Exp Appl Acarol (2017) 71:139–149
DOI 10.1007/s10493-017-0108-z

Ixodid ticks parasitizing wild carnivores in Romania

Gianluca D'Amico[1] · Mirabela Oana Dumitrache[1] · Ioana Adriana Matei[1] · Angela Monica Ionică[1] · Călin Mircea Gherman[1] · Attila David Sándor[1] · David Modrý[2,3,4] · Andrei Daniel Mihalca[1]

Received: 27 September 2016 / Accepted: 15 January 2017 / Published online: 26 January 2017

Abstract In Romania, data regarding hard-tick diversity and tick–host associations in wild carnivores are scarce. We aimed to identify tick species in wild carnivores and to establish reliable data on tick–host associations. The study was conducted in various Romanian localities from all five ecoregions found in the country. Fourteen species of wild carnivores were examined. Immature and adult ticks were collected and identified using the morphological keys. The frequency and mean intensity of tick infestation, overall and differentiated by species, developmental stage and host were calculated. Of 202 wild carnivores, 68 were parasitized by seven tick species (predominantly *Ixodes ricinus*, *I. hexagonus* and *Dermacentor reticulatus*). The mean intensity of tick infestation was similar in males (6.97, BCa 95% CI 5.15–9.88) and females (5.76, BCa 95% CI 4.15–9.17). The highest prevalence of infested animals was recorded in the pannonian and steppic ecoregions, 66.7 and 52.7%, respectively. In the continental ecoregion the prevalence was 26.7%, whereas in the pontic ecoregion it was 28%. The lowest value, 16.7% was recorded in the alpine ecoregion. In total 430 ticks were collected, and 24.8% (n = 50) of the animals were infested with more than one tick species. Fourteen new tick–host associations were recorded. Our results suggest that anthropogenic changes of the environment lead to the diminishing of the boundaries, between wild and domestic animals,

G. D'Amico and M. O. Dumitrache have equally contributed to this work.

✉ Ioana Adriana Matei
matei.ioana@usamvcluj.ro

1 Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Calea Mănăştur 3-5, 400372 Cluj-Napoca, Romania

2 Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého tř. 1/3, 612 42 Brno, Czech Republic

3 CEITEC–VFU, University of Veterinary and Pharmaceutical Sciences, Palackého tř. 1/3, 612 42 Brno, Czech Republic

4 Institute of Parasitology, Biology Centre of Academy of Sciences of the Czech Republic, v.v.i., České Budějovice, Czech Republic

increasing the exposure for both animals and humans, to infective agents, including tick-borne pathogens.

Keywords *Dermacentor* spp. · *Haemaphysalis* spp. · *Ixodes* spp. · *Rhipicephalus* spp. · Wildlife · Tick–host associations

Introduction

Ticks are a group of blood feeding ectoparasites of terrestrial vertebrates, with a worldwide distribution. Of the about 700 species of hard ticks (Ixodidae), many were demonstrated as vectors of agents causing diseases of veterinary and medical importance (de la Fuente et al. 2008). In Europe, around 65 species hard and soft ticks have been reported (Estrada-Peña and de la Fuente 2016). In Romania, according to the historical data reviews and updates, 25 species of hard ticks were reported (Coipan et al. 2011; Mihalca et al. 2012).

Because of the great impact of ticks on public health, their negative effect on livestock production, and their parasitism on companion animals, we are witnessing an increased interest for the tick-borne related medical conditions, and for the several past years, most of the studies are focused on synanthropic ticks and domestic hosts (Jongejan and Uilenberg 2004; Bermúdez et al. 2015). Despite this tendency, wild animals play a major role in the eco-epidemiology of tick-borne diseases, as they may act as reservoir or amplifying host for different pathogens (Lorusso et al. 2011). Different economic and ecological factors, as the changing of the agricultural practices or the environmental and land-use changes, lead to a broader distribution area of the wild hosts and vectors, facilitating the transfer of pathogens between wild and domestic animals (Bengis et al. 2004). Moreover, vector-mediated animal-to-human infection is considered the usual pattern of human infection (Bengis et al. 2004). In this frame, the study of tick species that parasitize wild animal populations is crucial in order to understand ticks' ecobiology and the dynamics of pathogens transmission.

Among wildlife, the wild carnivores are difficult to monitor and capture (Gese 2001), a reason why tick–host associations are less commonly studied. Due to their wide home range, these animals are exposed to many tick species, which makes them of particular interest in the context of parasite ecology (Labruna et al. 2005).

Romania is a preponderant rural country, and agriculture is the main economic activity, dominated by extensive rearing system of livestock. The country is populated by a great variety and a large number of wild carnivores. The traditional breeding of livestock and the high density of wild carnivores increase the contact between humans, and these domestic and wild animals (Mertens and Promberger 2001). In Romania, the only exhaustive review on hard-tick diversity, published more than 50 years ago (Feider 1965), and few subsequent sporadic new tick–host associations (Dumitrache et al. 2012; Mihalca et al. 2012) listed 10 species of ticks that parasitize wild carnivores. No targeted surveillance was performed. Therefore, the present study aimed to identify tick species collected from hunted or road-killed wild carnivores in order to complement and confirm the sparse information currently available and to establish reliable data on tick–host associations.

