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A new focus of the tick *Haemaphysalis concinna* in Western Poland

Dorota Dwużnik[1] · Ewa J. Mierzejewska[1] · Mohammed Alsarraf[1] · Anna Bajer[1]

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Abstract

The relict tick *Haemaphysalis concinna* has a fragmented and focal distribution in Central Europe and Asia. Although in the majority of neighboring countries the occurrence of this tick species is well-documented (i.e., in Germany, Czech Republic, Slovakia, Ukraine), to-date its occurrence in Poland has been registered only once, in 1953 in Troszyn in North-Western Pomerania, close to the German-Polish border. In the present study we report the first documented finding of *H. concinna* in Western Poland, confirmed both by collection of juvenile ticks from rodent hosts and questing ticks from vegetation. Trapping of rodents took place in the summer of 2018 in three locations in Western Poland (Słonin, Nowy Młyn 1, Nowy Młyn 2). Rodents were inspected for ectoparasites, which were detached and fixed in 70% ethanol. All the collected ticks were assigned to species and developmental stages using appropriate morphological keys, and representative individuals were genotyped by molecular methods. A total of 1482 feeding ticks were collected from 106 rodents from three sites. The common tick *Ixodes ricinus* was found in abundance on small rodents at all three sites; *Dermacentor reticulatus* ticks were identified at two sites in small numbers and, finally, numerous juvenile *H. concinna* (n = 427) were found at one of our study sites (Nowy Młyn 2). The highest prevalence and abundance of *H. concinna* were recorded on voles, *Microtus agrestis* and *M. oeconomus,* from this site in August. Additionally, questing nymphs and adult *H. concinna* were collected locally from vegetation (n = 20). Genotyping and phylogenetic analysis confirmed the species as *H. concinna*. A new focus of *H. concinna* has been described in Western Poland. Our long-term field work monitoring the expansion of the distribution of *D. reticulatus* in Poland, during which all collected ticks are identified, suggests that *H. concinna* is still very rare in the country.

Keywords *Haemaphysalis concinna* · Poland · Rodent · Genotyping

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✉ Dorota Dwużnik
dorota.dwuznik@biol.uw.edu.pl

[1] Department of Parasitology, Institute of Zoology, Faculty of Biology, University of Warsaw, 1 Miecznikowa Street, 02-096 Warsaw, Poland

Introduction

Nineteen species of ticks are known to occur in Poland comprising the established tick fauna of the region (Nowak-Chmura and Siuda 2012). The most common species of hard ticks (Ixodidae) are *Ixodes ricinus* and *Dermacentor reticulatus.* These two species are subjects of numerous field and laboratory studies, covering the majority of the territory of the country (Asman et al. 2017; Kiewra et al. 2014, 2016; Mierzejewska et al. 2015, 2016; Stańczak et al. 2015, 2018; Wodecka et al. 2014; Wójcik-Fatla et al. 2016; Zając et al. 2017). However, the relict tick *Haemaphysalis concinna* has never been the subject of such studies because it has been so rare in the Poland. A recent overview of the Polish tick fauna has reported only one previous finding of this tick species in Poland (Nowak-Chmura and Siuda 2012). A single female *H. concinna* was found in close proximity to the German-Polish border and Baltic Sea shore, in Troszyn, N. West Pomerania in 1953, 65 years ago (Lachmajer et al. 1956).

Interestingly, although the number of field studies on ticks (involving intensive dragging in different habitats and studies on the tick fauna of a wide range of hosts, including wildlife) has increased substantially over the last 20 years, no new foci of *H. concinna* have been reported in Poland. In contrast, the rapid expansion of *D. reticulatus* has been well documented in Europe (Rubel et al. 2016; Földvári et al. 2016) and also in Central and Western Poland (Mierzejewska et al. 2016, 2017). In a recent review of the distribution of *H. concinna* in Central Europe, Poland was still designated as a 'white spot' region with minimal records, while neighboring countries were recorded as 'red spot' foci reflecting the well-documented presence of the tick: for example in Germany, South Czech Republic, Slovakia, Ukraine and Belarus, South and Eastern Russia (Rubel et al. 2018). Thus, with more vigilant monitoring in the future it will not be surprising to find that more foci of *H. concinna* are recorded in Poland. Moreover, given the well documented climate change and the evidence that it has promoted tick expansion and tick population growth in Europe (Karbowiak 2014; Medlock et al. 2013; Sréter et al. 2005), substantial changes in the distribution of *H. concinna* are also likely to have taken place since its first and only report in the country 65 years ago.

Following our earlier study on the expansion of the distribution of *D. reticulatus* in the period 2012–2014, we also monitored this process subsequently between 2016 and 2018, in order to document further the continued colonization of Western and Central Poland with this important vector of *Babesia canis*. This study was performed by dragging of suitable open habitats in Spring (March–May) and Autumn (September–November) of each year (Dwużnik unpublished). Additionally, the study was broadened by targeted monitoring of the instars of *D. reticulatus*. For this reason we collected ticks from several rodent communities in Central and Western Poland in the period June–August, when juvenile *D. reticulatus* ticks can be found on rodent hosts (Karbowiak 2009; Paziewska et al. 2010). It was during this work that we encountered also various developmental stages of *H. concinna* in our sampling of both vegetation and on the rodents. In the present study we report a new location for the confirmed presence of *H. concinna* in Western Poland, identified both in the samples of collected questing ticks encountered on vegetation and also feeding ticks removed from rodent hosts.

Materials and methods

Field study

The study was completed in 2018 in Western Poland (Fig. 1). Juvenile ticks were collected from rodents from three sites: Słonin (52.119201N; 16.747274E); Nowy Młyn 1 (52.152127N; 16.119467E) and Nowy Młyn 2 (52.148505N; 16.114098E). Trapping was performed at all sites in June–July (the season for occurrence of *D. reticulatus* larvae) and in August in Nowy Młyn 2 and Słonin (*D. reticulatus* nymph season). The distance between Słonin and Nowy Młyn is about 50 km, and that between Nowy Młyn 1 and Nowy Młyn 2 is about 1 km. Additionally, Nowy Młyn 1 and Nowy Młyn 2 sites were separated by a road (Fig. 1).

The habitats at the three locations were similar, comprising submerged meadows and fallow lands in close proximity to water bodies (water ditch in Słonin, Dojca River in Nowy Młyn 1, Dojca River and large breeding pond in Nowy Młyn 2). Each of the trapping sites was located in the vicinity of forests. All locations were selected on the basis of habitat qualities typical of the preferred habitats for *Microtus* spp. voles which are the main hosts of *D. reticulatus* instars, this tick species being the main focus of our field work (Paziewska et al. 2010).

Small rodents were live-trapped and live-processed at the site of trapping, as described in our previous papers (Bajer et al. 2014; Grzybek et al. 2015; Tołkacz et al. 2017), including the removal of ticks and other ectoparasites from anesthetized animals. Rodents were then released near to their trapping points.

