

The diversity, distribution and host-parasite associations of trypanosomes in Western Australian wildlife

S. AVERIS¹, R. C. A. THOMPSON¹, A. J. LYMBERY¹, A. F. WAYNE², K. D. MORRIS³
and A. SMITH^{1*}

¹ WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and the State Agricultural Biotechnology Centre, School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Western Australia 6150, Australia

² Science Division, Department of Environment and Conservation, Manjimup, WA 6258, Australia

³ Science Division, Department of Environment and Conservation, Wildlife Place, Woodvale WA 6026, Australia

(Received 4 May 2009; revised 27 May 2009; accepted 15 June 2009; first published online 7 August 2009)

SUMMARY

Little is known regarding the diversity, distribution or host-parasite associations of *Trypanosoma* spp. in Australian wildlife. Here we report on an investigation based on divergence of the 18S rRNA gene of trypanosomes isolated from a range of hosts and varied geographical locations. A total of 371 individuals representing 19 species of native animals from 14 different locations were screened. In total, 32 individuals from 9 different species tested positive for the parasite. Phylogenetic analysis revealed considerable parasite diversity with no clear geographical distribution and no evidence of host specificity. In general, it appears that Australian *Trypanosoma* spp. are widespread, with several genotypes appearing in multiple host species and in varied locations including both mainland areas and offshore islands. Some host species were found to be susceptible to multiple genotypes, but no individuals were infected with more than a single isolate.

Key words: *Trypanosoma* spp., genetic diversity, Australia, wildlife, host-specificity, geographical distribution.

INTRODUCTION

Protozoan parasites of the genus *Trypanosoma* are widespread and commonly found in all groups of vertebrates. Some are associated with severe clinical disease in humans (*T. cruzi*, *T. brucei gambiense*, *T. b. rhodesiense*) and other animals (*T. evansi*), and can be particularly detrimental to new or novel host species (Pickering and Norris, 1996; Wyatt *et al.* 2008). Others are host specific, with little or no apparent negative effects associated with infection, and which appear to rarely become established in cycles involving new hosts (Hoare, 1972; Smith *et al.* 2005). Except for *T. equiperdum*, which is a sexually transmitted disease of horses, vertebrate trypanosomes are primarily transmitted by insect vectors, although other blood-sucking invertebrates including ticks and leeches may also play a role in transmission. They are separated into 2 distinct biological groups depending on their developmental pattern in the vector and mode of transmission. The stercorarian trypanosomes (e.g. *T. cruzi*) develop in the hind gut of an invertebrate vector and are deposited in faeces

during feeding, with infection occurring via contamination of the bite wound or mucosal membranes. The second group, the salivarian trypanosomes (e.g. *T. brucei*), develop in the mid-gut of an insect vector and, following migration to the salivary glands or proboscis, are injected directly into a host during feeding (Hoare, 1972; Brun *et al.* 1998).

While much is known of the trypanosomes that impact on human health or economic development, a great deal less is known of the trypanosomes associated with wildlife. Amongst the unique fauna of Australia, trypanosomes have been reported from numerous vertebrates, including the woylie (*Bettongia penicillata*), chuditch (*Dasyurus geoffroii*) (Smith *et al.* 2008), eastern grey kangaroo (*Macropus giganteus*), common wombat (*Vombatus ursinus*), platypus (*Ornithorhynchus anatinus*) (Noyes *et al.* 1999), long-necked tortoise (*Emydura signata*) (Jakes *et al.* 2001), quokka (*Setonix brachyurus*) (Clark and Spencer, 2006), swamp wallaby (*Wallabia bicolor*) (Hamilton *et al.* 2005a) and Gilbert's potoroo (*Potorous gilbertii*) (GenBank Accession no. DQ868978), as well as birds (Mackerras and Mackerras, 1960) frogs and fish (Mackerras and Mackerras, 1961) and other mammals (Mackerras, 1959) and reptiles (Mackerras, 1961). However, those parasites that have been identified from Australian vertebrates may be important for our understanding of both trypanosome evolution and disease processes. For example, Hamilton *et al.* (2005a) showed that trypanosomes from 3 different

* Corresponding author: WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and the State Agricultural Biotechnology Centre, School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Western Australia 6150, Australia. Tel: 08 9965 0908. E-mail: andrew.smith@murdoch.edu.au

Table 1. Geographical location within Western Australia, host species, sample size and trypanosome infection prevalence (%) of samples collected during the present investigation

(* Indicates data published previously by Smith *et al.* (2008). ** Indicates non-native species. NSW, New South Wales; SA, South Australia.)

Location	Species	Number of individuals tested	Number infected (% prevalence; 95%CI)
Faure Island	Boodie (<i>Bettongia lesueur</i>)	58	3 (5.2; 1.4–14.4)
Faure Island	Shark Bay mouse (<i>Pseudomys fieldi</i>)	18	3 (16.7; 4.7–41.4)
Faure Island	Western barred bandicoot (<i>Perameles bougainville</i>)	8	0
Faure Island	Banded-hare wallaby (<i>Lagostrophus fasciatus</i>)	9	0
Julimar	Chuditch (<i>Dasyurus geoffroii</i>)	39	2 (5.1; 0.9–17.5)
Julimar	Brush-tailed possum (<i>Trichosurus vulpecular</i>)	1	0
Lake Magenta	Brush-tailed possum (<i>Trichosurus vulpecula</i>)	6	0
Karakamia	Woylie (<i>Bettongia penicillata</i>)*	149	17 (11.4; 6.9–17.7)
Karakamia	Brush-tailed possum (<i>Trichosurus vulpecula</i>)	27	8 (29.6; 14.8–50.0)
Cape Peron	Bilby (<i>Macrotis lagotis</i>)	9	0
Upper Warren	Woylie (<i>Bettongia penicillata</i>)*	370	85 (23.0; 18.9–27.5)
Upper Warren	Brush-tailed possum (<i>Trichosurus vulpecula</i>)	7	3 (42.9; 12.9–77.5)
Upper Warren	Chuditch (<i>Dasyurus geoffroii</i>)	1	0
Barrow Is	Brush-tailed possum (<i>Trichosurus vulpecula</i>)	8	0
Barrow Is	Boodie (<i>Bettongia lesueur</i>)	13	2 (15.4; 2.8–43.4)
Barrow Is	Common planigale (<i>Planigale maculata</i>)	6	1 (16.8; 0.9–58.9)
Barrow Is	Western chestnut mouse (<i>Pseudomys namus</i>)	6	0
Barrow Is	Golden bandicoot (<i>Isoodon auratus</i>)	12	1 (8.3; 0.4–37.0)
Barrow Is	Spactadled-hare wallaby (<i>Lagorchestes conspicillatus</i>)	3	0
Barrow Is	Water rat (<i>Hydromys chrysogaster</i>)	1	0
Dryandra	Boodie (<i>Bettongia lesueur</i>)	5	1 (20.0; 1.0–65.7)
Dryandra	Bilby (<i>Macrotis lagotis</i>)	8	0
Paruna	Woylie (<i>Bettongia penicillata</i>)	4	1 (25.0; 1.3–75.1)
Paruna	Bandicoot (<i>Isoodon obesulus</i>)	5	0
Paruna	Brush-tailed possum (<i>Trichosurus vulpecula</i>)	6	0
Paruna	Bush rat (<i>Rattus fuscipes</i>)	4	0
Fitzgerald River	House mouse (<i>Mus musculus</i> **)	1	0
Fitzgerald River	Bandicoot (<i>Isoodon obesulus</i>)	2	0
Fitzgerald River	Chuditch (<i>Dasyurus geoffroii</i>)	1	0
Fitzgerald River	Dibbler (<i>Parantechinus apicalis</i>)	2	1 (50.0; 2.5–97.5)
Fitzgerald River	Ash-grey mouse (<i>Pseudomys albocinereus</i>)	2	1 (50.0; 2.5–97.5)
Fitzgerald River	Bush rat (<i>Rattus fuscipes</i>)	16	4 (25.0; 9.0–50.0)
Lorna Glen	Spinifex hopping mouse (<i>Notomys alexis</i>)	2	0
Lorna Glen	Bilby (<i>Macrotis lagotis</i>)	19	0
Lorna Glen	Brush-tailed possum (<i>Trichosurus vulpecula</i>)	3	0
Mt Caroline	Black-footed rock wallaby (<i>Petrogale lateralis</i>)	10	0
Scotia (NSW)	Woylie (<i>Bettongia penicillata</i>)	26	1 (3.8; 0.2–18.8)
Thistle Is (SA)	Bilby (<i>Macrotis lagotis</i>)	20	0
Thistle Is (SA)	Brush-tailed possum (<i>Trichosurus vulpecula</i>)	3	0

