonging to seven species) were analysed for the presence of *A. phagocytophilum* with molecular methods. The zoonotic ecotype-I of *A. phagocytophilum* was identified in a European wildcat (*Felis silvestris*) and its tick, a European pine marten (*Martes martes*) and a Eurasian red squirrel (*Sciurus vulgaris*). All PCR-positive samples were collected in 2019 and originated in the same geographic area. These results indicate that taxonomically diverse mammalian species can maintain the local enzootic cycle of the same genotype of *A. phagocytophilum*. To the best of our knowledge, this is the first report of the zoonotic variant of *A. phagocytophilum* in the wildcat and in the European pine marten in a broad geographical context, as well as in the red squirrel in Hungary. Since all these host species are well known for their urban and peri-urban presence, the results of this study verify their role in the synanthropic enzootic cycle of granulocytic anaplasmosis and tick-borne fever.

KEYWORDS

tick-borne fever, granulocytic anaplasmosis, Carnivora, Rodentia

Anaplasma phagocytophilum (Rickettsiales: Anaplasmataceae) is a tick-borne, Gram-negative bacterium species with a broad geographical distribution in the northern hemisphere (Stuen et al., 2013a). It develops in neutrophils, thus causing a disease known as granulocytic anaplasmosis in humans, dogs, cats, horses and tick-borne fever in ruminants (Stuen et al., 2013a). Based on serological and molecular evidence from countries in the geographical region of Hungary (Central and Southeastern Europe), co-infections of horses and dogs with other tick-borne pathogens and *A. phagocytophilum* might enhance the pathogenic effect of the latter (Mircean et al., 2012; Krämer et al., 2014; Huber et al., 2017; Kovačević Filipović et al., 2018; Tsachev et al., 2019; Drážovská et al., 2021).

In Europe, the main vector of *A. phagocytophilum* is *Ixodes ricinus* (Woldehiwet, 2010). This is a generalist, three-host tick species that may feed on a broad range of reservoirs of *A. phagocytophilum*, and thus may overbridge wild and domestic animals as well as humans, implying the risk of zoonotic spread. In the absence of its transovarial transmission by female ticks, *A. phagocytophilum* cannot survive across several tick generations, and the maintenance of its natural and urban enzootic cycles is based on transstadial transmission via

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successive tick developmental stages (Jaarsma et al., 2019). This necessitates the alternating participation of tick vectors and vertebrate hosts in the development of *A. phagocytophilum*, increasing the spectrum of evolutionary pressures and thus its genetic variation.

Based on its transmission dynamics, hosts, ecological and genetic properties, *A. phagocytophilum* was shown to belong to four major ecotypes, among which ecotype-I has zoonotic potential (Jahfari et al., 2014). This zoonotic ecotype has the broadest range of wildlife reservoirs but may also infect certain domestic animals (Jahfari et al., 2014). In particular, the most important synanthropic hosts of ecotype-I are dogs, cats, horses, and several wild living urban or peri-urban mammals, as exemplified by wild boars, red foxes and hedgehogs, but not birds and murine or cricetid rodents (Jahfari et al., 2014; Matei et al., 2019; Jaarsma et al., 2019). However, the epidemiological role of non-canid wild carnivores and non-murine/cricetid rodents remains to be elucidated, as relevant data were published only sporadically and the ecotype(s) these hosts may harbour is (are) not always reported (e.g., in Matei et al., 2021).

In Hungary, infection with *A. phagocytophilum* was reported in various hosts, including small mammals (Rigó et al., 2011), dogs (Hornok et al., 2013), hedgehogs (Földvári et al., 2014), birds (Hornok et al., 2014a), red foxes (Tolnai et al., 2015) and large game animals (Hornok et al., 2018a), as well as in the main tick vector, *Ixodes ricinus* (Sréter et al., 2004; Egyed et al., 2012; Hornok et al., 2014b). However, while this tick species is known to occur on wild felids, mustelids and sciurid rodents in the country (Hornok et al., 2020, 2022a), there are no data on the *A. phagocytophilum* infection status of these mammals, like in several other regions of Central and Eastern Europe. The aim of this study was to compensate for this lack of epidemiological data.

