International Journal for Parasitology: Parasites and Wildlife 25 (2024) 100982

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Tick-borne piroplasms and trypanosomes incidentally detected in eastern grey kangaroos (*Macropus giganteus*) during a mortality and morbidity event in southern New South Wales, Australia

Makenna Short [a], Kira Lowe [a], Michelle Michie [a], Ina Smith [a], Kim Blasdell [b], Alexander G. Maier [c], Alexander W. Gofton [d,*]

[a] CSIRO, Health and Biosecurity, Canberra, ACT, Australia
[b] CSIRO, Health and Biosecurity, Geelong, VIC, Australia
[c] Research School of Biology, Australian National University, Canberra, ACT, Australia
[d] CSIRO, Health and Biosecurity, Brisbane, QLD, Australia

ARTICLE INFO

Keywords:
Theileria
Babesia
Trypanosoma
Kangaroo
Haemoparasite
Australia
Tick

ABSTRACT

Tick-borne haemoparasites, including piroplasms and trypanosomes, are almost ubiquitous in Australian wildlife, with some associated with health impacts to individual animals and declining wildlife populations. An array of ecologically distinct piroplasm and trypanosome species occur throughout Australia although many of these species and their sylvatic ecologies are poorly characterised. Between May 2022 and October 2023, an anecdotally reported localised eastern grey kangaroo (*Macropus giganteus*) morbidity/mortality event occurred in coastal southern New South Wales, Australia, characterised by animals presenting with blindness, emaciation, lethargy, ataxia, and astasia. Here we used molecular techniques to identify tick-borne piroplasms (*Babesia* and *Theileria*) and trypanosomes in affected animals. Blood (n = 89) and liver (n = 19) samples were collected after the humane euthanasia of wild animals due to welfare concerns, and brief notes on the animal's health were recorded. In total, 20 (22.5%) animals were infected with tick-borne haemoparasites, including a novel *Theileria* sp. nov. (14, 15.7%), *Babesia macropus* (2, 2.2%), *Trypanosoma gilletti* (5, 5.6%), and *Trypanosoma vegrandis* (1, 1.1%). Liver samples were also screened for Wallal and Warego viruses due to animals' blindness, but were negative. This is the first report of *T. gilletti* and *T. vegrandis* in eastern grey kangaroos, although they have been previously reported in high numbers in ticks which commonly parasites this host. The novel *Theileria* sp. was previously reported in questing *Ixodes holocyclus* and in ticks from an opportunistically collected eastern grey kangaroo and red-necked wallaby (*Notamacropus rufogriseus*). However, we show for the first time this *Theileria* sp. can occur widely in eastern grey kangaroos. Ultimately, this small study did not intend, and is not able to draw inference regarding the pathogenicity of these haemoparasites to eastern grey kangaroos and it is likely that other factors, such as chronic *Phalaris* grass toxicity, had a role in this localised mortality/morbidity event.

1. Introduction

Tick-borne haemoparasites are almost ubiquitous in Australian wildlife with piroplasms (*Babesia* and *Theileria* spp.) and trypanosomes (*Trypanosoma* spp.) the most common. At least 24 distinct *Babesia* and *Theileria* genotypes have been described to date in Australian wildlife, including numerous poorly characterised genotypes awaiting formal classification (Barbosa et al., 2019; Egan et al., 2021). Most Australian endemic *Babesia* and *Theileria* species are contained within distinct monophyletic lineages due to the prolonged geographic isolation of Australia (Barbosa et al., 2019). Likewise, the known diversity of trypanosomes infecting endemic Australian wildlife has grown dramatically in recent years, with more than eight trypanosome species known to infect >28 host species (Cooper et al., 2017). Although Australia contains an evolutionary diverse range of trypanosomes, many Australian species fall within the monophyletic *Trypanosoma pestanai* clade, which is associated with tick-borne transmission and infection of mammalian hosts (Cooper et al., 2017; Koual et al., 2023).