marsupial host species in Australia were more closely related to trypanosomes from non-Australian mammals than to each other, suggesting that Australian marsupial trypanosomes have divergent evolutionary origins. Furthermore, it is not yet understood whether Australian trypanosomes have a negative impact on the host, and therefore whether they should be a concern for conservation initiatives (Smith *et al.* 2008; Department of Environment and Conservation, 2008).

Here we report an investigation into the diversity of trypanosomes associated with Australian wildlife based on divergence of the 18S rRNA gene. We also report on the diversity of trypanosomes within selected host species sampled across large geographical areas and varied environments, including

mainland and offshore island populations, in an attempt to determine whether Australian trypanosomes are host-specific, or if there is regional variation in host-parasite associations. The 18S rRNA gene was targeted as it is generally conserved throughout the eukaryotes, but due to different rates of evolution among different regions within the gene, it can be used to infer relationships across a wide phylogenetic range (Sogin *et al.* 1986).

MATERIALS AND METHODS

Collection and preparation of samples

Blood samples were obtained from 371 native mammals of 19 different species, trapped from 14

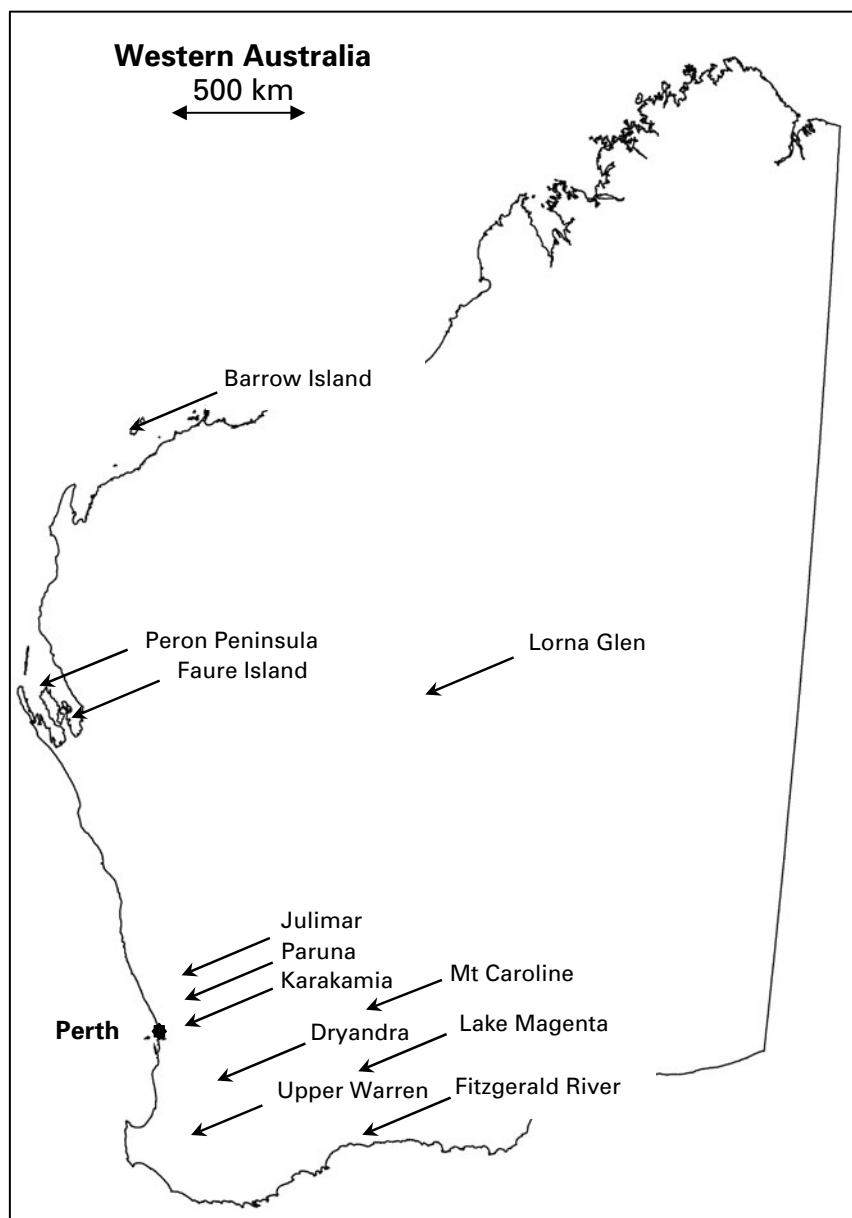


Fig. 1. Map of Western Australia showing approximate locations of the 12 sampling sites in relation to Perth city (filled circle). Sampling sites in New South Wales (Scotia) and South Australia (Thistle Island) are not shown.

different locations throughout Australia (12 within Western Australia) as part of the Western Australian Department of Environment and Conservation (DEC) fauna research and monitoring programs between March 2007–August 2008 (Table 1, Fig. 1). All animals were live-trapped using either Sheffield wire-cages or Elliot traps baited with a mixture of peanut butter, rolled oats and sardines. Blood samples ranged in size from 50 to 500 μl depending on the species and were obtained from either the lateral caudal vein (boodie *Bettongia lesueur*; woylie; brush-tailed possum *Trichosurus vulpecula*), marginal ear vein (chuditch; bandicoot *Isodon auratus*), or dorsal pedal vein (*Planigale* sp.; Shark Bay mouse *Pseudomys fieldi*; bush rat *Rattus fuscipes*; ash grey mouse *Pseudomys albocinereus*; dibbler *Parantechinus apicalis*). Whole blood was collected in 1.5 ml

Eppendorf tubes and centrifuged within 6 h at 10 600 g for 10 min to separate and remove sera. The resulting blood pellets were stored at -20°C . Genomic DNA was extracted from blood using the QIAamp Blood DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Wildlife sampling was carried out under Murdoch University Animal Ethics Approval Permit Numbers NS1182-06 and W2172-08, and DEC Animal Ethics Approval Permit Number DEC AEC/21/2008.