Spleen and liver samples were collected from seven species of wild mammals, including European wildcats (Felidae: *Felis silvestris*, $n = 4$); one raccoon dog (Canidae: *Nyctereutes procyonoides*); four species of Mustelidae: beech martens (*Martes foina*, $n = 3$), European pine martens (*Martes martes*, $n = 3$), least weasels (*Mustela nivalis*, $n = 2$) and one Eurasian otter (*Lutra lutra*); as well as Eurasian red squirrels (*Sciurus vulgaris*, $n = 4$). These animals were found dead due to natural causes or as road-kills, between 2015 and 2021, in northeastern Hungary, in the Aggtelek National Park and its surroundings. This region (48° 30′ N, 20° 36′ E) is a low, karstic area with altitudes of 150–604 m covered by various deciduous forests (mainly oak and hornbeam). The landscape is dominated by systems of karstic plateaus dissected by deep valleys. The climate is humid continental with long summers. The average annual temperature is rather low, 8.2 °C, which figure is typical only of higher elevations in Hungary. The annual precipitation was formerly between 600–700 mm but it significantly decreased in the last few years to about 400–500 mm. Biogeographically this region displays a mosaic-like transition between the higher mountains of the Carpathians and the lowlands of the Pannonian Basin (Varga, 1999). From a biogeographical point of view, the study area is part of the Pannonic region. The sampled species are widespread and regularly observed in the area. However, no specific population surveys have been carried out so far, so the exact size of the populations is currently unknown.

The fur covering of each animal was carefully checked for the presence of ticks which were collected into and stored in 96% ethanol. Tick species were identified according to standard keys (Estrada-Peña et al., 2017). The DNA extracts of ticks originating from *A. phagocytophilum* PCR-positive hosts were also examined in this study.

DNA was extracted from host tissue samples and from whole ticks (individually) with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction, including an overnight digestion in tissue lysis buffer and proteinase-K at 56 °C in case of the latter, as reported by Hornok et al. (2014b, 2018a). An extraction control (tissue lysis buffer) was also processed in each set of samples.

In the screening assay, the primers EHR16SD (5′-GGT ACC YAC AGA AGA AGT CC-30) and EHR16SR (5′-TAG CAC TCA TCG TTT ACA GC- 3′) were used, which amplify an approximately 350-bp-long fragment of the 16S rRNA gene from various members of Anaplasmataceae (Brown et al., 2001), modified as reported (Hornok et al., 2018b). From samples positive in the screening PCR, amplification of an approx. 600-bp-long fragment of the heat shock chaperonin (*GroEL*) gene of *A. phagocytophilum* was also attempted (Alberti et al., 2005). The primers EphplGroEL(569)F (5′-ATG GTA TGC AGT TTG ATC GC-3′) and EphGroEL(1142)R (5′-TTG AGT ACA GCA ACA CCA CCG GAA-3′) were used as reported (Hornok et al., 2022b). Sequence-verified *A. phagocytophilum* DNA from a dog (code VE39) was used as positive control.

Purification and sequencing of the PCR products were done by Biomi Ltd. (Gödöllő, Hungary). Obtained sequences were manually edited, then aligned with GenBank sequences by nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). Four species-specific sequences were submitted to GenBank (*A. phagocytophilum GroEL*: ON186490-ON186493).

The results are summarised in Fig. 1. The spleen and liver samples of two carnivores, a European wildcat (*F. silvestris*) and a European pine marten (*M. martes*), and only the spleen sample (but not the liver DNA extract) of a Eurasian red squirrel (*Sciurus vulgaris*) were positive in the 16S rRNA gene PCR to detect Anaplasmataceae. This is in line with the observation that the spleen is a more likely source of *A. phagocytophilum* detection than the liver (Matei et al., 2021).

