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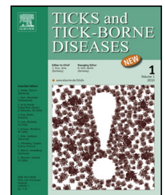

Original article

Natural hybridization between *Ixodes ricinus* and *Ixodes persulcatus* ticks evidenced by molecular genetics methods

S.Y. Kovalev[a,*], I.V. Golovljova[b], T.A. Mukhacheva[a]

[a] *Laboratory of Molecular Genetics, Ural Federal University, Yekaterinburg, Russia*
[b] *Department of Virology, National Institute for Health Development, Tallinn, Estonia*

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ABSTRACT

The recently shown phenomenon of natural hybridization between *Ixodes persulcatus* and *Ixodes pavlovskyi* ticks (Kovalev et al., 2015) stimulated similar studies in the sympatric zones of other tick species. In the present paper, 265 *Ixodes ricinus* and *I. persulcatus* ticks from Estonia were subjected to a search for interspecific hybrids based on nuclear (ITS2) and mitochondrial (cox1) markers as well as morphological features. Surprisingly, only 72.1% of ticks morphologically identified as *I. ricinus* actually were *I. ricinus* both at nuclear and mitochondrial markers, while the accuracy of morphological species identification for *I. persulcatus* was 99.3%. Among ticks morphologically identified as *I. ricinus*, 24.6% turned out to be interspecific hybrids and 3.3% were *I. persulcatus*. Generally, about 11% of the individuals studied were shown to be interspecific hybrids with different levels of nuclear DNA introgression. The analysis of hybrid populations proved the mating pair female *I. ricinus* × male *I. persulcatus* to form hybrids more efficiently, then female *I. persulcatus* × male *I. ricinus*. The same trend can be observed for backcrosses preferentially mating with *I. ricinus*. Hybridization between *I. ricinus* and *I. persulcatus* proved the existing view about their reproductive isolation to be untenable. Interspecific hybridization occurring between both closely (*I. persulcatus* and *I. pavlovskyi*) and more distantly (*I. ricinus* and *I. persulcatus*) related *Ixodes* species could introduce novel alleles that modify vector competence, host use or the ability to exploit diverse microhabitats.

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1. Introduction

The *Ixodes ricinus* (*Ixodes ricinus* – *Ixodes persulcatus*) species complex (Acari: Ixodidae) is a group of phylogenetically related species of hard ticks (Snow and Arthur, 1970). This complex includes at least fourteen species (e.g., *I. ricinus* L., *I. persulcatus* Schulze and *Ixodes pavlovskyi* Pomerantsev) distributed in almost all geographic regions of the world and is regarded as a monophyletic group (Xu et al., 2003). Several tick species within the complex are well known as main vectors of tick-borne diseases, such as tick-borne encephalitis, Lyme disease, Looping ill and babesiosis (Anderson, 1989; de la Fuente et al., 2008).

The geographical ranges of different tick species can often overlap thus forming large sympatric zones. For example, the western part of the distributional range of the taiga tick *I. persulcatus* (East European Plain, Baltic countries) overlaps with that of *I. ricinus*, which is distributed over most of Europe. The eastern part of the distributional range of *I. persulcatus* forms two disjunctive sympatric zones with *I. pavlovskyi* in Western Siberia and the Far East (Filippova, 1999).

The co-occurrence of two tick species in the same territory allows the possibility of their hybridization in nature. Recently, genetic evidence of hybridization between *I. persulcatus* and *I. pavlovskyi* in Western Siberia was obtained (Kovalev et al., 2015). Moreover, the research revealed not only first generation hybrids but also backcrosses and mtDNA introgression, which indicated the fertility of F1 hybrids and the lack of genetic isolation between *I. persulcatus* and *I. pavlovskyi*. The results obtained suggested that hybridization between different tick species in their sympatric zones may be considered as a common phenomenon. However, it has been shown only for phylogenetically closely related species such as *I. persulcatus* and *I. pavlovskyi*. To make the hypothesis tenable, the ability of more distantly related tick species, such as *I. ricinus* and *I. persulcatus*, to form interspecific hybrids in nature should be investigated. Previous studies have shown that *I. ricinus* and *I. persulcatus* do not have morphological obstacles to copulation and are encountered on the same hosts in their sympatric zones (Filippova, 1999). However, the sterility of the experimentally obtained F1 hybrids (Balashov et al., 1998) as well as unsuccessful efforts to morphometrically detect these hybrids in natural

* Corresponding author.
E-mail address: Sergey.Kovalev@urfu.ru (S.Y. Kovalev).