Materials and methods

Study area

Romania is a country located in south-eastern Central Europe, on the Lower Danube, in the north of the Balkan Peninsula and on the northwestern coast of the Black Sea. Romania is crossed by the Carpathians and shows a balance of one-third of each type of landform (plains, hills and mountains). Its geographic position and ecological characteristics make Romania the only country in Europe having five biogeographic regions: continental, steppic, alpine, pannonian and pontic (Petrişor et al. 2010).

The climate of Romania is generally temperate-continental, but country's relief influences it locally. Ocean influences of the Atlantic air masses are restricted to the Western and Central part of the country by the Carpathians, which act as a barrier. Mediterranean influences are present in the southwest of the Romanian Plain, ensuring a milder climate (Stefan et al. 2004).

The high habitat diversity reflects a rich biodiversity. In Romania 17 species of wild carnivores are listed, belonging to the following families: 4 Canidae (golden jackal - *Canis aureus*, grey wolf - *C. lupus*, red fox - *Vulpes vulpes*, raccoon dog - *Nyctereutes procyonoides*); 2 Felidae (wildcat - *Felis silvestris*, Eurasian lynx - *Lynx lynx*); 1 Ursidae (brown bear - *Ursus arctos*); 1 Phocidae (Mediterranean monk seal - *Monachus monachus*); 9 Mustelidae (stoat - *Mustela erminea*, least weasel - *M. nivalis*, European mink - *M. lutreola*, European polecat - *M. putorius*, marbled polecat - *Vormela peregusna*, pine marten - *Martes martes*, stone marten - *M. foina*, European badger - *Meles meles*, European otter - *Lutra lutra*) (Murariu 2010).

Sampling

The study was carried out from January 2010 to May 2016 on carcasses of wild carnivores hunted, road-killed or found dead in several localities (Fig. 1). Only other species then red foxes (*Vulpes vulpes*) were included in this study. Due to the large number of specimens (over 300), this species was evaluated separately and the results are included in two other studies. During the study period, the carcasses of wild carnivores collected in the aforementioned areas were brought to the Unit of Parasitology and Parasitic Diseases, of the Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (Romania). The carcasses were transported to the laboratory, fresh when possible or frozen, following the current laws on dead animals transport and zoonotic risks. A thorough inspection of skin and fur of each carcass was carried out. For each animal, all immature and adult ticks were carefully collected, and stored in labeled tubes with 96% ethanol, for further identification using morphological keys (Feider 1965; Filippova 1977; Walker et al. 2005).

Statistical analysis and mapping

The frequency, prevalence, 95% confidence interval, of ticks on wild carnivores, overall and differentiated by ticks species, developmental stage and by hosts species were calculated using Epi Info^{TM} 7 (CDC, USA) software. The mean intensity and Bootstrap BCa confidence interval were calculated in Quantitative Parasitology software (Reiczigel et al. 2013).The differences between variables were analyzed by Chi squared independence test,

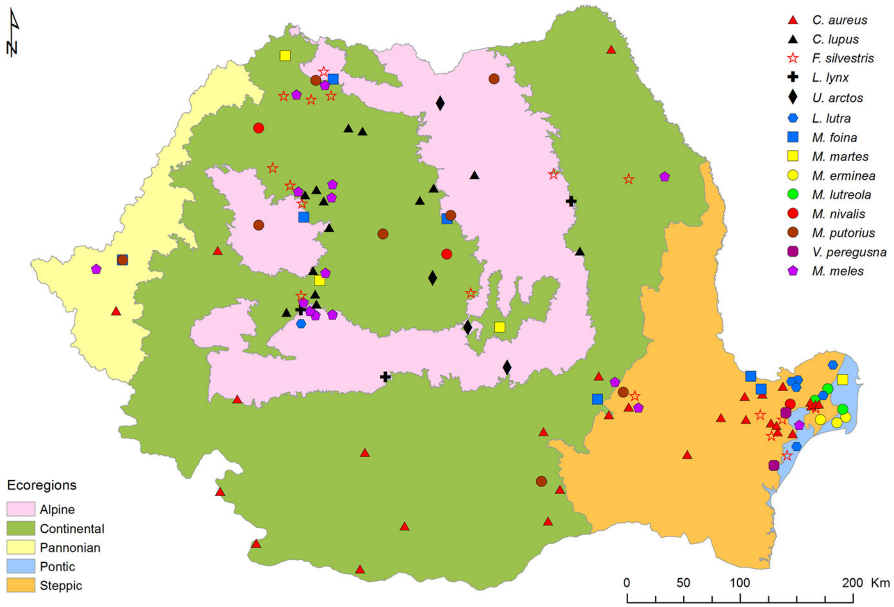

Fig. 1 Animal collection sites

respective by bootstrap t test for mean intensity. Mapping of sample collection sites was generated using the QGIS software (http://www.qgis.org).

