

## Novel genotypes of *Trypanosoma binneyi* from wild platypuses (*Ornithorhynchus anatinus*) and identification of a leech as a potential vector



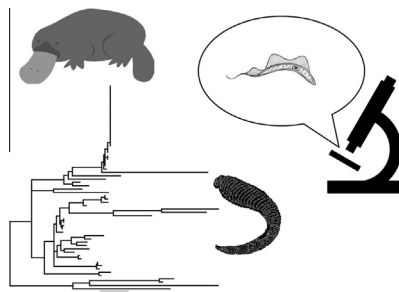
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### HIGHLIGHTS

- We detect high levels of *Trypanosoma binneyi* infections in wild Tasmanian platypuses.
- We detect multiple closely-related genotypes of *T. binneyi* in wild platypuses from Tasmania.
- We provide evidence to support the hypothesis that leeches are potential vectors of *T. binneyi*.
- In the sampled animals, no particular clinical signs were clearly associated with the infections.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Little is known about the prevalence and pathogenesis of trypanosomes in Australian monotremes, and few genetic characterisation studies have been conducted with these haemoparasites. During the present investigation, molecular and microscopic methods were used to screen peripheral blood ( $n = 28$ ) and ectoparasites ( $n = 10$  adult ticks;  $n = 5$  tick nymphs;  $n = 1$  leech; and  $n > 500$  tick eggs) collected from wild Tasmanian platypuses (*Ornithorhynchus anatinus*), for the presence of trypanosomatid-specific DNA and/or trypomastigotes. The genes for the small ribosomal subunit RNA (18S rDNA) and glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) were amplified and sequenced, prior to conducting phylogenetic analyses.

The detection rate of the parasite-specific 18S rDNA in platypus blood was 85.7% ( $n = 24/28$ ), and the leech was also positive at both loci. Microscopically, high parasitaemia and the presence of abundant trypomastigotes, morphologically consistent with *Trypanosoma binneyi* Mackerras (1959), were observed in the blood films. Phylogenetic analyses at the 18S locus revealed the existence of four trypanosomatid-like genotypes, with variable similarity to two previously-described genotypes of *T. binneyi* (range of genetic p-distance: 0.0–0.5%). For the gGAPDH locus, for which only one *T. binneyi* sequence is available in GenBank, three genotypes closely related *T. binneyi* were identified (range of genetic p-distance: 0.1–0.4%). The leech-derived trypanosome isolate was virtually identical (at the two loci studied) to the other parasites sequenced from infected platypuses; however, the molecular or morphological identification of the leech species was not possible.

Although further studies are required, the molecular detection of trypanosomes in an aquatic leech removed from a platypus, suggests the possibility that these haematophagous hirudineans may be a vector for *T. binneyi* (and closely related genotypes).

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## 1. Introduction

Trypanosomes (phylum Euglenozoa) are parasitic haemoprotezoa that infect both humans and animals, causing morbidity, mortality and economic losses worldwide (Englund et al., 1982). Trypanosomes, usually transmitted by arthropod or leech vectors, are the aetiological agents of serious human diseases such as African and South American trypanosomiasis (sleeping sickness and Chagas disease, respectively) and diseases of veterinary importance such as African bovine trypanosomiasis, which are characterised by a range of clinical signs including fatigue, fever, anaemia, and death (Hamilton et al., 2004).

In addition to their medical, veterinary and economic relevance, their ubiquitous biogeography, as well as the broad host range of trypanosomes, has prompted molecular studies on their phylogeny and evolution (Haag et al., 1998; Hamilton et al., 2007, 2004). To date, numerous trypanosome species/genotypes have been reported from native Australian eutherians, marsupials and monotremes (rev. in Thompson et al., 2014) (Table 1). In addition to the potential relevance of these parasites on the conservation status of some of the critically endangered species (Papparini et al., 2011; Thompson et al., 2014), collection of DNA sequences from evolutionary distinct and/or geographically

isolated hosts may shed light on the transmission, phylogeny, and evolution of these parasites. For these reasons, also exploiting the opportunity offered by a broader project underway, we decided to perform a survey in wild platypuses (*Ornithorhynchus anatinus*). In particular, we were interested in uncovering novel phenotypes or genotypes of trypanosomes, and their potential vectors.