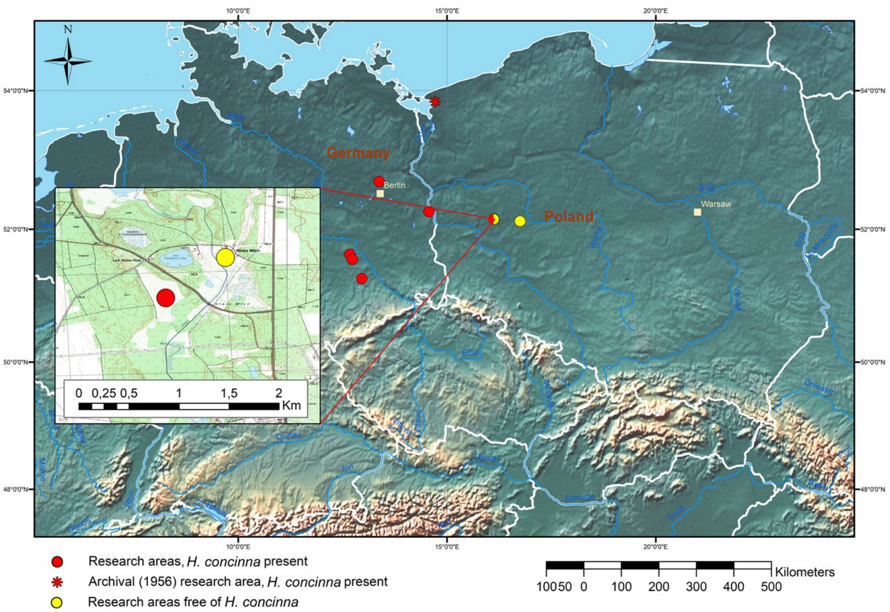


Fig. 1 Map of *Haemaphysalis concinna* occurrence in Poland

Additionally, during the inspection of traps in Nowy Młyn 2 site in August 2018 questing adult ticks and nymphs were collected along a 300 m transect from two researchers and their accompanying dog.

Tick identification

Ticks were fixed in 70% EtOH, transported to the laboratory of the Department of Parasitology, Faculty of Biology, University of Warsaw and identified to species and stage levels using a stereoscopic microscope Zeiss Stemi 508 equipped with a camera. All ticks were assigned to species and stages according to the key of Estrada-Peña et al. (2004). Photographical documentation was performed. Ticks were counted and two infestation parameters were calculated: prevalence (% infested rodents) and abundance (mean number of tick/individual). For questing ticks we calculated the mean density/100 m^2 of territory.

Genotyping of ticks

Genomic DNA was extracted from four ticks (4 nymphs) collected from rodents at the Nowy Młyn 2 site and identified as *H. concinna*, using the A&A Biotechnology DNA extraction kit (Gdańsk, Poland). Variable mitochondrial (mt) 16S rRNA gene fragment (440 bp) was amplified following Kulakova et al. (2014).

PCR products were sequenced by a private company (Genomed, Warsaw, Poland). DNA sequence alignments and analyses were conducted using MEGA 7. Consensus sequences were compared with sequences deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/).

For the phylogenetic analysis, the Akaike information criterion was used in jModel Test to identify the most appropriate model of nucleotide substitution. A representative tree for mt16S rDNA was constructed using MEGA 7 by the Maximum Likelihood method and the General Time Reversible model with Gamma distribution (Kumar et al. 2016).

Ethics approval

All of the procedures were conducted with the approval of the First Warsaw Local Ethics Committee for Animal Experimentation in Poland (ethical license number: 706/2015) according to the principles governing experimental conditions and care of laboratory animals required by the European Union and the Polish Law on Animal Protection.

Results

Ticks from rodents

A total of 106 rodents were examined for ectoparasites, including 22 *Apodemus agrarius*, 14 *A. flavicollis*, six *A. sylvaticus*, 51 *Microtus oeconomus*, six *M. agrestis*, one *M. arvalis*, three *Mus musculus* and three juvenile *Microtus* voles (*Microtus* spp.), which could not be identified to species level due to their very small body size (juvenile *M. agrestis* and *M. oeconomus* are morphologically almost identical).

Altogether 1482 feeding ticks were collected from these rodents at our three study sites, including 13 *D. reticulatus* (1 larva and 12 nymphs), 1042 *I. ricinus* (972 larvae and 70 nymphs) and 427 *H. concinna* (405 larvae and 22 nymphs). The *H. concinna* ticks were collected only at Nowy Młyn 2 site (Figs. 1 and 2). A representative larva and nymph, identified as *H. concinna,* are illustrated in Fig. 3.

Ixodes ricinus was the most common species of tick on rodents from the three sites (Table 1). Total prevalence of this species ranged only between 82 and 95%, mainly due to high infestation with *I. ricinus* larvae. Mean abundance was also similar between sites (NS). Juvenile *D. reticulatus* ticks were found only in two rodent communities in low numbers, resulting in an overall low prevalence and abundance (Table 1). Ticks identified as *H. concinna* were found at the Nowy Młyn 2 site and at this site constituted the second most numerous tick species: 427 *H. concinna* were collected from 39 rodents in comparison to