Amplification of the heat shock protein (*GroEL*) gene from these samples yielded identical sequences, with 100% (517/517 bp) identity to several sequences of ecotype-I of *A. phagocytophilum* deposited in GenBank (e.g., MN093180 from *I. ricinus*, The Netherlands: Jaarsma et al., 2019; MW366836 from wild boar-infesting ticks, Hungary: Hornok et al., 2022b). Interestingly, all PCR-positive samples were collected in the same year, 2019 and originated in the same geographic area around Szalonna (co-ordinates: 48.45093°N 20.74045°E). This means that sympatric,

				
Tick infestation (number, stage)		*Ixodes ricinus* (2F)	*I. ricinus* (2N), *Haemaphysalis concinna* (1N, 8L), *Dermacentor marginatus* (6L), *D. reticulatus* (1L)	no ticks found
PCR status of:	ticks (number)	+ (1×), - (1×)	- (18×)	NA
	liver	+	+	-
	spleen	+	+	+
GenBank accession number:		tissue: ON186490 tick: ON186493	tissue: ON186491	tissue: ON186492

Fig. 1. Summary of results from molecular analyses of *Anaplasma phagocytophilum*-infected hosts. The latter included one of four European wildcats (*Felis silvestris*), one of three European pine martens (*Martes martes*) and one of four Eurasian red squirrels (*Sciurus vulgaris*). In the first row, these host species are shown with their silhouettes in this order, respectively. Abbreviations: F – female, N – nymph, L – larva; NA – not available

taxonomically diverse mammals, a wildcat, a pine marten and a red squirrel were infected with the same *GroEL* variant of *A. phagocytophilum*. Unlike this genetic consistency between three mammalian species from different orders and families, among sympatric ruminants, i.e., red deer and sheep (which both tend to harbour ecotype-I of *A. phagocytophilum*: Jahfari et al., 2014) identical *msp*4 genotypes were demonstrated only among individuals of the same host species (Stuen et al., 2013b). While it was reported that *A. phagocytophilum* ecotype-I has a broad host spectrum on a continental scale (Jaarsma et al., 2019), the results of this study prove that diverse mammalian species can maintain the local enzootic cycle of the same genotype.

Considering ticks collected from the three *A. phagocytophilum* PCR-positive mammals, one of the two *I. ricinus* females collected from the wildcat also harboured this *GroEL* variant. This may reflect that either the source of *A. phagocytophilum*-infection in this wildcat was the PCR-positive tick collected from it, or the tick ingested blood during bacteraemia of its host. It might be relevant to note in the context of both possibilities that the probability of transstadial transmission of *A. phagocytophilum* appears to be stage dependent: based on prevalence rates after acquisition feeding, it is more likely that adults of *I. ricinus* will harbour (and thus inoculate) *A. phagocytophilum* than nymphs (Ogden et al., 2003). Unlike in the case of the wildcat, all 18 ticks (belonging to four species: Fig. 1) collected from the pine marten were PCR negative. The most likely explanation for this is that the pine marten with PCR-positive spleen and liver samples was not in the state of bacteraemia. This is supported by the fact that none of the ticks collected from this host in different states of engorgement were PCR positive, and *A. phagocytophilum* is known to cause waving bacteraemia (Granquist et al., 2010). On the other hand, no ticks were found on the PCR-positive red squirrel.

In summary, although the sample size was relatively small in this study and all seven mammalian species involved were represented by only one to four individuals, the results are new in an international context. First, to the best of our knowledge, this is the first report of the zoonotic variant of *A. phagocytophilum* in wildcat in a worldwide context, because the ecotype of this pathogen reported recently in the same host species was not identified (Matei et al., 2021). Similarly, while a broad range of mustelids were screened for *A. phagocytophilum* in Western Europe (Hofmeester et al., 2018), ecotype-I could only be identified in the European polecat (*Mustela putorius*) but not in European pine martens as shown here for the first time. In addition, the Eurasian red squirrel was reported to harbour both ecotypes I and II in Western Europe (Belgium: Ruyts et al., 2017), ecotypes I, II and IV in Central Europe (Czech Republic: Lesiczka et al., 2021) and here it was identified for the first time as the host of ecotype-I in Hungary. Since all three host species of the zoonotic ecotype of *A. phagocytophilum* are well known for their urban or peri-urban presence (Fingland et al., 2021; Urzi et al., 2021; Wereszczuk et al., 2021), these results verify their role in the synanthropic enzootic cycle and epidemiology of granulocytic anaplasmosis and tick-borne fever.

ACKNOWLEDGEMENT

This study was funded by Project no. TKP2020-NKA-01 implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 'Tématerületi Kiválósági Program 2020' (2020-4.1.1-TKP2020) funding scheme.