Detection and amplification of the 18S rRNA gene

A sample of 10 μl of DNA extract was used as a template in a nested PCR using trypanosome-specific oligonucleotides as described by Noyes *et al.*

Table 2. *Trypanosoma* species or isolate code, host species, geographical location and GenBank Accession number of the 44 genotypes included in the current analysis

(Novel isolates detected during the present investigation are indicated by *.)

Sample/Species	Host	Location	Accession number
BRA1	<i>Rattus fuscipes</i>	Australia *	FJ823118
BRA2	<i>Rattus fuscipes</i>	Australia *	FJ823117
BRA3	<i>Rattus fuscipes</i>	Australia *	FJ823110
AMA1	<i>Pseudomys albocinerus</i>	Australia *	FJ823119
DBA1	<i>Parantechinus apicalis</i>	Australia *	FJ823115
BDA1	<i>Bettongia lesueur</i>	Australia *	FJ823108
BDA2	<i>Bettongia lesueur</i>	Australia *	FJ823114
BDA3	<i>Bettongia lesueur</i>	Australia *	FJ823109
BDA4	<i>Bettongia lesueur</i>	Australia *	FJ823112
MMA1	<i>Planigale maculata</i>	Australia *	FJ823107
CHU1	<i>Dasyurus geoffroii</i>	Australia	EU518941
CHA1	<i>Dasyurus geoffroii</i>	Australia *	FJ823120
GBA1	<i>Isoodon auratus</i>	Australia *	FJ823111
SMA1	<i>Pseudomys fieldi</i>	Australia *	FJ823113
TRY1	<i>Bettongia penicillata</i>	Australia	EU518939
TRY2	<i>Bettongia penicillata</i>	Australia	EU518940
WYA1	<i>Bettongia penicillata</i>	Australia *	FJ823116
WYA2	<i>Bettongia penicillata</i>	Australia *	FJ823121
H25	<i>Macropus giganteus</i>	Australia	AJ009168
ABF	<i>Wallabia bicolor</i>	Australia	AJ620564
P63	<i>Potorous gilbertii</i>	Australia	DQ868978
Q3	<i>Setonix brachyurus</i>	Australia	DQ868976
AAP	<i>Vombatus ursinus</i>	Australia	AJ620558
<i>T. binneyi</i> ABW	<i>Ornithohynchus anatinus</i>	Australia	AJ620567
<i>T. binneyi</i> AAW	<i>Ornithohynchus anatinus</i>	Australia	AJ620565
<i>T. binneyi</i> ABA	<i>Ornithohynchus anatinus</i>	Australia	AJ620566
AEM	<i>Vombatus ursinus</i>	Australia	AJ620560
AAI	<i>Vombatus ursinus</i>	Australia	AJ620559
AEX	<i>Vombatus ursinus</i>	Australia	AJ620561
AHB	<i>Vombatus ursinus</i>	Australia	AJ620562
Wallaby10	<i>Petrogale penicillata</i>	Australia	AJ620563
ADE	<i>Mixophyes fleayi</i>	Australia	AJ620569
TL.AQ22	<i>Philaemon sp.</i>	Australia	AJ620574
TL.AV 43	<i>Micobdella sp.</i>	Australia	AJ620570
AAT	<i>Strepera sp.</i>	Australia	AJ620557
MMAC	<i>Macropus giganteus</i>	Australia	AF073876
<i>T. nabiasi</i>	<i>Spilopsylluscuniculi</i>	UK	AJ843896
<i>T. cruzi marinkellei</i>	<i>Phyllostomum discolor</i>	Brazil	AJ009150
<i>T. cruzi</i> Sylvio X10	<i>Homo sapiens</i>	Brazil	AJ009147
<i>T. cruzi</i> VINCH 89	<i>Triatoma infestans</i>	Chile	AJ009149
<i>T. cruzi</i> CAN III	<i>Homo sapiens</i>	Brazil	AJ009148
<i>T. levisi</i>	<i>Bandicota indica</i>	Indonesia	AB242273
<i>Leishmania tarentolae</i>	<i>Tarentolae annularis</i>	Brazil	M84225
<i>L. sp.</i>	<i>Homo sapiens</i>	Martinique	AF303938

(1999). In the first round, each 50 μ l reaction also contained 5 μ l of 10 \times buffer (20 mM Tris-HCl at pH 8.8; 10 mM KCl; 10 mM (NH₄)₂SO₄; 2 mM MgSO₄; 0.1% Triton[®] X-100 and 0.1 mg/ml nuclease-free BSA); 200 μ M dNTPs (Promega, Madison, USA); 1 U *Taq* DNA polymerase (Promega, Madison, USA); 0.5 U of the proof-reading enzyme *Pfu* (Promega, Madison, USA) and 8 pmol of the following external primers: TRY927F 5' GAAAC-AAGAAACACGGGAG 3', TRY927R 5' CTACT-GGGCAGCTTGGA 3', for 30 cycles of 94 °C 30 s; 55 °C 60 s; 72 °C 90 s. The products from the first amplification were diluted 1:10 in sterile deionized

water. Then 2 μ l of the product from each reaction was used as a template for the second-round PCR using the following internal primers: SSU561F 5' TGGGATAACAAAGGAGCA 3', SSU561R 5' CTGAGACTGTAACCTCAAAGC 3' and the same cycling conditions described above. Amplified products were separated on a 1.5% agarose gel and run at 100 V for 1 h in Tris-acetate-EDTA buffer and visualized under UV light. Samples producing a band of approximately 600 bp were considered positive. Prevalence of parasitic infection was expressed as the percentage of hosts with a positive DNA sample and 95% confidence intervals were calculated

Table 3. Isolate code and host as determined during the current investigation, with the % identity and accession details of the most similar *Trypanosoma* genotype registered on GenBank as determined by BLAST analysis