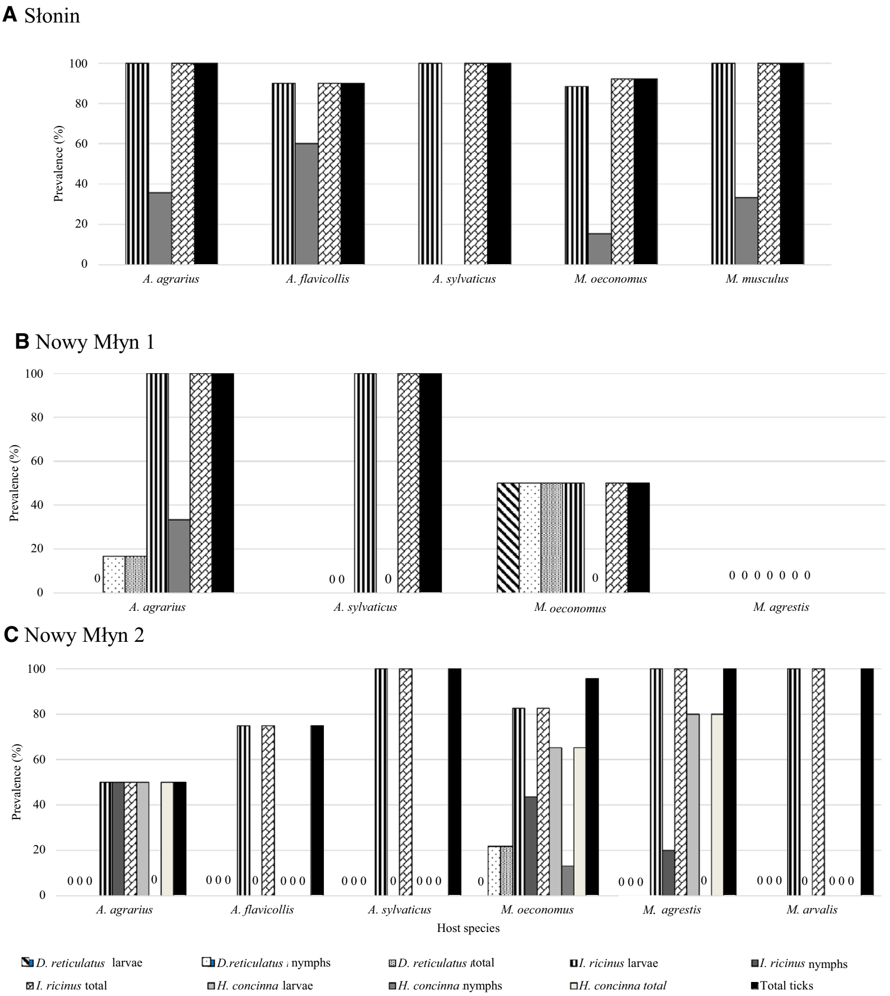


Fig. 2 Prevalence of three tick species (*Dermacentor reticulatus*, *Ixodes ricinus* and *Haemaphysalis concinna*) by site and host species (*Apodemus agrarius*, *A. flavicollis*, *A. sylvaticus*, *Microtus oeconomus*, *M. agrestis* and *Mus musculus*)

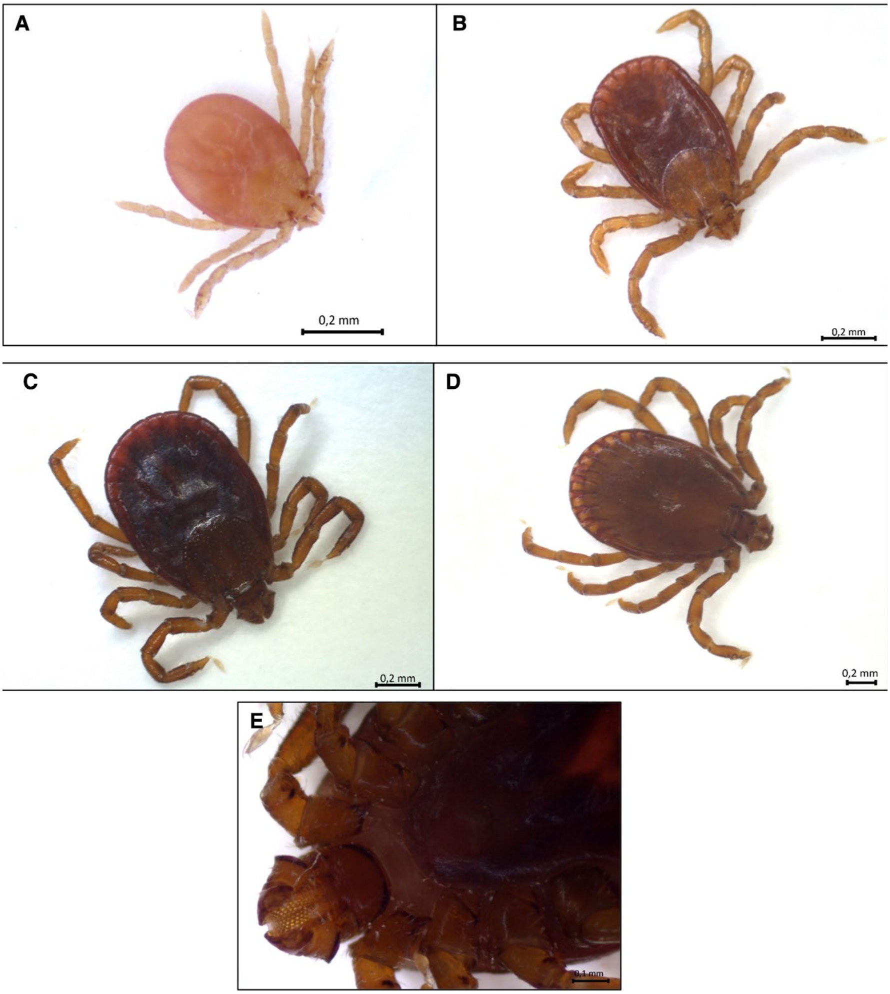


Fig. 3 Pictures of *Haemaphysalis concinna* larva (**A**), nymph (**B**), female (**C**), male (**D**) and hypostom (**E**)

443 *I. ricinus* and 10 *D. reticulatus*. Prevalence of infestation with *H. concinna* was above 50% at this site, and mean abundance exceeded 4 ticks/rodent, mainly due to the high number of *H. concinna* larvae.

Comparisons of the prevalence and abundance of the three tick species on the different species of rodents at individual sites are presented in Supplementary file 1 (Supplementary Tables 1–3). Prevalence of tick species, by host species and site, is presented in Fig. 2. Juvenile *I. ricinus* ticks were found on all inspected rodent species. Juvenile *D. reticulatus* ticks were found on *A. agrarius* and *M. oeconomus,* and *H. concinna* ticks were also found on *A. agrarius* and two vole species: *M. oeconomus* and *M. agrestis* (and on three unidentified juvenile *Microtus*) (Fig. 2). Both high prevalence (65–80%) and high mean abundance

Table 1 Comparison of the prevalence and mean (± SE) abundance of tick infestation (*Dermacentor reticulatus*, *Ixodes ricinus* and *Haemaphysalis concinna*) between trapping areas

Ticks species	Trapping area					
	Słonin, n = 56		Nowy Młyn, n = 11		Nowy Młyn 2, n = 39	
	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance
D. reticulatus larvae	0	0	9.1 (1)	0.24 ± 0.03	0	0
D. reticulatus nymphs	0	0	18.2 (2)	0.17 ± 0.19	12.8 (5)	0.06 ± 0.14
D. reticulatus total	0	0	18.2 (2)	0.29 ± 0.20	12.8 (5)	0.39 ± 0.49
I. ricinus larvae	92.9 (52)	12.09 ± 2.40	81.8 (9)	5.42 ± 4.72	79.5 (31)	8.76 ± 3.35
I. ricinus nymphs	28.6 (16)	5.33 ± 3.55	18.2 (2)	0.08 ± 0.70	30.8 (12)	0.39 ± 0.49
I. ricinus total	94.6 (53)	12.63 ± 2.71	81.8 (9)	5.50 ± 5.32	79.5 (31)	9.15 ± 3.77
H. concinna larvae	0	0	0	0	56.4 (22)	3.95 ± 3.88
H. concinna nymphs	0	0	0	0	7.7 (3)	0.14 ± 0.48
H. concinna total	0	0	0	0	56.4 (22)	4.09 ± 4.33
Total ticks	94.6 (53)	12.63 ± 5.48	81.8 (9)	5.79 ± 10.77	89.7 (35)	13.30 ± 7.62

