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Identification of *Theileria fuliginosa*-like species in *Ixodes australiensis* ticks from western grey kangaroos (*Macropus fuliginosus*) in Western Australia

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ABSTRACT

Piroplasms, including the genera *Babesia* and *Theileria*, are intra-erythrocytic protozoa that are generally transmitted by ticks and are the aetiological agents for piroplasmosis in animals, as well as humans, worldwide. In Australia, numerous studies have been conducted on piroplasms in domestic animals; however, less is known about these protozoa in ticks from native wildlife. The present study characterised piroplasms in *Ixodes australiensis* (n = 119) and *Amblyomma triguttatum* (n = 35) ticks collected from kangaroos in Western Australia (WA). Approximately 7.6% (9/119) (95% CI 2.8–12.2) of the *I. australiensis* ticks were positive for piroplasms using nested-PCR at the 18S rRNA locus, whereas no piroplasm 18S rDNA was detected in the *A. triguttatum* ticks. All sequences from *I. australiensis* ticks were identical. Using a 852 bp multiple nucleotide alignment at the 18S rRNA variable region, sequences shared 97.6%, 94.3%, 93.5% and 93.4% pairwise identity with *Theileria fuliginosa*, *Theileria brachyuri*, *Theileria penicillata*, and a *Theileria* sp. (K1), derived from a burrowing bettong or boodie (*Bettongia lesueur*), respectively. Phylogenetic analysis revealed that the *Theileria* sp. from *I. australiensis* clustered together in the marsupial-associated *Theileria* group, with *T. fuliginosa* as closest sister species. Hence, we conclude that this is the first observation of *T. fuliginosa*-like species in *I. australiensis* ticks parasitising kangaroos in WA.

1. Introduction

Piroplasms (Order: Piroplasmorida) are tick-borne haemoprotozoan parasites comprising three genera: *Babesia*, *Theileria*, and *Cytauxzoon*. Members of the genera *Babesia* and *Theileria* include a number of pathogens of veterinary and human health significance, which primarily affect domestic and wild animals. For example, *Babesia bigemina*, *Babesia bovis*, and *Babesia divergens* are the main causative agents of bovine babesiosis (Schnittger et al., 2012); *Babesia canis, Babesia gibsoni* and *Babesia vogeli* are responsible for canine babesiosis (Solano-Gallego et al., 2016); and *Theileria annulata*, *Theileria buffeli*, *Theileria lestoquardi, Theileria parva*, and *Theileria sergenti*, are the cause of theileriosis in a range of ruminants (Bishop et al., 2004). The feline tick-borne disease cytauxzoonosis is caused by *Cytauxzoon felis* (Cohn, 2013). Some piroplasm species, including *B. divergens* and *Babesia microti*, are zoonotic and are the cause of emerging human babesiosis in Europe and United States, respectively (Kjemtrup and Conrad, 2000).

In Australia, *Theileria orientalis*, vectored by *Haemaphysalis longicornis* ticks that were introduced via cattle importation from Japan in the 19th century (Hoogstraal et al., 1968), is a cause of outbreaks of theileriosis in livestock (Eamens et al., 2013; Gebrekidan et al., 2016; Islam et al., 2011; Izzo et al., 2010; Rogers and Callow, 1966); while *B. vogeli* infections are common in dogs, particularly in remote Indigenous communities (Brown et al., 2006). In addition, the death of a patient at Canberra Hospital in 2010, was the result of *B. microti* infection, representing the first human babesiosis case in Australia (Senanayake et al., 2012).

Several piroplasm species have been detected in a range of native wildlife in Australia, including *Babesia tachyglossi* and *Theileria tachyglossi* in echidnas (*Tachyglossus aculeatus*) (Backhouse and Bolliger, 1959; Priestley, 1915), *Babesia thylacis* in bandicoots (*Thylacis obesulus*) and northern quolls (*Dasyurus hallucatus*) (Bangs and Purnomo, 1996; Mackerras, 1959), *Babesia macropus* in the eastern grey kangaroos (*Macropus giganteus*) and agile wallabies (*Macropus agilis*), *Theileria fuliginosa* in western grey kangaroos (*Macropus fuliginosus*) (Clark and Spencer, 2007; Dawood et al., 2013; Donahoe et al., 2015), *Theileria gilberti* in Gilbert's potoroo (*Potorous gilbertii*) (Lee et al., 2009), *Theileria perameles* in long-nosed bandicoots (*Perameles nasuta*) and long-nosed potoroos (*Potorous tridactylus*) (Clark, 2004), *Theileria penicillata* in brush-tailed bettongs (woylies) (*Bettongia penicillata ogilbyi*), and *Theileria brachyuri* in quokkas (*Setonix brachyurus*) (Clark and Spencer, 2007). Furthermore, a recent investigation characterised *Theileria*

