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Phylogenetic and phylogeographic relationships in *Ixodes holocyclus* and *Ixodes cornuatus* (Acari: Ixodidae) inferred from COX1 and ITS2 sequences ☆

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ABSTRACT

We inferred the phylogenetic and phylogeographic relationships in ticks, which were identified morphologically as *Ixodes holocyclus* and *Ixodes cornuatus*, from mitochondrial cytochrome oxidase subunit 1 (COX1) and nuclear internal transcribed spacer 2 (ITS2) sequences. We obtained COX1 (640 bp) and ITS2 (527–568 bp) sequences from 429 ticks from 49 localities in Tasmania, Victoria, New South Wales and Queensland, Australia. Our analyses show that there are two species of *Ixodes* in eastern Australia that cause paralysis in dogs and other mammals: *I. holocyclus* and *I. cornuatus*. Further, we conclude that the morphological criteria used to differentiate female *I. holocyclus* and *I. cornuatus* are equivocal but *I. holocyclus* can be distinguished from *I. cornuatus* using COX1 and/or ITS2 sequences. Intraspecific genetic variation in *I. holocyclus* and *I. cornuatus* was less than 0.86% and 0.19% for COX1 and ITS2, respectively. *Ixodes holocyclus* could be genetically distinguished between different geographic ranges. There were no significant genetic differences between *I. cornuatus* from Tasmania and mainland Australia, but there are some COX1 haplotypes of *I. cornuatus* from the mainland that were not detected in Tasmanian and vice versa.

1. Introduction

Ixodes holocyclus and Ixodes cornuatus are endemic tick species to Australia and cause paralysis in a similar range of host species in livestock (Bagnall and Doube, 1975; Doube, 1975; Doube et al., 1977), pet animals (Ilkiw et al., 1987; Jackson et al., 2007) and humans (Pearn, 1977; Taylor and Murray, 1946). *Ixodes holocyclus* has a narrow, discontinuous distribution along the east coast of Australia from north Queensland to Bairnsdale in Victoria (Roberts, 1960; Jackson et al., 2007), while *I. cornuatus* is found in central and eastern Victoria, and in Tasmania (Roberts, 1960, 1970; Arundel and Sutherland, 1988; Jackson et al., 2007) (Fig. 1). The morphology of *I. holocyclus* and *I. cornuatus* has been studied extensively (Roberts, 1960, 1970; Mason et al., 1974; Jackson et al., 2002), but identification of specimens is still difficult. Morphological characters—the shape of papal article I, the spur on coxa I and the cornua—were used to distinguish female adults of *I. holocyclus* from *I. cornuatus* (Roberts, 1960, 1970). Differences in the chaetotaxy were also described between larvae of the two species (Kemp, 1980). However, due to limited morphological traits, lack of autapomorphies, intra-specific variation in key diagnostic features, and no description of nymphs of *I. cornuatus*, it is often difficult to distinguish *I. holocyclus* and *I. cornuatus* from each other (Roberts, 1970; Mason et al., 1974; Jackson et al., 2000). This difficulty has constrained studies on tick biology, clinical diagnosis and treatment of diseases caused by the two ticks.

Molecular markers have been used in phylogenetic and taxonomic investigations of *I. holocyclus* and *I. cornuatus* during the last two decades (Andrews et al., 1992; Jackson et al., 1998, 2000; Shaw et al., 2002). However, there are still uncertainties about whether or not *I. holocyclus*, as currently defined morphologically, represents a single species or a species complex and whether or not *I. cornuatus* from Tasmania and mainland Australia are different species. Jackson et al. (1998, 2000) analysed the genetic variation in 91 samples of *I. holocyclus* and *I. cornuatus* at 24 allozyme loci and concluded that the two species were genetically distinct, and *I. holocyclus* represented a species complex. In a later study on the nucleotide variation of the internal transcribed spacer 2 (ITS2) in 20 *I. holocyclus* individuals from 17 localities throughout eastern Australia, Shaw et al. (2002) argued that *I. holocyclus* was a single species, despite geographic isolation of some populations. Moreover, based on genetic polymorphism at 24 enzyme loci, Jackson et al. (1998) reported that *I. cornuatus* from Tasmania

☆ Nucleotide sequence data reported in this paper are available in GenBank under accession numbers HM545748–HM545846 (COX1) and HM581926–HM581931 (ITS2).