Table 2 Prevalence and mean (± SE) abundance of tick infestation (*Dermacentor reticulatus*, *Ixodes ricinus* and *Haemaphysalis concinna*) by host species, site and season

	Trapping site									
	Słonin				Nowy Młyn		Nowy Młyn 2			
	June–July		August		June–July		June–July		August	
	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance
Apodemus agrarius										
D. reticulatus larvae	0	0	0	0	0	0	0	0	0	0
D. reticulatus nymphs	0	0	0	0	16.7	0.17 ± 0.14	0	0	0	0
D. reticulatus total	0	0	0	0	16.7	0.17 ± 0.15	0	0	0	0
I. ricinus larvae	100 (8)	6.00 ± 4.52	100 (6)	8.50 ± 5.22	100 (6)	13.17 ± 5.22	0	0	50 (1)	11.00 ± 9.04
I. ricinus nymphs	50 (4)	0.75 ± 0.67	16.7 (1)	0.17 ± 0.78	33.3 (2)	0.33 ± 0.78	0	0	50 (1)	1.00 ± 1.35
I. ricinus total	100 (8)	6.75 ± 5.10	100 (6)	8.67 ± 5.86	100 (6)	13.50 ± 5.86	0	0	50 (1)	12.00 ± 10.20
H. concinna larvae	0	0	0	0	0	0	0	0	50 (1)	0.50 ± 1042
H. concinna nymphs	0	0	0	0	0	0	0	0	0	0
H. concinna total	0	0	0	0	0	0	0	0	50 (1)	0.50 ± 11.66
Total ticks	100 (8)	6.75 ± 10.34	100 (6)	8.67 ± 11.92	100 (6)	13.67 ± 11.92	0	0	50 (1)	12.50 ± 20.65
Apodemus flavicollis										
D. reticulatus larvae	0	0	0	0	nd	–	0	0	0	0
D. reticulatus nymphs	0	0	0	0	nd	–	0	0	0	0

Table 2 (continued)

	Trapping site									
	Słonin				Nowy Młyn		Nowy Młyn 2			
	June–July		August		June–July		June–July		August	
	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance
D. reticulatus total	0	0	0	0	nd	–	0	0	0	0
I. ricinus larvae	88.9 (8)	17.22 ± 4.26	100 (1)	1.00 ± 12.79	nd	–	66.7 (2)	5.67 ± 7.38	100 (1)	14.00 ± 12.7
I. ricinus nymphs	66.7 (6)	1.78 ± 0.63	0	0	nd	–	0	0	0	0
I. ricinus total	88.9 (8)	19.00 ± 4.80	100 (1)	1.00 ± 14.42	nd	–	66.7 (2)	5.67 ± 8.32	100 (1)	14.00 ± 14.41
H. concinna larvae	0	0	0	0	nd	–	0	0	0	0
H. concinna nymphs	0	0	0	0	nd	–	0	0	0	0
H. concinna total	0	0	0	0	nd	–	0	0	0	0
Total ticks	88.9 (8)	19.00 ± 9.73	100 (1)	1.00 ± 29.2	nd	–	66.7 (2)	5.67 ± 16.86	100 (1)	14.00 ± 29.20
Apodemus sylvaticus										
D. reticulatus larvae	0	0	0	0	0	0	0	0	0	0
D. reticulatus nymphs	0	0	0	0	0	0	0	0	0	0
D. reticulatus total	0	0	0	0	0	0	0	0	0	0

Table 2 (continued)

	Trapping site									
	Słonin				Nowy Młyn		Nowy Młyn 2			
	June–July		August		June–July		June–July		August	
	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance
I. ricinus larvae	100 (3)	20.33 ± 7.38	0	0	100 (2)	8.00 ± 9.04	100 (1)	16.00 ± 12.79	0	0
I. ricinus nymphs	0	0	0	0	0	0	0	0	0	0
I. ricinus total	100 (3)	20.33 ± 8.32	0	0	100 (2)	8.00 ± 10.19	100 (1)	16.00 ± 16.41	0	0
H. concinna larvae	0	0	0	0	0	0	0	0	0	0
H. concinna nymphs	0	0	0	0	0	0	0	0	0	0
H. concinna total	0	0	0	0	0	0	0	0	0	0
Total ticks	100 (3)	20.33 ± 16.86	0	0	100 (2)	8.00 ± 20.65	100 (1)	16. ± 29.20	0	0
Microtus oeconomus										
D. reticulatus larvae	0	0	0	0	50 (1)	0.50 ± 0.50	0	0	0	0
D. reticulatus nymphs	0	0	0	0	50 (1)	0.5 ± 0.23	100 (5)	2.00 ± 0.15	0	0
D. reticulatus total	0	0	0	0	50 (1)	1.00 ± 0.25	100 (5)	2.00 ± 0.16	0	0
I. ricinus larvae	66.7 (2)	1.00 ± 7.38	91.3 (21)	4.91 ± 2.67	50 (1)	0.50 ± 9.04	60 (3)	2.40 ± 5.71	88.9 (16)	14.61 ± 3.01
I. ricinus nymphs	33.3 (1)	0.33 ± 1.10	13 (3)	0.28 ± 0.40	0	0	20 (1)	0.2 ± 0.85	50 (9)	1.89 ± 0.45
I. ricinus total	66.7 (2)	1.33 ± 8.32	95 (22)	5.13 ± 3.01	50 (1)	0.50 ± 10.19	60 (3)	2.60 ± 6.45	88.9 (16)	16.50 ± 3.40

Table 2 (continued)

	Trapping site									
	Słonin				Nowy Młyn		Nowy Młyn 2			
	June–July		August		June–July		June–July		August	
	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance
H. concinna larvae	0	0	0	0	0	0	0	0	83.3 (15)	19.56 ± 3.47
H. concinna nymphs	0	0	0	0	0	0	0	0	16.7 (3)	1.22 ± 0.45
H. concinna total	0	0	0	0	0	0	0	0	83.3 (15)	20.79±
Total ticks	66.7 (2)	1.33 ± 16.86	95.7 (22	5.13 ± 6.10	50 (1)	1.50 ± 20.65	100 (5)	4.6 ± 13.06	94.4 (17)	37.28 ± 6.88
Microtus agrestis										
D. reticulatus larvae	nd	–	nd	–	0	0	0	0	0	0
D. reticulatus nymphs	nd	–	nd	–	0	0	0	0	0	0
D. reticulatus total	nd	–	nd	–	0	0	0	0	0	0
I. ricinus total	nd	–	nd	–	0	0	0	0	100 (5)	9.80 ± 6.45
I. ricinus larvae	nd	–	nd	–	0	0	0	0	100 (5)	9.60 ± 5.72
I. ricinus nymphs	nd	–	nd	–	0	0	0	0	20 (1)	0.20 ± 0.85
H. concinna larvae	nd	–	nd	–	0	0	0	0	80 (4)	8.20 ± 6.59
H. concinna nymphs	nd	–	nd	–	0	0	0	0	0	0

