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Differentiation of three species of ixodid tick, *Dermacentor andersoni*, *D. variabilis* and *D. albipictus*, by PCR-based approaches using markers in ribosomal DNA

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Abstract

Three species of *Dermacentor*, *Dermacentor albipictus, Dermacentor andersoni* and *Dermacentor variabilis*, commonly occur in Canada. *D. andersoni* and *D. variabilis* are morphologically similar and are important vectors of human and animal pathogens. A practical polymerase chain reaction (PCR) assay, based on the amplification of part of the second internal transcribed spacer ribosomal DNA (pITS-2 rDNA), was developed to distinguish *D. andersoni* from *D. variabilis*. In addition, single-strand conformation polymorphism (SSCP) analysis of the pITS-2 rDNA provided a reliable method of distinguishing specimens of the three species of ixodid tick. PCR and pITS-2 SSCP were also used to determine whether there was hybridization between *D. andersoni* and *D. variabilis* at two localities in Saskatchewan where they occur in sympatry. These molecular tools will be useful for the unequivocal identification of *D. andersoni* and *D. variabilis* at all life cycle stages, which is essential for studies on their ecology and on the transmission of tick-borne diseases. Also, pITS-2 SSCP may be of potential use for discriminating among the other morphologically similar species within the genus *Dermacentor*.

Keywords: *Dermacentor andersoni*; *Dermacentor variabilis*; *Dermacentor albipictus*; Species identification; Polymerase chain reaction (PCR); Single-strand conformation polymorphism (SSCP)

1. Introduction

The American dog tick, *Dermacentor variabilis*, and the Rocky Mountain wood tick, *Dermacentor andersoni*, are important vectors of human and animal pathogens in North America [1,2]. These two tick species require three hosts to complete their life cycle. Larvae and nymphs use small mammals, such as voles, chipmunks, deer mice, jumping mice, white-footed mice, and ground squirrels, as hosts. Adult ticks utilize medium sized to large mammals, including raccoons, skunks, horses, cattle and mule deer [2–5]. *D. andersoni* and *D. variabilis* have largely allopatric distributions, except in some parts of Saskatchewan (Canada), Montana, Nebraska, North Dakota and South Dakota (USA) [2,6–9], where they occur in sympatry. In some geographical areas, their distributions overlap with that of the winter tick, *Dermacentor albipictus* [3,6]. The winter tick requires only a single host (e.g., moose, caribou, elk, white-tailed deer, mule deer and cattle) to complete its life cycle [3,5].

These three species of *Dermacentor* can be distinguished from one another by differences in their morphology. Adult *D. albipictus* lack a dorsal prolongation on their spiracular plates and have fewer but larger goblets within the spiracular plates than *D. andersoni* or *D. variabilis*. The spiracular plates of *D. andersoni* have a more pronounced dorsal prolongation and contain fewer but larger goblets compared with *D. variabilis* [3]. However, there is variation in the features of the spiracular plate among specimens of *D. andersoni* and *D. variabilis*, which may make identification difficult, particularly for ticks collected at localities where the two species coexist. Hybrid adults, derived from laboratory experimental crosses between female *D. variabilis* and male *D. andersoni*, have also been shown to have spiracular plates that are intermediate in morphology

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between the two species [10]. Therefore, it is important that there are markers available that can be used to unequivocally distinguish specimens of *D. andersoni* from *D. variabilis*.

Molecular techniques have been used effectively to identify ticks to the genus and/or species level [11–16], and to examine the phylogeny and/or taxonomic status of some species [11,17]. The target regions used in these studies included the nuclear 18S ribosomal (r) RNA gene and second internal transcribed spacer (ITS-2), and the mitochondrial 12S and 16S ribosomal genes [11–17]. For example, Zahler et al. [11] examined the species status of *Dermacentor reticulatus* and *Dermacentor marginatus* using a comparison of the ITS-2 rDNA sequences of these two taxa, and those of *D. andersoni* and *D. variabilis*. The aim of the present study was to establish a practical and effective polymerase chain reaction (PCR)-based assay to distinguish *D. variabilis* from *D. andersoni*, based on interspecific differences in the ITS-2 sequences [11], and to determine whether there is any evidence of hybridization in areas where the two species occur in sympatry. Furthermore, the use of the mutation scanning technique, single-strand conformation polymorphism (SSCP), was evaluated as a diagnostic tool to distinguish among specimens of *D. andersoni*, *D. variabilis* and *D. albipictus*.

