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THE OCCURRENCE OF *DERMACENTOR RETICULATUS* TICK (ACARI: AMBLYOMMIDAE) IN NORTH-EAST SLOVENIA: ONE MORE EVIDENCE FOR ITS INCREASED DISTRIBUTION RANGE

Maja Gračner Jadresic[1], Roman Luštrik[2], Tomi Trilar[3]

[1] maja.gracner@gmail.com
[2] Department of biology, Biotechnical faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia, e-mail: romunov@gmail.com
[3] Slovenian Museum of Natural History, Prešernova 20, 1000 Ljubljana, Slovenia, e-mail: ttrilar@pms-lj.si

Abstract – The occurrence of *Dermacentor reticulatus* on the territory of Slovenia was not described before. By extensive 3.5 year sampling period, we obtained sufficient data allowing for the first time to clearly define the distribution rate and seasonal activity of adult *D. reticulatus* in this part of Europe, north-east Slovenia. A study was conducted from November 2003 to May 2007 to evaluate the comprehensive view of the annual seasonality of questing adult *D. reticulatus*. Ticks monitoring was conducted in three different localities/habitats: a) Muriša - wet meadows near oxbow lake of the river Mura; b) Murska šuma - swampy forest area between the confluence of the river Mura and Ledava, which is occasionally flooded; and c) Dolinski pašnik - meadows with old oak trees, forest edge and shrub communities. In total, 2511 ticks were collected by flagging. There were exclusively adults with female-biased sex ratio throughout the years in all sampling localities, namely for Muriša 59.5% (1287 of 2164), Murska šuma 62.7% (203 of 324) and Dolinski pašnik 60.8% (14 of 23). Most of the ticks (89.2%) were collected when the mean daily temperatures were ranging from 0 to 14.9ºC. The highest activity of *D. reticulatus* were noted in the spring, culminating in February-April/May, peaking in average in March and with considerably lower rate (3-8-times) in the second activity peak in autumn, culminating in the end of August/September-November. The greatest number of adults in autumn was collected in October or November. *Dermacentor reticulatus* was not active during the summer months. The highest quantity of ticks was recorded in meadows near oxbow lake of the river Mura where was caught 6.6-times more ticks than in swampy forest area Murska šuma, which was occasionally flooded after huge rainfall and

even 94-times more than in Dolinski pašnik. The confirmed occurrence of *D. reticulatus* in north-eastern Slovenia provided evidence that this species has extended its range. Our findings point to an enlargement of Pannonian distribution area of the species. This study indicates that *D. reticulatus* can be ranked as a typical element of the fauna in north-eastern Slovenia.

KEY WORDS: *Dermacentor reticulatus*, Slovenia, distribution, seasonality

Izvleček – POJAVLJANJE SEVERNEGA ORNAMENTIRANEGA KLOPA (*DERMACENTOR RETICULATUS*) (ACARI: AMBLYOMMIDAE) V SEVEROVZHODNI SLOVENIJI: ŠE EN DOKAZ ZA POVEČANJE NJEGOVE RAZŠIRJENOSTI

Pojavljanje severnega ornamentiranega klopa (*Dermacentor reticulatus*) v Sloveniji do sedaj ni bilo opisano. Z obsežnim tri in pol letnim vzorčenjem, od novembra 2003 do maja 2007, smo na treh lokalitetah (Muriša, Murska šuma in Dolinski pašnik) v severovzhodni Sloveniji v Prekmurju na območju Lendavskega Dolinskega pridobili dovolj podatkov, s katerimi smo dobili vpogled v razširjenost in sezonsko aktivnost severnega ornamentiranega klopa. Skupno smo nabrali 2511 odraslih osebkov klopov. Odrasli osebki severnega ornamentiranega klopa so bili na vseh lokalitetah najbolj aktivni spomladi, z vrhom v marcu. Aktivnost se je proti poletju počasi zmanjševala in v poletnih mesecih klopov nismo našli. Ponovno se je aktivnost povečala v jesenskih mesecih, z manjšim vrhom v oktobru ali novembru (3-8-krat manjša aktivnost kot spomladi). Vrsta je bila najbolj številčna ob Muriši, kjer je bilo ujetih 6,6-krat več odraslih osebkov kot v Murski šumi, ki je bila občasno poplavljena, in 94-krat več osebkov kot na Dolinskem pašniku. Razmerje med spoloma je bilo na vseh lokalitetah v prid samic, in sicer ob Muriši z 59,5 % (1287 od 2164), v Murski šumi 62,7 % (203 od 324) in na Dolinskem pašniku 60,8 % (14 od 23). Večina klopov (89,2%), je bilo nabranih pri povprečni dnevni temperaturi od 0 do 14,9ºC.