Isolate	Host	% Identity	Species/isolate	Accession no.
MMA1	<i>Planigale maculata</i>	98	<i>T. avium</i> SIM3	AF416563
BDA1	<i>Bettongia lesueur</i>	99	<i>T. sp.</i> MMAC	AF073876
BDA2	<i>Bettongia lesueur</i>	99	<i>T. avium</i> SIM3	AF416563
BDA3	<i>Bettongia lesueur</i>	98	<i>T. avium</i> SIM3	AF416563
BDA4	<i>Bettongia lesueur</i>	99	<i>T. corvi</i> ITMAP	AY461665
DBA1	<i>Parantechinus apicalis</i>	99	<i>T. lewisi</i> TryBiIDN203	AB242273
BRA1	<i>Rattus fuscipes</i>	100	<i>T. lewisi</i> TryBiIDN203	AB242273
BRA2	<i>Rattus fuscipes</i>	100	<i>T. sp.</i> H25	AJ009168
BRA3	<i>Rattus fuscipes</i>	100	<i>T. lewisi</i> TryBiIDN203	AB242273
AMA1	<i>Pseudomys albocinereus</i>	99	<i>T. lewisi</i> TryBiIDN203	AB242273
GBA1	<i>Isodon auratus</i>	97	<i>T. sp.</i> CHU1	EU518941
SMA1	<i>Pseudomys fieldi</i>	97	<i>T. sp.</i> CHU1	EU518941
WYA1	<i>Bettongia penicillata</i>	85	<i>T. sp.</i> TRY2	EU518940
WYA2	<i>Bettongia penicillata</i>	85	<i>T. sp.</i> TRY2	EU518940
CHA1	<i>Dasyurus geoffroii</i>	97	<i>T. lewisi</i> TryBiIDN203	AB242273

assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa *et al.* 2000).

Sequencing and phylogenetic analysis of Australian trypanosomes

PCR products were purified using Wizard SV PCR and gel purification kit (Promega, Madison, USA) according to the manufacturer's instructions. Sequencing reactions were initiated using the purified PCR products as templates with the following cycling conditions: initial cycle at 96 °C for 120 s followed by 25 cycles of 96 °C for 10 s, 54 °C for 5 s and 60 °C for 240 s. Sequences were analysed with FinchTV™ (Geospiza Inc, USA). Construction of the phylogenetic tree was carried out by comparing the resulting 18S rRNA sequences with known sequences in the GenBank database using ClustalW (Higgins *et al.* 1994). The host species, geographical origin and Accession number of all sequences used in the phylogenetic analyses are shown in Table 2. In order to acquire a better understanding of the phylogeny of Australian trypanosomes, the sequence alignment included representatives of a major disease-causing species of trypanosomes, *T. cruzi*. Phylogeny was determined by neighbour-joining, minimum evolution and maximum parsimony methods, implemented with MEGA Version 4.0 (Tamura *et al.* 2007). The robustness of branches within the resulting trees was tested by 100 cycles of bootstrap resampling. For distance estimation methods, evolutionary distances were computed using the maximum composite likelihood method. All positions containing gaps and missing data were eliminated from the dataset. There was a total of 77 positions in the final dataset. The trypanosomatid genera *Leishmania* was used as an outgroup to root the tree.

Phylogenetic analysis of *T. lewisi*-like trypanosomes

For phylogenetic analysis of the closely related *T. lewisi*-like trypanosomes obtained in this study, BRA1, BRA3, AMA1 and DBA1 sequences were aligned with other trypanosome 18S rRNA sequences in the GenBank database. The alignment was carried out with ClustalW followed by manual alignment. Phylogeny was determined by neighbour-joining, minimum evolution and maximum parsimony methods, implemented with MEGA Version 4.0. The robustness of branches within the resulting trees was tested by 100 cycles of bootstrap resampling. For distance estimation methods, evolutionary distances were computed using the maximum composite likelihood method. All positions containing gaps and missing data were eliminated from the dataset. There was a total of 444 positions in the final dataset. A consensus was made of the shortest trees.

RESULTS

Trypanosomes were found in 9 of the 19 native mammal species that were trapped in this study. Trypanosomes have been recorded previously in the woylie and chuditch (Smith *et al.* 2008), but this is the first record for the boodie, brush-tailed possum, golden bandicoot, Shark Bay mouse, bush rat, ash-grey mouse, dibbler and common planigale (*Planigale maculata*).

The partial 18S rRNA genes from Australian trypanosomes sequenced in this study were compared with data available in GenBank and showed high homology with 18S rRNA of other *Trypanosoma* spp. (Table 3). The neighbour-joining tree of evolutionary relationships among the 18S rRNA sequences produced a number of strongly supported clades, with similar support inferred using both

