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Short communication

High prevalence of *Trypanosoma vegrandis* in bats from Western Australia

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ABSTRACT

The present study describes the first report of *Trypanosoma vegrandis* in bats using morphology and sequence analysis of the 18S rRNA gene. The PCR prevalence of *T. vegrandis* in bats was 81.8% (18/22). The high prevalence of *T. vegrandis* in the present study suggests that bats may play an important role in the epidemiology of *T. vegrandis* in Australia. *T. vegrandis* appears to be geographically dispersed, has a wide distribution in Australia and low levels of host specificity.

1. Introduction

Bats (Order Chiroptera) are divided into two suborders, Yangochiroptera and Yinpterochiroptera (Springer, 2013). They are considered reservoirs of multiple infectious diseases and zoonotic pathogens (Calisher et al., 2006) and due to their broad distribution, social behavior and classification as the only mammals capable of sustained flight, have been implicated in the transmission of a range of microorganisms including rabies, severe acute respiratory syndrome (SARS), henipaviruses, ebola and pathogenic and non-pathogenic trypanosomes (Guyatt et al., 2003; Li et al., 2005; Halpin et al., 2000; Hamilton et al., 2012a,b). Trypanosomes are blood-borne parasites, and while the majority of species appear to asymptomatically infect the host, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* are known to cause severe disease in humans and *Trypanosoma cruzi* causes severe disease in both humans and animals, resulting in very large economic losses worldwide (Thompson et al., 2014). Bats are among the most common hosts of a large variety of trypanosomes in Africa, Asia, South America and Europe (Hoare, 1972; Baker, 1973; Marinkelle, 1976; Marinkelle, 1979; Gardner and Molyneux, 1988a,b; Molyneux, 1991; Hamanaka and Pinto Ada, 1993; Steindel et al., 1998; Grisard et al., 2003; Barnabe et al., 2003; Lisboa et al., 2008; Cottontail et al., 2009; Maia da Silva et al., 2009; Cavazzana et al., 2010; García et al., 2012; Hamilton et al., 2012a,b; Lima et al., 2012; Pinto et al., 2012; Lima et al., 2013; Marcili et al., 2013; Silva-Iturriza et al., 2013; Cottontail et al., 2014; Ramírez et al., 2014). However, knowledge of the genetic diversity, hosts, vectors, life-cycles, pathology, distribution and phylogenetic relationships of trypanosome species infecting bats is restricted to a few species.

Three trypanosome species have been identified in Australian bats; *Trypanosoma pteropi* from the black flying-fox (*Pteropus alecto*) (Breinl, 1913; Mackerras, 1959), *Trypanosoma hipposideri* from the dusky horse-shoe bat (also known as the dusky leaf-nosed bat) (*Hipposideros ater*) (Mackerras, 1959) and more recently a novel trypanosome most similar genetically to *Trypanosoma rangeli* and *Trypanosoma minasense* was identified in an adult female little red flying-fox (*Pteropus scapulatus*) (Mackie et al., 2015). In the present study, we screened Australian bats for trypanosomes using morphological and molecular methods.

2. Materials and methods

2.1. Samples

Both pteropid bats (Yangochiroptera) and microbats (Yinpterochiroptera) were screened (Table 1). Within the pteropids, black flying-fox (*P. alecto*) ($n = 5$) and little red flying-fox (*P. scapulatus*) ($n = 7$), blood and heart tissues were obtained from the Kimberley region of Western Australia. These bats were euthanased by local veterinary officers following reports of neurological disease, or interaction with members of the public resulting in bites or scratches. The carcasses were then necropsied in biological safety cabinets; the brain tissue sent to the CSIRO Australian Animal Health Laboratories (for Australian bat lyssavirus testing), and the remaining tissues stored at −80 °C. Blood samples from microbats were sampled at the request of local wildlife carers as part of routine veterinary investigation. Two species were screened; Gould's

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Table 1
Pteropid bats (Yangochiroptera) and microbats (Yinpterochiroptera) screened by PCR for the presence of *T. vegrandis* either in blood or heart tissue.