V članku sta prvič predstavljeni razširjenost in sezonska aktivnost severnega ornamentiranega klopa (*Dermacentor reticulatus*) v Sloveniji. Naše ugotovitve kažejo na povečanje panonskega območja razširjenosti in da predstavlja severni ornamentirani klop značilni favnistični element severovzhodne Slovenije.

KLJUČNE BESEDE: severni ornamentirani klop, *Dermacentor reticulatus*, Slovenija, razširjenost, sezonska aktivnost

1. Introduction

Dermacentor (Dermacentor) reticulatus (Fabricius 1794) is a hard tick species (Acari: Ixodida: Amblyommidae), which has a large geographical range across the temperate zone (Buczek et al. 2014) and is within its geographic range divided into two separate parts, Western and Eastern Europe. Western European part includes populations from the western Palaearctic region, in a temperate zone from England and France while the eastern European part extends to the Basin of Yenisei River in

Siberia and to Central Asia (Dautel et al. 2006, Karbowiak 2014). The tick species has not been found north of 53º-54ºN latitude nor in Mediterranean climate zone (Dautel et al. 2006). The currently known southern distribution of *D. reticulatus* in the Balkans is in Serbia near Belgrade at 44.77ºN 20.36ºE. The southernmost occurrence of this species is found in the Crimean Peninsula at 44.27ºN 34.03ºE (Rubel et al. 2016). The area of distribution as a whole is expanding and the expansion to new, previously free areas has been observed in several countries in Europe (Nowak 2011, Karbowiak 2014, Földvári et al. 2016, Rubel et al. 2016).

There are 36 species known in the genus *Dermacentor* (Barker and Murrell 2008), with two of them in Slovenia: *Dermacentor reticulatus* (Ornate Cow Tick) and *Dermacentor marginatus* (Ornate Sheep Tick). According to the data from the Ixodida study collection (PMSL-Ixodida) housed in Slovenian Museum of Natural History (Trilar, unpublished data) we are considering that in Slovenia *D. reticulatus* is distributed north of the river Sava (commonly found in the north-east Slovenia) and *D. marginatus* south of the river Sava.

Until the present research had been conducted, there were only few reports of adult *D. reticulatus* from north-east Slovenia, found for the first time in May 2001 from vegetation and later on Red Deer (*Cervus elaphus* Linnaeus 1758) in autumn 2002. There are also data of adult *D. reticulatus* found on dogs (*Canis familiaris* Linnaeus 1758) from Ljubljana in August 1995 and Brežice from year 2003 (south-east Slovenia). Dog could be with owner anywhere in Slovenia or Croatia, or even further, so we cannot know from where the ticks originally came from. *Dermacentor reticulatus* were from year 2003-2007 commonly found in the north-east Slovenia (Figure 3).

Dermacentor reticulatus is the second most reported tick species after *Ixodes ricinus* (Linnaeus 1758) in Central Europe (Rubel et al. 2016) and considered to be among important vectors of tick-borne diseases of animals and human (Široký et al. 2011, Földvári et al. 2016). Research results from Central and Eastern European countries are suggesting that *D. reticulatus* may have changed its spatial distribution and its geographic range (Sréter et al. 2005). Large areas of north-western and central Europe, formerly thought to be too cold for its survival and completion of life-cycle, have experienced a remarkable expansion of this species (Földvári et al. 2016). Given its vectorial capacity, expansion of *D. reticulatus* can affect the occurrence of certain diseases (Cochez et al. 2012) as canine babesiosis, tularemia, rickettsiosis, or Q fever and playing role in forming their natural foci (Řehaček et al. 1991, Hubálek et al. 1996, 1998, Parola et al. 2005, Duh et al. 2006, Dobec et al. 2009). Indeed, in Europe, *D. reticulatus* are the most important vectors of *Babesia canis canis*, the aetiological agent of canine babesiosis (Uilenberg et al. 1989, Lobetti 1998). In Slovenia it is expected the majority if not all cases of canine babesiosis to be caused by *B. c. canis* (Duh et al. 2004).

