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A novel *Ehrlichia* species in blood and *Ixodes ornithorhynchi* ticks from platypuses (*Ornithorhynchus anatinus*) in Queensland and Tasmania, Australia

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ABSTRACT

Worldwide, *Ehrlichia* spp. are emerging infectious organisms of domestic animals and people, however, most *Ehrlichia* spp. naturally infect wildlife reservoirs causing mainly asymptomatic infections. Australian ecosystems have been under-explored for these potentially pathogenic organisms, and recent studies have identified a range of novel *Ehrlichia*, and their sister genera, *Anaplasma* and ‘*Candidatus* Neoehrlichia’ species, from native Australian ticks. We used bacterial 16S rRNA (16S) next-generation sequencing and genus-specific PCR to profile the bacterial communities in platypus (*Ornithorhynchus anatinus*) blood samples and platypus ticks (*Ixodes ornithorhynchi*), and identified a high prevalence of *Ehrlichia* sequences. We also observed *Ehrlichia*-like intraneutrophilic inclusions (morulae) in PCR-positive stained platypus blood films that were consistent in morphology with other *Ehrlichia* spp. Bayesian phylogenetic analysis of 16S (1343 bp), *gltA* (1004 bp), and *groEL* (1074 bp) gene sequences group the platypus *Ehrlichia* with ‘*Candidatus* Ehrlichia khabarensis’ from far-eastern Russia, and demonstrate that the platypus *Ehrlichia* is clearly distinct from all other *Ehrlichia* spp. Enough genetic divergence exists to delineate this platypus *Ehrlichia* as a separate species that we propose to designate ‘*Candidatus* Ehrlichia ornithorhynchi’. There is no evidence that ‘*Candidatus* Ehrlichia ornithorhynchi’ causes disease in wild platypuses, however, the organism does seem to be widespread in Australia, being found in both Queensland and Tasmania. ‘*Candidatus* Ehrlichia ornithorhynchi’ is the second native Australian *Ehrlichia* described and adds to the rapidly growing diversity of recently described native Australian tick-borne bacteria.

1. Introduction

Members of the sister genera *Anaplasma*, *Ehrlichia*, and ‘*Candidatus* Neoehrlichia’ (family Anaplasmataceae) are tick-borne obligate intracellular Alphaproteobacteria that infect mammalian haematopoietic or endothelial cells, and are an emerging infectious threat to domestic animals and people worldwide. These bacteria commonly infect wildlife species (reservoirs) causing mostly asymptomatic infections, and are vectored between mammalian hosts by hard ticks (Acari: Ixodida). However, transmission by tick bite to naïve hosts, such as humans and domestic animals, can cause serious illness, and in severe cases death (Rar and Golovljova, 2011). Several species such as *A. phagocytophilum*, *E. chaffeensis*, *E. ewingii*, and ‘*Candidatus* N. mikurensis’ are responsible for emerging zoonoses, and species such as *E. ruminantium*, *A. marginale*

and *E. canis* cause significant disease in domestic animals (Atif, 2015; Colwell et al., 2011; Paddock and Childs, 2003; Rar and Golovljova, 2011; Silaghi et al., 2015; Stuen et al., 2013).

Globally, *Anaplasma*, *Ehrlichia*, and ‘*Candidatus* Neoehrlichia’ spp. are associated with a wide range of mammalian hosts and tick vectors, and novel genetic variants and new species are frequently described (Cruz et al., 2012; Guo et al., 2016; Kawahara et al., 2004; Pritt et al., 2011; Rar et al., 2015; Yabsley et al., 2008). However, until recently no members of these genera were thought to be native to Australia. Three *Anaplasma* spp. (*A. marginale*, *A. centrale*, and *A. platys*) have been introduced to Australia after European arrival, through the importation of domestic animals (cattle and dogs) and their associated ticks (*Haemaphysalis longicornis*, *Rhipicephalus australis*, and *R. sanguineus sensu lato*) (Angus, 1996; Callow, 1984). However, in Australia, these introduced

Abbreviations: 16S, 16S rRNA gene; *groEL*, 60 kDa heat shock chaperonin gene; *gltA*, citrate synthase gene; SNP, single nucleotide polymorphism

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Table 1Relative abundance of bacterial genera identified in ten female *Ixodes ornithorhynchi* ticks (F1-F10) used for next-generation sequencing 16S rRNA gene metagenomic profiling.

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Phylum: Actinobacteria										
<i>Actinomyces</i>	2.1									
<i>Corynebacterium</i>	23.1			22.6	12.2				34.5	0.1
<i>Agromyces</i>										0.2
<i>Leucobacter</i>								6.3		
<i>Kocuria</i>					11.3					
<i>Micrococcus</i>	4.2					0.1	0.2			
<i>Mycobacterium</i>		39.5						3.4		0.2
<i>Propionibacterium</i>	5.9		23.8	0.9	53.4		1.0		24.5	
Phylum: Firmicutes										
<i>Bacillus</i>	1.0					0.5	0.5		35.0	0.8
<i>Lactobacillus</i>	13.1									
Phylum: Proteobacteria, Class: Alphaproteobacteria										
<i>Ehrlichia</i>	47.8		70.7	73.3		99.4	97.7	89.3		98.3
<i>Novosphingobium</i>	2.8	5.3					0.6			
Phylum: Proteobacteria, Class: Betaproteobacteria										
<i>Delftia</i>		28.9		0.0	6.1			0.8	6.0	0.5
<i>Rhodiferax</i>				3.2						
<i>Variovorax</i>					17.0					
Phylum: Proteobacteria, Class: Gammaproteobacteria										
<i>Stenotrophomonas</i>		26.3	5.5					0.3		