REFERENCES

Alberti, A., Addis, M. F., Sparagano, O., Zobba, R., Chessa, B., Cubeddu, T., Parpaglia, M. L., Ardu, M. and Pittau, M. (2005): *Anaplasma phagocytophilum*, Sardinia, Italy. Emerg. Infect. Dis. **11,** 1322–1324.

Brown, G. K., Martin, A. R., Roberts, T. K. and Aitken, R. J. (2001): Detection of *Ehrlichia platys* in dogs in Australia. Aust. Vet. J. **79,** 554–558.

Drážovská, M., Vojtek, B., Mojžišová, J., Koleničová, S., Koľvek, F., Prokeš, M., Korytár, Ľ., Csanady, A., Ondrejková, A., Vataščinová, T. and Bhide, M. R. (2021): The first serological evidence of *Anaplasma phagocytophilum* in horses in Slovakia. Acta Vet. Hung. **69,** 31–37.

Egyed, L., Elő, P., Sréter-Lancz, Z., Széll, Z., Balogh, Z. and Sréter, T. (2012): Seasonal activity and tick-borne pathogen infection rates of *Ixodes ricinus* ticks in Hungary. Ticks Tick Borne Dis. **3,** 90–94.

Estrada-Peña, A., Mihalca, A. D. and Petney, T. N. (2017): Ticks of Europe and North Africa: A Guide to Species Identification. Springer International Publishing. 404 pp.

Fingland, K., Ward, S. J., Bates, A. J. and Bremner-Harrison, S. (2021): *A systematic review into the suitability of urban refugia for the Eurasian red squirrel* Sciurus vulgaris. Mamm. Rev. **52**, 26–38.

Földvári, G., Jahfari, S., Rigó, K., Jablonszky, M., Szekeres, S., Majoros, G., Tóth, M., Molnár, V., Coipan, E. C. and Sprong, H. (2014): *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum* in urban hedgehogs. Emerg. Infect. Dis. **20,** 496–498.

Granquist, E. G., Bårdsen, K., Bergström, K. and Stuen, S. (2010): Variant- and individual-dependent nature of persistent *Anaplasma phagocytophilum* infection. Acta Vet. Scand. **52,** 25.

Hofmeester, T. R., Krawczyk, A. I., van Leeuwen, A. D., Fonville, M., Montizaan, M. G. E., van den Berge, K., Gouwy, J., Ruyts, S. C., Verheyen, K. and Sprong, H. (2018): Role of mustelids in the life-cycle of ixodid ticks and transmission cycles of four tick-borne pathogens. Parasit. Vectors **11,** 600.

Hornok, S., Boldogh, S. A., Takács, N., Kontschán, J., Szekeres, S., Sós, E., Sándor, A. D., Wang, Y. and Tuska-Szalay, B. (2022a): Molecular epidemiological study on ticks and tick-borne protozoan parasites (Apicomplexa: *Cytauxzoon* and *Hepatozoon* spp.) from wild cats (*Felis silvestris*), Mustelidae and red squirrels (*Sciurus vulgaris*) in Central Europe, Hungary. Parasit. Vectors **15,** 174.

Hornok, S., Dénes, B., Meli, M. L., Tánczos, B., Fekete, L., Gyuranecz, M., de la Fuente, J., de Mera, I. G., Farkas, R. and Hofmann-Lehmann, R. (2013): Non-pet dogs as sentinels and potential synanthropic reservoirs of tick-borne and zoonotic bacteria. Vet. Microbiol. **167,** 700–703.

Hornok, S., Horváth, G., Takács, N., Farkas, R., Szőke, K. and Kontschán, J. (2018b): Molecular evidence of a badger-associated *Ehrlichia* sp., a *Candidatus* Neoehrlichia lotoris-like genotype and *Anaplasma marginale* in dogs. Ticks Tick Borne Dis. **9,** 1302–1309.

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

Hornok, S., Kováts, D., Horváth, G., Kontschán, J. and Farkas, R. (2020): Checklist of the hard tick (Acari: Ixodidae) fauna of Hungary with emphasis on host-associations and the emergence of *Rhipicephalus sanguineus*. Exp. Appl. Acarol. **80,** 311–328.

Hornok, S., Meli, M. L., Gönczi, E., Halász, E., Takács, N., Farkas, R. and Hofmann-Lehmann, R. (2014b): Occurrence of ticks and prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in three types of urban biotopes: forests, parks and cemeteries. Ticks Tick Borne Dis. **5,** 785–789.