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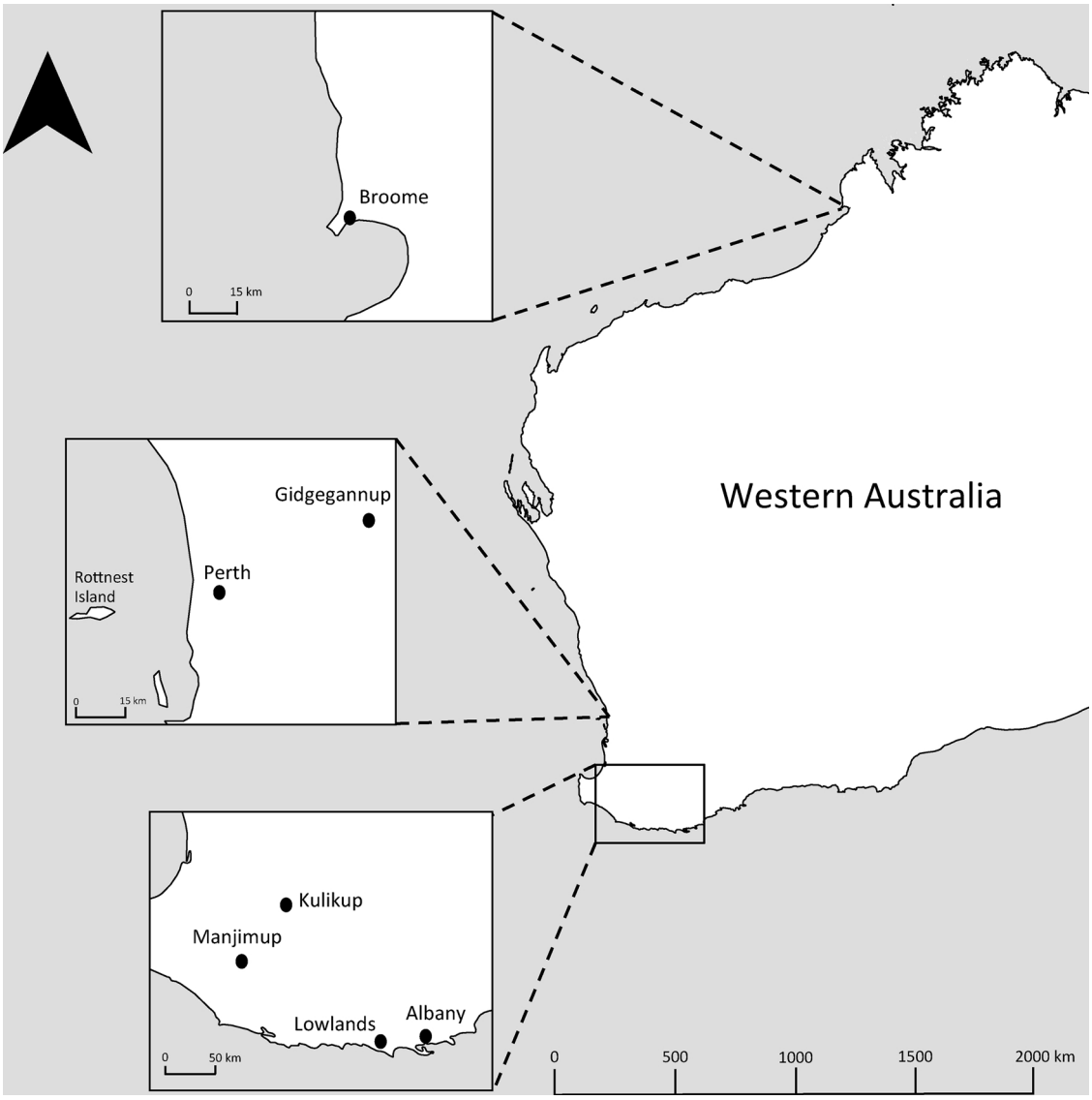

Fig. 1. Locations of tick samples collected in this study.

ornithorhynchi in platypuses (*Ornithorhynchus anatinus*) (Paparini et al., 2015), a piroplasm found to cause at least one case of haemolytic anaemia in these monotremes (Kessell et al., 2014; Macgregor et al., 2017). Other unnamed *Babesia* and *Theileria* spp. were also detected in woylies and a burrowing bettong (boodie) (*Bettongia lesueur*), respectively (Paparini et al., 2012). However, none of the wildlife piroplasms of Australian wildlife species have been confirmed to be zoonotic to date.

In contrast to the Australian native animal hosts, relatively little is known about piroplasms in Australian ticks, despite the fact that ticks are recognised vectors for numerous pathogenic piroplasm species worldwide (Schnittger et al., 2012). Therefore, the objective of the present investigation was to screen ticks parasitising kangaroos in WA for the presence of piroplasms using piroplasm-specific 18S rRNA gene primers and Sanger sequencing.

2. Materials and methods

2.1. Sample collection and tick identification

A total of 154 ticks were collected from approximately 23 western grey kangaroos (*Macropus fuliginosus*) and five red kangaroos (*Macropus rufus*) in several regions in WA by veterinarians and the public. Ninety per cent of the ticks were collected from the southwest regions of WA, including Albany, Kulikup, Lowlands and Manjimup; the remaining ticks were collected from Gidgegannup (~40 km from Perth CBD), and Broome (Fig. 1). Ticks were preserved in 70% ethanol before shipment to Murdoch University for morphological identification. The species were identified using morphological keys described by Roberts (1970). One-hundred-and-nineteen *Ixodes australiensis*, all of which were collected from *M. fuliginosus*; and 35 *Amblyomma triguttatum* ticks from both *M. fuliginosus* and *M. rufus* were identified.

2.2. DNA extraction and PCR

Ticks were washed with solutions of 10% sodium hypochlorite followed by 70% ethanol. The genomic DNA of the ticks was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), with the inclusion of extraction controls. Nested PCR was conducted using the BTF1/BTR1 and BTF2/BTR2 primers with the thermal cycle protocols as described previously (Jefferies et al., 2007), which amplify an 850-bp fragment of the 18S rRNA locus. *Babesia gibsoni*-infected canine DNA was used as a positive control in the PCR assays. No template controls (NTC) were also included in all PCR reactions.