Studying tick ecology is crucial for better understanding of the risk this tick species poses to animal and human populations. The present survey is undertaken in order to gain comprehensive view of the annual seasonality of *D. reticulatus* in Slovenia and fully proves for the first time that *D. reticulatus* occurs in Slovenia, in the area Lendavsko Dolinsko in Prekmurje (Pannonian biogeographical region), in less

than 1 km distance to the Slovenian-Hungarian and Slovenian-Croatian border, in the area where no natural populations of this species had been discovered until now.

2. Materials and methods

2.1 Study area

The study was conducted in three localities in the north-eastern part of Slovenia - Lendavsko Dolinsko area, Prekmurje: a) Muriša (46°29'08"N 16°33'11"E; 157m above sea level [a.s.l.]), b) Murska šuma (46°30'29"N 16°31'53"E; 155m a.s.l.) and c) Dolinski pašnik (46°31'46"N 16°29'54"E, 158m a.s.l.). Each locality represented different habitat type:

Oxbow lake Muriša: meadows near oxbow lake of the river Mura; the shoreline of oxbow lake was densely overgrown with True Sedge (*Carex* sp.), Common Reed (*Phragmites australis*) (Skoberne 1988) and with allochtonous plant species as Policeman's Helmet (*Impatiens glandulifera*) and Giant Goldenrod (*Solidago gigantea*),

Murska šuma: swampy forest area between the confluence of the river Mura and Ledava, occasionally flooded and overgrown by Common Hornbeam, Pedunculate Oak Forest (*Carpino betuli-Quercetum roboris*) and

Dolinski pašnik: meadows with old Pedunculate Oak trees (*Quercus robur*), forest edge and shrub communities (Gogala 2002). Part of the area was used as a football field.

Localities are separated by a distance from 1.8 to 5 km. Distance from the Slovenian-Hungarian and Slovenian-Croatian border is less than 1 km.

The study area is located in temperate zone with continental climatic impact of large temperature fluctuations, cold winters and hot summers with little rainfall, which are most abundant in July. The average annual temperature is 10-12°C. Average annual rainfalls for this area are 800-1000mm (Mršić 1997). Snow can appear from November-March but persists in each month for a short time. On 30 years average (from 1971-2000) were in north-eastern Slovenia 5-20 days of snow cover per year (data from the Slovenian Environment Agency).

2.2 Tick collection

Ticks collections were performed on a monthly basis for 1 consecutive day from November 2003 to May 2007. The choice of study days depended on weather conditions. No collection of ticks was arranged after snowfall or on a wet snowmelt days (in January and February 2005, in November 2005 till February 2006 and in December 2006 till January 2007) and after heavy rains with floods (in August 2005).

Ticks, questing for hosts, were collected by flagging method. A white linen cloth, 135 x 110cm, attached to a stick, was used. Each collection session lasted for 2 hours (one person collected two hours, two persons collected one hour each etc.), with an overall sampling effort of 12,240 min of tick collections. Ticks were collected between 11.00 a.m. and 5 p.m. Ticks attached to linen cloth were collected using exhauster and stored afterwards in vials containing 70% ethanol. Species determination was

carried out with the use of taxonomic key (Trilar, unpublished).

The material is stored in Study collection of ticks (PMSL-Ixodida) housed in Slovenian Museum of Natural History.

2.3 Climatic data

Climatic data were recorded in local climatological station Lendava. Data were kindly provided by the Environment Agency of the Republic of Slovenia. We obtained also data on site-sampling day (temperature in the shadow, sun and soil).