Results

A total of 202 individual wild carnivores, from 14 species (Table 1), originating in all five biogeographical regions were examined. Of these, 68 animals were parasitized by ticks.

The highest prevalence of infested animals was recorded in the pannonian and steppic ecoregions, 66.7% (95% CI 22.3–95.7) and 52.7% (95% CI 38.8–66.3) respectively ($p < 0.001$). In the continental regions the prevalence was 26.7% (95% CI 17.8–37.4), while in the pontic ecoregion it was 28% (95% CI 12.1–49.4). The lowest value, 16.7% (95% CI 5.6–34.7), was recorded in alpine biogeographical region ($p < 0.001$).

After examining all the infested wild carnivores, a number of 430 ticks were collected and their developmental stages and species were identified (Table 2). The tick burden varied between 1 and 36 ticks/animal, with a mean intensity of 6.31 (BCa 95% CI 4.99–8.28).

Detailed information on tick–host association and the exact number of examined and infested animals for each carnivore species are presented in Table 1.

Fifteen animals were infested with more than one tick species. Co-infestation with two species of ticks occurred as follows: *I. ricinus* + *I. hexagonus* (1 *C. aureus*, 2 *F. silvestris*, 1 *M. meles*), *I. ricinus* + *R. sanguineus* s.l. (1 *C. aureus*), *I. ricinus* + *Ha. concinna* (1 *C. aureus*), *I. ricinus* + *Ha. punctata* (1 *C. aureus*, 1 *C. lupus*), *I. ricinus* + *D. reticulatus* (3 *C. aureus*, 1 *L. lutra*).

I. hexagonus + *D. reticulatus* (2 *C. aureus*). In one situation, three ticks species were found on the same animal: *I. ricinus* + *R. rossicus* + *Ha. concinna* (1 *M. foina*).

Table 1 Wild carnivore species, their associated ticks and biogeographic distribution

Host (Total number of investigated)	Biogeographic region (No. of infested/No. of investigated)	Ticks[a]
Canis aureus (79)	Continental (6/28)	*I. ricinus* (2F, 2L); *I. hexagonus* (2F, 1N, 4L); *D. reticulatus* (9M + 10F)
	Pannonian (2/2)	*I. ricinus* (1F); *I. hexagonus* (1N); *D. reticulatus* (5M, 4F)
	Pontic (5/11)	*I. ricinus* (5M); *D. reticulatus* (1M, 1F); *Ha. punctata* (1M)
	Steppic (20/38)	*I. ricinus* (49M, 42F, 3N, 2L); *I. hexagonus* (2M, 9F, 22N, 8L); *D. reticulatus* (31M, 10F); *Ha. punctata* (2N); *Ha. concinna* (1N); *R. sanguineus* s.l. (1M)
Canis lupus (27)	Alpine (2/12)	*I. ricinus* (4M + 5F); *Ha. punctata* (1M)
	Continental (3/15)	*I. ricinus* (7M, 13F)
Felis silvestris (19)	Alpine (0/2)	–
	Continental (5/10)	*I. ricinus* (3F, 4N); *I. hexagonus* (2F, 3N, 28L)
	Pontic (1/1)	*I. ricinus* (5L)
	Steppic (4/6)	*I. ricinus* (9F); *I. hexagonus* (1M, 3F)
Lutra lutra (9)	Alpine (0/1)	–
	Pontic (0/4)	–
	Steppic (1/4)	*I. ricinus* (1N); *D. reticulatus* (1M)
Lynx lynx (8)	Alpine (1/7)	*I. ricinus* (2M, 14F)
	Continental (1/1)	*I. ricinus* (1M, 2F)
Martes foina (8)	Alpine (0/1)	–
	Continental (1/4)	*I. ricinus* (1F)
	Pannonian (0/1)	–
	Steppic (2/2)	*I. ricinus* (1M, 1F); *Ha. concinna* (1M); *R. rossicus* (1M); *R. sanguineus* s.l. (1M, 3F)
Martes martes (4)	Continental (0/2)	–
	Pannonian (1/1)	*I. ricinus* (6F)
	Pontic (0/1)	–
Meles meles (21)	Alpine (2/2)	*I. ricinus* (1F, 4N, 3L); *I. hexagonus* (15N)
	Continental (5/16)	*I. ricinus* (1M, 4F, 6N); *I. hexagonus* (2F, 1N, 8L)
	Pannonian (0/1)	–
	Pontic (1/1)	*I. ricinus* (2M, 1N)
	Steppic (1/1)	*I. ricinus* (1M, 1F, 10N)
Mustela erminea (3)	Pontic (0/3)	–
Mustela lutreola (5)	Pontic (0/3)	–
	Steppic (0/2)	–
Mustela nivalis (3)	Continental (0/3)	–
Mustela putorius (10)	Alpine (0/3)	–
	Continental (2/5)	*I. ricinus* (5L); *I. hexagonus* (1F, 4L)
	Pannonian (1/1)	*I. hexagonus* (1M, 4F)
	Steppic (1/1)	*I. ricinus* (2M, 1N)
Ursus arctos (4)	Alpine (0/2)	–
	Continental (0/2)	–