Table 2 (continued)

	Trapping site									
	Słonin				Nowy Młyn		Nowy Młyn 2			
	June–July		August		June–July		June–July		August	
	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance
H. concinna total	nd	–	nd	–	0	0	0	0	80 (4)	8.20 ± 7.38
Total ticks	nd	–	nd	–	0	0	0	0	100 (5)	18.00 ± 13.06
Microtus arvalis										
D. reticulatus larvae	nd	–	nd	–	nd	–	0	0	0	0
D. reticulatus nymphs	nd	–	nd	–	nd	–	0	0	0	0
D. reticulatus total	nd	–	nd	–	nd	–	0	0	0	0
I. ricinus larvae	nd	–	nd	–	nd	–	0	0	100 (1)	1.00 ± 12.79
I. ricinus nymphs	nd	–	nd	–	nd	–	0	0	0	0
I. ricinus total	nd	–	nd	–	nd	–	0	0	100 (1)	1.00 +/14.41
H. concinna larvae	nd	–	nd	–	nd	–	0	0	0	0
H. concinna nymphs	nd	–	nd	–	nd	–	0	0	0	0
H. concinna total	nd	–	nd	–	nd	–	0	0	0	0
Total ticks	nd	–	nd	–	nd	–	0	0	100 (1)	1.00 ± 29.20

Table 2 (continued)

	Trapping site									
	Słonin				Nowy Młyn		Nowy Młyn 2			
	June–July		August		June–July		June–July		August	
	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance
Microtus sp.										
D. reticulatus larvae	nd	–	nd	–	nd	–	0	0	0	0
D. reticulatus nymphs	nd	–	nd	–	nd	–	0	0	0	0
D. reticulatus total	nd	–	nd	–	nd	–	0	0	0	0
I. ricinus larvae	nd	–	nd	–	nd	–	0	0	33.3 (1)	4.00 ± 7.38
I. ricinus nymphs	nd	–	nd	–	nd	–	0	0	0	0
I. ricinus total	nd	–	nd	–	nd	–	0	0	33.3 (1)	4.00 ± 8.32
H. concinna larvae	nd	–	nd	–	nd	–	0	0	66.7 (2)	3.67 ± 8.51
H. concinna nymphs	nd	–	nd	–	nd	–	0	0	0	0
H. concinna total	nd	–	nd	–	nd	–	0	0	66.7 (2)	3.67 ± 9.52
Total ticks	nd	–	nd	–	nd	–	0	0	66.7 (2)	7.67 ± 16.86
Mus musculus										
D. reticulatus larvae	0	0	0	0	nd	–	nd	–	nd	–

Table 2 (continued)

	Trapping site									
	Słonin				Nowy Młyn		Nowy Młyn 2			
	June–July		August		June–July		June–July		August	
	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance	Prev. % (no. of infested ind.)	Mean abundance
D. reticulatus nymphs	0	0	0	0	nd	–	nd	–	nd	–
D. reticulatus total	0	0	0	0	nd	–	nd	–	nd	–
I. ricinus larvae	100 (3)	13.00 ± 7.38	0	0	nd	–	nd	–	nd	–
I. ricinus nymphs	33.3 (1)	0.33 ± 1.10	0	0	nd	–	nd	–	nd	–
I. ricinus total	100	13.33 ± 8.32	0	0	nd	–	nd	–	nd	–
H. concinna larvae	0	0	0	0	nd	–	nd	–	nd	–
H. concinna nymphs	0	0	0	0	nd	–	nd	–	nd	–
H. concinna total	0	0	0	0	nd	–	nd	–	nd	–
Total ticks	100 (3)	13.33 ± 16.86	0	0	nd	–	nd	–	nd	–

(4–16 ticks/host) of *H. concinna* infestation were noted on *Microtus* voles from Nowy Młyn 2 (Supplementary File 1: Supplementary Table 3). Interestingly, no *H. concinna* ticks were found on rodents in early summer (June–July) but both larvae and nymphs appeared on rodents in August (Table 2). At that time both prevalence and abundance of *H. concinna* were highest on *M. agrestis* and *M. oeconomus*, exceeding 83% and 21 ticks/host for the latter species (Table 2).

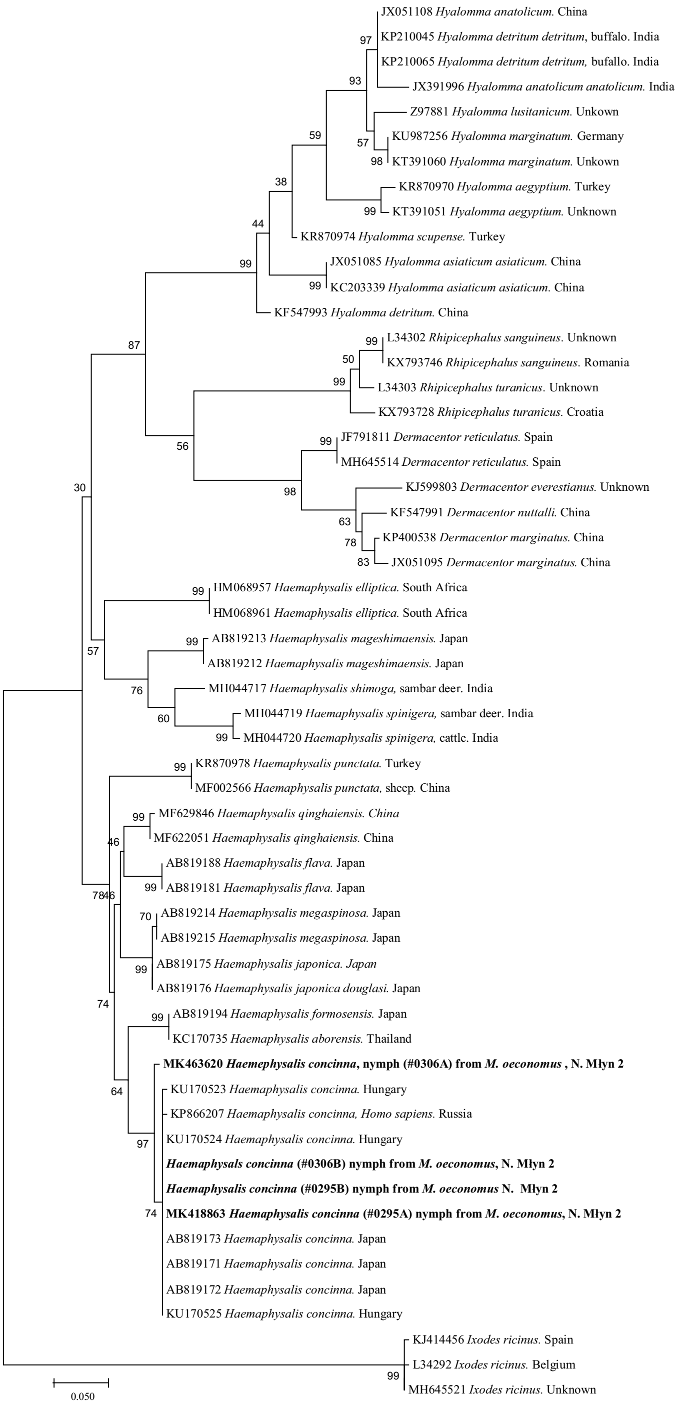
Questing ticks

In August 2018, 20 questing ticks were collected in Nowy Młyn 2 from two researchers and their accompanying dog, to whom they had attached while checking traps over a 300 m long transect. All ticks were morphologically identified as *H. concinna*, including 3 *H. concinna* females, one male and 16 nymphs. Representative female and male of *H. concinna* are presented in Fig. 3. The mean density of *H. concinna* in this location was estimated at 3.33 ticks/100 m^2.

