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# Ticks and Tick-borne Diseases

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## Original article

## Prevalence of different *Rickettsia* spp. in *Ixodes ricinus* and *Dermacentor reticulatus* ticks (Acari: Ixodidae) in north-eastern Poland

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## ABSTRACT

In two surveys conducted in April and May 2013, a total of 1148 unfed ticks belonging to two species, *Ixodes ricinus* and *Dermacentor reticulatus*, were collected by flagging lower vegetation in 15 different localities throughout the Białowieża Primeval Forest (Podlaskie voivodship; north-eastern Poland) and in its buffer zone. In order to establish their infection rate with *Rickettsia* spp. individual adult ticks and pooled nymphs were tested by real-time PCR targeting the *gltA* gene. For the further identification of rickettsial species, positive samples were subjected for nested and semi-nested PCR targeting *ompA* and 16S rRNA genes, respectively, followed by sequencing analysis. Rickettsial DNA was detected in at least 279 ticks (minimum infection rate [MIR], 23.9%), including 52 nymphal and adult *I. ricinus* (MIR 8.6%) and 222 adult *D. reticulatus* (41%). Three species of SFG rickettsiae were identified: *Rickettsia helvetica* and 'Candidatus *R. mendelii*' in *I. ricinus* and *R. raoultii* in *D. reticulatus* and *I. ricinus*. Moreover, unidentified *Rickettsia* spp. which showed 99.4% identity, among others, with the uncultured *Rickettsia* sp. isolated from *Cicadella viridis* leafhopper, *Rickettsia* endosymbiont of *Lasioglossum semilucens* bee and *R. bellii*, were detected in *I. ricinus*, while *Rickettsia* sp. 98.3–98.4% homologous to *Rickettsia* secondary endosymbionts of *Curculio* spp. weevils was found in *D. reticulatus*.

These results confirm the diversity of rickettsiae occurring in Poland. Further studies are needed to expand the knowledge on the species spectrum, prevalence and epidemiology of SFG rickettsiae in the country.

## 1. Introduction

*Ixodes ricinus* and *Dermacentor reticulatus* (Acari, Ixodidae) are the two main and most common tick species in Poland. The first one exists throughout the country where hundreds of locations have been recorded. It readily attacks people and is one of the arthropods of the greatest significance in the epidemiology of vector-borne diseases (Nowak-Chmura and Siuda, 2012). The second occurs mainly in the north-eastern and the eastern part of the country but recently its presence in south-western and western Poland has been recorded (Nowak, 2011; Kiewra and Czułowska, 2013; Mierzejewska et al., 2016). As *D. reticulatus* rarely attacks humans, its direct impact on public health and its relative contribution to the disease burden caused by tick-borne pathogens is relatively small in many regions of Europe, however, it should definitely not be ignored (Földvári et al., 2016).

In the Białowieża Primeval Forest (Puszcza Białowieska) and its buffer zone (Podlaskie voivodship, NE Poland) *I. ricinus* and *D. reticulatus* occur sympatrically. Previous studies conducted in this area

have shown that *I. ricinus* ticks were frequently infected with *Borrelia burgdorferi* sensu lato (Stańczak et al., 1999, 2000) and *Anaplasma phagocytophilum* (Grzeszczuk et al., 2004) while *D. reticulatus* harboured *A. phagocytophilum* (Matsumoto et al., 2009) and tick-borne encephalitis virus (TBEV) (Biernat et al., 2014, 2016a). Moreover, during preliminary studies conducted in the Białowieża Primeval Forest and throughout the Podlaskie voivodship both tick species were found to carry also *Rickettsia* spp. (Stańczak, 2006; Stańczak et al., 2008; Chmielewski et al., 2009). Taxonomy classifies rickettsiae (Rickettsiales, Rickettsiaceae) into 4 groups: the basal *R. bellii* and *R. canadensis* groups, the typhus group (TG) and the spotted fever group (SFG) rickettsiae. The obligate, intracellular bacteria of the last mentioned group are etiological agents of tick-borne rickettsioses, which belongs to the oldest-known transmissible diseases. So far, in Europe, at least 11 species have been identified as pathogens. Moreover, a few species of unconfirmed pathogenicity, e.g. *R. hoogstraalii* and *Rickettsia* genotype AvBAT, as well as many nonvalidated, partially described or uncultivated species were isolated or detected in different tick species