Infection with native trypanosomes and piroplasms is typically non-pathogenic in a wide range of Australian wildlife (Thompson et al.,

* Corresponding author.
E-mail address: alexander.gofton@csiro.au (A.W. Gofton).

https://doi.org/10.1016/j.ijppaw.2024.100982
Received 23 July 2024; Received in revised form 28 August 2024; Accepted 29 August 2024
Available online 30 August 2024

2014; Barbosa and Irwin, 2019). However, infection with some species can result in clinical disease, particularly in immunocompromised individuals or in conjunction with environmental stressors or other co-morbidities. Australian trypanosomes and piroplasms that have been associated with pathogenicity in native wildlife species include *Trypanosoma copemani* in brush-tailed bettongs (*Bettongia penicillata*) which has been associated with muscular inflammation and tissue degeneration in vital organs, *Trypanosoma gilletti* in koalas (*Phascolarctos cinereus*) which is associated with reduced haematology measurements, lower body condition scores, and increased levels of coinfection with chlamydiosis and koala AIDS, and *Babesia macropus* in eastern grey kangaroos (EGKs) (*Marcopus giganteus*) was can cause anaemia, lethargy, and neurological signs, and has been responsible for previous mass mortality events. (Smith et al., 2008; McInnes et al., 2011; Botero et al., 2013; Donahoe et al., 2015).

Recently, a comprehensive survey of tick-borne microorganisms in the south-coast of New South Wales (NSW), Australia, identified a range of piroplasms and trypanosomes in questing *Ixodes holocyclus* and *Haemaphysalis bancrofti* ticks, including *Babesia mackerrasorum*, *Theileria* sp. AU-1048, *Theileria* sp. nov. IH, *T. gilletti*, *Trypanosoma vegrandis*, and *Trypanosoma* sp. nov. HB (Gofton et al., 2022). The same study investigated several small wildlife hosts (rodents, possums, and bandicoots) but was unable to identify the vertebrate hosts of these tick-borne haemoparasites. This led to the hypothesis that these haemoparasites likely

Fig. 1. Location of eastern grey kangaroo samples collected by wildlife carers in the south coast of NSW between May 2022 and August 2023.

infect larger Macropodidae hosts such as EGKs which are highly abundant and commonly parasitised by both *I. holocyclus* and *H. bancrofti* (Barker and Barker, 2023; Laan et al., 2011).

Here we used molecular assays to detect piroplasms and trypanosomes in a population of EGKs from the south-coast of NSW during an anecdotally reported morbidity/mortality event that occurred between May 2022 and October 2023. Note that although our investigation includes animals affected by this morbidity/mortality event, this work is not intended as an investigation of disease pathology, and we do not have sufficient samples or data to make conclusions regarding the cause of the event or pathology of the affected animals.

2. Materials and methods

An anecdotally reported local EGK morbidity/mortality event was observed by local wildlife carers in the south coast of NSW between May 2022 and October 2023 (Fig. 1). This event was characterised by large numbers of animals being referred or reported to wildlife carers with bilateral blindness or photophobia, and showing signs of emaciation, lethargy, ataxia, and astasia (Fig. 2).

Blood samples from EGKs ($n = 89$) and red-necked wallabies (*Notamacropus rufogriseus*) ($n = 4$) were collected from wild animals on the mid-south coast of NSW, Australia, during this morbidity/mortality event (Fig. 1). Animals were humanely euthanised for welfare reasons by trained wildlife carers and/or veterinarians, and the authors had no role or influence on the humane euthanasia of wild animals. This research was conducted in accordance with the *Australian code for the care and use of animals for scientific purposes (2013)*.