Genotyping and phylogenetic analysis

Four mt 16S rDNA sequences were obtained from 4 nymphs morphologically identified as *H. concinna.* Three consensus sequences showed the highest similarity (404/405 = 99.75%) to a *H. concinna* sequence from Japan (GenBank: AB819173) and 99.51% (404/406) to a *H. concinna* sequence from Hungary (GenBank: KU170525). This first identified genotype has been deposited in the GenBank database under accession number MK418863. Fourth sequence (deposited with accession number MK463620) differed by five positions (one insertion, four A–T substitutions) from the three former sequences, representing second genotype, and showed the highest similarity—98.76% (399/404) to a sequence from Japan (Gen Bank: AB819171).

A phylogenetic tree incorporating sequences obtained in this study is presented in Fig. 4. All sequences grouped with *H. concinna* from Europe and Japan.


JX051108 Hyalomma anatolicum. China
KP210045 Hyalomma detritum detritum, buffalo. India
KP210065 Hyalomma detritum detritum, bufallo. India
JX391996 Hyalomma anatolicum anatolicum. India
Z97881 Hyalomma lusitanicum. Unkown
KU987256 Hyalomma marginatum. Germany
KT391060 Hyalomma marginatum. Unkown
KR870970 Hyalomma aegyptium. Turkey
KT391051 Hyalomma aegyptium. Unknown
KR870974 Hyalomma scupense. Turkey
JX051085 Hyalomma asiaticum asiaticum. China
KC203339 Hyalomma asiaticum asiaticum. China
KF547993 Hyalomma detritum. China
L34302 Rhipicephalus sanguineus. Unknown
KX793746 Rhipicephalus sanguineus. Romania
L34303 Rhipicephalus turanicus. Unknown
KX793728 Rhipicephalus turanicus. Croatia
JF791811 Dermacentor reticulatus. Spain
MH645514 Dermacentor reticulatus. Spain
KJ599803 Dermacentor everestianus. Unknown
KF547991 Dermacentor nuttalli. China
KP400538 Dermacentor marginatus. China
JX051095 Dermacentor marginatus. China
HM068957 Haemaphysalis elliptica. South Africa
HM068961 Haemaphysalis elliptica. South Africa
AB819213 Haemaphysalis mageshimaensis. Japan
AB819212 Haemaphysalis mageshimaensis. Japan
MH044717 Haemaphysalis shimoga, sambar deer. India
MH044719 Haemaphysalis spinigera, sambar deer. India
MH044720 Haemaphysalis spinigera, cattle. India
KR870978 Haemaphysalis punctata. Turkey
MF002566 Haemaphysalis punctata, sheep. China
MF629846 Haemaphysalis qinghaiensis. China
MF622051 Haemaphysalis qinghaiensis. China
AB819188 Haemaphysalis flava. Japan
AB819181 Haemaphysalis flava. Japan
AB819214 Haemaphysalis megaspinosa. Japan
AB819215 Haemaphysalis megaspinosa. Japan
AB819175 Haemaphysalis japonica. Japan
AB819176 Haemaphysalis japonica douglasi. Japan
AB819194 Haemaphysalis formosensis. Japan
KC170735 Haemaphysalis aborensis. Thailand
MK463620 Haemephysalis concinna, nymph (#0306A) from M. oeconomus , N. Mlyn 2
KU170523 Haemaphysalis concinna. Hungary
KP866207 Haemaphysalis concinna, Homo sapiens. Russia
KU170524 Haemaphysalis concinna. Hungary
Haemaphysals concinna (#0306B) nymph from M. oeconomus, N. Mlyn 2
Haemaphysalis concinna (#0295B) nymph from M. oeconomus N. Mlyn 2
MK418863 Haemaphysalis concinna (#0295A) nymph from M. oeconomus, N. Mlyn 2
AB819173 Haemaphysalis concinna. Japan
AB819171 Haemaphysalis concinna. Japan
AB819172 Haemaphysalis concinna. Japan
KU170525 Haemaphysalis concinna. Hungary
KJ414456 Ixodes ricinus. Spain
L34292 Ixodes ricinus. Belgium
MH645521 Ixodes ricinus. Unknown
0.050

◂ **Fig. 4** Molecular phylogenetic analysis of Ixodidae by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar 2000). The tree with the highest log likelihood (− 2079.4170) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+ G, parameter = 0.3748)). The rate variation model allowed for some sites to be evolutionarily invariable ([+ I], 20.7364% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 56 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 286 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016)

Discussion

A new well-established focus of *H. concinna* has been identified in Western Poland both through the collection of questing ticks from vegetation and feeding ticks from several rodent hosts. The identity of this tick species was confirmed by morphological criteria and by amplification and sequencing of mt 16S rDNA.

In comparison to the dynamic changes in distribution of many arthropod vectors, including ticks, the finding of one new focus of *H. concinna* 65 years after the first identification of this species in Western Poland may suggest a low overall ability of this tick to expand locally and to establish in a new territory. Looking at the distances involved, the new site is located about 100 km from the German-Polish border, where at least one known focus is present (Hagedorn 2013; Rubel et al. 2018). It is also located 200 km to the south of a previously described focus in Troszyn in NW Poland. There is no evidence that these sites could have served as the source of this newly recognized *H. concinna* population. Interestingly, the distribution of this tick species is very focal and rather restricted: no ticks of this species were found on rodents trapped 1 km south from the new focus at Nowy Młyn 1, nor 50 km east in Słonin. Accordingly, no questing *H. concinna* ticks were found during our previous monitoring surveys of *D. reticulatus* expansion in Central and Western Poland in 2012–2014 (Mierzejewska et al. 2016), nor during the present monitoring in 2016–2018 (Dwużnik, unpublished), when in this period a high number of sites (> 100) were regularly visited and sampled. Interestingly, in support of the rare occurrence of *H. concinna* in Poland, no ticks of this species were found on dogs from Central (Zygner and Wedrychowicz 2006; Mierzejewska et al. 2015), South (Kilar 2011) or Western Poland (Król et al. 2016a, b). Also in our long-term studies on rodent parasites in the Mazury Lake District (Bajer 2008; Bajer et al. 2001, 2002, 2005, 2014; Behnke et al. 2001, 2008a, b; Grzybek et al. 2015; Tołkacz et al. 2017), where the number of studied rodents is currently in excess of 4000, yet to-date no *H. concinna* have been found (Pawełczyk and Siński 2004; Welc-Falęciak et al. 2008; Paziewska et al. 2010).

