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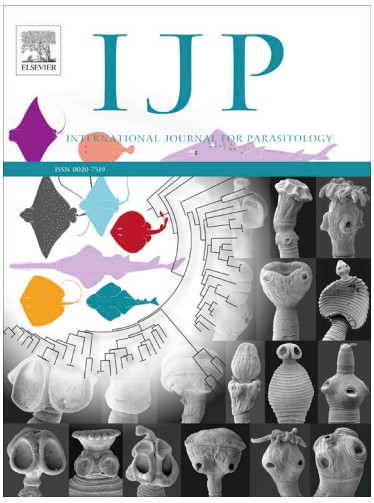

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Dermacentor reticulatus - a tick on its way from glacial refugia to a panmictic Eurasian population

Branka Bilbija[a], Cäcilia Spitzweg[b], Ivo Papoušek[a], Uwe Fritz[b], Gábor Földvári[c,d], Martin Mullett[e], Flora Ihlow[b], Hein Sprong[f], Kristína Civáňová Křížová[g], Nikolay Anisimov[h], Oxana A. Belova[i], Sarah I. Bonnet[j,k], Elizabeth Bychkova[l], Aleksandra Czułowska[m], Georg G. Duscher[n,o,], Manoj Fonville[f], Olaf Kahl[p], Grzegorz Karbowiak[q], Ivan S. Kholodilov[i], Dorota Kiewra[m], Stjepan Krčmar[r], Gulzina Kumisbek[s], Natalya Livanova[t], Igor Majláth[u], Maria Teresa Manfredi[v], Andrei D. Mihalca[w], Guadalupe Miró[x], Sara Moutailler[y], Igor V. Nebogatkin[z,aa], Snežana Tomanović[bb], Zati Vatansever[cc], Marya Yakovich[l], Sergio Zanzani[v], Pavel Široký[a,dd,*]

[a]*Department of Biology and Wildlife Diseases, FVHE, University of Veterinary Sciences Brno, Palackého 1946/1, 61242 Brno, Czech Republic*

[b]*Museum of Zoology, Senckenberg Dresden, A. B. Meyer Building, 01109 Dresden, Germany*

[c]*Institute of Evolution, Centre for Ecological Research, 1121 Budapest, Konkoly-Thege Miklós út 29-33, Hungary*

[d]*Centre for Eco-Epidemiology, National Laboratory for Health Security, 1121 Budapest, Konkoly-Thege Miklós út 29-33, Hungary*

[e]*Phytophthora Research Centre, Faculty of Forestry and Wood Technology, Department of Forest Protection and Wildlife Management, Mendel University in Brno, Zemědělská 3, 61300 Brno, Czech Republic*

[f]*National Institute of Public Health and Environment (RIVM), Centre for Infectious Disease Control (CIb), Laboratory for Zoonoses and Environmental Microbiology (Z&O), Mailbox 63, room V353, Antonie van Leeuwenhoeklaan 9, P.O. Box 1, 3720 BA Bilthoven, The Netherlands*

[g]*Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*

[h]*Institute of Environmental and Agricultural Biology (X-BIO), University of Tyumen, Volodarskogo 6, 625003 Tyumen, Russia*

[i]*FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis) prem. 8, k.17, pos. Institut Poliomyelita, poselenie Moskovskiy, 108819 Moscow, Russia*

[j]*Functional Genetics of Infectious Diseases Unit, Institut Pasteur, CNRS UMR 2000, Université de Paris, 75015 Paris, France*

[k]*Animal Health Department, INRAE, 37380 Nouzilly, France*

[l]*Laboratory of Parasitology, State Scientific and Production Association "Scientific and Practical Center of the National Academy of Sciences of Belarus on Bioresources", 27, Akademicheskaya Str., 220072 Minsk, Republic of Belarus*

[m]*Department of Microbial Ecology and Acaroentomology, Faculty of Biological Sciences, University of Wroclaw, Przybyszewskiego str. 63, 51-148 Wroclaw, Poland*

[n]*Department of Pathobiology, Institute of Parasitology, University of Veterinary Medicine Vienna, Vienna, Austria*

[o]*AGES-Austrian Agency for Health and Food Safety, Spargelfeldstrasse 191, Vienna, 1220, Austria*

[p]*tick-radar GmbH, 10555 Berlin, Germany*

[q]*Witold Stefański Institute of Parasitology of Polish Academy of Sciences, Twarda street 51/55, 00-818 Warsaw, Poland*

[r]*Department of Biology, Josip Juraj Strossmayer University of Osijek, Cara Hadrijana 8/A, HR-31000 Osijek, Croatia*

[s]*Asfendiyarov Kazakh National Medical University, School of Pharmacy, Department of Engineering disciplines, Tole Bi, 94, Almaty, Kazakhstan*

[t]*Institute of Systematics and Ecology of Animals, Frunze str. 11, Novosibirsk, 630091, Russia*

[u]*Pavol Jozef Safarik University in Kosice, Faculty of Science, Institute of Biology and Ecology, Department of Animal Physiology, Srobarova 2, 041 54 Kosice, Slovakia*

[v]*Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, via dell'Università 6, 26900 Lodi, Italy*

[w]*Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Manastur 3-5, Cluj-Napoca 400372, Romania*

[x]*Animal Health Dept. Veterinary School, Universidad Complutense de Madrid, Spain*

[y]*Anses, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, F-94700, France*

[z]*I.I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Bogdana Khmelnytskovo 15, 01030 Kyiv, Ukraine*

[aa]*Public Health Center of the Ministry of Health of Ukraine, Kyiv, Ukraine*

[bb]*University of Belgrade, Institute for Medical Research, National Institute of Republic of Serbia, Dr. Subotića 4, Belgrade, Serbia*

[cc]*Kafkas University, Faculty of Veterinary Medicine, Dept. of Parasitology, Kars, Turkey*

[dd]*CEITEC-Central European Institute of Technology, University of Veterinary Sciences Brno, Palackého 1946/1, 612 42 Brno, Czech Republic*

*****Corresponding author. Pavel Široký, Department of Biology and Wildlife Diseases, FVHE, University of Veterinary Sciences Brno, Palackého 1946/1, 61242 Brno, Czech Republic.

Tel.: +420 541 562 637. *E-mail address*: sirokyp@vfu.cz

This study is dedicated to the late Maria Margarida Santos Silva.

Note: Supplementary files associated with this article.

Abstract:

The ornate dog tick (*Dermacentor reticulatus*) shows a recently expanding geographic distribution. Knowledge on its intraspecific variability, population structure, rate of genetic diversity and divergence, including its evolution and geographic distribution, is crucial to understand its dispersal capacity. All such information would help to evaluate the potential risk of future spread of associated pathogens of medical and veterinary concern. A set of 865 *D. reticulatus* ticks was collected from 65 localities across 21 countries, from Portugal in the west to Kazakhstan and southern Russia in the east. Cluster analyses of 16 microsatellite loci were combined with nuclear (ITS2, 18S) and mitochondrial (12S, 16S, COI) sequence data to uncover the ticks' population structures and geographical patterns. Approximate Bayesian computation was applied to model evolutionary relationships among the found clusters. Low variability and a weak phylogenetic signal showing an east-west cline were detected both for mitochondrial and nuclear sequence markers. Microsatellite analyses revealed three genetic clusters, where the eastern and western cluster gradient was supplemented by a third, northern cluster. Alternative scenarios could explain such a tripartite population structure by independent formation of clusters in separate refugia, limited gene flow connected with isolation by distance causing a "bipolar pattern", and the northern cluster deriving from admixture between the eastern and western populations. The best supported demographic scenario of this tick species indicates that the northern cluster derived from admixture between the eastern and western populations 441 (median) to 224 (mode) generations ago, suggesting a possible link with the end of the Little Ice Age in Europe.

Keywords: Divergence, Ixodida, Glacial refugia, Multigene sequence analysis, Microsatellites, Palaearctic, Vectors

1. Introduction

Ticks of the genus *Dermacentor* are virtually cosmopolitan and widespread in the northern hemisphere (Guglielmone et al., 2010). After *Ixodes* spp., the genus *Dermacentor* is the second most abundant ixodid taxon in the western Palearctic region (Guglielmone et al., 2014; Rubel et al., 2016). Two species are endemic to Europe, *Dermacentor marginatus* (Sulzer, 1776) and *Dermacentor reticulatus* (Fabricius, 1794). The latter is distributed from northern Portugal, Spain, and southern Great Britain in the west, through eastern Europe, the Caucasus, and southern Siberia to central Asia and China in the east (Filipova, 1997; Földvári et al., 2016; Rubel et al., 2016, 2020; Zhao et al., 2021).

During the last few decades, the distribution of *D. reticulatus* has substantially expanded in north-western and central Europe into areas which were previously considered unsuitable for its survival and completion of its life-cycle (Kolonin, 2009; Földvári et al. 2016; Rubel et al., 2016; Zając et al., 2020). Climate oscillation, landscape and agricultural development, the distribution of host populations, and human-mediated introductions could have facilitated the spread of this species (Matjila et al., 2005; Karbowiak, 2014, 2021; Araya-Anchetta et al., 2015; Cunze et al., 2022). Also, its tolerance to starvation, low mortality, survival under water, and its high reproduction rate (review in Földvári et al., 2016) possibly contribute to the successful establishment of *D. reticulatus* populations across many European countries. *Dermacentor reticulatus* is a three-host tick with a wide host spectrum. Larvae and nymphs feed on small mammals, while adults prefer medium-sized and large mammals, occasionally including humans. Preimaginal stages were also recorded from birds (Guglielmone et al., 2014; Pfäffle et al., 2015; Földvári et al., 2016). Larvae and nymphs are endophilic; they live in or near shelters of their hosts, and are usually not found questing on vegetation, while adults have an exophilic lifestyle (Kahl and Dautel, 2013; Pfäffle et al., 2015; Földvári et al., 2016).

Because the species is a proven vector for many pathogens of human and veterinary health importance, the spread of *D. reticulatus* is monitored by the European Centre for Disease Prevention and Control (ECDC: https://www.ecdc.europa.eu/en/publications-data/dermacentor-reticulatus-current-known-distribution-september-2021). *Dermacentor reticulatus* has been reported as a carrier and vector for a wide range of viruses, bacteria, and protozoans (Rubel et al., 2016), suggesting the species represents a threat for livestock health and production, and public health, as well as for wild host species (Jongejan and Uilenberg, 2004; McCoy, 2008). Therefore, it is important to obtain insights into the species' population

structure and its evolution to understand its dispersal capacity, and to evaluate the potential risk of associated pathogen spread. Newly populated areas, previously free of the presence of this tick, are now exposed to new pathogens and an increased disease risk.