Table 1 continued

Host (Total number of investigated)	Biogeographic region (No. of infested/No. of investigated)	Ticks[a]
Vormela peregusna (2)	Pontic (0/1)	–
	Steppic (0/1)	–

Double underlined tick species are new host association worldwide; single underlined tick species are new reports for Romania

[a] Figures within parentheses represent the number of specimens identified and abbreviations are as follows: *L* larva, *N* nymph, *F* female, *M* male

Table 2 Species, developmental stages and distribution by ecoregion of ticks collected from wild carnivores in Romania

Tick species	Ecoregion	Larvae	Nymphs	Males	Females	Total	No. of hosts
Dermacentor reticulatus	C, Pa, Po, S	0	0	47	25	72	11
Haemaphysalis concinna	S	0	1	1	0	2	2
Haemaphysalis punctata	A, Po, S	0	2	2	0	4	3
Ixodes hexagonus	A, C, Pa, S	49	43	4	23	119	18
Ixodes ricinus	A, C, Pa, Po, S	17	30	75	105	227	48
Rhipicephalus rossicus	S	0	0	1	0	1	1
Rhipicephalus sanguineus s.l.	S	0	0	2	3	5	2

A Alpine, *C* Continental, *Pa* Pannonian, *Po* Pontic, *S* Steppic

All seven tick species were found in the steppic ecoregion, and three of them (*R. sanguineus* s.l., *R. rossicus,* and *Ha. concinna*) were present exclusively here. This study reports 14 new tick–host association in Romania: five for *I. ricinus;* two for *I. hexagonus;* one for *Ha. punctata;* two for *Ha. concinna;* two for *D. reticulatus;* one for *R. rossicus;* one for *R. sanguineus* s.l. (Table 2).

Discussion

This study presents the results of the first large-scale survey of ticks parasitizing wild carnivores in Romania. Fourteen species of the seventeen wild carnivore species listed in Romania were included. Carcasses originated from different localities, covering all five biogeographic regions. Ixodid ticks were collected from 68 wild carnivores, belonging to 9 species. Some tick–host associations reported in this study reinforce information that have already been reported (Feider 1965; Dumitrache et al. 2012; Mihalca et al. 2012). However, 14 new tick–host associations in Romania were identified. One of the main strengths of our survey resides in the high number of both animals and investigated species. It is widely known that a major constraint of studies on the ecology of wildlife parasites is the limited access to samples (Otranto et al. 2015a, b). Each animal species examined offers new insights on tick–host relation and relevant information on ticks' eco-biology, as each carnivore species has different activity patterns and behavior (Bermúdez et al. 2015). Moreover, ticks' strategy for finding a host depends on their species and sometimes on their life stages (Bermúdez et al. 2015). Wild carnivores are preferred hosts of endophilic ixodid ticks, but they also may be suitable host for some exophilic tick species (Sobrino et al. 2012).

The golden jackal (*Canis aureus*) is one of the most widespread canid species, found in many areas in Europe, Africa and Asia, but in the same time one of the less studied species with an almost unknown ecology. The first study of hard ticks from wild golden jackals in Europe was performed in Hungary (2012) and included 4 animals (Hornok et al. 2012). Since then, only few reports of ticks parasitizing *C. aureus* were published. A report of a road-kill golden jackal, parasitized by *D. reticulatus* and *Ha. concinna* and bearing *Hepatozoon canis,* represented the first report of this tick-borne pathogen in Austria (Duscher et al. 2013). As the presence of *He. canis* should overlap the distribution of its primary tick vector *R. sanguineus* s.l., and this tick is considered as being sporadically imported in Austria, this report raised again new questions about the possibility that also other tick species could be vector-competent. Another concern is related to the importance of golden jackals as a natural way of pathogen introduction and spreading into non-endemical areas (Duscher et al. 2013), especially because in the next few years an ongoing expansion of golden jackals from the Balkan to central Europe is expected (Arnold et al. 2012). Another study performed in Hungary on 15 carcasses reported infestation with *I. ricinus*, *D. reticulatus* and *Ha. concinna* in 7 of the investigated animals (Farkas et al. 2014).