Such a limited distribution of *H. concinna* in Poland is surprising, given that this tick species is known to be capable of inhabiting different habitats and climatic zones (Rubel et al. 2018) and is widespread in Germany (Hagedorn 2013) and Austria (where it is the dominant tick species on dogs in summer months; Duscher et al. 2013).

As *H. concinna* is a proven or suggested vector of a wide range of pathogens, including tick-borne encephalitis, *Anaplasma phagocytophilum* and *Rickettsia* spp. (reviewed in Rubel et al. 2018), the finding of this new focus in Poland is of considerable local concern. This tick species may feed on both human and domestic animal hosts, as found also in our study although ticks had been removed before they started feeding. Our results are therefore of public health relevance, contributing to the understanding of the risk of tick-borne diseases in regions where this tick species is endemic, and we emphasize the need for increased awareness of its presence and continued monitoring for potential expansion of its range.

Conclusions

In the present study we report a new focus of *H. concinna* in Western Poland, identified both by the collection of questing ticks from vegetation and feeding ticks from several rodent hosts. Moreover, we confirm that this is still a rare and uncommon species in other regions of Poland.

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References

Asman M, Nowak-Chmura M, Solarz K, Szilman E, Semla M, Zyśk B (2017) *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi* sensu lato, and *Toxoplasma gondii* in *Ixodes ricinus* (Acari, Ixodidae) ticks collected from Słowiński National Park (Northern Poland). J Vector Ecol 42:200–202. https://doi.org/10.1111/jvec.12258

Bajer A (2008) Between-year variation and spatial dynamics of *Cryptosporidium* spp. and *Giardia* spp. infections in naturally infected rodent populations. Parasitology 135:1629–1649

Bajer A, Pawełczyk A, Behnke JM, Gilbert FS, Siński E (2001) Factors affecting the component community structure of haemoparasites in bank voles (*Clethrionomys glareolus*) from the Mazury Lake District region of Poland. Parasitology 122:43–54

Bajer A, Bednarska M, Pawełczyk A, Behnke JM, Gilbert FS, Siński E (2002) Prevalence and abundance of *Cryptosporidium parvum* and *Giardia* spp. in wild rural rodents from the Mazury Lake District region of Poland. Parasitology 125:21–34

Bajer A, Behnke JM, Pawełczyk A, Kuliś K, Sereda MJ, Siński E (2005) Medium-term temporal stability of the helminth component community structure in bank voles (*Clethrionomys glareolus*) from the Mazury Lake District region of Poland. Parasitology 130:213–228

Bajer A, Welc-Faleçiak R, Bednarska M, Alsarraf M, Behnke-Borowczyk J, Siński E, Behnke JM (2014) Long-term spatiotemporal stability and dynamic changes in the haemoparasite community of bank voles (*Myodes glareolus*) in NE Poland. Microb Ecol 68:196–211

Behnke JM, Barnard CJ, Bajer A, Bray D, Dinmore J, Frake K, Osmond J, Race T, Siński E (2001) Variation in the helminth community structure in bank voles (*Clethrionomys glareolus*) from three comparable localities in the Mazury Lake District region of Poland. Parasitology 123:401–414

Behnke JM, Bajer A, Harris PD, Newington L, Pidgeon E, Rowlands G, Sheriff C, Kuliś K, Siński E, Gilbert FS, Barnard CJ (2008a) Temporal and between-site variation in helminth communities of bank voles (*Myodes glareolus*) from NE Poland. 1. Regional fauna and component community levels. Parasitology 135:985–997

Behnke JM, Bajer A, Harris PD, Newington L, Pidgeon E, Rowlands G, Sheriff C, Kuliś-Malkowska K, Siński E, Gilbert FS, Barnard CJ (2008b) Temporal and between-site variation in helminth communities of bank voles (*Myodes glareolus*) from NE Poland. 2. The infracommunity level. Parasitology 135:999–1018