Araya-Anchetta et al. (2015) stated that there is no ideal universal approach for population genetic studies, however, microsatellite markers are known to be a powerful, efficient, and economic tool (Barbará et al., 2007). Indeed, microsatellite markers have been successfully applied in studies on tick phylogeny, population structure, variability, and taxonomy, together with nuclear (18S, 28S, ITS2) and mitochondrial (12S, 16S) DNA sequences (Zahler et al., 1995; Mangold et al., 1998; Dobson and Barker, 1999; Fukunaga et al., 2000; Klompen et al., 2000; Murrell et al., 2001; Nava et al., 2009; Rumer et al., 2011; Bogdanov et al., 2017; Amzati et al., 2018). For *D. reticulatus*, previous molecular genetic studies were based on 5.8S, ITS2, 16S, 12S, and COI sequences (Movila et al., 2013; Bogdanov et al., 2017; Kloch et al., 2017) and revealed little genetic variability. A single study used 14 microsatellite loci previously developed for the North American *Dermacentor* species, *Dermacentor andersoni*, *Dermacentor variabilis*, and *Dermacentor albipictus,* to infer the population structure of *D. reticulatus* (Paulauskas et al., 2018). This study was based on a smaller sample size (*n*=254; 26 sampling sites) and revealed an east-west differentiation with a contact zone of the western and eastern clusters in Poland.

The main goal of our study, based on thorough whole-range sampling, was to combine microsatellite data with sequences of partial mitochondrial and nuclear genes to determine the present population structure of *D. reticulatus*. In addition, the rate of genetic diversity and divergence, including its geographic distribution, was analysed. And finally, the obtained data were used to reconstruct the species' evolutionary history based on competing historic demographic evolutionary scenarios.

2. Materials and methods

2.1. Sampling

In total, 3616 samples of adult *D. reticulatus* were collected from 65 localities across 21 countries, stretching from Portugal in the west to Ukraine, Russia, and Kazakhstan in the east. Ticks were either collected by flagging from the vegetation (*n*=2104), or collected from hosts (*n*=67) during adult peak activity (spring and autumn) between 2016 and 2018. The dataset was supplemented with 1445 samples of extracted DNA from previous studies

(Sprong et al., 2019). As unbalanced sample sizes can considerably impact microsatellite analyses using STRUCTURE (Puechmaille, 2016), the sample sizes were reduced at some tick-abundant localities, resulting in a subset of 865 ticks selected for processing (Supplementary Table S1).

2.2. DNA extraction and molecular markers used in the study

2.2.1. DNA extraction

DNA extraction was carried out by alkaline hydrolysis (Kubelová et al., 2011). Subsequently, DNA concentrations were measured using an IMPLEN NanoPhotometer® P330 (Implen, Munich, Germany). Extracted DNA was stored at -20 °C.

2.2.2. Nuclear and mitochondrial markers

A number of samples from the east, west and centre of the species distribution were chosen in order to get an insight into the haplotype distribution. For a representative subset of 42 samples, including four each from Portugal and the United Kingdom, 11 each from Belarus and the Czech Republic, nine from Russia, and three from Kazakhstan, a combination of two nuclear loci (18S and ITS2) and three mitochondrial (COI, 16S, and 12S) markers were sequenced to examine population divergence. PCR mixtures are explained in Supplementary Table S2. PCRs were performed as previously described (Mangold et al., 1998; Beati and Keirans, 2001; Rees et al., 2003; Lv et al., 2014), except for 16S and COI, for which thermocycling conditions were modified (Supplementary Table S3). As the quality of COI sequences initially obtained from the primers TY-J-1449 and C1-N-2312 differed, another reverse primer (HCO2198; Folmer et al., 1994) and modified PCR conditions were applied. Similarly, a shorter thermocycling protocol (Supplementary Table S3) was used for 16S PCRs to yield higher quality results. PCR products were sequenced by Macrogen (Amsterdam, the Netherlands).

2.2.3. Microsatellite markers

To investigate the population structure of *D. reticulatus*, a genomic library was developed by GenoScreen (Lille, France) from 10 pooled samples collected in Lednice (Czech Republic). A total of 563 markers were designed, 436 of which were dinucleotides,

109 trinucleotides, and 18 tetranucleotides. Loci with a higher number of repetitions, longer expected PCR product and with forward and reverse primers of similar melting temperatures were chosen for further investigation. According to these criteria, a subset of 88 microsatellite loci was subjected to gradient PCR to identify ideal thermocycling conditions. PCRs and thermocycling conditions are described in Supplementary Tables S2 and S4. For each marker, the best temperature from the gradient was selected by inspection of the PCR band intensity after agarose gel electrophoresis. Out of 88 loci tested, those which were amplifiable and contained the precise number of repetitions in most of the samples were selected and further tested for genotyping of a reduced representative set of samples (Supplementary Table S1). Using this approach, 17 markers were selected as most promising for analysis of the full sample set. Eleven of the selected markers were dinucleotide, five trinucleotide, and one was a tetranucleotide (Supplementary Table S5). Fragment length analysis was carried out with fluorescently-labelled forward primers and organised into four panels (Supplementary Table S5). They were compiled based both on the labels, i.e. dyes, and on the microsatellite fragment lengths. Fragment length analyses were multiplexed using a DS-33 matrix and a G5 dye set filter on an ABI 3130 Genetic Analyzer (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA) at Masaryk University, Brno, Czech Republic. The software PEAK SCANNER v.1 (Applied Biosystems) was used to score fragment lengths. The presence of the microsatellite was confirmed by sequencing a limited set of samples in which PCRs were performed using unlabelled primers with the same annealing temperatures, and thermocycling and sequencing conditions (Supplementary Tables S2, S4, S5). Cycle sequencing reaction products were purified using the Performa DTR V3 96-Well Short Plate (Edge Biosystems, Gaithersburg, MD, USA) with each well filled with 400 µl of Sephadex™ (GE Healthcare; 1:20 solution). Sequencing was performed on an ABI 3730 DNA Analyzer (Applied Biosystems). Sequences were checked using BIOEDIT 7.2.5 (Hall, 1999). The locus Dret32 was excluded from further analysis because it frequently could not be amplified and showed many irregularities in the repeat number. The remaining 16 loci were successfully analysed for all 865 samples.

2.3. Data analyses

2.3.1. Analyses of nuclear and mitochondrial genes

Nuclear and mitochondrial DNA sequences were aligned in Geneious 11.0.3. (Kearse et al., 2012) and compared with the GenBank nucleotide database using the BLAST algorithm

(Altschul et al., 1990). Haplotype networks were analysed using the TCS algorithm as implemented in POPART 1.7 (Leigh and Bryant, 2015). The gametic haplotypes and individual genotypes for the nuclear markers 18S and ITS2 were estimated using the Expectation-Maximization (EM) algorithm with default settings of ARLEQUIN 3.5.2.2 (Excoffier and Lischer, 2010). The EM algorithm was run with 50 starting points and a maximum of 1,000 iterations.

2.3.2. Microsatellite analyses

The microsatellite data were checked for the presence of null alleles using the software MICRO-CHECKER version 2.2.3. (van Oosterhout et al., 2004) and converted to .arp, .str, and .gen formats with CONVERT 1.31 (Glaubitz, 2004). ARLEQUIN was used to test for linkage disequilibrium, Hardy-Weinberg equilibrium, and to calculate the number of alleles per locus (n_A), the average number of alleles per locus ($n_{\bar{A}}$), the number of private alleles (n_p), observed (H_o) and expected (H_e) heterozygosity, AMOVA between STRUCTURE clusters and F_{ST} values. FSTAT 2.9.3.2. (Goudet, 1995) was used for allelic richness calculation (*AR*). To explore the relationship between genetic and geographic distances (i.e. isolation by distance), a Mantel test was performed using GenAlEx 6.5 (Peakall and Smouse, 2012). Matrices of Nei's genetic distance and untransformed geographic distance were compared using 10,000 permutations.

To gain insights into population structure and whether the geographical distribution of samples corresponded with genetic groups, the software STRUCTURE 2.3.4 (Hubisz et al., 2009) was used. The software searches the data set for genetic groups that are, as far as possible, in Hardy–Weinberg and linkage equilibrium, assigning the individual genotypes to clusters (*K*) optimized for these criteria and allowing for individual ancestries from more than one cluster. Calculations were run for *K*=1-10, applying the admixture model and correlated allele frequencies, and calculations for each *K* were repeated 10 times using a Markov chain Monte Carlo (MCMC) of 1,000,000 generations for each run, including a burn-in of 250,000 generations. The most likely number of clusters according to Evanno et al. (2005) was determined in STRUCTURE HARVESTER v0.6.94 (Earl and Bridgett, 2012). Since the ΔK method cannot identify *K*=1 (Evanno et al., 2005), the population structure was additionally examined using MAVERICK 1.0 (Verity and Nichols, 2016). Calculations in MAVERICK were run for *K*=1-10 using the admixture model. Calculations for each *K* were repeated 10 times using a burn-in of 500 generations, followed by an MCMC chain of 5,000 generations.

The threshold of cluster membership probability was determined separately, using hybrid simulations in HYBRIDLAB 1.0 (Nielsen et al., 2006). The simulation was run with 10 parent individuals per cluster, generating the same number for F_1 and F_2 hybrids and backcrossed individuals (Nielsen et al., 2006). The software DISTRUCT (Rosenberg, 2004) was used to display the STRUCTURE results. Since STRUCTURE has underlying population genetic assumptions and it is sensitive to uneven sample sizes (Puechmaille, 2016), additional Principal Component Analyses (PCAs) were run. PCAs compare samples solely according to their genotypic similarity without making population genetic or geographic presumptions. PCAs were run in R using the package ADEGENET 2.1.2 (Jombart, 2008). PCAs were three-dimensionally visualized using the package RGL 0.100.19 (Adler, D., Murdoch, D., Nenadic, O., Urbanek, S., Chen, M., Gebhardt, A., Urbanek, S., 2019. RGL: 3D visualization using OpenGL. R Package Version, 0.100.19. https://CRAN.R-project.org/packa ge=rgl) for R. Maps were compiled in QGIS 2.18.14. (QGIS Development Team., 2009. QGIS Geographic Information System. Open Source Geospatial Foundation. http://qgis.org).