In our study, a significant percent of the examined *C. aureus* (41.8%) presented tick infestation, with 6 tick species, both endophilic and exophilic. *I. ricinus* was the predominant species, and all developmental stages were found parasitizing on host. This tick–host association was previously reported in Hungary (Hornok et al. 2012; Farkas et al. 2014) and also in Romania (Dumitrache et al. 2012; Mihalca et al. 2012), where *I. ricinus,* a species with a low host specificity, is considered the most widespread (Mihalca et al. 2012) and abundant hard-tick species (Dumitrache et al. 2012). Similarly to *I. ricinus*, *D. reticulatus* was present in all four biogeographical regions where positive animals were recorded, but only adult stages were found. The absence of the immature stages could be explained partly by the nidicolous behavior of larvae and nymphs, their short activity period and their preference for small mammal hosts (Földvári et al. 2016). The expansion of *D. reticulatus* pose both veterinary and public health concerns, as vector for *Babesia canis,* a pathogen causing a severe canine vector-borne disease, but also for several *Rickettsia* spp. and tick-borne encephalitis virus (Földvári et al. 2016). Another new tick–host association for our country, and to the best of our knowledge worldwide, is the presence of the endophilic species *I. hexagonus* on *C. aureus*, recorded in our case from continental, pannonian and steppic ecoregions. Different carnivore species and hedgehogs are considered to be main hosts for *I. hexagonus* (Gern et al. 1991). The absence of reports of *I. hexagonus* in golden jackals is probably due to the limited studies existing on this carnivore species within the distribution range of this tick. Despite of being reported twice as parasitizing on *C. aureus* in the neighboring Hungary (Hornok et al. 2012; Farkas et al. 2014), in Romania, *Ha. concinna* has not been mentioned on this host before. The parasitism of *C. aureus* with *Ha. punctata,* reinforces the previous report of this tick–host association (Mihalca et al. 2012). All developmental stages of *R. sanguineus* s.l. are considered to have an endophilic behavior, segregated in the proximity of host dwellings (Gray et al. 2013). Taking in consideration the wide geographical distribution of

R. sanguineus s.l., the habitat of this tick species often represented by the human dwellings and that dogs are its preferred domestic host (Gray et al. 2013), the presence of *R. sanguineus* s.l. on golden jackals suggests that this tick might play an important role in the biological connections between wild and anthropic environments.

Canis lupus is the most widespread large carnivore in Europe, recovering after crossing a period of population decline and eradication (Randi 2011). In Romania, gray wolves are represented by one of the largest populations in Europe, and their conservation status of "least concern" was maintained throughout time (Boitani 2000). Few reports of *I. ricinus* parasitizing wolves in Romania were previously published (Dumitrache et al. 2012; Mihalca et al. 2012) and similarly to these, we identified only adult stages, as immature stages prefer other hosts (Matuschka et al. 1991). Interestingly, as grazing domestic animals and wild ungulates (Nosek 1971) are considered the preferred hosts, one male of *Ha. punctata* was found, representing the first report of this tick species parasitizing on *C. lupus*.

The wildcat has a very wide geographical distribution, being the most common wild felid in Europe. Despite the decreasing population trend, its conservation status is evaluated as "least concern" (IUCN 2015). In our study, *Felis silvestris* was found as a suitable host for *I. hexagonus* and *I. ricinus*. Both tick species have been previously reported in Romania on wildcats (Mihalca et al. 2012). *I. hexagonus* and *R. turanicus* were found parasitizing on a wildcat in Italy (Lorusso et al. 2011), while in Spain *R. sanguineus* s.l. and *R. pusillus* were reported on the same host (Sobrino et al. 2012).

There is scarce information regarding tick species parasitizing on *Lynx lynx*. Our study reveals only the presence of adults of *I. ricinus* on this host. Despite the absence of any other similar reports, positive serology for *Anaplasma phagocytophilum* antibodies reported in a survey from Sweden on 102 lynx (Ryser-Degiorgis et al. 2005), is in line with the present findings.

Limited information exists on ticks parasitizing mustelids in Europe, despite the large number of extensive research investigating the ecology and management of this family. In Romania, data regarding ticks of mustelids are similarly scarce. All the 5 species of Mustelidae (*Lutra lutra*, *Martes foina*, *Martes martes*, *Meles meles*, *Mustela putorius*) infested with ticks harbored at least one individual of *I. ricinus*, regardless the biogeographic region of provenience, confirming the ubiquitous distribution of this tick species in Romania (Dumitrache et al. 2012; Mihalca et al. 2012) and its well-known low host specificity (Anderson and Magnarelli 1993).

Interestingly, considering the activity pattern of the Eurasian otter (*Lutra lutra*), and the only *I. hexagonus* reported as tick species parasitizing on this host (Sherrard-Smith et al. 2012), our study reveals the presence of two exophilic ticks on *Lutra lutra*. To the best of our knowledge, the presence of *D. reticulatus* in the Eurasian otter represents the first report on this host. The presence of this tick species parasitizing the otter, a semi-aquatic mustelid (Pagacz 2016), confirms *D. reticulatus* adaptive biology and its natural capacity to live under water up to several months (Földvári et al. 2016). *Ixodes ricinus* was found only once in Eurasian otters in Germany after investigations of more than 500 carcasses (Christian 2012).