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Fig. 1. Collection sites of the ticks studied in the present paper.

populations (Filippova, 2002) may have hampered the search for hybrids for a long time. In 2014, though, discriminant analysis of larval morphological features suggested the existence of hybrid larvae in the sympatric populations of *I. ricinus* and *I. persulcatus* (Bugmyrin et al., 2015).

In the present paper, we have investigated a sympatric population of *I. ricinus* and *I. persulcatus* from Estonia for the presence of interspecific hybrids, using the strategy proposed previously for *I. persulcatus* and *I. pavlovskyi* (Kovalev et al., 2015). Here, for the first time, we present molecular genetic evidence for the existence of hybrids between *I. persulcatus* and *I. ricinus* in their sympatric zone.

2. Materials and methods

2.1. Material collection and morphological species identification

265 ticks (nymphs – 67, adults – 106 females and 92 males) were collected by flagging in Estonia in 2006, 2007 and 2008, near the following settlements: Alajõe (59°00′ N, 27°26′ E), Järvselja (58°16′ N, 27°19′ E), Kilingi-Nõmme (58°09′ N, 24°57′ E), Laeva (58°29′ N, 26°24′ E), Lemmaku (59°00′ N, 27°09′ E), Tähtvere (58°24′ N, 26°41′ E), Tudulinna (59°05′ N, 26°57′ E), Väike-Pungerja (59°12′ N, 27°23′ E), Vaikla (59°04′ N, 27°21′ E), and Mõniste (57°61′ N, 26°61′ E) (Fig. 1). Morphological identification of the tick species was performed using available morphological keys (Filippova, 1977).

2.2. DNA extraction

Individual ticks were homogenized in 300 ml of PBS by TissueLyser (Retsch, Haan, Germany). The suspensions (200 μl) were used for DNA extraction by the guanidinium thiocyanate–phenol–chloroform method using the TriPure isolation system (Roche Diagnostics, Lewes, UK) according to the manufacturer's recommendations. Sterile water was included as a negative control for every DNA preparation set.

2.3. Real-time PCR and sequencing

The research strategy aimed at detection of interspecific hybrids as well as revealing mtDNA introgression is published in detail previously (Kovalev et al., 2015) and consists of two real-time PCRs (RT-PCRs) with species-specific probes for both mitochondrial and nuclear markers, subsequent cloning, sequencing and phylogenetic analysis.

Primers and species-specific TaqMan probes were designed based on the alignment of the ITS2 and *cox1* sequences of *I. ricinus* and *I. persulcatus* available in GenBank; their specificity was successfully tested on allopatric populations of both species. The first RT-PCR reaction was targeted on the mitochondrial gene *cox1* (first subunit of cytochrome c oxidase), primers and probes used are given in Table 1, PCR conditions were as described previously (Kovalev et al., 2015).

The same tick DNA samples were subjected to a second RT-PCR targeted on the nuclear ribosomal internal transcribed spacer 2 – ITS2 (Table 1). To increase the sensitivity of the assay, the full-length ITS2 sequence was amplified for all samples prior to RT-PCR, during 20 cycles of PCR, total volume 5 μl, conditions were as described previously (Fukunaga et al., 2000). This reaction mixture after a preliminary ITS2 amplification was used as a template for RT-PCR. Each PCR experiment contained sterile water as a negative control and an experimentally mixed DNA of "pure" *I. persulcatus* and *I. ricinus* (1:1) as a positive control.

The RT-PCR targeted on the ITS2 was carried out in 25 μl, the reaction mixture containing 4 μl of the forward primer ITS2_B-TicksRP-F (5 μM), 1 μl of the reverse primer ITS2_B-TicksRP-R (5 μM) and 1 μl of each of the probes (5 μM), $MgCl_2$ (3 mM), dNTPs (100 μM each), and 1.5 U of TaqF DNA polymerase (InterLabService, Moscow, Russia). The molar concentration of the forward primer ITS2_B-TicksRP-F was in 4-fold excess because of its degeneracy in three positions (Table 1). RT-PCR conditions were as follows: 35 cycles of denaturation at 94 °C for 15 s, primer annealing and elongation at 68 °C for 1 min.

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