2.3.3. Modelling of evolutionary history

Approximate Bayesian computation (ABC), conducted in DIYABC v2.1.0 (Cornuet et al., 2014), was used to model the evolutionary relationships among the STRUCTURE clusters. In ABC the observed microsatellite data are compared with simulated datasets based on competing evolutionary scenarios. The scenarios consist of a composition of events such as separation of populations from one another, merging of populations or changing of effective population size. Each scenario is also characterised by the time of the events (in number of generations), effective population size, admixture rate and mutational model. To compare scenarios, relative posterior probabilities are assigned to scenarios from their relative vicinity (of the appropriate simulated datasets) to the observed dataset in a multidimensional space of summary statistics.

The principal question about relationships among the clusters revealed by STRUCTURE was assessed via a set of 10 scenarios. The scenarios tested whether all population clusters derived separately from an ancestral population or whether an individual cluster derived from other clusters or from admixture between two clusters (Table S6). The dataset contained all samples that were assigned to a single cluster by STRUCTURE (Supplementary Table S1: 121 northern cluster, 105 western cluster, 131 eastern cluster).

Demographic priors of the tested scenarios included the time of the split or admixture event (corresponding to the number of generations ago; 1 - 10,000), the duration of the bottleneck event (number of generations; 0 - 20), the effective number of founders of a population (2 - 100), the rate of admixture (0 - 1), and the effective population size (10 - 10,000). Two million datasets were simulated for each scenario. The generalized stepwise model (GSM) and the default DIYABC values for the priors of the mutation model parameters were used, with the exception of the mean mutation rate, which was extended to a minimum of 1×10^{-5}.

For each simulation, summary statistics (mean number of alleles for one sample and between two samples, mean heterozygosity, F_{ST} between two samples, mean index of classification between two samples, and $(\delta\mu)^2$ distance between two samples), were used for comparison to the observed dataset using Euclidian distances. The posterior probability of each scenario was then estimated by polychotomous logistic regression on the 1% of simulated datasets closest to the observed dataset (Cornuet et al., 2008, 2010). Posterior distributions of parameters, model checking using the posterior based error and summary statistics not used in model selection, and confidence in scenario choice using 1000 pseudo-observed test data sets, were calculated using the options in DIYABC v2.1.0.

2.4. Data availability statement

Raw data on the microsatellite loci length scored are available at MendeleyData (DOI: 10.17632/kjdzs9tvhk.1).

3. Results

3.1. Low haplotype diversity along an east-west cline

Variability was low both for mitochondrial and nuclear markers when tested across the whole distribution range of *D. reticulatus*. The highest variability was found for one mitochondrial marker (COI) with 10 haplotypes differing by a maximum of four mutations. Haplotype 1 was found almost throughout the entire range of *D. reticulatus* and was by far the most widespread (Fig. 1). Haplotypes 9 and 10 were exclusively recorded from the eastern part of the range, while the remaining haplotypes were mostly found in the central part of the

studied area. For the nuclear ITS2 gene, five haplotypes were discovered that differed by a maximum of five mutation steps. 12S, 16S, and 18S were difficult to amplify, and success could only be achieved for a few samples that showed very low variability with only two haplotypes for 12S and three each for 16S and 18S. Due to the weak phylogenetic signal, it was impossible to infer phylogenetic relationships.

3.2. Three major population clusters are structured by geography

All analysed loci were polymorphic (Supplementary Table S5). Dret41, with only three alleles across all localities, showed the lowest variability. For Dret05, Dret09, and Dret25, a possible presence of null alleles was revealed for a substantial number of localities, while null alleles were rarely recorded for the remaining loci. No linkage disequilibrium was detected for any pair of loci after a Bonferroni correction was applied. Significant deviations from Hardy–Weinberg equilibrium after sequential Bonferroni corrections were found, mostly at loci in which we detected the presence of null alleles. All loci were used for further processing and the samples were genotyped using the 16 microsatellite loci resulting in a data set of 865 samples.

The Mantel test for isolation by distance showed a significant positive correlation between Nei's genetic distance and geographic distance (R^2=0.0126, P<0.001). Thus, individuals that were close to each other geographically were also close to each other genetically, and vice versa. The STRUCTURE analyses revealed that three genetic clusters (K=3; Supplementary Fig. S1) were the most probable structure for the given dataset. One cluster occurred mostly in the eastern part of the distribution range (eastern cluster), another in the west (western cluster), and a third cluster (northern cluster) was predominantly confined to Essex (UK), Belgium, and the Netherlands (Fig. 2). Many individuals with admixed ancestries were identified, particularly between the eastern and western clusters. The result of the MAVERICK analyses confirmed K=3 to best reflect population structure (Supplementary Fig. S2). The HYBRIDLAB simulation unveiled that the thresholds for cluster affiliation corresponded to 86%, 87%, and 71% for the eastern, western, and northern cluster, respectively (Supplementary Table S7). The HYBRIDLAB produces a user-selected number of multilocus F1 hybrid genotypes between any pair of potentially hybridizing populations. The program estimates allele frequencies at each locus in each of the parental populations. Then, multilocus F1 hybrid genotypes are created by randomly drawing one allele at each locus as a

function of their calculated frequency distributions from each of the hybridizing populations suggested by the user (Nielsen et al., 2006).

The PCA (Supplementary Fig. S3), coloured by the STRUCTURE cluster assignments, revealed considerable overlap of clusters with the numerous hybrids. Considering axes one and three, the western and eastern clusters overlap. To get a better understanding of whether or not the PCA shows distinct groups of samples, the PCA results were visualized three-dimensionally (3D; Supplementary Fig. S4), showing that all three STRUCTURE clusters are distinctly visible as groups in the PCA. Yet, they overlap to a small extent and numerous hybrid individuals occur between them.

3.3. Genetic diversity is highest in the eastern cluster

Genetic diversity, the number of alleles (n_A), the average number of alleles per locus ($n_{\bar{A}}$) and the allelic richness (*AR*) were highest in the eastern cluster (n_A= 151, $n_{\bar{A}}$ = 9.438, *AR*= 7.359), while the lowest values occurred in the northern cluster (n_A= 95, $n_{\bar{A}}$ = 5.938, *AR*= 4.943) (Supplementary Table S8). Even though the eastern and northern clusters had an almost equal number of samples (131 and 121, respectively), the eastern cluster had an almost 1.5 times higher allelic richness and a higher number of private alleles (n_p = 38 i.e., 25.17%) than the western (n_p = 12 i.e., 10.91%) and northern clusters (n_p = 7 i.e., 7.37%). The majority of private alleles in the eastern cluster was found in individuals from eastern Europe and Asia. Out of 131 individuals of the eastern cluster, 121 (92.37%) had at least one private allele; 68 (56.20%) of them were from eastern Europe and Asia. In the western cluster, private alleles were only detected in 25 (23.81%) individuals out of 105. From this set of 25 samples, 16 (64%) were from western Europe, with 13 (52%) of them from Wales (United Kingdom). The lowest number of private alleles was found in the northern cluster, as well as the smallest percentage of individuals with those alleles: only 20 individuals out of 121 (16.51%) had private alleles, and 16 out of these 20 (80%) belonged to the Belgian sites.

The values of expected and observed heterozygosity were highest in the eastern cluster (*He* = 0.68903 and *Ho* = 0.46859). In the western and northern clusters, the values were lower, although they did not considerably differ from the eastern cluster, ranging from 0.64758 and 0.58034 for *He*, 0.42757 and 0.35644 for *Ho*, for the western and northern cluster, respectively. Regarding the F_{ST} values, which were all relatively low, the western and northern clusters differed most (F_{ST} = 0.10470). A similar value was found for the eastern and

northern clusters (F_{ST} = 0.09645). It was found that the most similar clusters were the western and eastern ones with F_{ST} = 0.04986. Assessed by Analysis of Molecular Variance (AMOVA), 8.19% of the molecular variance occurred between the clusters, 35.45% within the clusters, and 56.36% among individuals, reflecting weak differentiation.

3.4. The northern cluster is derived from an admixture of the eastern and western clusters

Scenario 7 was best supported by the highest posterior probability (*P*=0.2476; 95% credibility interval 0.2335-0.2617; Supplementary Table S9). In this scenario, the northern population derived from admixture between the eastern and western populations, and both the eastern and western populations independently derived from an ancestral population. Posterior probabilities for the most probable scenario indicate the northern population arose a median of 441 and a mode of 224 generations ago after undergoing a relatively strong bottleneck event (i.e. of long duration with few founders) (Supplementary Table S9). Visual inspection of the model checking results confirmed the winning scenario (scenario 7) and its priors fit the real observed dataset well (Supplementary Fig. S5).

4. Discussion

The near-absence of any phylogeographic structuring and a very limited intraspecific variability of ticks is evident from the majority of previous studies based on both mitochondrial (Kain et al., 1999; Casati et al., 2008; Kovalev and Mukhacheva, 2012; Kulakova et al., 2014; Kloch et al., 2017; Aguilar-Domínguez et al., 2019) and nuclear DNA sequences (Kovalev and Mukhacheva, 2012; Kulakova et al., 2014; Tarragona et al., 2018). Conversely, a high haplotype diversity was discovered for *Amblyomma ovale* in Brazil (Bitencourth et al., 2019) and *Haemaphysalis longicornis* in China (Liu et al., 2019). Weak geographic differentiation could result from a high rate of genetic exchange. Increased gene flow, assessed by microsatellite markers, was indeed recorded for ticks having a broad range of feeding strategies; e.g. in South Africa and Zimbabwe for *Rhipicephalus microplus*, a one-host tick feeding mainly on artiodactyls, mostly cattle (Baron et al., 2018; Sungirai et al., 2018), and for *Amblyomma aureolatum* in Brazil, a three-host tick species feeding on various mammals and birds (Ogrzewalska et al., 2016). Our study confirmed weak, but discernible, geographic differentiation for *D. reticulatus*. Yet, understanding the observed differentiation pattern is not straightforward.

The main pitfalls for an unequivocal interpretation of the obtained population structure of *D. reticulatus* represent, particularly: (i) the patchy information about the past distribution of the species; (ii) the low variability of nuclear and mitochondrial haplotypes; (iii) the weak geographic differentiaton inferred from microsatellite variation combined with evidence for isolation-by-distance effects; and (iv) the wide host spectrum corresponding to independence on particular host species and the resulting unspecific passive but potentially wide-ranging dispersal capability.