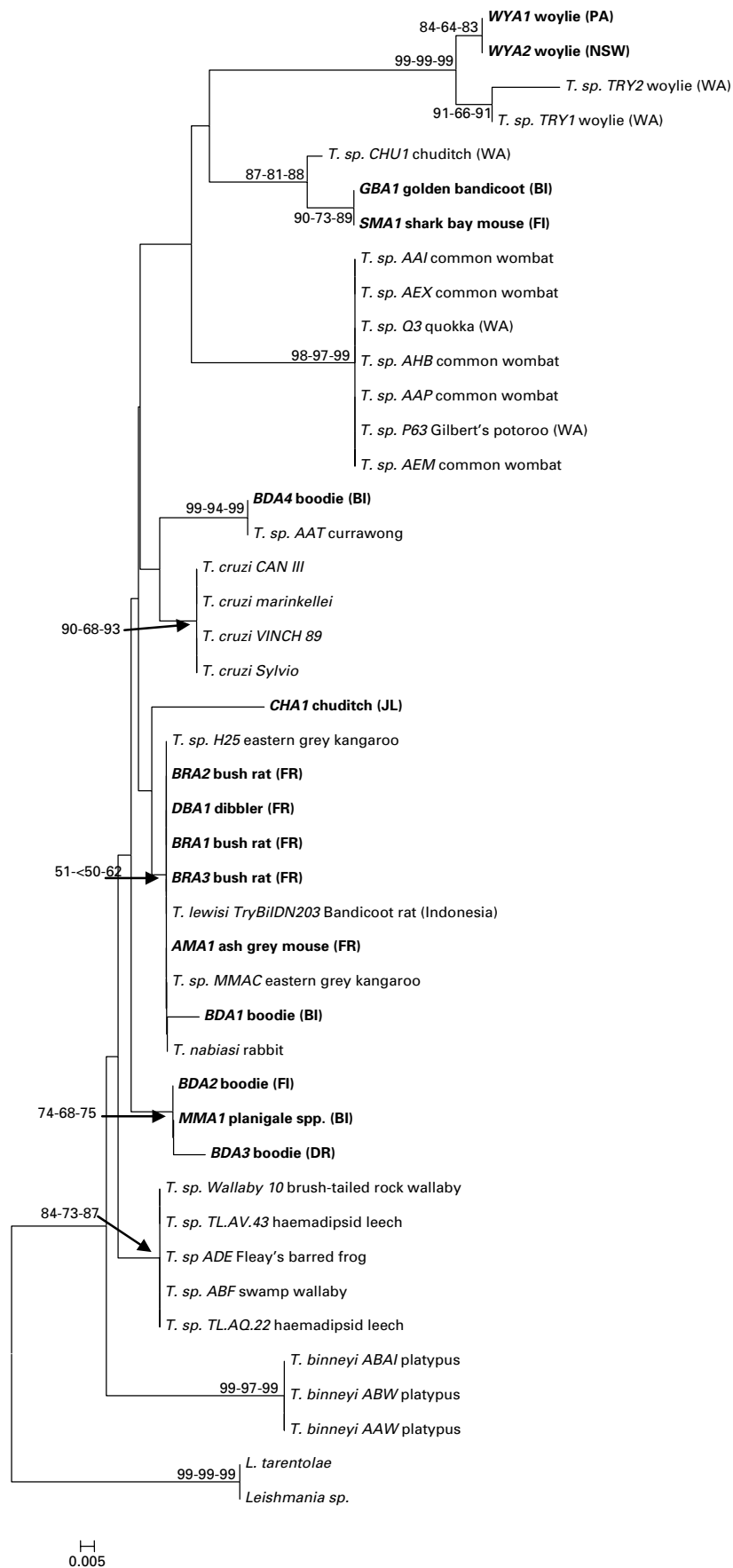


Fig. 2. Phylogram showing the relationship between 44 taxa at the 18S rRNA gene. The relationship was inferred using the neighbour-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown next to the branches or indicated by arrows. The distances for

Table 4. Comparison of *Trypanosoma lewisi*-like nucleotide sequences obtained during this study with *T. lewisi* from an Indonesian bandicoot rat

(Gaps and regions of alignment between each sequence and *T. lewisi* are shown.)

Isolate	Total bases sequenced	BLAST result	Gaps over sequenced region	Region of sequence overlap
BRA1	499	<i>T. lewisi</i> AB242273	0/499	936-1434
BRA3	499	<i>T. lewisi</i> AB242273	0/499	936-1434
DBA1	492	<i>T. lewisi</i> AB242273	2/492	890-1379
AMA1	499	<i>T. lewisi</i> AB242273	1/499	936-1434

maximum parsimony and minimum evolution (Fig. 2). A total of 13 novel genotypes were detected; 4 in the boodie, 2 in the woylie, 2 in the bush rat, 1 in the chuditch, common planigale, ash-grey mouse and dibbler, and a single genotype that was detected in both the Shark Bay mouse and golden bandicoot.

Trypanosome sequences from 3 woylie populations in the south west of Western Australia, and 1 population in Scotia Reserve in New South Wales, form a distinct subclade (WYA1, WYA2, TRY1 and TRY2). Two genotypes obtained from bush rats within the Fitzgerald River National Park (BRA1 and BRA3) were identical to each other and showed 100% identity to *T. lewisi*, a widely distributed trypanosome usually associated with the black rat (*R. rattus*). Two additional genotypes, dibbler (DBA1) and ash-grey mouse (AMA1) showed 99% identity with *T. lewisi* and also grouped with other *T. lewisi*-like trypanosomes (Table 3, Fig. 2). The similarity of these 4 genotypes across the variable V7–V8 region is given in Table 4, and their relationship to other *T. lewisi*-like trypanosomes from outside Australia (Hamilton *et al.* 2005*b*) in Fig. 3.

Other 18S rRNA sequences showed little congruence with either host or geographical distribution (Fig. 2). *Trypanosoma* genotypes from a common planigale (MMA1), and 2 boodies (BDA2 and BDA3) grouped together on 1 dichotomous branch with the other branch occupied by all other Australian genotypes isolated in this study. The boodie and common planigale genotypes, although closely related, originate in 3 different geographical locations, Faure Island, Barrow Island and Dryandra on the mainland. In contrast, genotype BDA4, also from a Barrow Island boodie, appears distantly related and forms a cluster with an isolate from an Australian

currawong (*Strepera* spp.), whilst the other isolate from a Barrow Island boodie (BDA1) falls within the *T. lewisi* group. The chuditch genotype CHA1 showed 97% homology to *T. lewisi*, whereas an isolate reported previously by Smith *et al.* (2008) from the same population (CHU1) appears distinct and more similar to *T. benetti* isolated from an American falcon.

DISCUSSION

The main purpose of our study was to assess the diversity of trypanosomes associated with a range of Australian (particularly Western Australian) wildlife, and to search for patterns of host and geographical specificity. The overall pattern appears to be one of widespread distribution, with trypanosome genotypes occurring in many different host species, often at high prevalences, across the state as well as on offshore islands.

Although we have not found multiple infections within any single individual host, there is evidence to suggest that some species can be infected by multiple genotypes within the same location, for example 2 distinct genotypes occur in chuditch within Julimar Conservation Park. There is also evidence to suggest that some genotypes are widespread and occur in multiple host species, while others are more likely to be associated with certain hosts or within certain geographical areas. For example, isolates detected in woylies from geographically different populations, including a population in New South Wales, consistently group together within a single clade. Those from the closely related boodie appear not only to be genetically different from genotypes from woylies, but to vary considerably both within and between

neighbour-joining analysis were computed using the maximum composite likelihood method. Multiple bootstrap values at nodes are in order: NJ, MP, ME. All positions containing gaps and missing data were eliminated from the dataset. There was a total of 77 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4. Clades without bootstrap values have bootstrap values of <50%. Host species are indicated after *Trypanosoma* species or isolate code. Genotypes sequenced during this study are shown in bold. The letters in parentheses refer to sampling locality: BI Barrow Island; FR Fitzgerald River National Park; JI Julimar Conservation Park; DR Dryandra; FI Faure Island; PA Paruna; NSW New South Wales (see Fig. 1). Other Western Australian genotypes reported previously are indicated as WA.