Samples were collected in the field immediately following humane euthanasia and donated for this study. Approximately 1–2 ml of blood

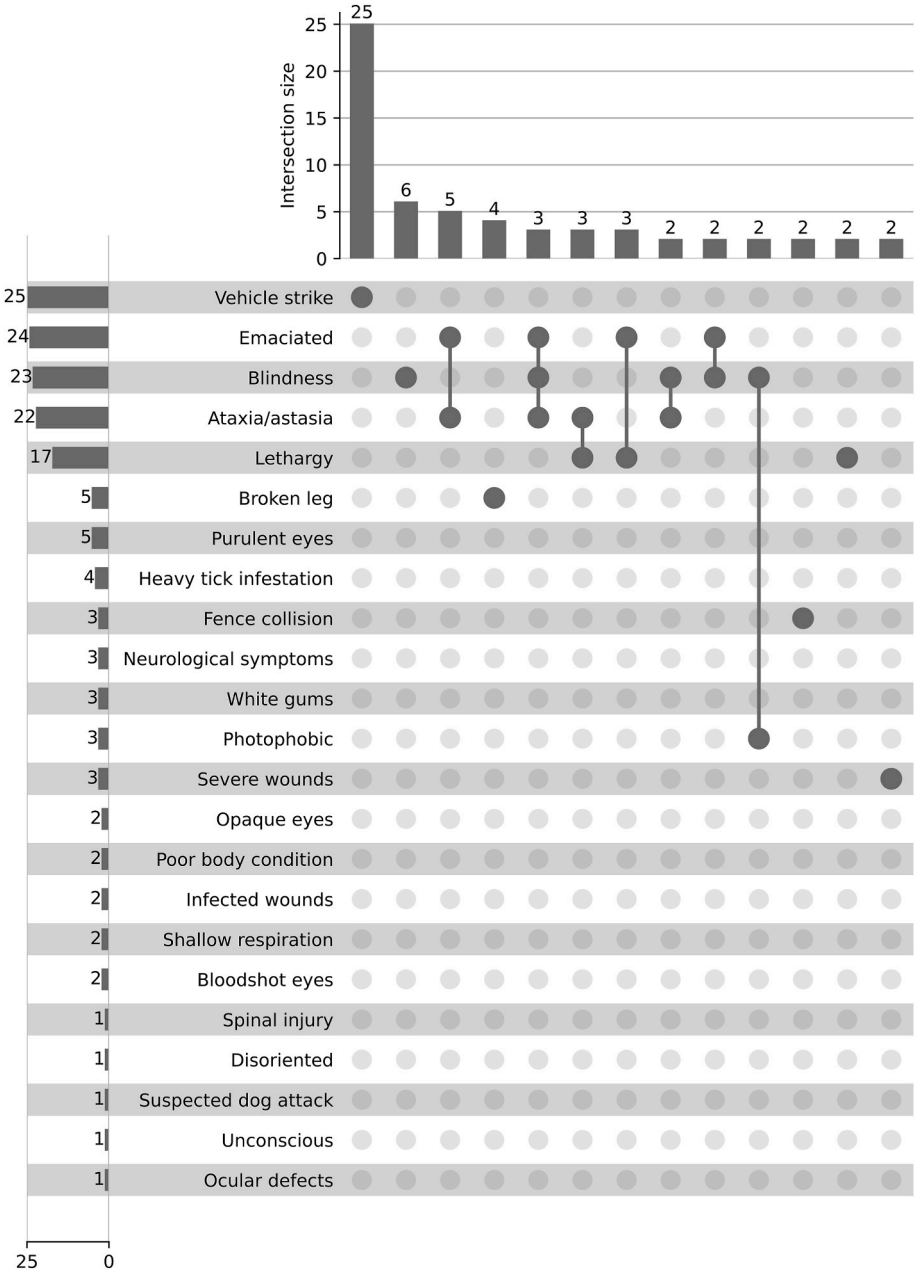

Fig. 2. Upset plot showing the frequency of reported health observations, and the intersections of these observations for eastern grey kangaroos in this study. The vertical axis represents the frequency of each individual health observation (all shown), the horizontal axis shows the frequency of co-occurring health observation (intersections) (frequencies >1 shown), and the matrix shows which sets of health observations are involved in each intersection.