2.4 Data visualization and analysis

Data was stored in Excel.xlsx files and imported into R (R Core Team, 2015) for further analysis. Figures were done using ggplot2 (Wickham 2009). Modeling was explored using base R functions and lme4 (Bates et al. 2015) package. Data manipulation was handled by packages readxl and tidyr. Modeling approach, while not described here, is part of a reproducible supplemental material generated using package knitr (Xie 2015).

3. Results and discussion

There are 16 tick species (Acarina: Ixodida) distributed across Slovenia (Tovornik 1987a, b, 1988a, b, c, 1989, 1990, 1991, Trilar 2004), with *D. reticulatus* among them.

The occurrence of *D. reticulatus* on the territory of Slovenia was not described before. By extensive 3.5 year sampling period, we obtained sufficient data allowing for the first time to clearly define the distribution rate and seasonal activity of adult *D. reticulatus* in this part of Europe, north-east Slovenia.

From November 2003 till May 2007 were with flagging method collected altogether 2511, exclusively adult stages of *D. reticulatus*, among them 2164 in Muriša, 324 in Murska šuma and 23 in Dolinski pašnik area. The absence of immature life stages of *D. reticulatus* in samples obtained by flagging is usual observation (Široký et al. 2011, Földvári et al. 2016). Nymphs and larvae generally parasitize on Insectivora and Rodentia, occasionally on Aves (Baker 1999) and probably also living in host's burrows, protected from unfavourable climate (Meyer-König et al. 2001). Therefore, the presence of these developmental stages should be checked also herein.

The seasonal activity of adult *D. reticulatus* has been extensively studied in Europe and although the abundance and dynamics of these ticks vary, two activity peaks have been reported: in spring and autumn (Razumova 1988, Martinod and Gilot 1991, Széll et al. 2006, Bartosik et al. 2011, Buczek et al. 2014, Földvári et al. 2016). Also our field study showed a bimodal pattern of activity of this species. The highest activity peaks were noted in the spring, culminating in February-April/May, peaking in average in March and with considerably lower rate (3-8-times) in the second activity peak in autumn, culminating in the end of August/September-November. The greatest number of adults in autumn was collected in October or November (Figure 1).

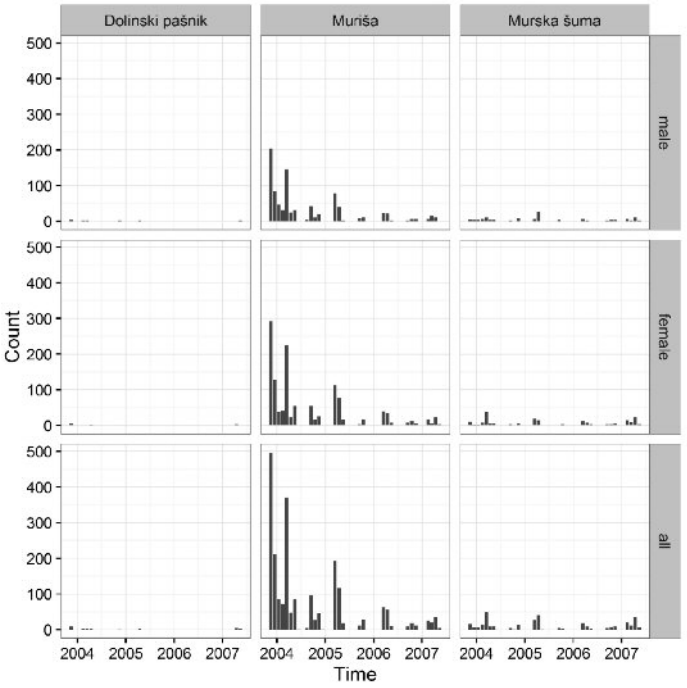

Fig. 1: Count of collected individuals for individual locations (columns) per sex (rows). Bottom row represents total count.