Despite a weak phylogeographic pattern of *D. reticulatus,* based on evidence from DNA sequences, a general east-west differentiation is discernible in the population structure (Fig. 1). The COI sequences from Russia and Kazakhstan correspond to unique haplotypes already described by Bogdanov et al. (2017), who found one isolated cluster for this geographic region. East-west divergence also occurs in 16S rDNA sequences, with one widespread western haplotype (16S haplotype 1) and a more geographically restricted eastern haplotype (16S haplotype 3) that differed, however, only by a single mutation. For 16S rDNA, Kloch et al. (2017) analysed samples from an area extending from Berlin to Kiev (>1,200 km) and found one haplotype that was widely distributed, and four rare haplotypes confined to eastern sites. The present study, covering ~6,000 km from east to west, confirmed an east-west distribution pattern of haplotypes, with one 16S haplotype spread almost throughout the whole area (16S haplotype 1). Using 16S rDNA and ITS2 sequences from Central Europe, Bajer et al. (2016) found that Ukrainian localities had the largest number of unique haplotypes. Our analysis of ITS2 shows that one haplotype (ITS2 haplotype 1) occurs across the entire distribution range, and an east-west differentiation is reflected by the occurrence of ITS2 haplotype 5 only in the east (Russia and Kazakhstan) and ITS2 haplotypes 2, 3, and 4 only in the west. Our trials with 18S and 12S sequence data showed little variation, comparable to Mangold et al. (1998), but less variability compared with Movila et al. (2013), who detected four haplotypes from three sampling sites. Our results are representative, but definitely could be affected by a lower sample size. It is likely that sequencing more samples would find more haplotypes.

An east-west pattern to the population structure of *D. reticulatus* was expected, considering the large geographic distances involved and previous studies using DNA sequences or more localized sampling. However, contrary to expectations, three genetic clusters were revealed, with the discovery of a novel cluster in the north, in addition to a western and an eastern cluster (Fig. 2).

Paulauskas et al. (2018) suggested that the population structure of *D. reticulatus* corresponds to two clusters, one from the west and another from the east of the range, with a contact zone in Poland. This differentiation is, however, less evident in their study due to a small sample size and restricted geographic range. Nevertheless, their results suggested extensive gene flow.

Our novel evidence for a tripartite differentiation could be explained by the following alternative scenarios. Although vast areas of Eurasia were not ice-covered during the Last Glacial Maximum (LGM ~ 21 thousands of years ago (ka)) and possibly offered *D. reticulatus* a wide spectrum of cold-tolerant hosts, empirical evidence suggests that *D. reticulatus* is more thermophilous and hygrophilous, and its survival under cold pristine Pleistocene steppe conditions (Földvári et al., 2016; Tkadlec et al., 2018) is unlikely. Accordingly, *D. reticulatus* was most likely confined to southern refugia during the glacial period, surviving on local hosts there. Hence, it can be assumed that the genetic divergence of the western and the eastern clusters originate from the differentiation of *D. reticulatus* populations in distinct southern glacial refugia in the west and east. With the Holocene climatic warming, the western and eastern clusters of *D. reticulatus* may have spread out of their respective refugia to colonize more northerly regions, resulting in the present patterns.

However, the presence of a northern cluster without any obvious geographic barrier is surprising. One explanation could be that this cluster arose from a northern refugium corresponding to the landmass between what is now the Benelux region and the United Kingdom during the LGM (Lambeck, 2005), contrary to our general assumption that *D. reticulatus* is too thermophilous to survive there. Some of the known hosts of *D. reticulatus*, such as foxes (Edwards et al., 2012), bank voles, wood mice (Cordy, 1991), and red deer (Lister, 1984), were active in northern areas not covered by ice approximately 20,000 years ago (Fig. 3). Hypothetically, *D. reticulatus* might also have fed on other contemporary mammals in the area, as illustrated by the discovery of a *Dermacentor* sp. from the ear of a Pleistocene woolly rhino (Schille, 1916). However, our ABC modelling did not support this hypothesis. The most probable scenario reveals that the northern cluster arose from admixture between the eastern and western clusters. This event was found to be relatively recent, in terms of evolutionary history, having occurred between 200 and 400 generations ago (mode 224, median 441). If one generation is considered to be 1 year, or more frequently 2 years, then this is in the range of only 200−800 years ago. This time-scale suggests a possible link to the ending of the Little Ice Age in Europe which occurred between the 14th and 19th centuries,

together with a higher frequency of supreme hydroclimatic events (Matthews and Briffa, 2005). This scenario is in line with the eastern and western clusters spreading slowly from their respective refugia after the LGM, as stated above, and meeting more recently, after the end of the Little Ice Age, to create a novel, unique population in the Benelux area. Such an event would undoubtedly result in a reduced number of individuals and thus genes, a fact reflected in the strong bottleneck event suggested by the ABC scenario (few founders and long duration of reduced population size) and the genetic traits of the current day northern cluster (e.g. lowest allelic richness and fewest number of private alleles of all the clusters).

Another explanation of the east-to-west differentiation is that the isolation by distance causes a "bipolar pattern" in population structure. If this option were true, the present differentiation would not result from divergence in distinct glacial refugia, but from limited gene flow across large distances.

Distribution and local abundance of ticks closely depend on the vagility of their hosts (Hoogstraal and Aeschlimann, 1982). The spectrum of *D. reticulatus* hosts belongs to the widest among ixodid ticks (Földvári et al., 2016). Ungulates and carnivores are the most important large hosts of *D. reticulatus* adults in terms of contributing to spread and shaping its recent range, particularly since they can carry many ticks over long distances (Buczek et al., 2017). During the LGM, the distribution of the European wild boar (*Sus scrofa*) was limited to the Iberian, Apennine and Balkan Peninsulas, and southwestern France (Scandura et al., 2011), its diversity hot spots being south-eastern Spain, southern France, southern Italy and Greece (Vilaça et al., 2014). Fossil records from LGM refugia supported these findings (Sommer and Nadachowski, 2006). Adult female wild boar with piglets can travel over 500 km in 2 months (Jerina et al., 2014). Female roe deer (*Capreolus capreolus*) can migrate 84 km during the migration season and establish in a new area (Mysterud, 1999). Wolves (*Canis lupus*), one of the most important tick hosts (Hodžić et al., 2020), are known for their long-distance movements (Mech, 1974). In Europe, the reported home range of a wolf is 460.5 km^2 in 6 months, with daily migrations of 14.7±6.7 km (Karamanlidis et al., 2017). Andersen et al. (2015) confirmed that the first grey wolf noted in northern Jutland (Denmark) immigrated from the border area between Germany, the Czech Republic and Poland, i.e. over a distance of 800 km. This indicates that an individual grey wolf can migrate distances of 800–1200 km within a short period. Furthermore, Klitgaard et al. (2017) reported 21 males of *D. reticulatus* on a golden jackal (*Canis aureus*) carcass from western Jutland in February 2017. In 2019 it was recognised that the golden jackal population is undergoing a population explosion in

Europe, causing significant expansion throughout the continent (Lanszki et al., 2022). The current expansion started from three basal populations, two from the Balkans and one from the Caucasus (Spassov and Acosta-Pankov, 2019). The importance of the raccoon dog (*Nyctereutes procyonoides*) in the spread of *D. reticulatus* has been demonstrated in Poland. It is an invasive species which appeared in Poland in the mid-20th century and spread from east to west. Annually, the raccoon dog can move from 150 to almost 500 km. The direction and time of expansion of the raccoon dog correlates with changes in the range and abundance of *D. reticulatus* (Karbowiak, 2021). Such range expansions of mammalian hosts can accelerate the dispersal of *D. reticulatus*. All of these large mammals are important tick hosts and their long-distance movements exemplify the ease with which ticks can cover these distances as 'passengers'. Not only does this allow ticks to spread to new areas, but it also allows them to easily mix with other populations, leading to the current blurred population structure of *D. reticulatus*.

Most of the studied localities were dominated by admixed individuals with a small proportion of ticks belonging purely to either the western, eastern, or northern clusters, depending on the region. In central and eastern Europe, from Germany in the west to Belarus in the east, ticks belonging to all three clusters, as well as admixed ticks, were frequently detected (Fig. 2B, C). This pattern was disturbed by some putatively translocated individuals from the eastern cluster westwards up to north-western Spain, and by ticks belonging to the western cluster occurring eastwards up to Belarus and Ukraine. Ticks belonging to the northern cluster were concentrated in Great Britain, Belgium, and the Netherlands. However, they were also rarely detected in central Europe, with the easternmost occurrence in Belarus (Fig. 2).

The occurrence of individuals of each cluster over almost the entire distribution range covering various habitat types suggests the absence of clear barriers to dispersal and gene flow. The high number of admixed individuals is likely caused by host migration and, more recently, increasing human traffic and travelling with dogs as human companions (Duscher et al., 2010; Hamel et al., 2011). The biology of the tick plays a crucial role in its potential for dispersal. Ixodid ticks feed once per active life-stage, and feeding can last more than 2 weeks for adults, allowing long-distance dispersal when attached to migrating hosts. In *D. reticulatus*, larvae feed for 3–6 days, nymphs for 5–12 days, and adult females for 7–16 days, while adult males may remain on the host for a long time, even if no female is present (Slovak et al., 2002; Šimo et al., 2004). Engorged and fertilised females may lay up to 7200 eggs in

the new locality (Šimo et al., 2004). *Dermacentor reticulatus* can be active throughout the year with biphasic peak activity during spring and autumn; individuals are dormant during periods of snow cover and the driest and hottest periods (Guglielmone et al., 2014). Adults can overwinter both in unfed or engorged states (Belozerov, 1982; Kiewra et al., 2016; Drehmann et al., 2020) and survive more than 2.5 years of starvation (Razumova, 1998). It has been also observed that ticks can spend the entire winter on hosts (Karbowiak et al., 2014). Their eggs can survive underwater for several months (Hoogstraal, 1967) and thus may be spread by floods into new areas. Adults of *D. reticulatus* also can repeatedly switch from inactivity to host-seeking activity, improving their chances of finding a suitable host.