was collected either into 2 ml EDTA-treated vacutainers or into 2 ml screw cap tubes containing 1 ml of 2x strength DNA/RNA Shield™ (Zymo Research) and stored as soon as practicable at −20 °C. Liver samples (n = 19) were also collected from some EGKs and stored in DNA/RNA Shield™ (Zymo Research) at −20 °C as soon as practicable until provided to the authors. Wildlife carers and veterinarians also collected brief subjective observations on the health of the animals and reason for euthanasia. Additional samples were unavailable due to constraints placed on wildlife carers and veterinaries collecting samples in the field.

Genomic DNA was extracted from 100 µl of frozen blood or 25 mg of clotted blood with the DNeasy Blood and Tissue Kit (QIAGEN, Germany), quantified with the Qubit fluorometer and analysed by nested PCRs targeting *Babesia* and *Theileria* 18S rRNA (Jefferies et al., 2007) and *Trypanosoma* 18S rRNA (McInnes et al., 2009). PCR assays were performed in 25 µl volumes containing PCR buffer (KAPA Biosystems, South Africa), 2.5 mM $MgCl_2$, 1 mM dNTPs, 400 nM of each primer, and 0.5 U KAPA Taq DNA polymerase (KAPA Biosystems, South Africa). Primary PCRs used 2 µl of genomic DNA as template, and nested PCRs used 1 µl of primary product as a template. No-template controls and extraction reagent blank controls were included in all PCR assays. PCR products were electrophoresed through 1% agarose gels and positive PCR products excised, purified and Sanger sequenced using both 5′ and 3′ nested PCR primers. Forward and reverse chromatograms were aligned in Geneious Prime v2020.2.5 (Kearse et al., 2012), and consensus sequences aligned using MAFFT (Katoh et al., 2002) with reference sequences from GenBank. Maximum likelihood phylogenetic analyses were performed in IQ-TREE v2.2.0 (Minh et al., 2020) with model selection (Kalyaanamoorthy et al., 2017) and 1000 ultrafast bootstrap replicates (Hoang et al., 2018).

Due to the presentation of many animals with blindness, the presence of Wallal virus (WALV) and Warrego virus (WARV) was also investigated using a limited number of liver samples preserved in DNA/RNA shield. RNA was isolated from EGK liver samples (n = 19) using the RNeasy Blood and Tissue kit (QIAGEN), and screened for WALV and WARV using RT-qPCR with the SuperScript™ III One-Step RT-PCR kit as previously described (Hooper et al., 1999).

3. Results

Overall, 89 EGKs were sampled by wildlife carers or veterinarians between May 2022 and October 2023. The majority of animals (55.1%) had abnormal health observations including emaciation (26.9%), blindness (25.8%), ataxia/astasia (24.7%), and lethargy (19.1%), or a combination of such observations (Fig. 2). 44.9% were euthanised due to vehicle strikes, fence collisions, broken limbs, or other wounds or injuries, and were reported without any further health observations (Fig. 2). However, post factum anecdotal reporting from wildlife carers indicated that there had been a noticeable large increase in vehicle strikes and fence collisions over this period largely due to blind animals wandering onto roads (pers. comms: WIRES Mid-South Coast Branch). Three out of the four red-necked wallabies sampled were euthanised after vehicle or fence collisions, with one animal having health observations that included blindness, emaciation, and lethargy.

Screening of liver samples (n =19) for WALV and WARV did not identify any positive samples, and no piroplasms or trypanosomes were found in red-necked wallaby samples. Overall, 16 (17.9%) EGK blood samples were positive for piroplasms, with *Babesia macropus* identified in two samples (2.2%) with 18S rRNA sequences identical to reference sequences in GenBank (KM206780). *Babesia macropus* infected animals were observed to have clinical signs consistent with *B. macropus*-infection that included lethargy, bloodshot eyes, and pale gums (Dawood et al., 2013; Donahoe et al., 2015).