Abundance of *D. reticulatus* varied markedly between localities in interval 23-2164. It was most abundant in zones along rivers Mura and Ledava. The highest quantity of ticks was recorded in meadows near Muriša, oxbow lake of the river Mura, where 6.6-times more ticks were caught than in swampy forest area Murska šuma, which was occasionally flooded after huge rainfall and even 94-times more than in Dolinski pašnik. Meadows, deciduous forest and swampy mixed woods placed near water bodies or large stagnant waters are typical habitats of *D. reticulatus*. The most important factor for occurrence of this tick species is a combination of a high level ground water, along with drying soil (Karbowiak 2014). These habitats are in north-east Slovenia probably representing a suitable habitat for hosts of *D. reticulatus*. On the other hand, there are studies of quite abundant distribution of this tick species even in shrubby ecotones, much far from the rivers (Široký et al. 2011) or on higher hills (up to an altitude of 1000 m a.s.l.) (Hornok and Farkas 2009) which speaks also for the relative importance of host availability.

In subsequent years of sampling the abundance of ticks was decreasing, especially in localities Muriša and Dolinski pašnik (Figure 1). For instance, in locality Muriša was in late autumn 2003 (November, December) noted the highest activity peak of *D. reticulatus* in spring or autumn in the all 3.5 year collection period (altogether with 708 individuals which represented 28% of all collected ticks in all localities). In late autumn 2004 was in the same locality caught 661 ticks less than in previous year (Figure 1). We believe that the reason was not due to temperature. Temperatures

(measured in shadow) were on the day of sampling in November/December 2003 and in November/December 2004; 6.6/3.6ºC and 13.1/3.2ºC, respectively, which is in the range of *D. reticulatus* activity (Bartosik et al. 2011, Buczek et al. 2014). We think that decrease could be explained either by oversampling of ticks or by changes in habitat. At Muriša the use of arable land started to increase and surrounding vegetation by oxbow lake has frequently been removed presumably by fishermen. In Dolinski pašnik 70% (16 of 23) of all ticks were collected in year 2003-2004 (Figure 1). The main causes could be due to habitat degradation. Part of the locality Dolinski pašnik was already dedicated to football field, furthermore in the year 2006 nearby started construction works for the highway. A problem could be also abandonment of grazing cattle in Dolinski pašnik, thereby reducing the number of potential tick hosts. It is also possible that our 3.5-year sampling period contributed to every year reduction of the numbers of this tick species in sampling areas. Finally, decreasing amount of ticks in both sampling localities could be also due to longer snow cover in late autumn-winter 04/05 and 05/06.

Dermacentor reticulatus is a psychrophilic tick and is very tolerant to low temperatures (Hubálek et al. 2003). The lower thermal threshold of activity of adult *D. reticulatus* is at air/soil temperatures 0.7/-0.1ºC (Buczek et al. 2014). Its activity starts after disappearance of snow cover at 2-4ºC and can be found active in January and February (Karbowiak 2014). The highest activity of adult *D. reticulatus* at daily mean temperatures is ranging from 4-13ºC (Bartosik et al. 2011). During a 24-hour monitoring in Wales the minimum temperature at which *D. reticulatus* were recorded active was 3.3ºC and the minimum overnight temperature was -5.4ºC (Földvári et al. 2016). The upper range of the threshold temperature for tick activity is 39ºC (Bartosik et al. 2011). Warmer and humid conditions are preferred for its development and reproduction (Zähler et al. 1996). The present investigation confirmed the activity of adult *D. reticulatus* throughout winter months (January and February) in Muriša and Murska šuma in year 2004, at daily temperatures (measured in shadow) ranging from 5-11.2°C. Mean monthly air/soil temperatures for January and February 2004 were -1.0/-0.4ºC and 2.5/-0.1ºC, respectively (data from the Slovenian Environmental Agency, Climatological station Lendava). In winter months was altogether caught 177 specimens, with 157 in Murska šuma. The winter occurrence of *D. reticulatus* was not reported later during the field study, which may be attributed to weather conditions, e.g. longer persistence of snow cover. The mean temperatures and hours of sunshine in winter months did not differ over the sampling years. Difference in the number of days of persistence of snow cover was noted, namely, in winter months 2004 with 9 days, 2005 (28 days), 2006 (32 days) and 2007 (4 days) (data from the Slovenian Environmental Agency). Most of the ticks (89.2%) were collected when the mean daily temperatures were ranging from 0 to 14.9ºC. In particular, 91 (3.6%) were collected with temperatures at ≤0ºC, 1029 (41%) at 0.5-4.9ºC, 676 (27%) at 5-9.9ºC, 535 (21.2%) at 10-14.9ºC, 175 (7%) at 15-19.9ºC, and 5 (0.2%) at ≥20ºC. *Dermacentor reticulatus* was not active during the summer months.