2. Materials and methods

2.1. Ticks

Adult ticks were collected by flagging grassy and shrubby vegetation along walking tracks in provincial parks in Saskatchewan (Blackstrap, Saskatchewan Landing and Buffalo Pound) and Alberta (Cypress Hills), Canada. Ticks were identified morphologically as either *D. andersoni*, *D. variabilis* or *D. albipictus* (Table 1), based on the shape of the spiracular plates, and on the relative size and number of the goblets within the spiracular plates [6]. Adult *D. albipictus* were included in the study for comparative purposes. Each tick was frozen at −70 °C until required for the molecular work. Also included for comparison were unfed *D. albipictus* larvae derived from the eggs of two engorged females collected from moose near Prince George (British Columbia) and Calgary (Alberta).

Table 1
Number of adult *Dermacentor* used in this study

Collection site (provincial park)	No. of adult individuals		
	D. andersoni	*D. variabilis*	*D. albipictus*
Blackstrap	–	33	–
Saskatchewan Landing	48	38	2
Buffalo Pound	34	39	–
Cypress Hills	20	–	–
Total	102	110	2

2.2. DNA purification and PCR

Genomic DNA (gDNA) was extracted and purified from either one or two legs, or the complete body of adult ticks using the DNeasy Tissue Kit™ (Qiagen). The use of only one or two legs permits the remainder of the specimen to be preserved for morphological examination or to be tested for the presence of pathogenic organisms. The leg(s) or complete body of an individual tick was/were placed into a 1.5 ml micropestle tube (Kontes) with 180 µl of ATL Buffer (Qiagen) and homogenized using a micropestle attached to a cordless drill. Proteinase K (20 µl of 15 µg/µl) was then added to the micropestle tube and the sample incubated overnight at 55 °C. Two hundred microlitres of AL Buffer (Qiagen) were added to the sample, vortexed and incubated for 10 min at 70 °C. Then, 200 µl of 100% ethanol were added, and the solution was applied to a spin column. After rinsing the columns with the wash buffers AW1 and AW2 (Qiagen), gDNA was eluted with 100 µl AE buffer (Qiagen) and stored at −70 °C. The gDNA from two whole individual *D. albipictus* larvae was extracted and purified using the same methodology.

Part of the ITS-2 (pITS-2) rDNA was amplified from gDNA using the forward primer DAVF (5′-TCACATATCAAGAGAGCCTT-3′) and reverse primer DAVR (5′-ACGTACTTCGAAGGCAAACA-3′), designed based on previously published sequences of *D. andersoni* and *D. variabilis* (GenBank accession nos. AY365355–AY365363 and S83088; [11,18]). The PCR was performed in 25 µl containing 200 µM of each dNTP (Promega), 3 mM $MgCl_2$, 50 pmol of each primer and 0.5 U of *Taq* polymerase (Promega) using a thermal cycler (Bio-Rad iCycler) with the following conditions: 95 °C, 5 min (initial denaturation); 30 cycles of 95 °C, 30 s (denaturation), 52 °C, 30 s (annealing), and 74 °C, 30 s (extension); followed by 74 °C for 5 min (final extension). A negative (i.e. without gDNA) control was included in each PCR run. Individual amplicons were subjected to electrophoresis on SYBR-safe™ (Molecular Probes) stained 2% agarose-TBE (EMD Biosciences; 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) gels. A 100 bp TrackIt™ DNA ladder (Invitrogen) was used on gels as a size standard.

2.3. SSCP analysis

Amplicons from representative samples of each species were also subjected to SSCP analyses. In thin-walled tubes, individual amplicons (1 µl) were mixed with 4 µl of DNase-free water and 5 µl of loading buffer (Gel Tracking Dye™, Promega), then denatured at 95 °C for 5 min prior to snap cooling in ice water for 5 min. Each sample (5 µl) was loaded into the wells of precast GMA™ S-50 gels (Elchrom Scientific) and subjected to electrophoresis for 18 h at 74 V and 7.4 °C (constant) in a horizontal SEA2000™ apparatus (Elchrom Scientific) connected to a temperature-controlled circulating water bath. Following electrophoresis, gels were stained for 30 min with