Bat ID	Species	Sample type	Positive via *T. vegrandis* specific PCR	Sequenced product
15-3	*Chalinolobus gouldii*	Blood	Positive	Yes
15-4	*Chalinolobus gouldii*	Blood	Positive	Yes
15-5	*Chalinolobus gouldii*	Blood	Positive	Yes
15-6	*Chalinolobus gouldii*	Blood	Positive	Yes
15-7	*Chalinolobus gouldii*	Blood	Positive	Yes
15-8	*Chalinolobus gouldii*	Blood	Positive	Yes
15-9	*Chalinolobus gouldii*	Blood	Positive	Yes
15-10	*Chalinolobus gouldii*	Blood	Positive	No
15-11	*Nyctophilus geoffroyi*	Blood	Positive	Yes
14-2549	*Pteropus alecto*	Blood	Negative	–
14-2382	*Pteropus scapulatus*	Blood	Positive	Yes
14-2259 #1	*Pteropus scapulatus*	Heart tissue	Positive	Yes
14-2259 #2	*Pteropus scapulatus*	Heart tissue	Positive	No
14-2259 #4	*Pteropus scapulatus*	Heart tissue	Positive	Yes
14-2347	*Pteropus scapulatus*	Heart tissue	Negative	–
14-2382	*Pteropus scapulatus*	Heart tissue	Positive	Yes
14-2430	*Pteropus scapulatus*	Heart tissue	Positive	No
14-2549	*Pteropus alecto*	Heart tissue	Negative	–
14-2566	*Pteropus alecto*	Heart tissue	Positive	No
14-3739 #1	*Pteropus alecto*	Heart tissue	Positive	Yes
14-3739 #2	*Pteropus alecto*	Heart tissue	Positive	No
15-0092	*Chalinolobus gouldii*	Heart tissue	Negative	–

wattled bats (*Chalinolobus gouldii*) ($n = 9$) and a lesser long-eared bat (*Nyctophilus geoffroyi*) ($n = 1$). All microbats were from the outer Perth metropolitan area and had been taken to carers as a result of habitat loss or suspected misadventure. Bats were anaesthetized with isoflurane and approximately 12 μl of blood was drawn from a blood vessel in the uropatagium (wing membrane vein) using a 26 gauge needle, before immediately being diluted 1:10 in phosphate buffered saline.

2.2. Microscopy

Bat blood samples were examined for the presence of trypanosomes using Wright–Giemsa stained (Hematek® Stain Pak) thin-blood smears. Slides were stained using a Hema-Tek Slide Stainer (Ames Company Division, Miles Laboratories Pty Ltd., Springvale Victoria, Australia). The slides were then air-dried and a coverslip mounted using DePeX mounting medium Gurr (Merck Pty. Ltd., Kilsyth, Victoria, Australia). Digital light micrograph images of any trypanosomes observed in blood films were taken at ×1000 magnification. Morphological measurements (total length, breadth, nucleus to anterior, kinetoplast to nucleus, posterior to kinetoplast and free flagellum of the trypanosomes were made using Image-Pro Express software (Media Cybernetics, Inc., Bethesda, Maryland, U.S.A.) and the means and standard errors (±) were calculated. Morphological features were measured using Image J and compared with available morphological measurements for trypanosomes infecting Australian mammals.

2.3. DNA extraction

Whole genomic DNA was extracted from bat blood using a MasterPure™ DNA Purification Kit (EPICENTRE® Biotechnologies, Madison, Wisconsin, U.S.A.) and from bat tissue using a Qiagen DNeasy blood and tissue kit (Qiagen, Victoria, Australia). DNA was eluted in 50 μl of water and stored at −20 °C until use. A DNA extraction blank (with no blood or tissue added) was included with each batch of DNA extractions.

2.4. 18S rRNA amplification and sequencing

Both generic *Trypanosoma* sp. primers and *T. vegrandis*-specific primers were used. For amplification of *T. vegrandis,* external forward primer S-823 and external reverse primer S-662 were used as previously described by McInnes et al. (2009) and Maslov et al. (1996) respectively. The PCR product from these external primers was run on a 1.5% agarose gel stained with SYBR safe (Invitrogen, U.S.A.). The gel band was excised and purified using a Promega Wizard SV Gel and PCR Clean-up System (Promega, Wisconsin, U.S.A.) and 1 μl of gel-purified PCR was used in the secondary reaction. The secondary reaction consisted of the internal nested primers TRYall 1 Forward 5′ ACCGTTTCGGCTTTTGTTGG 3′ and the *T. vegrandis* specific TVEG Reverse 5′ AAATCGTCTTCGCTTTAACTTT 3′ which amplify an ∼468 bp fragment of the *T. vegrandis* 18S rRNA. PCR amplification with the *T. vegrandis*-specific primers was performed with the final mix containing 1 μl of first round gel purified PCR product, 0.8 μM of each primer, 0.02 U/μl Kapa Taq (Kapa Biosystems, Massachusetts, U.S.A.), a final concentration of 2.5 mM of each dNTP and 2.5 μl of 10× buffer (Kapa Biosystems, Massachusetts, U.S.A.) with 1.5 mM $MgCl_2$ (Kapa Biosystems, Massachusetts, U.S.A.) and Baxter Ultra-Pure H2O to a volume of 25 μl. Cycling conditions consisted of an initial PCR round of 94 °C for 5 min, 50 °C for 2 min and 72 °C for 4 min followed by 45 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. The PCR was completed with a final extension of 7 min at 72 °C.

For amplification of *Trypanosoma* spp. external forward primer S-LF and external reverse primer S-762 was used in the primary PCR while two pairs of internal primers S-825 and S-LIR (producing a ∼959 bp) and S-823 and S-662 (producing a ∼900 bp) were used in secondary reactions as previously described by Maslov et al. (1996) and McInnes et al. (2009).