The sex ratio (Figure 2) is an important parameter, which characterizes the state and dynamics of natural populations of animals. Most ixodid tick species can be characterized

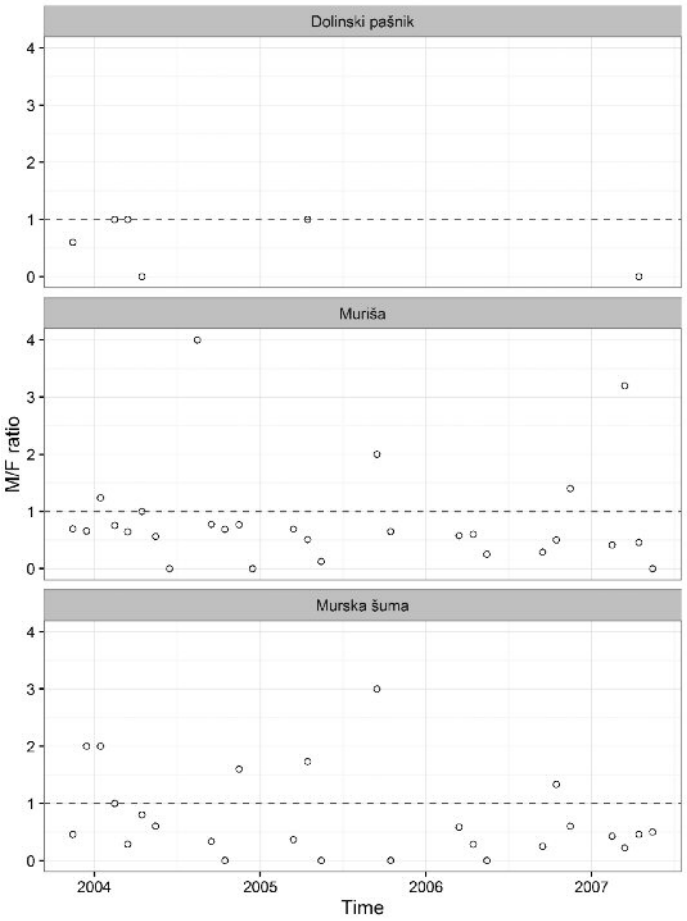

Fig. 2: Sex ratio per individual collection for different locations. Some ratios are not represented by a real number because only one sex was found.

by a 1:1 sex ratio of their progeny with biased sex ratios later in adult stage (Fourie et al. 1996). We recorded female-biased sex ratio throughout the years in all sampling localities, namely for Muriša 59.5% (1287 of 2164), Murska šuma 62.7% (203 of 324) and Dolinski pašnik 60.8% (14 of 23). Higher proportion of females were obtained also in studies, e.g. Široký et al. 2011 (in Czech Republic); Hornok 2009, Hornok and Farkas 2009 (in Hungary); Buczek et al. 2014, Nowak 2011 (in Poland), Krčmar et al. 2014a (in Croatia), among others. Female-biased sex ratio in field samples of *D. reticulatus* may be influenced by mating strategies (Fourie et al. 1996). Metastriate ticks, including *Dermacentor* spp., mate exclusively on their hosts (Kiszewski et al. 2001). Bartosik et al. (2011) reported that *D. reticulatus* males remain on the host longer than females, which may explain their lower numbers found on vegetation. Besides that, females need to find a host for ingestion of blood, essential for egg development, which increase their questing. Consequently, males are less likely to be found by cloth-dragging (Fourie et al. 1996). Prevalence rates may be affected also by different capa-

bilities of the sexes to survive unfavourable conditions, e.g. *D. reticulatus* females were found to be more resistant to dehydration than males (Meyer-König et al. 2001), which may be another reason why *D. reticulatus* females quest more.