In the platypus, early observations of un-named trypanosomes were made by Owen in 1933–1934 (Owen undated, ca. 1934–5). However, it was not until 1959 (and then 1974) that a description and a name were provided for *Trypanosoma binneyi* (Mackerras, 1959; McMillan and Bancroft, 1974). Preliminary molecular (and microscopic) studies conducted on this protozoan species revealed morphological peculiarities, a high frequency of infections, and a phylogenetic association with the cartilaginous fish-derived sequences of *Trypanosoma boissoni* (Jakes et al., 2001; Noyes et al., 1999). In the present study, we describe the molecular systematics of novel genotypes of *T. binneyi*, found in wild-trapped Tasmanian platypuses. This study also provides the first molecular identification of *T. binneyi*-like DNA in a leech, collected from a platypus. This observation suggests that, as for most members of the aquatic trypanosomes, leeches may act as a vector also of this species of parasite.

**Table 1**

Trypanosome species recorded in naturally-infected Australian animals (adapted from Thompson et al., 2014).

Subclass	Group	Common name	Species	Trypanosome species	
Monotremes	Order: Monotremata	Platypus	<i>Ornithorhynchus anatinus</i>	<i>T. binneyi</i>	
Marsupials	Orders: Dasyuromorphia and Peramelemorphia (Polyprotodont Marsupials)	Chuditch (Western quoll)	<i>Dasyurus geoffroii</i>	<i>T. vegrandis</i>	
		Tiger quoll	<i>Dasyurus maculatus</i>	<i>T. copemani</i>	
		Golden bandicoot	<i>Isoodon auratus</i>	<i>T. sp.</i>	
		Northern brown bandicoot	<i>Isoodon macrourus</i>	<i>T. thylacis</i>	
		Southern brown bandicoot	<i>Isoodon obesulus</i>	<i>T. vegrandis</i> , <i>T. copemani</i>	
		Eastern barred bandicoot	<i>Perameles gunnii</i>	<i>T. sp.</i>	
		Dibbler	<i>Parantechinus apicalis</i>	<i>T. sp.</i>	
		Common planigale	<i>Planigale maculata</i>	<i>T. sp.</i>	
		Common wombat	<i>Vombatus ursinus</i>	<i>T. copemani</i>	
		Koala	<i>Phascolarctos cinereus</i>	<i>T. copemani</i> , <i>T. irwini</i> , <i>T. gilletti</i>	
		Brush-tailed possum	<i>Trichosurus vulpecula</i>	<i>T. sp. H25</i> , <i>T. copemani</i>	
		Boodie (Burrowing bettong)	<i>Bettongia lesueur</i>	<i>T. sp.</i> , <i>T. sp. H25</i>	
		Woylie (Brush-tailed bettong)	<i>Bettongia penicillata</i>	<i>T. copemani</i> , <i>T. vegrandis</i> , <i>T. sp. H25</i>	
		Order: Diprotodontia (Diprotodont Marsupials)	Gilbert's potoroo	<i>Potorous gilbertii</i>	<i>T. copemani</i>
			Banded hare wallaby	<i>Lagostrophus fasciatus</i>	<i>T. sp. H25</i>
			Swamp Wallaby	<i>Wallabia bicolor</i>	<i>T. sp.</i>
			Brush-tailed rock wallaby	<i>Petrogale penicillata</i>	<i>T. sp.</i>
			Tammar wallaby	<i>Macropus eugenii</i>	<i>T. vegrandis</i>
			Western grey kangaroo	<i>Macropus fuliginosus</i>	<i>T. vegrandis</i>
			Eastern grey kangaroo	<i>Macropus giganteus</i>	<i>T. sp. H25</i>
Quokka	<i>Setonix brachyurus</i>		<i>T. copemani</i>		
Eutherians(Placental mammals)	Order: Chiroptera (Bats)		Grey-headed flying fox	<i>Pteropus poliocephalus</i>	<i>T. pteropi</i>
			Dusky horseshoe bat	<i>Hipposideros ater</i>	<i>T. hipposideri</i>
	Order: Rodentia (Rodents)	Water rat	<i>Hydromys chrysogaster</i>	<i>T. lewisi</i>	
		Bush rat	<i>Rattus fuscipes</i>	<i>T. lewisi</i> , <i>T. sp.</i>	
		Djoongari (Shark Bay mouse)	<i>Pseudomys fieldi</i>	<i>T. sp.</i>	

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