Hornok, S., Sugár, L., Fernández de Mera, I. G., de la Fuente, J., Horváth, G., Kovács, T., Micsutka, A., Gönczi, E., Flaisz, B., Takács, N., Farkas, R., Meli, M. L. and Hofmann-Lehmann, R. (2018a): Tick- and fly-borne bacteria in ungulates: the prevalence of *Anaplasma phagocytophilum*, haemoplasmas and rickettsiae in water buffalo and deer species in Central Europe, Hungary. BMC Vet. Res. **14,** 98.

Hornok, S., Szekeres, S., Horváth, G., Takács, N., Bekő, K., Kontschán, J., Gyuranecz, M., Tóth, B., Sándor, A. D., Juhász, A., Beck, R. and Farkas, R. (2022b): Diversity of tick species and associated pathogens on peri-urban wild boars – first report of the zoonotic *Babesia cf. crassa* from Hungary. Ticks Tick Borne Dis. **13,** 101936.

Huber, D., Reil, I., Duvnjak, S., Jurković, D., Lukačević, D., Pilat, M., Beck, A., Mihaljević, Ž., Vojta, L., Polkinghorne, A. and Beck, R. (2017): Molecular detection of *Anaplasma platys, Anaplasma phagocytophilum* and *Wolbachia* sp. but not *Ehrlichia canis* in Croatian dogs. Parasitol. Res. **116,** 3019–3026.

Jaarsma, R. I., Sprong, H., Takumi, K., Kazimirova, M., Silaghi, C., Mysterud, A., Rudolf, I., Beck, R., Földvári, G., Tomassone, L., Groenevelt, M., Everts, R. R., Rijks, J. M., Ecke, F., Hörnfeldt, B., Modrý, D., Majerová, K., Votýpka, J. and Estrada-Peña, A. (2019): *Anaplasma phagocytophilum* evolves in geographical and biotic niches of vertebrates and ticks. Parasit. Vectors **12,** 328.

Jahfari, S., Coipan, E. C., Fonville, M., van Leeuwen, A. D., Hengeveld, P., Heylen, D., Heyman, P., van Maanen, C., Butler, C. M., Földvári, G., Szekeres, S., van Duijvendijk, G., Tack, W., Rijks, J. M., van der Giessen, J., Takken, W., van Wieren, S. E., Takumi, K. and Sprong, H. (2014): Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe. Parasit. Vectors **7,** 365.

Kovačević Filipović, M. M., Beletić, A. D., Ilić Božović, A. V., Milanović, Z., Tyrrell, P., Buch, J., Breitschwerdt, E. B., Birkenheuer, A. J. and Chandrashekar, R. (2018): Molecular and serological prevalence of *Anaplasma phagocytophilum, A. platys, Ehrlichia canis, E. chaffeenses, E. ewingii, Borrelia burgdorferi, Babesia canis, B. gibsoni* and *B. vogeli* among clinically healthy outdoor dogs in Serbia. Vet. Parasitol. Reg. Stud. Rep. **14,** 117–122.

Krämer, F., Schaper, R., Schunack, B., Połozowski, A., Piekarska, J., Szwedko, A., Jodies, R., Kowalska, D., Schüpbach, D. and Pantchev, N. (2014): Serological detection of *Anaplasma phagocytophilum, Borrelia burgdorferi* sensu lato and *Ehrlichia canis* antibodies and *Dirofilaria immitis* antigen in a country-wide survey in dogs in Poland. Parasitol. Res. **113,** 3229–3239.

Lesiczka, P. M., Hrazdilová, K., Majerová, K., Fonville, M., Sprong, H., Hönig, V., Hofmannová, L., Papežík, P., Růžek, D., Zurek, L., Votýpka, J. and Modrý, D. (2021): The role of peridomestic animals in the eco-epidemiology of *Anaplasma phagocytophilum*. Microb. Ecol. **82,** 602–612.

Matei, I. A., Estrada-Peña, A., Cutler, S. J., Vayssier-Taussat, M., Varela-Castro, L., Potkonjak, A., Zeller, H. and Mihalca, A. D. (2019): A review on the eco-epidemiology and clinical

management of human granulocytic anaplasmosis and its agent in Europe. Parasit. Vectors **12,** 599.