Dermacentor reticulatus are expanding their habitats and changing the spatial distribution as they occur at far more sites than previously known (Karbowiak 2014, Földvári et al. 2016). To Slovenia the closest data of occurrence of this species are from Hungary, where is *D. reticulatus* the second most common tick species (Sréter et al. 2005). In south western part of Hungary (close to the Slovenian-Hungarian border) were found only in places with partly dry vegetation, and up to an altitude of 900–1000m a.s.l. (Hornok and Farkas 2009), while in north eastern Slovenia at altitude 160m a.s.l. and in swampy mixed woods placed near water bodies or large stagnant waters. We assume that our findings point to an enlargement of Hungarian distribution area of the species, as well as Krčmar et al. (2014) and their findings of it in Croatian part of Baranja region (Figure 3). The expansion of *D. reticulatus* to new and previously uninhabited areas has been observed in Europe (Karbowiak 2014, Földvári et al. 2016). A range of factors could be responsible for the changes in the

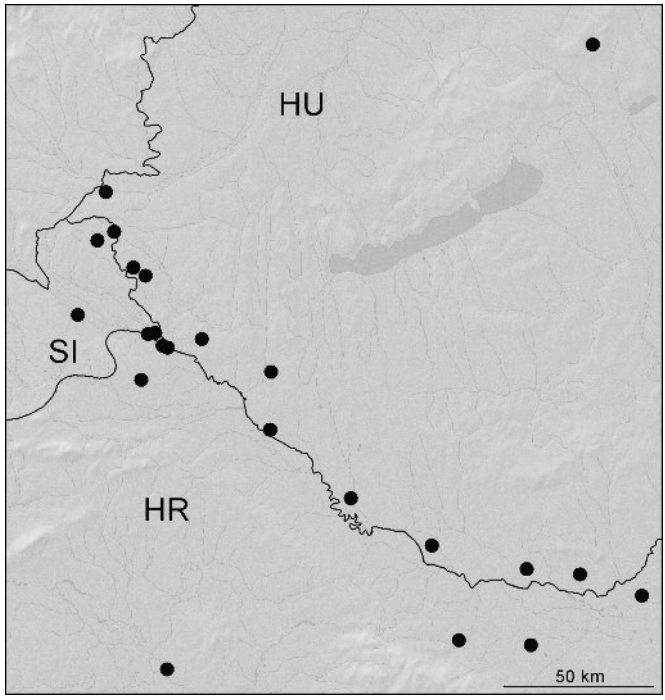

Fig. 3: Collection sites of *Dermacentor reticulatus* at the south western edge of Pannonian lowland. The data are taken from this work and from literature (Vesenjak-Hirjan and Šooš 1976, Borčić et al. 1978, Hornok and Farkas 2009, Dobec et al. 2009, Krčmar 2012, Krčmar et al. 2014). The map is created by GPS Visualizer (Schneider 2003-2014)

distribution of this tick species, including global warming (Gray et al. 2009), the shifting use of landscape (e.g. reduction of the use of pesticide and other chemicals, reforestation) (Bullová et al. 2009), the transformation of ploughed land into areas covered with permanent vegetation (Heile et al. 2006), the increase of numbers of wild animals (as a result of nature conservation), the introduction of ticks into new regions through tourism, transport (Sréter et al. 2005, Karbowiak 2014), as well as the relationship between the occurrence of *D. reticulatus* and the habitats of their important hosts (Nowak 2011) and also other unknown factors.

Conclusions

Studying tick ecology is essential step towards a better understanding of the transmission dynamics and the risk these arthropods pose to animal and human populations. *Dermacentor reticulatus* tick is a vector and final host of *B. c. canis*, recently expanding in central Europe (Duh et al. 2004, 2006, Földvári et al. 2007, Leschnik et al. 2008, Adaszek et al. 2011, Kubelová et al. 2011). Expansion of *D. reticulatus* might be followed by an expansion of tick-borne diseases (Kiewra and Czulowska 2013, Földvári et al. 2016). The confirmed occurrence of *D. reticulatus* in north-eastern Slovenia provided evidence that this species has extended its range. It indicates a need of future systematic sampling to assess a speed of expansion as well as infection rate of this tick species.

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