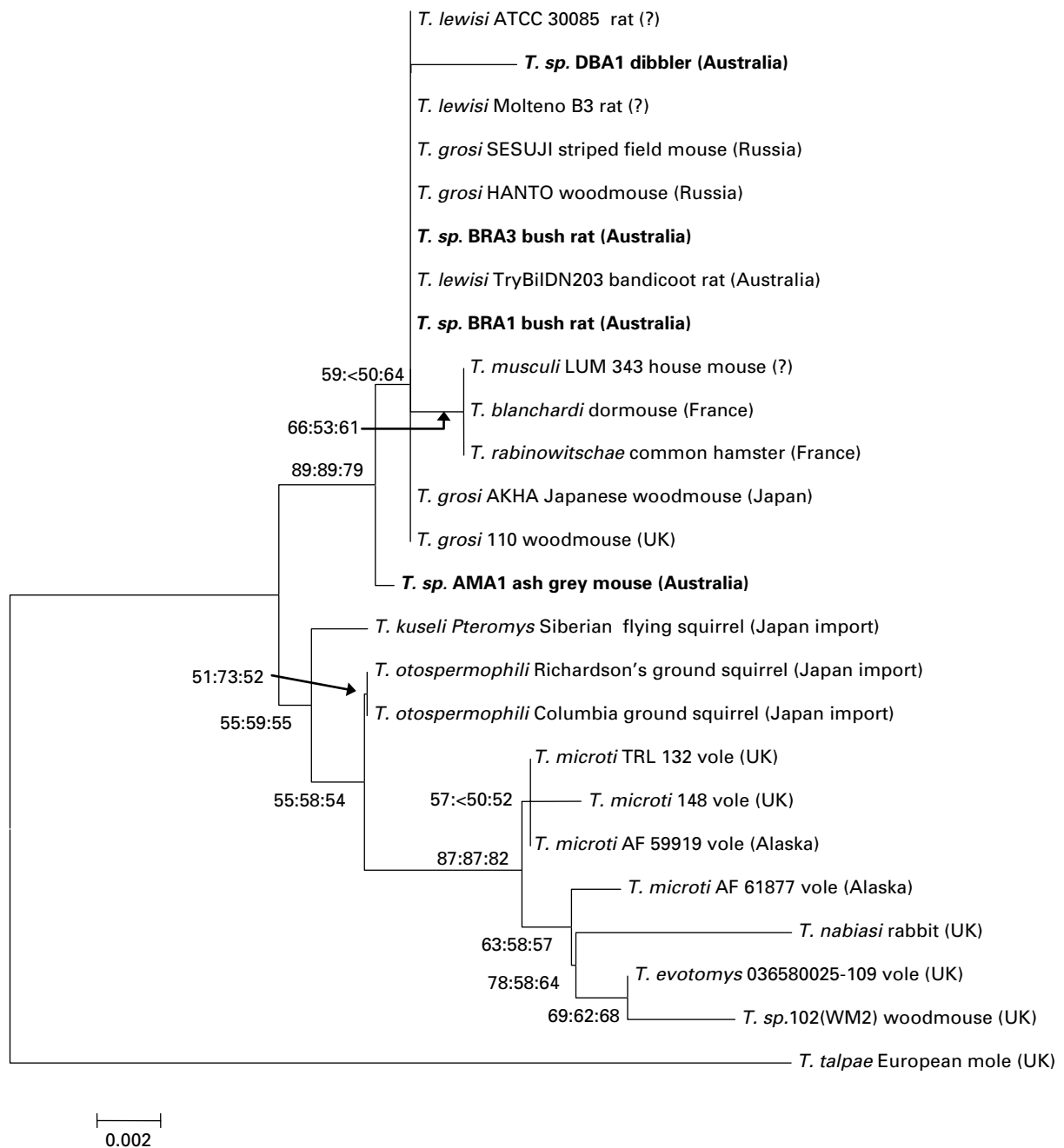


Fig. 3. Phylogram showing the relationship between *Trypanosoma lewisi*-like genotypes (DBA1, AMA1, BRA1 and BRA3) isolated during the current investigation and those from regions outside Australia after Hamilton *et al.* (2005 *a*) inferred using the Neighbour-Joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent evolutionary history of the taxa. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Multiple bootstrap values at nodes are in order: NJ, MP, ME. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). There was a total of 444 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4. Host species, isolate code and country of origin are indicated for each sequence. Genotypes sequenced during this study are shown in bold.

geographically distinct populations, suggesting that some host species may be susceptible to infection from multiple *Trypanosoma* spp. and therefore possibly susceptible to exotic species should they be introduced into Australia. Furthermore, based on the section of 18S rRNA gene sequenced, it also

appears that several host species within the Fitzgerald River area, including the bush rat, dibbler and ash-grey mouse, were all infected with a single or closely related *T. lewisi*-like group of parasites, suggesting there may be areas where specific trypanosomes, or their vectors, are more prevalent. Our results

repeatedly position the kangaroo isolate H25 within the *T. lewisi*-like clade, albeit with relatively low bootstrap support, whereas it has previously been positioned within the *T. cruzi* group (Smith *et al.* 2008; Stevens *et al.* 1999). This may be an artefact of the single gene region that we were able to sequence or it may be due to the increased number of Australian taxa in the *T. lewisi* clade, which was the closest group to the *T. cruzi* clade in previous analyses (Hamilton *et al.* 2005a).

We recognize the limitations of using only the 18S rRNA locus and the need to target additional genes to specifically address the phylogenetic re-positioning of the H25 isolate and the relationships between isolates from native wildlife and *T. lewisi*. Considerable effort was put into the amplification of both the internal transcribed spacer (ITS) and glyceraldehyde phosphate dehydrogenase (GAPDH) genes without success. This was principally due to logistic constraints of sampling in remote areas over long periods of time and often in extremely high temperatures.

The wide geographical distribution of several genotypes seen in our study may have been influenced by the relatively recent translocation of endangered species within Western Australia. For example, boodie populations on Faure Island and Dryandra were both originally sourced from Dore Island, 1 of only 3 extant wild populations, suggesting a potentially similar geographical origin for their associated parasites. The wild population of boodies on Barrow Island, however, have been isolated from the mainland for approximately 8000 years (Dortch and Morse, 1984), so it is surprising to note the similarity between genotypes originating there with genotypes obtained from bush rats in Fitzgerald River on the south coast of Western Australia and a kangaroo from Victoria.

Recent phylogenetic studies based on the 18S rRNA gene have identified extensive genetic variation within in the genus *Trypanosoma* (Stevens *et al.* 2001). The diversity of genotypes presented here suggests that this is also true of trypanosomes associated with Australian wildlife. The general lack of host specificity also supports the conclusions of Hamilton *et al.* (2007) who suggested that co-speciation in association with vertebrate hosts appears to have played little or no role in the evolution of trypanosome diversity. However, the fact that we do not find a single genotype infecting all cohabiting hosts in any one area indicates that there are factors maintaining a degree of host specificity. This may well be due to the association of different vectors with different host species.

The vectors of Australian trypanosomes are not known, although Noyes *et al.* (1999) and Hamilton *et al.* (2005a) have proposed that haemadipsid (jawed) leeches could act as vectors of some mammalian *Trypanosoma* spp. in Australasia, as they do elsewhere. Jakes *et al.* (2001) have proposed

tabanid flies as possible vectors for kangaroo trypanosomes, although ixodid ticks are also commonly found on kangaroos and are thought to be involved in the transmission of trypanosomes in Japan (Thekisoe *et al.* 2007). Phylogenetic grouping of genotypes from several ecologically similar wildlife hosts including bush rat, dibbler, and ash-grey mouse, together with the flea-transmitted *T. lewisi*, a known stercorarian *Trypanosoma* sp., suggests a potentially similar mode of transmission (Hamilton *et al.* 2007). However, ecological similarity of hosts does not explain the positioning of the genotype isolated from a Barrow Island boodie within the *T. lewisi*-like group, as they are both geographically isolated and live in large communal burrow systems similar to rabbit warrens.