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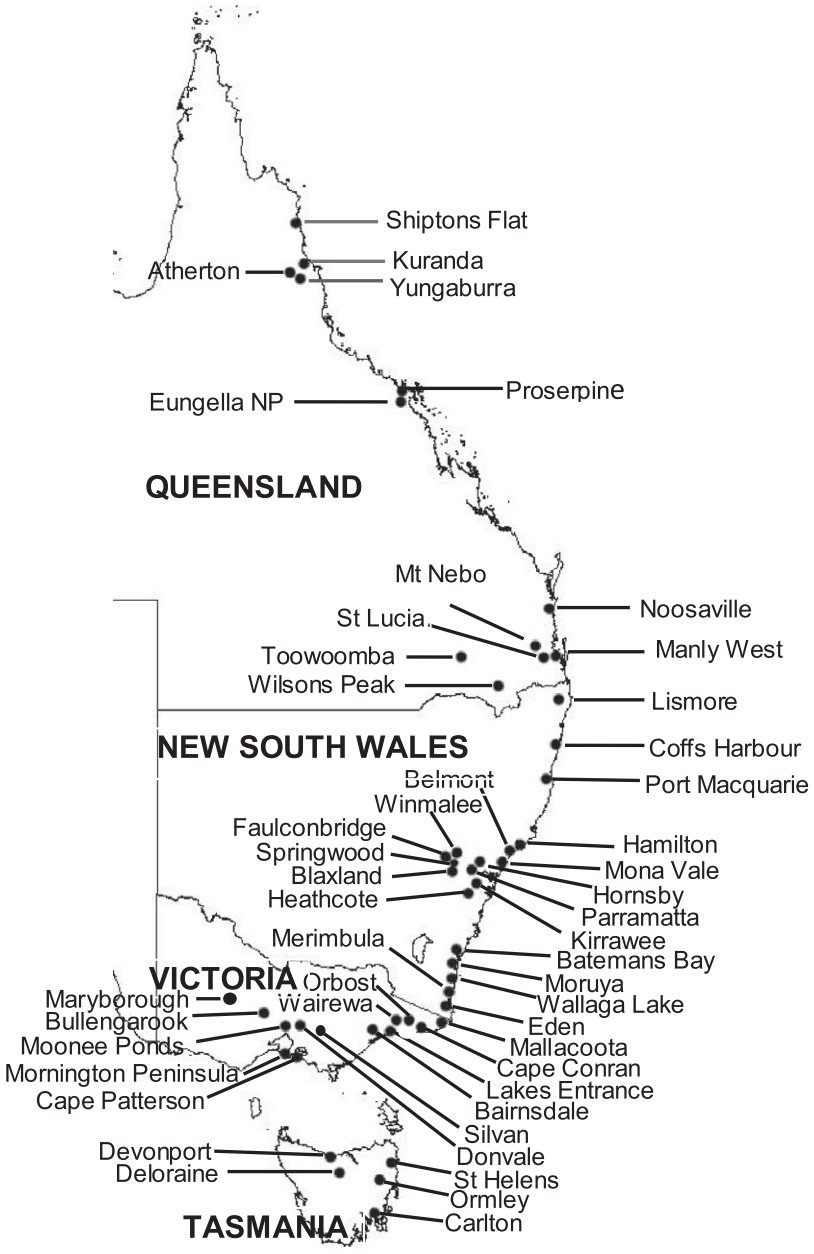

Fig. 1. Geographical localities from where *Ixodes holocyclus* and *Ixodes cornuatus* were collected on mainland Australia and in Tasmania.

and the Australian mainland were different species, but they later concluded that they were a single species based on a study involving more samples (Jackson et al., 2000). Despite these research efforts genetic variants between/within geographically distinct localities of *I. holocyclus* and *I. cornuatus* remained uncertain.

Nuclear rRNA and/or ITS sequences have been applied to phylogenetic analyses of tick species (Mclain et al., 1995; Crampton et al., 1996; Zahler et al., 1997; Barker, 1998; Fukunaga et al., 2000; Murrell et al., 2001; Shaw et al., 2002). mtDNA has also been used as a species-level marker for phylogenetic and taxonomic studies of ticks due to its higher mutation rate, maternal inheritance and haploid nature (Norris et al., 1996; Kain et al., 1999; Murrell et al., 1999, 2001). In addition, sequences of the mitochondrial cytochrome oxidase subunit I (COX1) gene have been used for phylogenetic analyses of morphologically similar tick species (Murrell et al., 2000; Murrell and Barker, 2003; Mitani et al., 2007). Thus, both COX1 and ITS sequences might resolve uncertainties relating to the phylogenetic and phylogeographic status of *I. holocyclus* and *I. cornuatus* in Tasmania and mainland Australia. The present study was aimed at a comparative analysis of phylogeographic structure, genetic diversity and divergence of lineages of *I. holocyclus* and *I. cornuatus* based on a large sample size using sequences of COX1 and ITS2.

2. Materials and methods

2.1. Tick collection and preparation

Ticks were studied from 49 localities throughout their geographic range in mainland Australia and Tasmania (Table 1, Fig. 1). We collected ticks from dogs exhibiting clinical signs of tick