All controls (negative and positive PCR controls and DNA extraction blanks) produced appropriate PCR results. A representative sample of 13/18 *T. vegrandis*-specific PCR positive amplicons was gel purified using a Promega Wizard SV Gel and PCR Clean-up System (Promega, Wisconsin, U.S.A.) and submitted to the Australian Genome Research Facility (Ltd.) for unidirectional sequencing using the *T. vegrandis*-specific internal reverse primer TVEG-Reverse.

2.5. Phylogenetic analysis

Nucleotide sequences generated for the 18S rRNA locus were imported into Geneious R7 and aligned with a number of closely related 18S trypanosome sequences from GenBank, with *T. irwini* (FJ649479) used as an outgroup (Table 2). Alignments based on 421 bp, were obtained by MUSCLE (Edgar, 2004) using the default

Table 2
GenBank accession number of the 18S rDNA sequences used in the phylogenetic analysis.

Isolates	Accession number
T. irwini	FJ649479
Trypanosoma copemani AB-2013-G2	KC753531
Trypanosoma copemani isolate AAP (wombat)	AJ620558
T. copemani isolate Charlton (koala)	GU966588
Trypanosoma copemani isolate AB-2013-G1	KC753530
T. gilletti isolate Lanie (koala)	GU966589
T. vegrandis AP-2011b isolate 4 clone 6 (woylie)	JN315392
T. vegrandis AB-2013-G3 (woylie)	KC753533
T. vegrandis AB-2013-G6 (woylie)	KC753535
T. vegrandis AP-2011b isolate 27 clone 4 (woylie)	JN315394
T. vegrandis AP-2011b isolate 28 clone 11 (woylie)	JN315387
T. vegrandis AB-2013-G5 (woylie)	KC753534
T. vegrandis AB-2013-G4 (woylie)	KC753532
T. vegrandis AB-2013-G7 (woylie)	KC753536

settings, and then curated by Gblocks (Castresana, 2000), remotely, choosing the low stringency options available on the Phylogeny.fr platform (Dereeper et al., 2008). The curated alignments were then exported in MEGA 6 (Tamura et al., 2013) for the selection of the most appropriate nucleotide substitution model (Tamura–Nei), using the dedicated function. The evolutionary history was inferred by distance (NJ) (in MEGA 6) and by maximum likelihood method implemented in the PhyML program (v3.0), on the Phylogeny.fr platform (Dereeper et al., 2008). Specifically, the (Tamura–Nei) substitution model was selected assuming an estimated proportion of invariant sites and 4 gamma-distributed rate categories to account for rate heterogeneity across sites. The gamma shape parameter was estimated directly from the data. Reliability for internal branch was assessed using the bootstrapping method (500 bootstrap replicates). Genetic distances were generated in MEGA 6 based on the Tamura–Nei algorithm.

2.6. Statistical analysis

Prevalences were expressed as the percentage of samples positive by PCR, with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa et al., 2000).

3. Results

3.1. Microscopy

The morphology of *T. vegrandis* in blood films from the bats, represented short stumpy lanceolate to oval shaped trypanosomes, with absent undulating membranes (Fig. 1). The trypanosomes generally lacked a clearly defined kinetoplast and nucleated region and were observed as polymorphic (Fig. 1A–D). The posterior end was drawn out and tapered into a point while the anterior end was slightly rounded with either an absent or short free flagellum. A kinetoplast and nuclear region was identifiable in one trypanosome isolated from bat 15-4 (Fig. 1A). The kinetoplast in this isolate was round and situated close to the anterior nucleus The morphological parameters for this isolate were 13.8 μm in total length, 6.7 μm posterior to kinetoplast, 1.0 μm in breadth, 0.7 μm for kinetoplast to nucleus, 0.8 μm for nucleus to anterior and the free flagellum measured 2.8 μm. In addition, morphological measurements for total length and free flagellum were made from 13 and 10 trypanosomes respectively from bat isolate 15-4. The total length ranged between 6.7–13.8 μm with a mean of 9.0 ± 0.54 μm while the free flagellum ranged between 1.6–3.7 μm with a mean of 2.6 ± 0.2 μm.

3.2. Prevalence of trypanosomes in bat populations

The overall PCR prevalence of trypanosomes in bats was 81.8% (18/22) (65.7–97.9% 95CI). For pteropids, the prevalence was 85.7% (59.8–11.6% 95CI) for the little red flying-fox (*P. scapulatus*) and 60% (17.1–102.9% 95CI) for the black flying-fox (*P. alecto*). For microbats, the prevalence was 88.9% (68.4–109.4% 95CI) for Gould's wattled bats (*C. gouldii*) and the single blood sample from a lesser long-eared bat (*N. geoffroyi*) was also positive.