Anthropogenic factors such as changes in agricultural practices and deforestation have a considerable impact on the dispersal and distribution of *D. reticulatus* (Karbowiak, 2014; Mierzejewska et al., 2015). Human practices increasing the abundance of some wildlife host populations (e.g. deer) have further contributed to their spread (Gray et al., 2009). Among climate variables, temperature and precipitation are leading factors affecting the distribution of ticks. *Dermacentor reticulatus* prefers warmer and wetter regions with greater diurnal and seasonal variation in temperature but with lower seasonality in precipitation than, e.g., the partly sympatric *I. ricinus* (Tkadlec et al., 2018). Increasing winter temperatures enable overwintering and extend the active period of adults (Karbowiak, 2014), while warming associated with desiccation might have an inverse impact on tick survival. Apart from direct influence on ticks, climate change can also indirectly shape their distribution by affecting biotic interactions, vegetation types, land cover, abundance, habitat preferences and dispersal capacities of host species (Sobéron and Peterson, 2005). Regardless of which factor is most influential for tick dispersal, the distribution range of *D. reticulatus* is currently obviously expanding (Kahl and Dautel, 2013; Drehmann et al., 2020; Rubel et al., 2020; Cunze et al., 2022; Daněk et al., 2022).

The spread of ticks from each particular cluster reflects this current expansion and it is expected that this will lead to increased admixture of tick populations and will further obscure the ancestral population structure, eventually resulting in one panmictic Eurasian population of *D. reticulatus*. According to its dynamics, it is very probable that the continuing range expansion of this important vector will lead to increasing concern about the future spread of associated *Dermacentor*-borne pathogens, together with their ecological, veterinary and medical consequences.

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References

Aguilar-Domínguez, M., Sánchez-Montes, S., Esteve-Gassent, M.D., Barrientos-Salcedo, C., Pérez de León, A., Romero-Salas, D., 2019. Genetic structure analysis of *Amblyomma mixtum* populations in Veracruz State, Mexico. Ticks. Tick. Borne. Dis., 10, 86–92. https://doi.org/10.1016/j.ttbdis.2018.09.004

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Amzati, G.S., Pelle, R., Muhigwa, J.B.B., Kanduma, E.G., Djikeng, A., Madder, M., Kirschvink, N., Marcotty, T., 2018. Mitochondrial phylogeography and population structure of the cattle tick *Rhipicephalus appendiculatus* in the African Great Lakes region. Parasit Vectors. 11, 329–348. https://doi.org/10.1186/s13071-018-2904-7

Andersen, L.W., Harms, V., Caniglia, R., Kluth, G., Madsen, A.B., Nowak, C., Pertoldi, C., Randi, E., Stronen, A.V., 2015. Long-distance dispersal of a wolf, *Canis lupus*, in Northwestern Europe. Mammal Res. 60, 163–168. https://doi.org/10.1007/s13364-015-0220-6

Araya-Anchetta, A., Busch, J.D., Scoles, G.A., Wagner, D.M., 2015. Thirty years of tick population genetics: A comprehensive review. Infect. Genet. Evol. 29, 164–179. https://doi.org/10.1016/j.meegid.2014.11.008

Bajer, A., Mierzejewska, E.J., Karbowiak, G., Slivinska, K., Alsarraf, M., Rodo, A., Kowalec, M., Dwużnik, D., Kloch, A., 2016. Genetic diversity of a tick, *Dermacentor reticulatus*, in an expansion zone in central Europe. Ann. Parasitol. 62, Supplement, 180.

Barbará, T., Palma-Silva, C., Paggi, G.M., Bered, F., Fay, M.F., Lexer, C., 2007. Cross-species transfer of nuclear microsatellite markers: Potential and limitations. Mol. Ecol. 16, 3759–3767. https://doi.org/10.1111/j.1365-294X.2007.03439.x

Baron, S., van der Merwe, N.A., Maritz-Olivier, C., 2018. The genetic relationship between *R. microplus* and *R. decoloratus* ticks in South Africa and their population structure. Mol. Phylogenet. Evol. 129, 60–69. https://doi.org/10.1016/j.ympev.2018.08.003

Beati, L., Keirans, J.E., 2001. Analysis of the systematic relationship among ticks of the genera *Rhipicephalus* and *Boophilus* (Acar: Ixodida) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J. Parasitol. 87, 32–48.

Belozerov, V.N., 1982. Diapause and Biological Rhithms in Ticks. In F. D. Obenchain, R. Galun (Eds.), Physiology of Ticks. Pergamon Press, Oxford. pp. 469-500 https://doi.org/10.1016/c2013-0-03261-6

Bitencourth, K., Amorim, M., de Oliveira, S.V., Voloch, C.M., Gazêta, G.S., 2019. Genetic diversity, population structure and rickettsias in *Amblyomma ovale* in areas of epidemiological interest for spotted fever in Brazil. Med. Vet. Entomol. 33, 256–268. https://doi.org/10.1111/mve.12363

Bogdanov, A.S., Makenov, M.T., Medyanikova, L.V., Shchouchinova, L.D., Yakimenko, V.V., 2017. Variability of mitochondrial cytochrome oxidase first subunit gene (COI) fragments in several tick species of the marginatus group (Ixodidae, Amblyomminae, *Dermacentor*). Biol. Bull. Russ. Acad. Sci. 44, 379–83. https://doi.org/10.1134/S1062359017030025

Buczek, A., Zając, Z., Woźniak, A., Kulina, D., Bartosik, K., 2017. Locomotor activity of adult *Dermacentor reticulatus* ticks (Ixodida: Ixodidae) in natural conditions. Ann. Agric. Environ. Med. 24, 271–275. https://doi.org/10.5604/12321966.1230736

Casati, S., Bernasconi, M.V., Gern, L., Piffaretti, J.C., 2008. Assessment of intraspecific mtDNA variability of European *Ixodes ricinus* sensu stricto (Acari: Ixodidae). Infect. Genet. Evol. 8, 152–158. https://doi.org/10.1016/j.meegid.2007.11.007

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1659.

Cordy, J.-M., 1991. Palaeoecology of the late glacial and early postglacial of Belgium and neighbouring areas. In N. Barton, A. J. Roberts, D. A. Roe, Eds., The late glacial in northwest Europe: Human adaptation and environmental change at the end of the Pleistocene. CBA Research Report, Council for British Archaeology, UK. pp. 40-47. https://doi.org/10.1177/095968369200200218

Cornuet, J.-M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J.M., Estoup, A., 2014. DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. Bioinformatics. 30, 1187–1189. https://doi.org/10.1093/bioinformatics/btt763

Cornuet, J.-M., Ravigné, V., Estoup, A., 2010. Inference on population history and model

checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). BMC Bioinform. 11, 401.

Cornuet, J.-M., Santos, F., Beaumont, M.A., Robert, C.P., Marin, J.M., Balding, D.J., Guillemaud, T., Estoup, A., 2008. Inferring population history with DIYABC: A user-friendly approach to approximate Bayesian computation. Bioinformatics. 24, 2713–2719.

Cunze, S., Glock, G., Kochmann, J., Klimpel, S., 2022. Ticks on the move–climate change-induced range shifts of three tick species in Europe: current and future habitat suitability for *Ixodes ricinus* in comparison with *Dermacentor reticulatus* and *Dermacentor marginatus*. Parasitol. Res. 121, 2241–2252. https://doi.org/10.1007/s00436-022-07556-x

Daněk, O., Hrazdilová, K., Kozderková, D., Jirků, D., Modrý, D., 2022. The distribution of *Dermacentor reticulatus* in the Czech Republic re-assessed: citizen science approach to understanding the current distribution of the *Babesia canis* vector. Parasit Vectors. 15, 132. https://doi.org/10.1186/s13071-022-05242-6

Dobson, S.J., Barker, S.C., 1999. Phylogeny of the hard ticks (Ixodidae) inferred from 18S rRNA indicates that the genus *Aponomma* is paraphyletic. Mol. Phylogenet. Evol. 11, 288–295.

Drehmann, M., Springer, A., Lindau, A., Fachet, K., Mai, S., Thoma, D., Schneider, C.R., Chitimia-Dobler, L., Bröker, M., Dobler, G., Mackenstedt, U., Strube, C., 2020. The spatial distribution of *Dermacentor* ticks (Ixodidae) in Germany—Evidence of a continuing spread of *Dermacentor reticulatus*. Front. Vet. Sci. 7, 1–8. https://doi.org/10.3389/fvets.2020.578220

Duscher, G., Edelhofer, R., Leschnik, M., Joachim, A., 2010. Travel-related diseases of dogs and cats. Kleintierpraxis. 55, 204–222.

Earl, D.A., Bridgett, M., 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361. https://doi.org/10.1007/s12686-011-9548-7

Edwards, C.J., Soulsbury, C.D., Statham, M.J., Ho, S.Y.W., Wall, D., Dolf, G., Iossa, G., Baker, P.J., Harris, S., Sacks, B.N., Bradley, D.G., 2012. Temporal genetic variation of the red fox, *Vulpes vulpes*, across Western Europe and the British Isles. Quat. Sci.

Rev. 57, 95–104. https://doi.org/10.1016/j.quascirev.2012.10.010

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol. Ecol. 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., Laval, G., 2010,. ARLEQUIN SUITE VER 3.5.: A new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Filippova, N.A., 1997. Ixodid ticks of subfamily Amblyomminae. Fauna of Russia and neighbouring countries, Nauka, St. Petersburg. In Russian.

Földvári, G., Široký, P., Szekeres, S., Majoros, G., Sprong, H., 2016. *Dermacentor reticulatus*: A vector on the rise. Parasit. Vectors. 9, 314–342. https://doi.org/10.1186/s13071-016-1599-x

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Marine Biol. Biotechnol. 3, 294–299.

Fukunaga, M., Yabuki, M., Hamase, A., Oliver, J.H., Nakao, M., 2000. Molecular phylogenetic analysis of ixodid ticks based on the ribosomal DNA spacer, internal transcribed spacer 2, sequences. J. Parasitol. 86, 38–43. https://doi.org/10.2307/3284905

Glaubitz, J.C., 2004. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol. Ecol. Notes. 4, 309–310. https://doi.org/10.1111/j.1471-8286.2004.00597.x

Goudet, J., 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. J. Hered. 86, 485–486.