Duscher GG, Feiler A, Leschnik M, Joachim A (2013) Seasonal and spatial distribution of ixodid tick species feeding on naturally infested dogs from Eastern Austria and the influence of acaricides/repellents on these parameters. Parasit Vectors 6:76. https://doi.org/10.1186/1756-3305-6-76
Estrada-Peña A, Bouattour A, Camicas J-L, Walker AR (2004) Ticks of domestic animals in the Mediterranean region: a guide to identification of species. University of Zaragoza, Saragossa
Földvári G, Široký P, Szekeres S, Majoros G, Sprong H (2016) *Dermacentor reticulatus*: a vector on the rise. Parasit Vectors 9:314
Grzybek M, Bajer A, Bednarska M, Alsarraf M, Behnke-Borowczyk J, Harris P, Price SJ, Brown GS, Osborne S-J, Siński E, Behnke JM (2015) Long-term spatiotemporal stability and dynamic changes in helminth infracommunities of bank voles (*Myodes glareolus*) in NE Poland. Parasitology 142:1722–1743
Hagedorn P (2013) Untersuchung von Zeckenals Marker für die GefährdungDurch von Ihnen Übertragene Krankheiten. Dissertation
Karbowiak G (2009) Kleszcz łąkowy *Dermacentor reticulatus*- występowanie, biologa i rola jako wektora chorób odkleszczowych. Habilitation thesis. Ed. Instytut Parazytologii im. Witolda Stefańskiego PAN, Warsaw (in Polish)
Karbowiak G (2014) The occurrence of the *Dermacentor reticulatus* tick—its expansion to new areas and possible causes. Ann Parasitol 60:37–47
Kiewra D, Stańczak J, Richter M (2014) *Ixodesricinus* ticks (*Acari*, Ixodidae) as a vector of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* in Lower Silesia, Poland—preliminary study. Ticks Tick Borne Dis 5:892–897. https://doi.org/10.1016/j.ttbdis.2014.07.004
Kiewra D, Czułowska A, Lonc E (2016) Winter activity of *Dermacentor reticulatus* (Fabricius, 1794) in the newly emerging population of Lower Silesia, south-west Poland. Ticks Tick Borne Dis 7:1124–1127. https://doi.org/10.1016/j.ttbdis.2016.08.012
Kilar P (2011) Ticks attacking domestic dogs in the area of the Rymanów district, Subcarpathian province, Poland. Wiad Parazytol 57:189–191
Król N, Kiewra D, Lonc E, Janaczyk B, Chodorowska-Skubiszewska A, Dzięcioł M, Gola M, Gruszka R, Jackowska-Szlachcic E, Jagiełło M, Kawski S, Łukaszewski Z, Mizgalski P, Narajowska T, Niedzielska J, Noczyński M, Rudkowska M, Rzepka D, Samulska K, Senze M, Sieczko P, Silny A, Staniewska A, Stańczyk J, Stańczyk W, Stasiak M, Włodarczyk M, Zimniak S (2016a) *Dermacentor reticulatus* (Fabricius, 1794) and *Babesia canis* (Piana et Galli-Valerio, 1895) as the parasites of companion animals (dogs and cats) in the Wrocław area, south-western Poland. Ann Parasitol 62:125–130. https://doi.org/10.17420/ap6202.44
Król N, Obiegala A, Pfeffer M, Lonc E, Kiewra D (2016b) Detection of selected pathogens in ticks collected from cats and dogs in the Wrocław Agglomeration, South-West Poland. Parasit Vectors 9:351. https://doi.org/10.1186/s13071-016-1632-0
Kulakova NV, Khasnatinov MA, Sidorova EA, Adel'shin RV, Belikov SI (2014) Molecular identification and phylogeny of *Dermacentor nuttalli* (*Acari*: Ixodidae). Parasitol Res 113:1787–1793
Kumar S, Stecher G, Tamura K (2016) MEGA 7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
Lachmajer J, Skierska B, Wegner Z (1956) Kleszcze rodzaju *Haemaphysalis* Koch (Ixodidae) znalezione na terenie Polski. Biul Państw Inst Med Morsk Trop J W Gdańsku 7:189–195
Medlock JM, Hansford KM, Bormane A, Derdakova M, Estrada-Peña A, George JC, Golovljova I, Jaenson TG, Jensen JK, Jensen PM, Kazimirova M, Oteo JA, Papa A, Pfister K, Plantard O, Randolph SE, Rizzoli A, Santos-Silva MM, Sprong H, Vial L, Hendrickx G, Zeller H, Van Bortel W (2013) Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasit Vectors 6:1
Mierzejewska EJ, Welc-Falęciak R, Karbowiak G, Kowalec M, Behnke JM, Bajer A (2015) Dominance of *Dermacentor reticulatus* over *Ixodes ricinus* (Ixodidae) on livestock, companion animals and wild ruminants in Poland. Exp Appl Acarol 66:83–101
Mierzejewska EJ, Estrada- Peña A, Alsarraf A, Kowalec M, Bajer A (2016) Mapping of tick *Dermacentor reticulatus* expansion in Poland in 2012-2014. Ticks Tick Borne Dis 7:94–106
Mierzejewska EJ, Estrada-Peña A, Bajer A (2017) Spread of *Dermacentor reticulatus* is associated with the loss of forest area. Exp Appl Acarol 72:399–413. https://doi.org/10.1007/s10493-017-0160-8
Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, New York
Nowak-Chmura M, Siuda K (2012) Ticks of Poland. Review of contemporary issues and latest research. Ann Parasitol 58:125–155
Pawełczyk A, Siński E (2004) Prevalence of *Ixodes ricinus* infection with *Borrelia burgdorferi s.l.*: seasonal and annual variations. Wiad Parazytol 50:253–258 **(in Polish)**

Paziewska A, Zwolińska L, Harris PD, Bajer A, Siński E (2010) Utilisation of rodent species by larvae and nymphs of hard ticks (Ixodidae) in two habitats in NE Poland. Exp Appl Acarol 50:79–91
Rubel F, Brugger K, Pfeffer M, Chitimia-Dobler L, Didyk YM, Leverenz S, Dautel H, Kahl O (2016) Geographical distribution of *Dermacentor marginatus* and *Dermacentor reticulatus* in Europe. Ticks Tick Borne Dis 7:224–233
Rubel F, Brugger K, Walter M, Vogelgesang JR, Didyk YM, Fu S, Kahl O (2018) Geographical distribution, climate adaptation and vector competence of the Eurasian hard tick *Haemaphysalis concinna*. Ticks Tick Borne Dis 9:1080–1089. https://doi.org/10.1016/j.ttbdis.2018.04.002
Sréter T, Széll Z, Varga I (2005) Spatial distribution of *Dermacentor reticulatus* and *Ixodes ricinus* in Hungary: evidence for change? Vet Parasitol 128:347–351
Stańczak J, Cieniuch S, Lass A, Biernat B, Racewicz M (2015) Detection and quantification of *Anaplasma phagocytophilum* and *Babesia* spp. in *Ixodes ricinus* ticks from urban and rural environment, northern Poland, by real-time polymerase chain reaction. Exp Appl Acarol 66:63–81. https://doi.org/10.1007/s10493-015-9887-2
Stańczak J, Biernat B, Racewicz M, Zalewska M, Matyjasek A (2018) Prevalence of different *Rickettsia* spp. in *Ixodesricinus* and *Dermacentor reticulatus* ticks (*Acari*: Ixodidae) in north-eastern Poland. Ticks Tick Borne Dis 9:427–434. https://doi.org/10.1016/j.ttbdis.2017.12.010
Tołkacz K, Bednarska M, Alsarraf M, Dwużnik D, Grzybek M, Welc-Faleciak R, Behnke JM, Bajer A (2017) Prevalence, genetic identity and vertical transmission of *Babesia microti* in three naturally infected species of vole, *Microtus* spp. (Cricetidae). Parasit Vectors 10:66. https://doi.org/10.1186/s13071-017-2007-x
Welc-Falęciak R, Bajer A, Behnke JM, Siński E (2008) Effects of host diversity and the community composition of ixodid ticks (Ixodidae) on *Babesia microti* infection. Int J Med Microbiol 298:235–242. https://doi.org/10.1016/j.ijmm.2007.12.002
Wodecka B, Rymaszewska A, Skotarczak B (2014) Host and pathogen DNA identification in blood meals of nymphal *Ixodes ricinus* ticks from forest parks and rural forests of Poland. Exp Appl Acarol 62:543–555. https://doi.org/10.1007/s10493-013-9763-x
Wójcik-Fatla A, Zając V, Sawczyn A, Sroka J, Cisak E, Dutkiewicz J (2016) Infections and mixed infections with the selected species of *Borrelia burgdorferi* sensu lato complex in *Ixodes ricinus* ticks collected in eastern Poland: a significant increase in the course of 5 years. Exp Appl Acarol 68:197–212. https://doi.org/10.1007/s10493-015-9990-4
Zając V, Wójcik-Fatla A, Sawczyn A, Cisak E, Sroka J, Kloc A, Zając Z, Buczek A, Dutkiewicz J, Bartosik K (2017) Prevalence of infections and co-infections with 6 pathogens in *Dermacentor reticulatus* ticks collected in eastern Poland. Ann Agric Environ Med 24:26–32. https://doi.org/10.5604/12321966.1233893
Zygner W, Wedrychowicz H (2006) Occurrence of hard ticks in dogs from Warsaw area. Ann Agric Environ Med 13:355–359