Matei, I. A., Ivan, T., Ionică, A. M., D'Amico, G., Deak, G., Nadas, G. C., Novac, C. S., Gherman, C. M. and Mihalca, A. D. (2021): *Anaplasma phagocytophilum* in multiple tissue samples of wild carnivores in Romania. J. Wildl. Dis. **57,** 949–953.

Mircean, V., Dumitrache, M. O., Györke, A., Pantchev, N., Jodies, R., Mihalca, A. D. and Cozma, V. (2012): Seroprevalence and geographic distribution of *Dirofilaria immitis* and tick-borne infections (*Anaplasma phagocytophilum, Borrelia burgdorferi* sensu lato, and *Ehrlichia canis*) in dogs from Romania. Vector Borne Zoonotic Dis. **12,** 595–604.

Ogden, N. H., Casey, A. N., Woldehiwet, Z. and French, N. P. (2003): Transmission of *Anaplasma phagocytophilum* to *Ixodes ricinus* ticks from sheep in the acute and post-acute phases of infection. Infect. Immun. **71,** 2071–2078.

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

Ruyts, S. C., Frazer-Mendelewska, E., Van Den Berge, K., Verheyen, K. and Sprong, H. (2017): Molecular detection of tick-borne pathogens Borrelia afzelii, Borrelia miyamotoi and Anaplasma phagocytophilum in Eurasian red squirrels (Sciurus vulgaris). Eur. J. Wildl. Res. **63,** 9–12.

Sréter, T., Sréter-Lancz, Z., Széll, Z. and Kálmán, D. (2004): *Anaplasma phagocytophilum*: an emerging tick-borne pathogen in Hungary and Central Eastern Europe. Ann. Trop. Med. Parasitol. **98,** 401–405.

Stuen, S., Granquist, E. G. and Silaghi, C. (2013a): *Anaplasma phagocytophilum* – a widespread multi-host pathogen with highly adaptive strategies. Front. Cell. Infect. Microbiol. **3,** 31.

Stuen, S., Pettersen, K. S., Granquist, E. G., Bergström, K., Bown, K. J. and Birtles, R. J. (2013b): *Anaplasma phagocytophilum* variants in sympatric red deer (*Cervus elaphus*) and sheep in southern Norway. Ticks Tick Borne Dis. **4,** 197–201.

Tolnai, Z., Sréter-Lancz, Z. and Sréter, T. (2015): Spatial distribution of *Anaplasma phagocytophilum* and *Hepatozoon canis* in red foxes (*Vulpes vulpes*) in Hungary. Ticks Tick Borne Dis. **6,** 645–648.

Tsachev, I., Baymakova, M. and Pantchev, N. (2019): Seroprevalence of *Anaplasma phagocytophilum, Ehrlichia* spp. and *Borrelia burgdorferi* infections in horses: first report from Northern Bulgaria – Short communication. Acta Vet. Hung. **67,** 197–203.

Urzi, F., Šprem, N., Potočnik, H., Sindičić, M., Konjević, D., Ćirović, D., Rezić, A., Duniš, L., Melovski, D. and Buzan, E. (2021): Population genetic structure of European wildcats inhabiting the area between the Dinaric Alps and the Scardo-Pindic mountains. Sci. Rep. **11,** 17984.

Varga, Z. (1999): Bio-geographical outline of the invertebrate fauna of the Aggtelek Karst and surrounding areas. In: Mahunka, S. (ed.) The Fauna of the Aggtelek National Park. Natural History of the National Parks of Hungary. Hungarian Natural History Museum, Budapest. pp. 21–28.

Wereszczuk, A., Hofmeester, T. R., Csanády, A., Dumić, T., Elmeros, M., Lanszki, J., Madsen, A. B., Müskens, G., Papakosta, M. A., Popiołek, M., Santos-Reis, M., Zuberogoitia, I. and Zalewski, A. (2021): Different increase rate in body mass of two marten species due to climate warming potentially reinforces interspecific competition. Sci. Rep. **11,** 24164.

Woldehiwet, Z. (2010): The natural history of *Anaplasma phagocytophilum*. Vet. Parasitol. **167,** 108–122.