Gray, J.S., Dautel, H., Estrada-Peña, A., Kahl, O., Lindgren, E., 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip. Perspect. Infect. Dis. 2009, 1–12. https://doi.org/10.1155/2009/593232

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Peña, A., Horak, I.G., 2014. The Hard Ticks of the World: (Acari: Ixodida: Ixodidae). Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7497-1

Guglielmone, A.A., Robbins, R.G., Horak, I.G., Shao, R., Barker, S.C., 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. Zootaxa. 2528, 1–28.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hamel, D., Röhrig, E., Pfister, K., 2011. Canine vector-borne disease in travelled dogs in Germany-A retrospective evaluation of laboratory data from the years 2004-2008. Vet. Parasitol. 181, 31–36. https://doi.org/10.1016/j.vetpar.2011.04.020

Hodžić, A., Georges, I., Postl, M., Duscher, G.G., Jeschke, D., Szentiks, C.A., Ansorge, H., Heddergott, M., 2020. Molecular survey of tick-borne pathogens reveals a high prevalence and low genetic variability of *Hepatozoon canis* in free-ranging grey wolves (*Canis lupus*) in Germany. Ticks. Tick. Borne. Dis. 11, 101389. https://doi.org/10.1016/j.ttbdis.2020.101389

Hoogstraal, H., 1967. Ticks in relation to human diseases caused by *Rickettsia* species. Annu. Rev. Entomol. 12, 377–420. https://doi.org/10.1146/annurev.en.12.010167.002113

Hoogstraal, H., Aeschlimann, A., 1982. Tick-host specificity. Bull. Soc. Entomol. Suisse. 55, 5–32.

Hubisz, M.J., Falush, D., Stephens, M., Pritchard, J.K., 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Res. 9, 1322–1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Jerina, K., Pokorny, B., Stergar, M., 2014. First evidence of long-distance dispersal of adult female wild boar (*Sus scrofa*) with piglets. Eur. J. Wildl. Res. 60, 367–370. https://doi.org/10.1007/s10344-014-0796-1

Jombart, T., 2008. ADEGENET: a R package for the multivariate analysis of genetic markers. Bioinform. App. Note. 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitology 129, 3–14. https://doi.org/10.1017/S0031182004005967

Kahl, O., Dautel, H., 2013. Seasonal life cycle organisation of the ixodid tick *Dermacentor reticulatus* in central Europe implications on its vector role and distribution. Med. Kuzbass. 13, 84–87.

Kain, D.E., Sperling, F.A.H., Daly, H.V., Lane, R.S., 1999. Mitochondrial DNA sequence variation in *Ixodes pacificus* (Acari: Ixodidae). Heredity 83, 378–386. https://doi.org/10.1038/sj.hdy.6886110

Karamanlidis, A.A., De Gabriel Hernando, M., Georgiadis, L., Kusak, J., 2017. Activity, movement, home range and habitat use of an adult gray wolf in a Mediterranean landscape of northern Greece. Mammalia 81, 95–99. https://doi.org/10.1515/mammalia-2015-0091

Karbowiak, G., 2014. The occurrence of the *Dermacentor reticulatus* tick – Its expansion to new areas and possible causes. Ann. Parasitol. 60, 37–47.

Karbowiak, G., 2022. Changes in the occurrence range of hosts cause the expansion of the ornate dog tick *Dermacentor reticulatus* (Fabricius, 1794) in Poland. Biologia 77, 1513–1522. https://doi.org/10.1007/s11756-021-00945-0

Karbowiak, G., Demiaszkiewicz, A., Pyziel, A., Wita, I., Moskwa, B., Werszko, J., Bień, J., Goździk, K., Lachowicz, J., Cabaj, W., 2014. The parasitic fauna of the European bison (*Bison bonasus*) (Linnaeus, 1758) and their impact on the conservation. Part 1: The summarising list of parasites noted. Acta Parasitol. 59, 363–371. https://doi.org/10.2478/s11686-014-0252-0

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Theirer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinform. App. Note. 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

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

Klitgaard, K., Chriél, M., Isbrand, A., Jensen, T.K., Bødker, R., 2017. Identification of *Dermacentor reticulatus* ticks carrying *Rickettsia raoultii* on migrating jackal, Denmark. Emerg. Infect. Dis. 23, 2072–2074. https://doi.org/10.3201/eid2312.170919

Kloch, A., Mierzejewska, E.J., Karbowiak, G., Slivinska, K., Alsarraf, M., Rodo, A., Kowalec, M., Dwużnik, D., Didyk, Y.M., Bajer, A., 2017. Origins of recently emerged

foci of the tick *Dermacentor reticulatus* in Central Europe inferred from molecular markers. Vet. Parasitol., 237, 63–69. https://doi.org/10.1016/j.vetpar.2017.02.020

Klompen, J.S.H., Black, W.C., Keirans, J.E., Norris, D.E., 2000. Systematics and biogeography of hard ticks, a total evidence approach. Cladistics 16, 79–102. https://doi.org/10.1111/j.1096-0031.2000.tb00349.x

Kovalev, S.Y., Mukhacheva, T.A., 2012. Phylogeographical structure of the tick *Ixodes persulcatus*: A novel view. Ticks. Tick. Borne. Dis. 3, 212–218. https://doi.org/10.1016/j.ttbdis.2012.03.005

Kubelová, M., Tkadlec, E., Bednář, M., Roubalová, E., Široký, P., 2011. West-to-east differences of *Babesia canis canis* prevalence in *Dermacentor reticulatus* ticks in Slovakia. Vet. Parasitol. 180, 191–196. https://doi.org/10.1016/j.vetpar.2011.03.033

Kulakova, N.V., Khasnatinov, M.A., Sidorova, E.A., Adel'shin, R.V., Belikov, S.I., 2014. Molecular identification and phylogeny of *Dermacentor nuttalli* (Acari: Ixodidae). Parasitol. Res. 113, 1787–1793. https://doi.org/10.1007/s00436-014-3824-x

Lambeck, K., 2005. Isostasy. In Schwartz, M. L. (Eds.), Encyclopedia of Coastal Sciences, Springer-Verlag, Dordrecht, pp. 565-571. https://doi.org/10.2307/1783272

Lanszki, J., Hayward, M.W., Ranc, N., Zalewski, A., 2022. Dietary flexibility promotes range expansion: the case of golden jackals in Eurasia. J. Biogeogr. 49, 993-1005. https://doi.org/10.1111/jbi.14372

Leigh, J.W., Bryant, D., 2015. POPART: full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116. https://doi.org/10.1111/2041-210X.12410

Lister, A.M., 1984. Evolutionary and ecological origins of British deer. Proc. Royal Soc. Edinburgh B Biol. Sci. 82, 205–229. https://doi.org/10.1017/s0269727000003754

Liu, T., Feng, X., Zhang, Y., Liu, J., Bao, R., 2019. Genetic diversity of *Haemaphysalis longicornis* from China and molecular detection of *Rickettsia*. Exp. Appl. Acarol. 79, 221–231. https://doi.org/10.1007/s10493-019-00423-y

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L., Lin, X., 2014. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). Parasit. Vectors. 7, 93–104. https://doi.org/10.1186/1756-3305-7-93

Mangold, A.J., Bargues, M.D., Mas-Coma, S., 1998. 18S rRNA gene sequences and phylogenetic relationships of European hard-tick species (Acari : Ixodidae). Parasitol. Res. 84, 31–37.

Matjila, T.P., Nijhof, A.M., Taoufik, A., Houwers, D., Teske, E., Penzhorn, B.L., de Lange, T., Jongejan, F., 2005. Autochthonous canine babesiosis in the Netherlands. Vet. Parasitol., 131, 23–29. https://doi.org/10.1016/j.vetpar.2005.04.020

Matthews, J.A., Briffa, K.R., 2005. The 'Little ice age': Re-evaluation of an evolving concept. Geografiska Annaler, Series A: Phys. Geogr. 87, 17–36. https://doi.org/10.1111/j.0435-3676.2005.00242.x

McCoy, K.D., 2008. The population genetic structure of vectors and our understanding of disease epidemiology. Parasite 15, 444–448.

Mech, L. D., 1974. *Canis lupus*. Mamm. Species. 37, 1–6. https://doi.org/10.2307/3503924

Mierzejewska, E.J., Alsarraf, M., Behnke, J.M., Bajer, A., 2015. The effect of changes in agricultural practices on the density of *Dermacentor reticulatus* ticks. Vet. Parasitol. 211, 259–265. https://doi.org/10.1016/j.vetpar.2015.05.023

Movila, A., Morozov, A., Sitnicova, N., 2013. Genetic polymorphism of 12S rRNA gene among *Dermacentor reticulatus* Fabricius ticks in the Chernobyl nuclear power plant exclusion zone. J. Parasitol. 99, 40–43. https://doi.org/10.1645/ge-3225.1

Murrell, A., Campbell, N.J.H., Barker, S.C., 2001. A total-evidence phylogeny of ticks provides insights into the evolution of life cycles and biogeography. Mol. Phylogenet. Evol. 21, 244–258. https://doi.org/10.1006/mpev.2001.1018

Mysterud, A., 1999. Seasonal migration pattern and home range of roe deer (*Capreolus capreolus*) in an altitudinal gradient in southern Norway. J. Zool. 247, 479–486. https://doi.org/10.1017/S0952836999004070

Nava, S., Guglielmone, A.A., Mangold, A.J., 2009. An overview of systematics and evolution of ticks. Front. Biosci. 14, 2857–2877.