3.3. Phylogenetic analysis of trypanosome isolates from bats at the 18S locus

Amplicons were only produced using the *T. vegrandis*-specific primers. No other trypanosome species were detected. Sequences were obtained for thirteen of the eighteen *T. vegrandis*-specific PCR positives (Table 1). Bat-derived trypanosome sequences from all four species were 100% identical to each other and one representative isolate (15-5) from a Gould's wattled bat was included in the phylogenetic analysis. Based on Gblocks, of the 421 bp of sequence available, 397 bp were phylogenetically useful. Concordant tree topologies from ML and NJ analysis were obtained (data not shown). Phylogenetic analysis of the relationships between the bat-derived *T. vegrandis* isolate with *T. vegrandis* 18S genotypes on GenBank and closely related trypanosome species (Fig. 2 NJ tree shown) confirmed that all the sequences isolated from bats belonged to the *T. vegrandis* clade. The bat-derived *T. vegrandis*, grouped most closely with *T. vegrandis* genotype 5 from a woylie/brushtailed bettongs (*Bettongia penicillata*), (KC753534) and exhibited four single nucleotide polymorphisms (SNP's) from this genotype across 421 bp of sequence.

4. Discussion

In the present study, morphological and molecular analysis was used to identify *T. vegrandis* infecting bats from the outer Perth metropolitan area in the south–west of Western Australia and the Kimberley region in the north of Western Australia. *T. vegrandis* has previously been identified in woylies/brushtailed bettongs, western grey kangaroos (*Macropus fuliginosus*), southern brown bandicoots (*Isoodon obesulus*), the tammar wallaby (*Macropus eugenii*) and western quolls (*Dasyurus geoffroii*) in Western Australia (Averis et al., 2009; Paparini et al., 2011; Botero et al., 2013; Thompson et al., 2013). More recently it has been identified in koalas (*Phascolarctos cinereus*) from south–east Queensland and northern New South Wales (Barbosa et al., unpublished). Data from the present and previous studies therefore suggest that *T. vegrandis* is geographically dispersed, has a wide distribution in Australia and low levels of host specificity.

Three trypanosome species have been described in Australian bats; *T. pteropi, T. hipposideri* and an un-named species (Breinl, 1913; Mackerras, 1959; Mackie et al., 2015). *T. hipposideri* was described as very small and slender with a large kinetoplast located near the posterior end and a delicate short free flagellum at the anterior end and measured between 10.5–13.0 μm in total length and 1.5- 2 μm in breadth. *T. pteropi,* first described by Breinl (1913) in flying-foxes (*Pteropus gouldii*), represented a slender trypanosome with a long pointed posterior end, which had an under-developed undulating membrane and a long free flagellum. The original measurements by Breinl (1913) for total length was between 20 and 22 μm, 2.5–3.0 μm in breadth, 2.5–3.0 μm from the posterior to the kinetoplast and 11–12 μm in free flagellum. Mackerras (1959) later described trypanosomes isolated from a flying-fox (*Pteropus* sp.) as morphologically similar to *T. pteropi*, however they were slightly smaller with a sharply pointed anterior

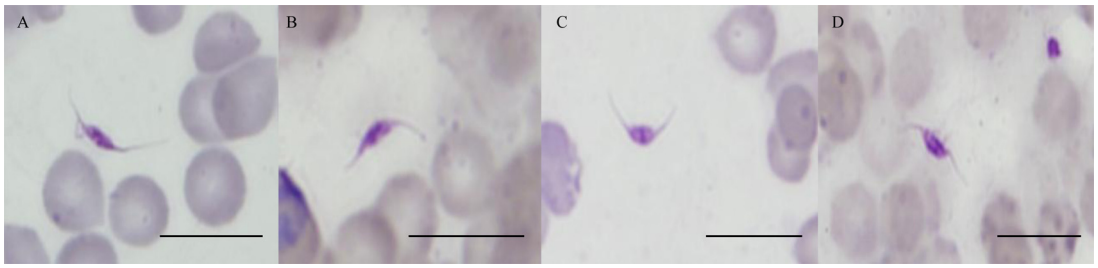

Fig. 1. Light photomicrograph of trypanosome stages of *T. vegrandis* in a Modified Wright's stained blood films from a bat (isolate 15-4). Scale bars represent 10 μm.

end and measured 18–20 μm in total length, 2–4 μm in breadth, 1.5–4 μm from the posterior end to the kinetoplast, 4–5 μm from the kinetoplast to the nucleus with the free flagellum measuring 8–10 μm (Mackerras, 1959). In the most recent study, the novel trypanosome identified in the adult female little red flying fox (Mackie et al., 2015), measured 20.4–30.8 μm in length and 1.3–2.3 μm in breadth, with tapered ends, and is therefore much larger than *T. vegrandis*.