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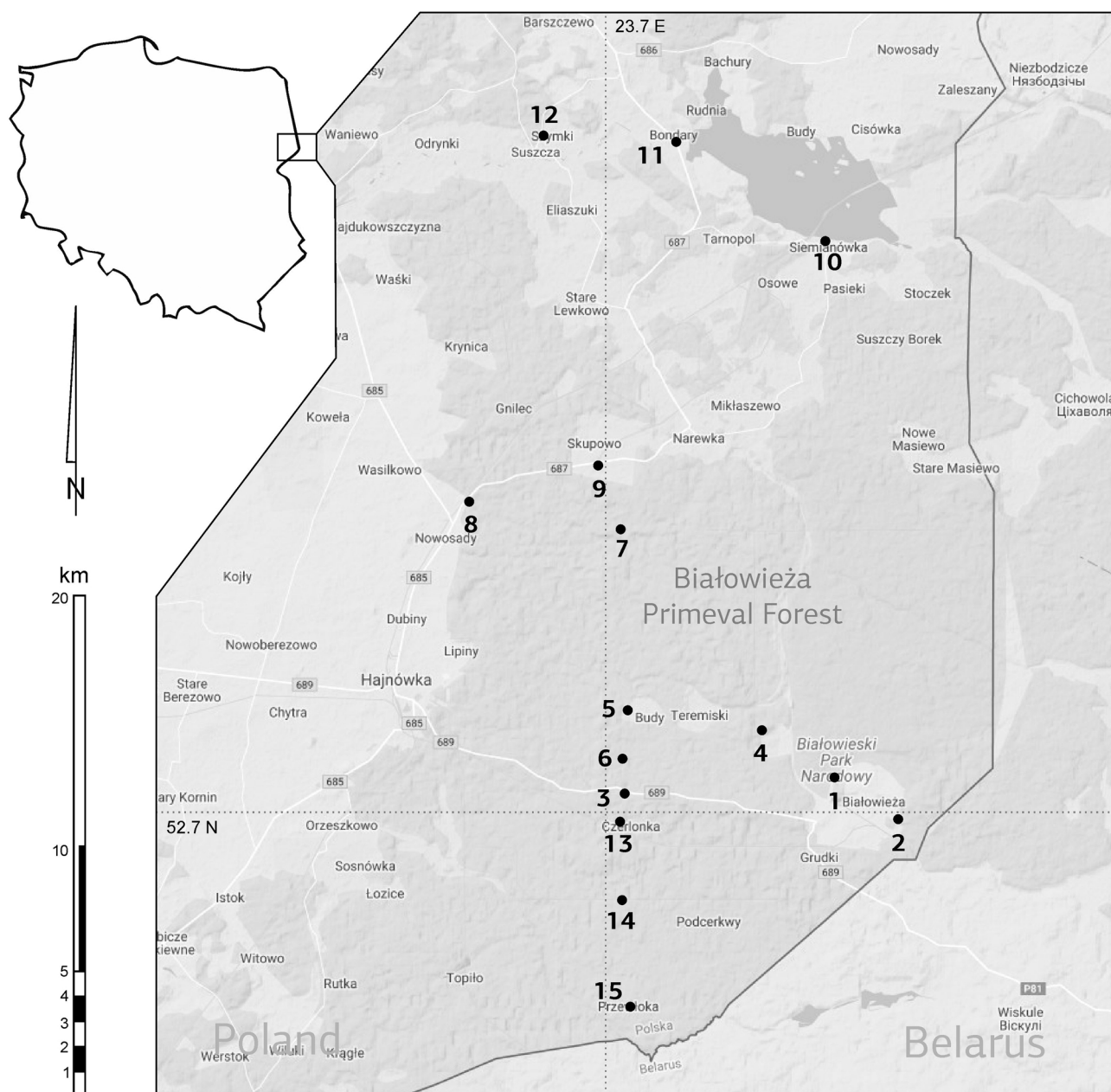


Fig. 1. Distribution of the tick collection sites in the Białowieża Primeval Forest and its buffer zone (April & May 2013) (Numbers 1–15 correspond to the numbers of particular tick collection sites given in Table 2).

(Parola et al., 2013). Recently, ‘*Candidatus Rickettsia mendelii*’ has been detected in *I. ricinus* in the Czech Republic (Hajduskova et al., 2016) and a novel *R. raoultii* strain Jongejan in *D. reticulatus* in Austria (Vijnveld et al., 2016).

In Poland, four species belonging to the SFG rickettsiae: *R. raoultii*, *R. slovacica*, *R. helvetica* and *R. massiliae* have been reported so far in *I. ricinus* and/or *D. reticulatus* (Stańczak et al., 2008; Chmielewski et al., 2009; Rymaszewska and Piotrowski, 2013; Mierzejewska et al., 2015). All but the last are proved pathogens for humans (Nilsson et al., 2005; de Sousa et al., 2013; Jia et al., 2014).

Here, besides *R. raoultii* and *R. helvetica*, we report for the first time in Poland the occurrence of ‘*Candidatus Rickettsia mendelii*’ and new strains of *Rickettsia*, which seem to be secondary endosymbionts, in *I. ricinus* and *D. reticulatus* from the Białowieża Primeval Forest.

## 2. Material and methods

### 2.1. Study area

The Białowieża Primeval Forest (Puszcza Białowieska) (Fig. 1) stretches on both sides of the border between Poland and Belarus. It is the best preserved natural forest complex in lowland Europe and in lots of fragments still retains characters of a primeval forest. It covers 141,885 ha with a buffer zone of 166,708 ha and maintains a diverse complex of mixed forests and a range of associated non-forest habitats, including wet meadows, river valleys and other wetlands. Its western part, situated in Poland [52°29′ – 52°57′N, 23°31′ – 24°21′E], covers the area of 62,500 ha (647 km<sup>2</sup>), a sixth of which belongs to Białowieża National Park (Białowieżski Park Narodowy), the oldest national park in the country. The large and integral forest area creates a favourable conditions for diverse and rich wildlife including large mammals: red deer (*Cervus elaphus*), elks (*Alces alces*) and the largest free-roaming

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