It is now well accepted that parasites and pathogens can have a significant impact on wildlife population dynamics (Hudson *et al.* 1992, 1998; Tompkins and Begon, 1999; Albon *et al.* 2002; Newey and Thirgood, 2004; Hawlena *et al.* 2007; Møller and Nielsen, 2007; Pedersen *et al.* 2007; Burthe *et al.* 2008). Parasites that infect a new or naïve host can be particularly pathogenic due to the absence of an appropriate immune response. *T. lewisi*, usually considered to be a non-pathogenic trypanosome, has been shown to vary significantly and often unpredictably in virulence and resultant clinical impact in rats, with higher incidences of anaemia and weight loss, and significantly greater mortality rates in juveniles than adults (Brown, 1914, 1915). In addition, the virulence of non-pathogenic strains has been shown experimentally to increase following passage through cold-blooded animals (Wendelstadt and Fellmer, 1909, cited by Brown, 1914). Wyatt *et al.* (2008) also presented evidence that the extinction of the Christmas Island rat (*Rattus macleari*) was caused by *T. lewisi*, which was inadvertently introduced onto the island by ship rats (*R. rattus*) c1900.

T. lewisi was first reported in Australia by Bancroft (1888) and was subsequently reported in bush rats and water rats (*Hydromys chrysogaster*) (Mackerras, 1959). Its presence within native wildlife raises questions regarding its origin, specifically whether it was introduced along with black and brown rats on ships arriving from Europe or was present before their arrival. It also raises questions regarding the impact of infection on other native wildlife species in terms of apparent plasticity in virulence. For instance, the variants reported here may reflect a recent evolutionary adaptation by *T. lewisi* to new hosts, a process that could result in the development of increased virulence on contact with either the original or additional new hosts (Brown, 1914). The sudden emergence of a virulent form of *T. lewisi* could conceivably result in localized epidemics amongst naïve host species with potentially detrimental effects, although there is no direct evidence to support this at

present. Furthermore, virulence of trypanosome infection is known to vary depending on the presence of concomitant infections and the general condition of the host (Cox, 2001; Brown *et al.* 2000). For example, Smith *et al.* (2008) reported a higher prevalence and intensity of trypanosome infection in a declining population of woylies compared to that observed in a stable non-declining population in the southwest of Western Australia. They suggested that the impact of infection was only of significance for animals in the declining population because they were also exposed to high levels of the parasite *Toxoplasma gondii* (Arrea *et al.* 1998). The physiological stress caused by the presence of introduced predators such as cats and foxes, as well as stress resulting from poor nutrition, competition for food and resources, and the effects of habitat degradation, are all thought to contribute to both host susceptibility and severity of infection (McCallum and Dobson, 2002; Davey *et al.* 2006; Pedersen and Grieves, 2008).

A more comprehensive understanding of the diversity of *Trypanosoma* spp. associated with native wildlife will contribute to the conservation efforts and translocation programmes of endangered species in Western Australia. Assessment of the current range of trypanosome infections will also allow inferences to be made regarding the susceptibility of host species to the introduction of non-native pathogenic trypanosomes, such as *T. evansi*, thus helping to guide biosecurity initiatives (Thompson *et al.* 2003). Furthermore, it is important to understand the current range of infections that exist within wildlife populations to be able to predict their suitability to act as competent reservoirs for introduced zoonoses in the event that climate change (Polley and Thompson, 2009) should result in Australia becoming a suitable environment for establishment (Thompson *et al.* 2009). As in other parts of the world, increasing encroachment of humans on wildlife habitats enhances the opportunity for novel pathogens to spread to humans, their livestock and pets.

We would like to thank staff at the Australian Wildlife Conservancy and the Department of Environment and Conservation, and Halina Burmej from Murdoch University, for assistance in collecting and processing field samples and for providing access to field sites, and to Nicole Willers for supplying *P. lateralis* samples. This work was funded by the Australian Research Council and the Department of Environment and Conservation under ARC-Linkage Project ID: LP0775356.

REFERENCES

- Albon, S. D., Stien, A., Irvine, R. J., Langvatn, R., Ropstad, E. and Halvorsen, O.** (2002). The role of parasites in the dynamics of a reindeer population. *Proceedings of the Royal Society of London, B* **269**, 1625–1632.
- Arrea, G. C., Carmona, M. C., Bermudez, O. M. G. and Abrahams, E.** (1998). Effect of *Trypanosoma lewisi* (Kinetoplastida: Trypanosomatidae) on the infection of white rats with *Toxoplasma gondii* (Eucoccidia: Sarcocystidae) oocysts. *Revista De Biología Tropical* **46**, 1121–1123.
- Bancroft, T. L.** (1888). Note on Haematomonas in rat's blood. *Proceedings of the Royal Society of Queensland* **5**, 31–32.
- Brown, W. H.** (1914). A note on the pathogenicity of *Trypanosoma lewisi*. *The Journal of Experimental Medicine* **19**, 406–410.
- Brown, W. H.** (1915). Concerning changes in the biological properties of *Trypanosoma lewisi* produced by experimental means, with special reference to virulence. *The Journal of Experimental Medicine* **21**, 345–364.
- Brown, M. J. F., Loosli, R. and Schmid-Hempel, P.** (2000). Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos* **91**, 421–427.
- Brun, R., Hecker, H. and Lun, Z. R.** (1998). *Trypanosoma evansi* and *T. equiperdum*: Distribution, biology, treatment and phylogenetic relationship (A review). *Veterinary Parasitology* **79**, 95–107.
- Burthe, S. J., Telfer, S., Begon, M., Bennett, M., Smith, A. and Lambin, X.** (2008). Cowpox virus infection in natural field vole, *Microtus agrestis*, populations: significant negative impacts on survival. *Journal of Animal Ecology* **77**, 110–119.
- Clark, P. and Spencer, P. B. S.** (2006). Haematological characteristics of two wild populations of quokka (*Setonix brachyurus*). *Comparative Clinical Pathology* **15**, 82–86.
- Cox, F. E. G.** (2001). Concomitant infections, parasites and immune response. *Parasitology* **122**, S23–S38.
- Davey, C., Sinclair, A. R. E., Pech, R. P., Arthur, A. D., Krebs, C. J., Newsome, A. E., Hik, D., Molsher, R. and Allcock, K.** (2006). Do exotic vertebrates structure the biota of Australia? An experimental test in New South Wales. *Ecosystems* **9**, 992–1008.
- Department of Environment and Conservation** (2008). Diagnosis of recent woylie declines in southwestern Australia: progress report of the Woylie Conservation Research Project (Final Draft). 1–314. Western Australian Government Department of Environment and Conservation, Perth, Western Australia.
- Dortch, C. E. and Morse, K.** (1984). Prehistoric stone artefacts on some offshore islands in Western Australia. *Australian Archaeology* **19**, 31–47.
- Hamilton, P. B., Gibson, W. C. and Stevens, J. R.** (2007). Patterns of co-evolution between trypanosomes and their hosts deduced from ribosomal RNA and protein-coding gene phylogenies. *Molecular Phylogenetics and Evolution* **44**, 15–25.
- Hamilton, P. B., Stevens, J. R., Gidley, J., Holz, P. and Gibson, W. C.** (2005a). A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (Haemadipsidae). *International Journal for Parasitology* **35**, 431–443.
- Hamilton, P. B., Stevens, J. R., Holz, P., Boag, B., Cooke, B. and Gibson, W. C.** (2005b). The inadvertent introduction into Australia of *Trypanosoma*