In the present study, *T. vegrandis* from bats ranged between 6.7–13.8 μm in total length (mean 9.0 μm), with one isolate measuring 1.0 μm in breadth at the level of the nucleus. The overlap in size between *T. hipposideri* and *T. vegrandis* suggests that these may be the same species. However, in contrast to *T. hipposideri,* the trypanosomes identified in bats in the present study generally lacked a clearly defined kinetoplast and nucleated region. Unfortunately genetic sequences from *T. pteropi* and *T. hipposideri* are not available and therefore cannot be compared genetically with *T. vegrandis. T. vegrandis* was first described in woylies as a small curved trypanosome with a pointed posterior and an anterior nucleus in which the kinetoplast is closely situated. In woylies, *T. vegrandis* measured 6.9–10.5 μm in total length (mean 8.3 μm), (1.0–1.6 μm in breadth (mean 1.3 μm), with a free flagellum length of 1.2–2.8 μm (mean 1.8 μm), a posterior to kinetoplast length of 2.7–3.8 μm (mean 3.3 μm), a kinetoplast to nucleus length of 0.8–1.9 μm (mean 1.2 μm) and a nucleus to anterior length of 1.5–3.2 μm (mean N 2.1 μm) (Thompson et al., 2013). In the present study, the mean total length (9.0 μm) and mean free flagellum length (2.6 μm) of the bat isolates are morphologically similar to *T. vegrandis* from woylies. Only one bat isolate was observed with a defined kinetoplast and nuclear region and similar to the woylie isolates, contain an anterior nucleus with a kinetopast situated adjacently. For this bat isolate, the posterior to kinetoplast length (6.7 μm) and total length (13.8 μm) were larger compared to the woylie isolates, yet smaller in kinetoplast to nucleus (0.7 μm) and nucleus to anterior (0.8 μm).

The morphological differences observed between the marsupial and mammalian *T. vegrandis* isolates are possibly due to this species being polymorphic in nature with reports of multiple genotypes (Botero et al., 2013; Thompson et al., 2013). Trypanosomes have few morphological features detectable using light microscopy, which can adequately delimit species (Gibson, 2009) and the very small size of *T. vegrandis* (8.3 μm in length and 1.3 μm in width), renders this even more difficult. In the present study, morphological analysis was also limited due to the low levels of true blood stream trypomastigotes. This may have been due to the life-cycle phase at the time of sampling. Typically trypanosomes within the vertebrate host exhibit *T. cruzi*-like multiplication, with division taking place in the amastigote stage, followed by epimastigote forms that later develop into trypomastigote blood stream stages (Hoare, 1972). The small trypanosome stages observed in the bat blood (Fig. 1B–D) may represent epimastigote or transitional forms, while the more defined trypanosome (Fig. 1A) may represent the true blood stream life-cycle. Additional research however is required to understand the life-cycle stages of *T. vegrandis*.

The identity of *T. vegrandis* was confirmed using sequence and phylogenetic analysis of the 18S rRNA gene, which clearly showed that the bat-derived isolates grouped within the *T. vegrandis* clade. No other trypanosome species were identified. The bat-derived *T. vegrandis* isolates grouped most closely with *T. vegrandis* genotype 5 from a woylie.

In the present study, the PCR prevalence of *T. vegrandis* in Australian bats was 81.8% (65.7–97.9% 95CI), which was much higher than the 4% (0.1–20.4% 95CI) reported in pteropids by Mackerras (1959). Previous studies have reported a prevalence of

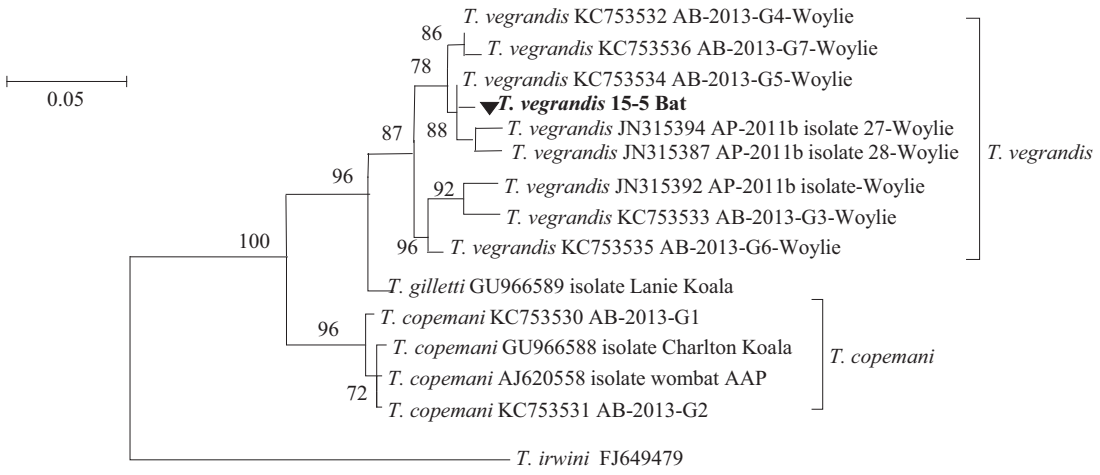

Fig. 2. Neighbour Joining (NJ) analysis of the relationships between *Trypanosoma* spp. and bat-derived *T. vegrandis* at the 18S rRNA locus (421 bp). Percentage support (>50%) from 500 replicates is indicated at the left of the supported node.