The records of *I. ricinus* and *I. hexagonus* on the European badger (*Meles meles*) in Romania confirm other reports across Europe (Gern et al. 1991; Gern and Sell 2009). Our study reports also for the first time in Romania, the presence of *I. ricinus* on the European pine marten (*Martes martes*), despite the fact this host-ectoparasite association is commonly described in Europe (Sobrino et al. 2012).

The stone marten (*Martes foina)* harbored the greatest variety (4) of tick species: *I. ricinus*, *Ha. concinna*, *R. rossicus*, *R. sanguineus* s.l., with one individual harboring co-infestation with three tick species: *I. ricinus*, *Ha. concinna*, *R. rossicus*. The finding of *R. rossicus* on the stone marten represents a new host record. However, the area of collection is included in the normal distribution pattern of this tick species (Dumitrache et al. 2014). The existing reports show domestic dog as the most suitable host for *R. rossicus* adults, at least in some geographical regions (Sándor et al. 2014), but further investigations are needed to better understand the role of wildlife in the biology of this tick species.

The finding of *R. sanguineus* s.l. on stone marten suggests that this host could play an important role in the spreading and maintaining of this tick species, otherwise known to occur locally and temporarily in areas with improper environmental conditions (Duscher et al. 2014). In northern European countries where the climatic conditions are not suitable for this tick species, *R. sanguineus* s.l. can adapt to indoor conditions in mustelids dens and establish small colonies able to resist at least one winter season (Jaenson et al. 1994).

Previous data regarding ticks' parasitism on the European polecat (*Mustela putorius*) in Romania showed the presence on this mustelid of *I. ricinus* (Mihalca et al. 2012) and *I. hexagonus* (Feider 1965). Our findings confirm the existing reports. No ticks were found on five (*Mustela erminea, M. lutreola, M. nivalis, Vormela peregusna, Ursus arctos*) of the investigated carnivores species. However, previous reports in Romania suggest the involvement of the European mink (*M. lutreola*) in the natural cycle of *Borrelia burgdorferi* s.s. (Gherman et al. 2012).

Most of the co-infested carnivores ($n = 13$) were parasitized by *I. ricinus* in association with different other tick species. The constant presence of *I. ricinus* in these co-infestation or parasitizing as single species is probably due to its wide host range, that includes approximately 300 species (Anderson 1990). Co-feeding on the same individual could result in co-infections of the host animal with pathogens vectored by the parasitizing tick species and/or transmission of disease agents between vector species (Dziemian et al. 2014). Establishing tick co-infestation patterns and species associations could lead to a better understanding of the eco-epidemiology of tick-borne diseases (Anderson et al. 2013). However, as co-infestation are influenced by different specific factors (season, niche segregation, competition among tick species) (Anderson et al. 2013; Dziemian et al. 2014), further targeted studies on this topic are required.

Despite previous reports of other tick species on wild carnivores in Romania (i.e. *I. crenulatus*, *I. acuminatus*/*I. redikorzevi*, *I. rugicollis*) (Mihalca et al. 2012), they are absent in the current studies. This might be related to different sampling geographical areas and seasonality.

The consequences of anthropogenic changes of the environment (urbanization, industrialization, fragmentation) lead to the diminishing of the boundaries between wild and domestic animals (Otranto et al. 2015a, b) with consequent increased exposure to infective agents, including tick-borne pathogens. Each species has its own environmental alcove that often is shared with other species, including humans (Otranto et al. 2015a, b). In this frame, the study of tick species associated with wild carnivores has both veterinary and public health importance, especially in areas where this type of surveillance is limited or absent.

Acknowledgements GD, MOD, IAM, AMI, CMG, ADS, DM, ADM are members of the COST Action TD1303. This study was supported by the CNCS-UEFISCDI Grant Agency Romania, grant number TE 36/2015 for ADS and AMI. DM is a member of the COST Action CZ LD14048 we would like to thank Cristi Domşa for the map in Fig. 1.

References

Anderson JF (1990) Epizootiology of Lyme borreliosis. Scand J Infect Dis S77:23–34

Anderson JF, Magnarelli LA (1993) Epizootiology of Lyme disease-causing borreliae. Clin Dermatol 11:339–351

Anderson K, Ezenwa VO, Jolles AE (2013) Tick infestation patterns in free ranging African buffalo (Syncercus caffer): effects of host innate immunity and niche segregation among tick species. Int J Parasitol Parasites Wildl 2:1–9

Arnold J, Humer A, Heltai M, Murariu D, Spassov N, Hacklaender K (2012) Current status and distribution of golden jackals *Canis aureus* in Europe. Mammal Rev 42:1–11

Bengis RG, Leighton FA, Fischer JR, Artois M, Mörner T, Tate CM (2004) The role of wildlife in emerging and re-emerging zoonoses. Rev Sci Tech 23:497–511