Nielsen, E.E., Bach, L.A., Kotlicki, P., 2006. HYBRIDLAB (Version 1.0): A program for generating simulated hybrids from population samples. Mol. Ecol. Notes. 6, 971–973. https://doi.org/10.1111/j.1471-8286.2006.01433.x

Ogrzewalska, M., Schwarcz, K., Bajay, M.M., Bajay, S.K., Pinheiro, J.B., Zucchi, M.I.,

Pinter, A., Labruna, M.B., 2016. Characterization of genetic variability and population structure of the tick *Amblyomma aureolatum* (Acari: Ixodidae). J. Med. Entomol. 53, 843–850. https://doi.org/10.1093/jme/tjw049

van Oosterhout, C., Hutchinson, W.F., Willis, D.P., Shipley, P., 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes. 4, 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Paulauskas, A., Galdikas, M., Galdikaitė-Brazienė, E., Stanko, M., Kahl, O., Karbowiak, G., Radzijevskaja, J., 2018. Microsatellite-based genetic diversity of *Dermacentor reticulatus* in Europe. Infect. Genet. Evol. 66, 200–209. https://doi.org/10.1016/j.meegid.2018.09.029

Peakall, R., Smouse, P.E., 2012. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28, 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pfäffle, M., Littwin, N., Petney, T., 2015. Host preferences of immature *Dermacentor reticulatus* (Acari: Ixodidae) in a forest habitat in Germany. Ticks. Tick. Borne. Dis. 6, 508–515. https://doi.org/10.1016/j.ttbdis.2015.04.003

Puechmaille, S.J., 2016. The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol. Ecol. Resour. 16, 608–627. https://doi.org/10.1111/1755-0998.12512

Razumova, I.V., 1998. The activity of *Dermacentor reticulatus* Fabr. (Ixodidae) ticks in nature. Medicinskaja Parazitologia 4, 8–14. (in Russian)

Rees, D.J., Dioli, M., Kirkendall, L.R., 2003. Molecules and morphology: evidence for cryptic hybridization in African *Hyalomma* (Acari : Ixodidae). Mol. Phylogenet. Evol. 27, 131–142. https://doi.org/10.1016/S1055-7903(02)00374-3

Rosenberg, N.A., 2004. DISTRUCT: A program for the graphical display of population structure. Mol. Ecol. Notes. 4, 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Rubel, F., Brugger, K., Belova, O.A., Kholodilov, I.S., Didyk, Y.M., Kurzrock, L., García-Pérez, A.L., Kahl, O., 2020. Vectors of disease at the northern distribution limit of the genus *Dermacentor* in Eurasia: *D. reticulatus* and *D. silvarum*. Exp. Appl.

Acarol. 82, 95–123. https://doi.org/10.1007/s10493-020-00533-y

Rubel, F., Brugger, K., Pfeffer, M., Chitimia-Dobler, L., Didyk, Y.M., Leverenz, S., Dautel, H., Kahl, O., 2016. Geographical distribution of *Dermacentor marginatus* and *Dermacentor reticulatus* in Europe. Ticks. Tick. Borne. Dis., 7, 224–233. https://doi.org/10.1016/j.ttbdis.2015.10.015

Rumer, L., Sheshukova, O., Dautel, H., Mantke, O.D., Niedrig, M., 2011. Differentiation of medically important Euro-Asian tick species *Ixodes ricinus*, *Ixodes persulcatus*, *Ixodes hexagonus*, and *Dermacentor reticulatus* by polymerase chain reaction. Vector-Borne Zoonotic Dis. 11, 899–905. https://doi.org/10.1089/vbz.2009.0191

Scandura, M., Iacolina, L., Apollonio, M., 2011. Genetic diversity in the European wild boar *Sus Scrofa*: phylogeography, population structure and wild x domestic hybridization. Mamm. Rev. 41, 125–137. https://doi.org/10.1111/j.1365-2907.2010.00182.x

Schille, F., 1916. Entomologie aus der Mammut- und Rhinoceros-Zeit Galiziens. Entomologische Zeitschrift, 30, 42–44.

Šimo, L., Kocáková, P., Sláviková, M., Kubeš, M., Hajnická, V., Vančová, I., Slovák, M., 2004. *Dermacentor reticulatus* (Acari, Ixodidae) female feeding in laboratory. Biologia 59, 655–660.

Slovák, M., Labuda, M., Marley, S. E., 2002. Mass laboratory rearing of *Dermacentor reticulatus* ticks (Acarina, Ixodidae). Biologia. 57, 261–266.

Soberón, J., Peterson, A.T., 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. Biodivers. Inform. 2, 1–10. https://doi.org/10.17161/bi.v2i0.4

Sommer, R.S., Nadachowski, A., 2006. Glacial refugia of mammals in Europe: evidence from fossil records. Mamm. Rev. 36, 251–265.

Spassov, N., Acosta-Pankov, I., 2019. Dispersal history of the golden jackal (*Canis aureus moreoticus* Geoffroy, 1835) in Europe and possible causes of its recent population explosion. Biodivers. Data J. 7, e34825. https://doi.org/10.3897/BDJ.7.e34825

Sprong, H., Fonville, M., van Leeuwen, A.D., Devillers, E., Ibañez-Justicia, A., Stroo, A., Hasford, K., Cull., B., Medlock, J., Heyman, P., Cochez, C., Weis, L., Silaghi, C., Moutailler, S., 2019. Detection of pathogens in *Dermacentor reticulatus* in

northwestern Europe: evaluation of a high-throughput array. Heliyon. 5, e01270. https://doi.org/10.1016/j.heliyon.2019.e01270

Sungirai, M., Baron, S., van der Merwe, N.A., Moyo, D.Z., de Clercq, P., Maritz-Olivier, C., Madder, M., 2018. Population structure and genetic diversity of *Rhipicephalus microplus* in Zimbabwe. Acta Trop. 180, 42–46. https://doi.org/10.1016/j.actatropica.2018.01.003

Tarragona, E.L., Sebastian, P.S., Saracho Bottero, M.N., Martinez, E.I., Debárbora, V.N., Mangold, A.J., Guglielmone, A.A., Nava, S., 2018. Seasonal dynamics, geographical range size, hosts, genetic diversity and phylogeography of *Amblyomma sculptum* in Argentina. Ticks. Tick. Borne. Dis. 9, 1264–1274. https://doi.org/10.1016/j.ttbdis.2018.04.009

Tkadlec, E., Václavík, T., Kubelová, M., Široký, P., 2018. Negative spatial covariation in abundance of two european ticks: diverging niche preferences or biotic interaction? Ecol. Entomol. 43, 804–812. https://doi.org/10.1111/een.12668

Verity, R., Nichols, R.A., 2016. Estimating the number of subpopulations (K) in structured populations. Genetics 203, 1827–1839. https://doi.org/10.1534/genetics.115.180992

Vilaça, S.T., Biosa, D., Zachos, F., Iacolina, L., Kirschning, J., Alves, P.C., Paule, V., Gortazar, C., Mamuris, Z., Jedrzejewska,B., Borownik, T., Sidorovich, V.E., Kusak, J., Costa, S., Schley, L., Hartl, G.B., Apollonio, M., Bertorelle, G., Scandura, M., 2014. Mitochondrial phylogeography of the European wild boar: the effect of climate on genetic diversity and spatial lineage sorting across Europe. J. Biogeogr. 41, 987–998. https://doi.org/10.1111/jbi.12268

Zając, Z., Woźniak, A., Kulisz, J., 2020. Density of *Dermacentor reticulatus* ticks in eastern Poland. Int. J. Environ. Res. Public Health. 17 (8), 2814. https://doi.org/10.3390/ijerph17082814

Zahler, M., Gothe, R., Rinder, H., 1995. Genetic evidence against a morphologically suggestive conspecificity of *Dermacentor reticulatus* and *D. marginatus* (Acari: Ixodidae). Int. J. Parasitol. 25, 1413–1419. https://doi.org/10.1016/0020-7519(95)00081-X

Zhao, G.-P., Wang, Y.-X., Fan, Z.-W., Ji, Y., Liu, M.-J., Zhang, W.-H., Li, X.-L., Zhou, S.-X., Li, H., Liang, S., Liu, W., Yang, Y., Fang, L., 2021. Mapping ticks and tick-borne

pathogens in China. Nat. Commun.. 12, 1075–1087. https://doi.org/10.1038/s41467-021-21375-1

Legend to figures

Fig. 1. Eurasian haplotype distribution of two nuclear (ITS2, 18S) and three mitochondrial (12S, 16S, COI) markers for *Dermacentor reticulatus*. Sampling sites are indicated by red circles and the associated samples with corresponding haplotype numbers are arranged around. Insets show the haplotype networks for each marker created in TCS 1.21 (Clement et al. (2000)). Circle sizes in the network represent the numbers of individuals, and the colours represent cluster affiliations of the samples; for size and colour assignments see the legend. Maps were created with QGIS Desktop 2.18.4.

Fig. 2. Genetic cluster of 865 Eurasian *Dermacentor reticulatus* ticks from 65 sites inferred with STRUCTURE using 16 microsatellite loci (A) and their geographic distribution (B). Distinct clusters are indicated by colours. (A) Each individual is represented as a vertical band with segments representing particular cluster affiliations. Samples are sorted by sampling sites (numbered according to Supplementary Table S1) from west to east. (B) Circles represent a sampling site with the genetic clusters of all individuals from that site. Grey circles and slices represent individuals with admixed ancestries. The offset shows an enlarged version of Europe for a more detailed view. (C) For better understanding of admixture between clusters, this maps shows only hybrids for specific sampling sites. Various shades of grey represent admixture between different clusters. Symbol sizes correspond to the site sample sizes. Maps were created with QGIS Desktop 2.18.4.

Fig. 3. Extent of the ice shield in northwestern Europe and landmass during the last glacial maximum (LGM) approximately 20,000 years ago. Borders of the ice shields are presented with dashed lines, and the ice shield with a pale colour. Circles represent sampling sites of *Dermacentor reticulatus* with the genetic clusters of all individuals from that site. Grey circles and slices represent individuals with admixed ancestries. Symbol sizes correspond to sample sizes. The map was created using QGIS 2.18.14., with the layers representing the ice shield and landmass downloaded from https://crc806db.uni-koeln.de/layer/show/324/.

Legends to supplementary figures

Supplementary Fig. S1. Values for Δ*K* (above) and mean LnPD (below) for STRUCTURE analyses in *Dermacentor reticulatus*.

Supplementary Fig. S2. Values for Δ*K* for MAVERICK analyses in *Dermacentor reticulatus*.