- nabiasi*, the trypanosome of the European rabbit (*Oryctolagus cuniculus*), and its potential for biocontrol. *Molecular Ecology* **14**, 3167–3175.
- Hawlena, H., Bashary, D., Abramsky, Z. and Krasnov, B.** (2007). Benefits, costs and constraints on anti-parasitic grooming in adult and juvenile rodents. *Ethology* **113**, 394–402.
- Higgins, D., Thompson, J., Gibson, T., Thompson, J. D., Higgins, D. J. and Gibson, T. J.** (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Research* **22**, 4673–4680.
- Hoare, C. A.** (1972). *The Trypanosomes of Mammals: a Zoological Monograph*. Blackwell Scientific Publications, Oxford, UK.
- Hudson, P. J., Dobson, A. P. and Newborn, D.** (1992). Do parasites make prey vulnerable to predation-red grouse and parasites. *Journal of Animal Ecology* **61**, 681–692.
- Jakes, K. A., O'Donoghue, P. J. and Adlard, R. D.** (2001). Phylogenetic relationships of *Trypanosoma chelodina* and *Trypanosoma binneyi* from Australian tortoises and platypuses inferred from small subunit rRNA analysis. *Parasitology* **123**, 483–487.
- Mackerras, M. J.** (1959). The haematozoa of Australian mammals. *Australian Journal of Zoology* **7**, 105–132.
- Mackerras, M. J.** (1961). The haematozoa of Australian reptiles. *Australian Journal of Zoology* **9**, 61–122.
- Mackerras, M. J. and Mackerras, I. M.** (1960). The haematozoa of Australian birds. *Australian Journal of Zoology* **8**, 226–260.
- Mackerras, M. J. and Mackerras, I. M.** (1961). The haematozoa of Australian frogs and fish. *Australian Journal of Zoology* **9**, 123–140.
- McCallum, H. and Dobson, A.** (2002). Disease, habitat fragmentation and conservation. *Proceedings of the Royal Society of London, B* **269**, 2041–2049.
- Møller, A. P. and Nielsen, J. T.** (2007). Malaria and risk of predation: a comparative study of birds. *Ecology* **88**, 871–881.
- Newey, S. and Thirgood, S.** (2004). Parasite-mediated reduction in fecundity of mountain hares. *Proceedings of the Royal Society of London, B* **271**, S413–S415.
- Noyes, H. A., Stevens, J. R., Teixeira, M., Phelan, J. and Holz, P.** (1999). A nested PCR for the ssrRNA gene detects *Trypanosoma binneyi* in the platypus and *Trypanosoma* sp. in wombats and kangaroos in Australia. *International Journal for Parasitology* **29**, 331–339.
- Pedersen, A. B., Jones, K. E., Nunn, C. L. and Altizer, S.** (2007). Infectious diseases and extinction risk in wild mammals. *Conservation Biology* **21**, 1269–1279.
- Pedersen, A. B. and Grievies, T. J.** (2008). The interaction of parasites and resources cause crashes in a wild mouse population. *Journal of Animal Ecology* **77**, 370–377.
- Pickering, J. and Norris, C. A.** (1996). New evidence concerning the extinction of the endemic murid *Rattus macleri* from Christmas Island, Indian Ocean. *Australian Mammalogy* **19**, 19–25.
- Polley, L. and Thompson, R. C. A.** (2009). Parasite zoonoses and climate change: molecular tools for shifting boundaries. *Trends in Parasitology* **25**, 285–291.
- Rózsa, L., Reiczigel, J. and Majoros, G.** (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology* **86**, 228–232.
- Smith, A., Telfer, S., Burthe, S., Bennett, M. and Begon, M.** (2005). Trypanosomes, fleas and field voles: ecological dynamics of a host-vector-parasite interaction. *Parasitology* **131**, 355–365.
- Smith, A., Clark, P., Averis, S., Lymbery, A. J., Wayne, A. F., Morris, K. D. and Thompson, R. C. A.** (2008). Trypanosomes in a declining species of threatened Australian marsupial, the brush-tailed bettong *Bettongia penicillata* (Marsupialia: Potoroidae). *Parasitology* **135**, 1329–1335.
- Stevens, J. R., Noyes, H. A., Dover, G. A. and Gibson, W. C.** (1999). The ancient and divergent origins of the human pathogenic trypanosomes, *Trypanosoma brucei* and *T. cruzi*. *Parasitology* **118**, 107–116.
- Stevens, J. R., Noyes, H. A., Schofield, C. J. and Gibson, W. C.** (2001). The molecular evolution of Trypanosomatidae. *Advances in Parasitology* **48**, 1–56.
- Tamura, K., Dudley, J., Nei, M. and Kumar, S.** (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.
- Thekiso, O. M., Honda, T., Fujita, H., Battsetseg, B., Hatta, T., Fujisaki, K., Sugimoto, C. and Inoue, N.** (2007). A trypanosome sp. isolated from naturally infected *Haemaphysalis hystricis* ticks in Kagoshima Prefecture, Japan. *Parasitology* **134**, 967–974.
- Tompkins, D. M. and Begon, M.** (1999). Parasites can regulate wildlife populations. *Parasitology Today* **15**, 311–313.
- Thompson, R. C. A., Kutz, S. J. and Smith, A.** (2009). Parasite zoonoses and wildlife: emerging issues. *International Journal of Environmental Research and Public Health* **6**, 678–693.
- Thompson, R. C. A., Owen, I. L., Puana, I., Banks, D., Davis, T. M. E. and Reid, S. A.** (2003). Parasites and biosecurity: the example of Australia. *Trends in Parasitology* **19**, 410–416.
- Wyatt, K. B., Campos, P. F., Gilbert, M. T. P., Kolokotronis, S. O., Hynes, W. H., DeSalle, R., Daszak, P., MacPhee, R. D. E. and Greenwood, A. D.** (2008). Historic mammalian extinction on Christmas Island (Indian Ocean) correlates with introduced infectious disease. *PLoS ONE* **3**, 1–9.