Bermúdez CSE, Esser HJ, Miranda R, Moreno RS (2015) Wild carnivores (Mammalia) as hosts for ticks (Ixodida) in Panama. Syst Appl Acarol 20:13–19

Boitani L (2000) Action Plan for the conservation of the wolves (*Canis lupus*) in Europe. In Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). Nature and Environment, No.113.Council of Europe Publishing, Strasbourg, France, pp 76-88

Christian A (2012) Tick infestation (*Ixodes*) on the Eurasian Otter (*Lutra lutra*) – a long-term study. Soil Org 84:481–487

Coipan EC, Vladimirescu AF, Ciolpan O, Teodorescu I (2011) Tick species (Acari: Ixodoidea) distribution, seasonality and host associations in Romania. Trav Mus Natl Hist Nat Grigore Antipa LIV 2:301–317

de la Fuente J, Estrada-Peña A, Venzal JM, Tocan KM, Sonenshine DE (2008) Overview: ticks as vectors of pathogens that cause disease in humans and animals. Front Biosci 13:6938–6946

Dumitrache MO, Gherman CM, Cozma V, Mircean V, Györke A, Sándor AD, Mihalca AD (2012) Hard ticks (Ixodidae) in Romania: surveillance, host associations, and possible risks for tick-borne diseases. Parasitol Res 110:2067–2070

Dumitrache MO, Kiss B, Dantas-Torres F, Latrofa MS, D'Amico G, Sándor AD, Mihalca AD (2014) Seasonal dynamics of *Rhipicephalus rossicus* attacking domestic dogs from the steppic region of southeastern Romania. Parasit Vectors 7:97

Duscher GG, Kübber-Heiss A, Richter B, Suchentrunk F (2013) A golden jackal (*Canis aureus*) from Austria bearing *Hepatozoon canis*–import due to immigration into a non-endemic area? Ticks Tick Borne Dis 4:133–137

Duscher GG, Fuehrer HP, Kübber-Heiss A (2014) Fox on the run–molecular surveillance of fox blood and tissue for the occurrence of tick-borne pathogens in Austria. Parasit Vectors 7:521

Dziemian S, Michalik J, Piłacińska B, Bialik S, Sikora B, Zwolak R (2014) Infestation of urban populations of the Northern white-breasted hedgehog, Erinaceus roumanicus, by Ixodes spp. ticks in Poland. Med Vet Entomol 28:465–469

Estrada-Peña A, de la Fuente J (2016) Species interactions in occurrence data for a community of tick-transmitted pathogens. Sci Data 3:1–13

Farkas R, Solymosi N, Takács N, Hornyák Á, Hornok S, Nachum-Biala Y, Baneth G (2014) First molecular evidence of *Hepatozoon canis* infection in red foxes and golden jackals from Hungary. Parasit Vectors 7:303

Feider Z (1965) Fauna of the Popular Republic of Romania. Volume 5/2. Acaromorpha, Suprafamily Ixodoidea. Academia Republicii Populare Române, Bucureşti

Filippova NA (1977) Arachnida class: ixodid ticks of the subfamily Ixodinae. In Paukoobraznye Fauna SSSR, vol. 4. Nov. Ser. Nauka, Leningrad

Földvári G, Široký P, Szekeres S, Majoros G, Sprong H (2016) *Dermacentor reticulatus*: a vector on the rise. Parasit Vectors 9:314

Gern L, Sell K (2009) Isolation of *Borrelia burgdorferi* sensu lato from the skin of the European badger (*Meles meles*) in Switzerland. Vector Borne Zoonotic Dis 9:207–208

Gern L, Toutoungi LN, Hu CM, Aeschlimann A (1991) *Ixodes* (*Pholeoixodes*) *hexagonus*, an efficient vector of *Borrelia burgdorferi* in the laboratory. Med Vet Entomol 5:431–435

Gese EM (2001) Monitoring of terrestrial carnivore populations. In Gittleman JL, Funk SM, MacDonald DW, Wayne RK (eds) Carnivore Conservation, Cambridge University Press & The Zoological Society of London, 2001 pp 372–396

Gherman CM, Sándor AD, Kalmár Z, Marinov M, Mihalca AD (2012) First report of *Borrelia burgdorferi* sensu lato in two threatened carnivores: the Marbled polecat, *Vormela peregusna* and the European mink, *Mustela lutreola* (Mammalia: Mustelidae). BMC Vet Res 8:137

Gray J, Dantas-Torres F, Estrada-Peña A, Levin M (2013) Systematics and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Ticks Tick Borne Dis 4:171–180

Hornok S, Fuente J, Horváth G, Fernández de Mera I, Wijnveld M, Tánczos B, Farkas R, Jongejan F (2012) Molecular evidence of *Ehrlichia canis* and *Rickettsia massiliae* in ixodid ticks of carnivores from South Hungary. Acta Vet Hung 61:42–50