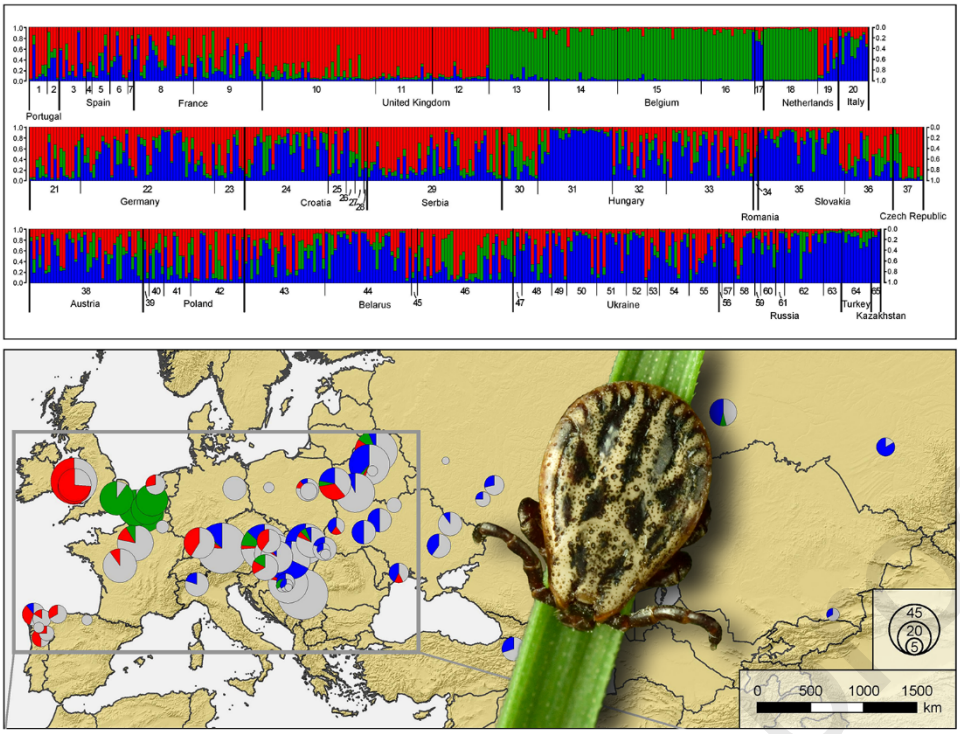
Supplementary Fig. S3. Principal Component Analyses (PCAs) using microsatellite data of 865 *Dermacentor reticulatus* ticks from 65 sites coloured matching STRUCTURE results (A) Axes 1 and 2 and (B) axes 2 and 3. Axis 1 explains 2.08%, axis 2 1.90%, and axis 3 1.80% of the complete genetic variability. Ellipses indicate 95% confidence intervals of applied groups.

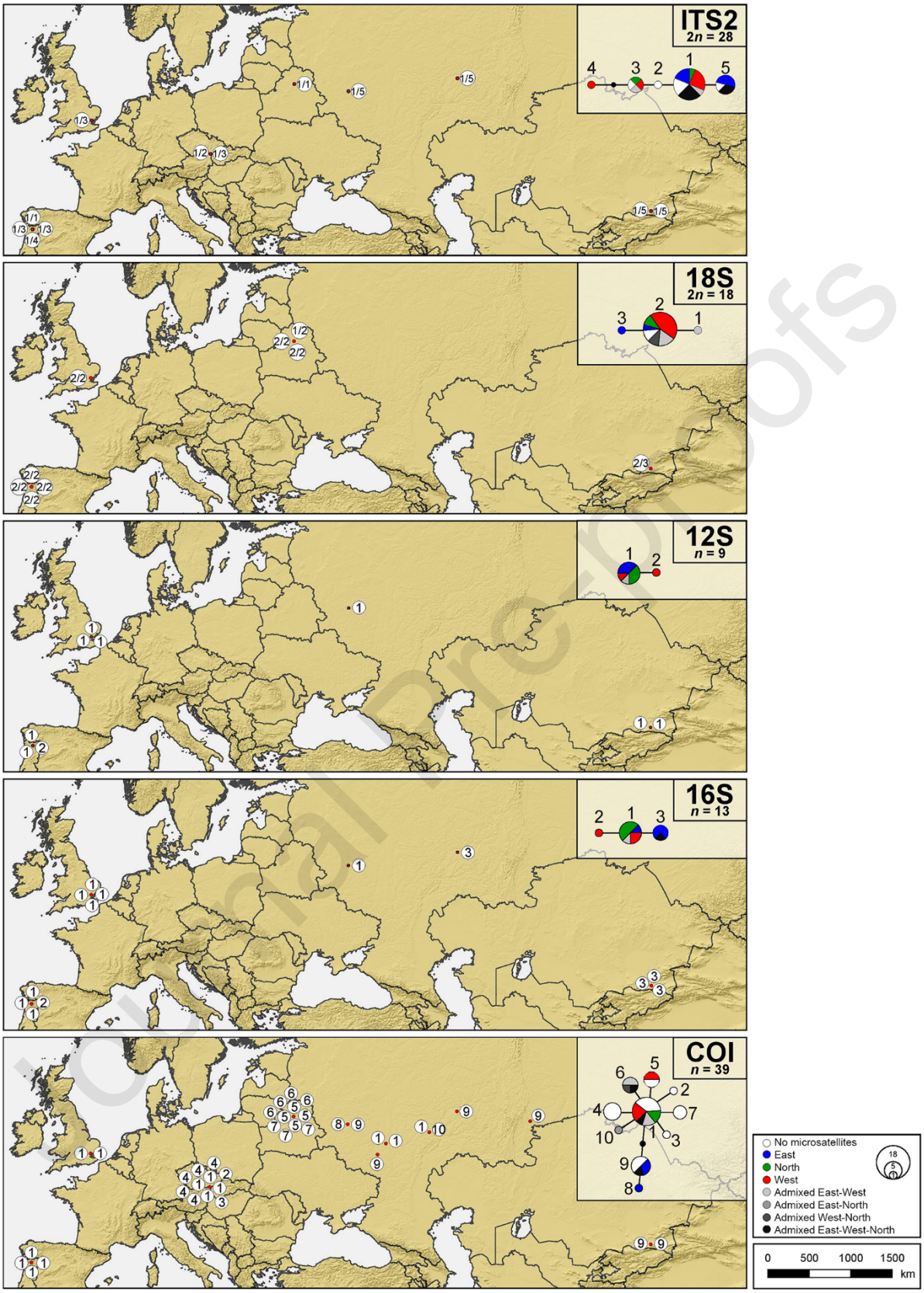
Supplementary Fig. S4. Three-dimensional representation of Principal Component Analyses (PCAs) using microsatellite data of (A) 865 *Dermacentor reticulatus* ticks from 65 sites coloured according to STRUCTURE cluster, and (B) 423 *D. reticulatus* ticks of three STRUCTURE clusters, with admixed individuals excluded.

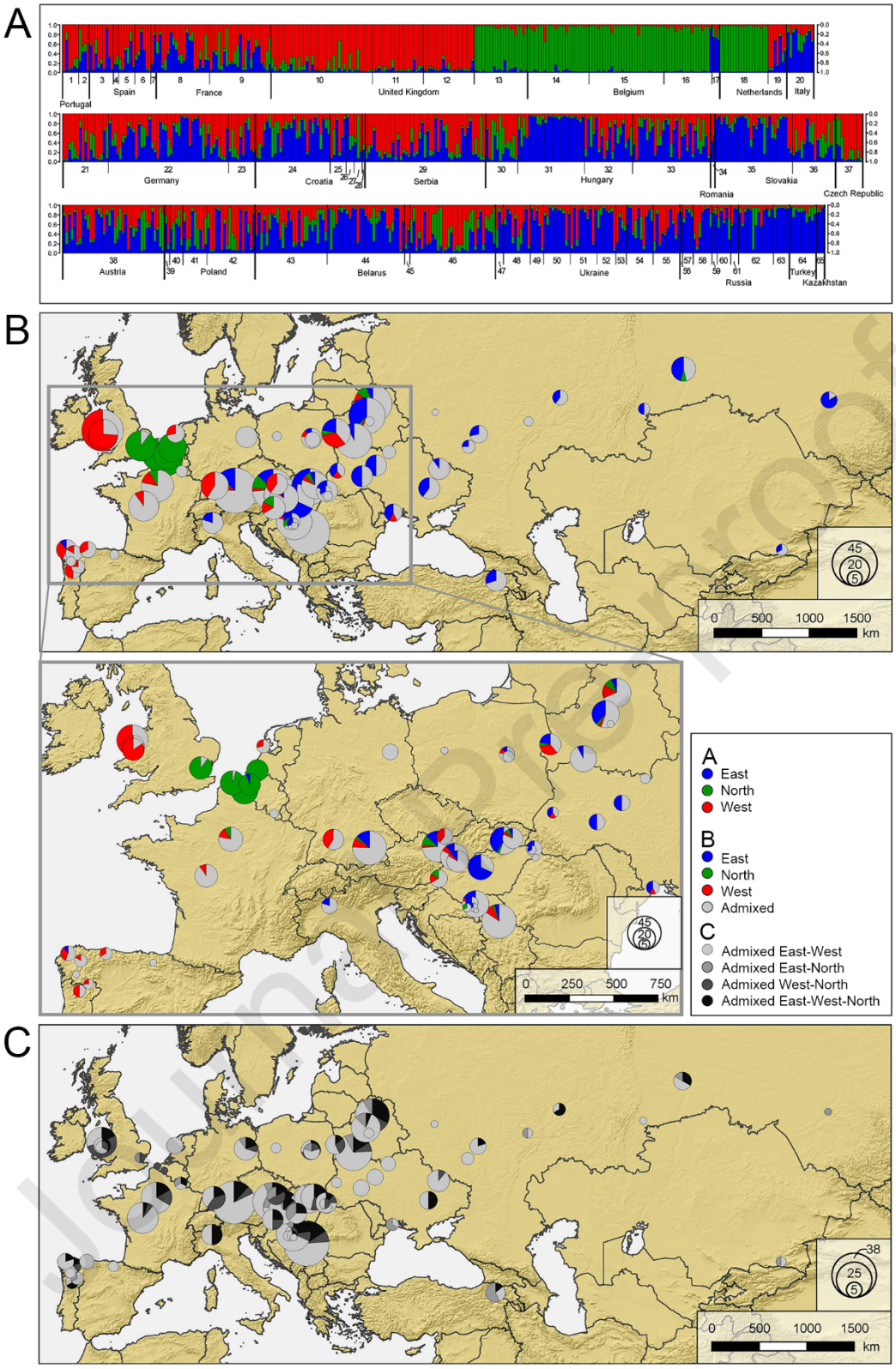
Supplementary Fig. S5. Model checking of the DIYABC scenario.

Highlights

- Low haplotype diversity in Eurasian *Dermacentor reticulatus* populations forms a bipolar pattern along an east-west cline.
- Microsatellite analysis differentiates the dataset into three clusters.
- The northern cluster arose only ca. 200-800 years ago.
- The spread of clusters reflects the current range expansion, leading to increased admixture.



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