20% (0.0–40.2% 95CI) in woylies (Paparini et al., 2011) and up to 32% (27.1–36.6% 95CI) in various other marsupial species (Botero et al., 2013). The very high prevalence of *T. vegrandis* in Australian bats suggests that bats may be the primary host for *T. vegrandis*, however further research is required to confirm this. In other countries, a range of both zoonotic and bat-specific trypanosome species have been identified in bats. For example, studies in Colombian and Brazilian bats have reported trypanosome prevalences of 2.4–80% using microscopy (D'Alessandro et al., 1971; Marinkelle, 1972; Marinkelle, 1976). Molecular studies have identified a trypanosome prevalence of 61.2% (53.6–68.5% 95CI) in bats in Colombia (Ramírez et al., 2014) and 11.6% (7.7–16.6% 95CI) in Panamian bats (Pinto et al., 2012). Recently a novel trypanosome was identified in the blood of an adult female little red flying-fox (*P. scapulatus*) from south-eastern Queensland (Mackie et al., 2015). The novel trypanosome was most similar to *T. rangeli* and *T. minasense* (1% genetic distance at the 18S rRNA locus and 14–15% at the gGAPDH locus respectively) (Mackie et al., 2015).

Further investigations comprising larger sample sizes and additional species of bats are required to determine and compare the prevalence of *T. vegrandis* and other trypanosome species in bats more accurately. However the high prevalence of *T. vegrandis* in the present study suggests that bats may play an important role in the epidemiology of *T. vegrandis* in Australia. The impact of *T. vegrandis* on bat and marsupial health is currently unknown, but mixed *T. vegrandis* and *Trypanosoma copemani* co-infections were found more frequently in declining compared to stable woylie populations ($p = 0.001$) (Botero et al., 2013). All of the flying-foxes in the present study were clinically infected with Australian bat lyssavirus, and the microbats were from a wildlife care facility and so may be viewed as a population under a degree of stress. Further studies are essential to better understand the clinical significance of *T. vegrandis*, particularly in populations subject to stress from environmental factors or infectious agents, which may lead to expression of trypanosome-associated disease or increased shedding of the organism.

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References

Averis, S., Thompson, R.C.A., Lymbery, A.J., Wayne, A.F., Morris, K.D., Smith, A., 2009. The diversity, distribution and host–parasite associations of trypanosomes in Western Australian wildlife. Parasitology 136, 1269–1279.

Baker, J.R., 1973. First European record of *Trypanosoma* (*Megatrypanum*) sp. of bats. Nat. New Biol. 241, 96.

Barnabe, C., Brisse, S., Tibayrenc, M., 2003. Phylogenetic diversity of bat trypanosomes of subgenus *Schizotrypanum* based on multilocus enzyme electrophoresis, random amplified polymorphic DNA, and cytochrome b nucleotide sequence analyses. Infect. Genet. Evol. 3, 201–208.

Botero, A., Thompson, C.K., Peacock, C., Clode, P.L., Nicholls, P.K., Wayne, A.F., Lymbery, A.J., Thompson, R.C.A., 2013. Trypanosomes genetic diversity, polyparasitism and the population decline of the critically endangered Australian marsupial, the brushtailed bettong or woylie (*Bettongia penicillata*). Int. J. Parasitol. 2, 77–89.

Breinl, A., 1913. Parasite protozoa encountered in the blood of Australian native animals. Aust. Inst. Trop. Med., 30–38.

Calisher, C.H., Childs, J.E., Field, H.E., Holmes, K.V., Schountz, T., 2006. Bats: important reservoir hosts of emerging viruses. Clin. Microbiol. Rev. 19, 531–545.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552.

Cavazzana Jr., M., Marcili, A., Lima, L., da Silva, F.M., Junqueira, A.C., Veludo, H.H., Viola, L.B., Campaner, M., Nunes, V.L., Paiva, F., Coura, J.R., Camargo, E.P., Teixeira, M.M.G., 2010. Phylogeographical ecological and biological patterns shown by nuclear (ssrRNA and gGAPDH) and mitochondrial (Cyt b) genes of trypanosomes of the subgenus *Schizotrypanum* parasitic in Brazilian bats. Int. J. Parasitol. 40, 345–355.

Cottontail, V.M., Wellinghausen, N., Kalko, E.K., 2009. Habitat fragmentation and haemoparasites in the common fruit bat, *Artibeus jamaicensis* (*Phyllostomidae*) in a tropical lowland forest in Panamá. Parasitology 136, 1133–1145.