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

Jongejan F, Uilenberg G (2004) The Global Importance of Ticks. Parasitol 129:S3–S14

Labruna MB, Jorge RS, Sana DA, Jácomo ATA, Kashivakura CK, Furtado MM, Ferro C, Perez SA, Silveira L, Santos JRTS, Marques SR, Morato RG, Nava A, Adania CH, Teixeira RHF, Gomes AAB, Conforti VA, Azevedo FCC, Prada CS, Silva JCR, Batista AF, Marvulo MFV, Morato RLG, Alho CJR, Pinter A, Ferreira PM, Ferreira F, Marques SR (2005) Ticks (Acari: Ixodida) on wild carnivores in Brazil. Exp Appl Acarol 36:149–163

Lorusso V, Lia RP, Dantas-Torres F, Mallia E, Ravagnan S, Capelli G, Otranto D (2011) Ixodid ticks of road-killed wildlife species in southern Italy: new tick–host associations and locality records. Exp Appl Acarol 55:293–300

Matuschka FR, Fischer P, Musgrave K, Richter D, Spielman A (1991) Hosts on which nymphal *Ixodes ricinus* most abundantly feed. Am J Trop Med Hyg 44:100–107

Mertens A, Promberger C (2001) Economic aspects of large carnivore-livestock conflicts in Romania. Ursus 173-180

Mihalca AD, Dumitrache MO, Magdaş C, Gherman CM, Domşa C, Mircean V, Ghira IV, Pocora V, Ionescu DT, Sikó Barabási S, Cozma V, Sándor AD (2012) Synopsis of the hard ticks (Acari: Ixodidae) of Romania with update on host associations and geographical distribution. Exp Appl Acarol 58:183–206

Murariu D (2010) Systematic list of the Romanian vertebrate fauna. Trav Mus Natl Hist Nat Grigore Antipa 53:377–411

Nosek J (1971) The ecology, bionomics, and behaviour of *Haemaphysalis* (*Aboimisalis*) *punctata* tick in central Europe. Z Parasitenkd 37:198–210

Otranto D, Cantacessi C, Dantas-Torres F, Brianti E, Pfeffer M, Genchi C, Guberti V, Capelli G, Deplazes P (2015a) The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part II: Helminths and arthropods. Vet Parasitol 213:24–37

Otranto D, Cantacessi C, Pfeffer M, Dantas-Torres F, Brianti E, Deplazes P, Genchi C, Guberti V, Capelli G (2015b) The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part I: Protozoa and tick-borne agents. Vet Parasitol 213:12–23

Pagacz S (2016) The effect of a major drainage divide on the gene flow of a semiaquatic carnivore, the Eurasian otter. J Mammal. doi:10.1093/jmammal/gyw066

Petrişor AI, Ianoş I, Tălângă C (2010) Land cover and use changes focused on the urbanization processes in Romania. Environ Eng Manag J 9:765–771

Randi E (2011) Genetics and conservation of wolves *Canis lupus* in Europe. Mammal Rev 41:99–111

Reiczigel J, Rozsa L, Reiczigel A, Fabian I (2013) Quantitative Parasitology (QPweb), http://www2.univet.hu/qpweb. Accessed 6 August 2016

Ryser-Degiorgis MP, Hofmann-Lehmann R, Leutenegger CM, Segerstad CHA, Mörner T, Mattsson R, Lutz H (2005) Epizootiologic investigations of selected infectious disease agents in free-ranging Eurasian lynx from Sweden. J Wildl Dis 41:58–66

Sándor AD, Dumitrache MO, D'amico G, Kiss BJ, Mihalca AD (2014) *Rhipicephalus rossicus* and not *R. sanguineus* is the dominant tick species of dogs in the wetlands of the Danube Delta. Romania. Vet Parasitol 204:430–432

Sherrard-Smith E, Chadwick E, Cable J (2012) Abiotic and biotic factors associated with tick population dynamics on a mammalian host: *Ixodes hexagonus* infesting otters. Lutra lutra. PLoS One 7:e47131

Sobrino R, Millán J, Oleaga Á, Gortázar C, de la Fuente J, Ruiz-Fons F (2012) Ecological preferences of exophilic and endophilic ticks (Acari: Ixodidae) parasitizing wild carnivores in the Iberian Peninsula. Vet Parasitol 184:248–257

Stefan S, Ghioca M, Rimbu N, Boroneant C (2004) Study of meteorological and hydrological drought in southern Romania from observational data. Int J Climatol 24:871–881

Walker JB, Keirans JE, Horak IG (2005) The genus *Rhipicephalus* (Acari, Ixodidae): a guide to the brown ticks of the world. Cambridge University Press

Yamaguchi N, Kitchener A, Driscoll C, Nussberger B (2015) *Felis silvestris*. The IUCN Red List of Threatened Species 2015: e.T60354712A50652361: http://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T60354712A50652361.en. Accessed 18 August 2016