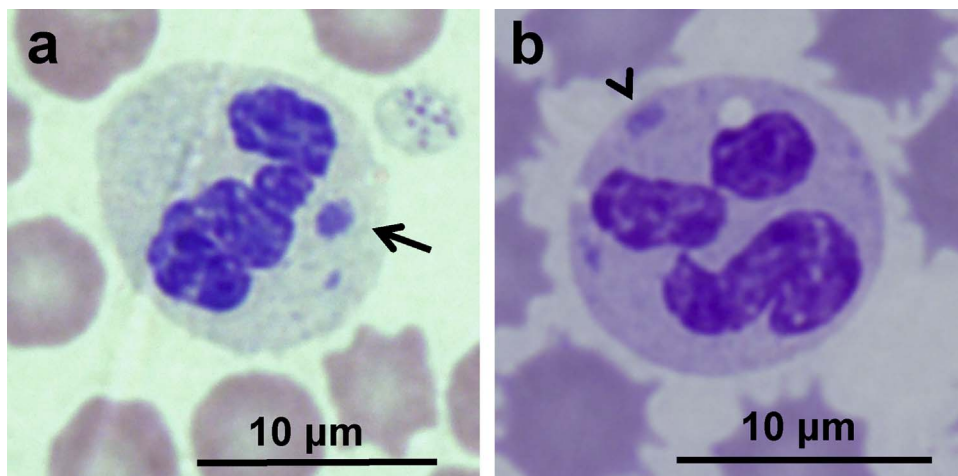


Fig. 1. Light micrographs of Modified Wrights-stained platypus (*Ornithorhynchus anatinus*) blood films with a) *Ehrlichia*-like intracellular inclusions (morulae) (arrow) and b) Döhle bodies (arrowhead) in neutrophils. Original magnification x 1000.

Anaplasma spp. only infect introduced domestic animals, are only vectored by introduced ticks, and to the authors' knowledge have never been detected in native wildlife or ticks (Angus, 1996; Callow, 1984).

Recent investigations, however, have identified, for the first time, a diverse range of indigenous Australian *Anaplasma*, *Ehrlichia*, and 'Candidatus Neoehrlichia' spp. in native Australian ticks. These newly described species and genetic variants include 'Candidatus N. australis' and 'Candidatus N. arcana' that were identified in *Ixodes holocyclus* ticks in Queensland (QLD) and New South Wales (NSW) (Gofton et al., 2015a, 2016), 'Candidatus E. occidentalis' that was identified in *Amblyomma triguttatum* ticks in Western Australia and South Australia, and *A. bovis* genotype Y11 that was detected in *Am. t. triguttatum* and *Am. t. ornatisimum* ticks in Western Australia (Gofton et al., 2017). Notably, these novel *Ehrlichia* and 'Candidatus Neoehrlichia' spp. identified in Australia are phylogenetically closely related to zoonotic species found overseas, such as *E. ruminantium*, *Ehrlichia* sp. Panola Mtn., and "Candidatus N. mikurensis" (Gofton et al., 2016; Gofton et al., 2017). Although research is ongoing, little is known about the epidemiology and ecology of these newly discovered Australian *Anaplasma*, *Ehrlichia*, and 'Candidatus Neoehrlichia' spp. and it is currently unknown whether they are pathogenic to people or animals.

Compared to other continents, Australia has unique mammal and

tick fauna that have co-evolved in relative isolation since the complete separation of the continent from the Gondwana landmass ~ 40 million years ago (Long, 2017). One such unique Australian mammal is the platypus (*Ornithorhynchus anatinus*), which is a semi-aquatic monotreme (egg-laying mammal), endemic to eastern Australia including Tasmania (TAS). Platypuses harbour the tick *Ixodes ornithorhynchi*, which has evolved a highly specific host-parasite relationship for platypuses and does not parasitise any other species (Roberts, 1970). Little is known about the ecology or behaviour of *I. ornithorhynchi*, however, it is thought that all life stages are nidicolous, remaining within platypus burrows when not feeding; *I. ornithorhynchi* are found throughout the entire enzootic range of the platypus (Roberts, 1970).

As part of a wider study investigating the microbiome of native Australian ticks, we used next-generation sequencing (NGS) bacterial 16S rRNA (16S) metagenomic techniques to profile the microbiome of *I. ornithorhynchi*. Preliminary NGS data identified a high prevalence of *Ehrlichia*-like 16S rRNA (16S) gene sequences in *I. ornithorhynchi* ticks from platypuses. Prompted by these findings, we then used PCR-based methods to screen *I. ornithorhynchi* samples and platypus blood samples from two allopatric platypus populations in QLD and TAS for *Ehrlichia* and describe the phylogeny of the novel *Ehrlichia* sp. identified.

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