Cottontail, V.M., Kalko, E.K., Cottontail, I., Wellinghausen, N., Tschapka, M., Perkins, S.L., Pinto, C.M., 2014. High local diversity of *Trypanosoma* in a common bat species, and implications for the biogeography and taxonomy of the *T. cruzi* clade. PLoS One 9 (9), e108603.

D'Alessandro, A., Barreto, B., Duarte, R.C.A., 1971. Distribution of triatominae transmitted trypanosomiasis in Colombia and new records of the bugs and infections. J. Med. Entomol. 8, 159–172.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.M., Gascuel, O., 2008. Phylogeny: fr: robust phylogenetic analysis for the nonspecialist. Nucleic Acids Res. 36, W465–W469.

Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5, 113.

García, L., Ortiz, S., Osorio, G., Torrico, M.C., Torrico, F., Solari, A., 2012. Phylogenetic analysis of Bolivian bat trypanosomes of the subgenus *Schizotrypanum* based on cytochrome B sequence and minicircle analyses. PLoS One 7, e36578.

Gardner, R.A., Molyneux, D.H., 1988a. *Schizotrypanum* in British bats. Parasitology 97, 43–50.

Gardner, R.A., Molyneux, D.H., 1988b. *Trypanosoma* (*Megatrypanum*) incertum from *Pipistrellus pipistrellus*: development and transmission by cimicid bugs. Parasitology 96, 433–447.

Gibson, W., 2009. Species-specific probes for the identification of the African tsetse-transmitted trypanosomes. Parasitology 136, 1501–1507.

Grisard, E.C., Sturm, N.R., Campbell, D.A., 2003. A new species of trypanosome, *Trypanosoma desterrensis* sp. n., isolated from South American bats. Parasitology 127 (Pt 3), 265–271.

Guyatt, K.J., Twin, J., Davis, P., Holmes, E.C., Smith, G.A., Smith, I.L., Mackenzie, J.S., Young, P.L., 2003. A molecular epidemiological study of Australian bat lyssavirus. J. Gen. Virol. 84, 485–496.

Halpin, K., Young, P.L., Field, H.E., Mackenzie, J.S., 2000. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. J. Gen. Virol. 81, 1927–1932.

Hamanaka, S.I., Pinto Ada, S., 1993. Growth and differentiation on a trypanosome of the subgenus *Schizotrypanum* from the bat *Phyllostomus hastatus*. Rev. Soc. Bras. Med. Trop. 26 (4), 225–230.

Hamilton, P.B., Cruickshank, C., Stevens, J.R., Teixeira, M.M.G., Mathews, F., 2012a. Parasites reveal movement of bats between the new and old worlds. Mol. Phylogenet. Evol. 63, 521–526.

Hamilton, P.B., Teixeira, M.M.G., Stevens, J.R., 2012b. The evolution of *Trypanosoma cruzi*: the 'bat seeding' hypothesis. Trends Parasitol. 28, 136–141.

Hoare, C.A., 1972. The trypanosomes of mammals: a zoological monograph. Blackwell Scientific Publishing, Oxford, England.

Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J.H., Wang, H., Crameri, G., Hu, Z., Zhang, H., Zhang, J., McEachern, J., Field, H., Daszak, P., Eaton, B.T., Zhang, S., Wang, L.F., 2005. Bats are natural reservoirs of sars-like coronaviruses. Science 310, 676–679.

Lima, L., Maia da Silva, F., Neves, L., Attias, M., Takata, C.S., Campaner, M., de Souza, W., Hamilton, P.B., Teixeira, M.M.G., 2012. Evolutionary insights from bat trypanosomes: morphological, developmental and phylogenetic evidence of a new species, *Trypanosoma* (*Schizotrypanum*) *erneyi* sp. nov., in African bats closely related to *Trypanosoma* (*Schizotrypanum*) *cruzi* and allied species. Protist 163, 856–872.

Lima, L., Espinosa-Álvarez, O., Hamilton, P.B., Neves, L., Takata, C.S., Campaner, M., Attias, M., de Souza, W., Camargo, E.P., Teixeira, M.M., 2013. *Trypanosoma livingstonei*: a new species from African bats supports the bat seeding hypothesis for the *Trypanosoma* cruzi clade. Parasites Vectors 6 (1), 221.

Lisboa, C.V., Pinho, A.P., Herrera, H.M., Gerhardt, M., Cupolillo, E., Jansen, A.M., 2008. Trypanosoma cruzi (*Kinetoplastida, Trypanosomatidae*) genotypes in neotropical bats in Brazil. Vet. Parasitol. 156, 314–318.

Mackerras, M.J., 1959. The haematozoa of Australian mammals. Aust. J. Zool. 7, 105–135.

Mackie, J.T., Stenner, R., Gillett, A., Barbosa, A., Ryan, U., Irwin, P., 2015. Trypanosomiasis in an Australian little red flying-fox (*Pteropus scapulatus*). Vet. Pathol. (In press).

Maia da Silva, F., Marcili, A., Lima, L., Cavazzana Jr., M., Ortiz, P.A., Campaner, M., Takeda, G.F., Paiva, F., Nunes, V.L., Camargo, E.P., Teixeira, M.M., 2009. *Trypanosoma rangeli* isolates of bats from Central Brazil: genotyping and phylogenetic analysis enable description of a new lineage using spliced-leader gene sequences. Acta Trop. 109 (3), 199–207.

Marcili, A., da Costa, A.P., Soares, H.S., Acosta Ida, C., de Lima, J.T., Minervino, A.H., Melo, A.T., Aguiar, D.M., Pacheco, R.C., Gennari, S.M., 2013. Isolation and phylogenetic relationships of bat trypanosomes from different biomes in Mato Grosso, Brazil. J. Parasitol. 99 (6), 1071–1076.

Marinkelle, C.J., 1972. Colombian triatominae and their infestation with trypanosomatid flagellates. Mitt. Inst. Colombo-Alemaín Invest. Qentif Colombia 6, 13–29.

Marinkelle, C.J., 1976. The biology of the trypanosomes of bats. In: Lumdsen, W.H.R., Evans, D.A. (Eds.), Biology of the Kinetoplastida. Academic, New York, pp. 175–216.

Marinkelle, C.J., 1979. *Trypanosoma* (*Megatrypanum*) *megachiropterum* sp. n. from the flying-fox Pteropus tonganus Quoy, Gaimard. J. Protozool. 26, 352–353.

Maslov, D.A., Lukes, J., Jirku, M., Simpson, L., 1996. Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for the

evolution of parasitism in the trypanosomatid protozoa. Mol. Biochem. Parasitol. 75, 197–205.
McInnes, L.M., Gillett, A., Ryan, U.M., Austen, J., Campbell, R.S.F., Hanger, J., Reid, S.A., 2009. *Trypanosoma irwini* n. sp (Sarcomastigophora: Trypanosomatidae) from the koala (*Phascolarctos cinereus*). Parasitology 136, 875–885.
Molyneux, D.H., 1991. Trypanosomes of bats. In: Kreier, J.P., Baker, J.R. (Eds.), Parasitic Protozoa. Academic, pp. 195–223.
Paparini, A., Irwin, P.J., Warren, K., McInnes, L.M., De Tores, P., Ryan, U.M., 2011. Identification of novel trypanosome genotypes in native Australian marsupials. Vet. Parasitol. 183, 21–30.
Pinto, C.M., Kalko, E.K., Cottontail, I., Wellinghausen, N., Cottontail, V.M., 2012. TcBat a bat-exclusive lineage of *Trypanosoma cruzi* in the Panama Canal Zone, with comments on its classification and the use of the 18S rRNA gene for lineage identification. Infect. Genet. Evol. 12, 1328–1332.
Ramírez, J.D., Tapia-Calle, G., Muñoz-Cruz, G., Poveda, C., Rendón, L.M., Hincapié, E., Guhl, F., 2014. Trypanosome species in neo-tropical bats: biological, evolutionary and epidemiological implications. Infect. Genet. Evol. 22, 250–256.
Rózsa, L., Reiczigel, J., Majoros, G., 2000. Quantifying parasites in samples of hosts. J. Parasitol. 86, 228–232.
Silva-Iturriza, A., Nassar, J.M., García-Rawlins, A.M., Rosales, R., Mijares, A., 2013. Trypanosoma evansi kDNA minicircle found in the Venezuelan nectar-feeding bat *Leptonycteris curasoae* (*Glossophaginae*), supports the hypothesis of multiple origins of that parasite in South America. Parasitol. Int. 62 (2), 95–99.
Springer, M.S., 2013. Phylogenetics: Bats United, Microbats Divided. Curr. Biol. 23, 999–1001.
Steindel, M., Grisard, E.C., de Carvalho Pinto, C.J., Cordeiro, F.D., Ribeiro-Rodrigues, R., Romanha, A.J., 1998. Characterization of trypanosomes from the subgenus Schizotrypanum isolated from bats, *Eptesicus sp.* (Chiroptera: Vespertilionidae), captured in Florianopolis, Santa Catarina State, Brazil. J. Parasitol. 84 (3), 601–607.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30 (12), 2725–2729.
Thompson, C.K., Botero, A., Wayne, A.F., Godfrey, S.S., Lymbery, A.J., Thompson, R.C.A., 2013. Morphological polymorphism of *Trypanosoma copemani* and description of the genetically diverse T. vegrandis sp. Nov. from the critically endangered Australian potoroid, the brushtailed bettong (*Bettongia penicillata* (Gray, 1837)). Parasite Vectors 6, 121.
Thompson, C.K., Godfrey, S.S., Thompson, R.C., 2014. Trypanosomes of Australian mammals: a review. Int. J. Parasitol. Parasites Wildl. 3, 57–66.