A *Theileria* sp. was also identified in 14 samples (15.7%) with 18S rRNA sequences identical to sequences from *Theileria* sp. nov. AU_1048 that was previously reported in blood-fed *Haemaphysalis* sp. and *I. holocyclus* ticks from a red-necked wallaby and an EGK, respectively, from the mid-north coast of NSW (Storey-Lewis et al., 2018), and from questing *I. holocyclus* ticks from the same geographic region as EGKs in this study (Gofton et al., 2022) (Fig. 3). Sequences from this *Theileria* sp. were >4.9% dissimilar to other *Theileria* sequences and phylogenetic analysis clustered all of these novel *Theileria* sp. sequences into a discrete monophyletic clade that was distinct to its closest relatives *T. fuliginosus* and *T. worthingtonorum* (Fig. 3). The predominant health observations for animals positive for this novel *Theileria* sp. were blindness, emaciation, lethargy, and ataxia/astasia. However, 4/14 positive animals were involved in vehicle strikes and these signs were not observed.

Trypanosomes were also identified in six samples (6.7%). *Trypanosoma gilletti* was identified in five samples (5.6%), with 18S rRNA sequences identical to reference sequences in GenBank, including from questing *I. holocyclus* ticks from the same region (MZ502203) (Fig. 4). One sample (1.1%) contained *T. vegrandis*, with 18S rRNA sequences identical to reference *T. vegrandis* sequences (MZ502214) from the same region (Gofton et al., 2022) (Fig. 4). Phylogenetic analysis revealed that *T. gilletti* sequences from EGKs were highly conserved (>99.2% sequence identity) with other *T. gilletti* samples from diverse hosts including the koala and long-nosed potoroo (*Potorous tridactylus*) (Fig. 4). Conversely, there was significant variability in *T. vegrandis* 18S rRNA sequences, including >4.4% sequence dissimilarity between *T. vegrandis* SCW67 and *T. vegrandis* AB-2013 G3. Two animals were also found to be co-infected with the *Theileria* sp. nov. AU_1048 and trypanosomes (one each with *T. gilletti* and *T. vegrandis*). The predominant clinical signs for animals positive for *T. gilletti* were blindness, emaciation, lethargy, and heavy tick infestations, although two individuals also suffered broken limbs from fence collisions but were otherwise healthy. The individuals co-infected with *Theileria* sp. nov. AU_1048 and *T. gilletti* or *T. vegrandis* had clinical signs that included emaciation, lethargy, and white gums, and blindness, emaciation, and ataxia/astasia, respectively.

4. Discussion

This work described the molecular identification of piroplasms and trypanosomes in EGK blood samples during an anecdotally reported morbidity/mortality event in south-coast NSW. Overall, four haemoparasite species were identified including a *Theileria* sp. nov. AU_1048, *B. macropus*, *T. gilletti*, and *T. vegrandis*. This morbidity/mortality event was characterised by large numbers of animals showing signs of blindness, lethargy, emaciation, and ataxia/astasia, as well as other health observations such as white gums, heavy tick infestations, and purulent eyes (Fig. 2).

Previous epidemics of blindness in EGKs in NSW and Victoria, Australia, have been caused by *Culicoides*-transmitted viral infections with WALV or WARV (Belaganahalli et al., 2014; Hooper et al., 1999). However, here screening of RNA from 19 EGK liver samples did not find any evidence that these viruses contributed to this morbidity/mortality event.

The identification of *B. macropus* in two samples is not unexpected as it is a common parasite of EGKs and the primary cause of previous EGK mass morbidity/mortality events that were characterised by anaemia, lethargy, and neurological signs including depression, ataxia, bruxism, and ocular deficits (Donahoe et al., 2015). However, in previous such events *B. macropus* was identified in 100% of tested individuals (Donahoe et al., 2015), compared to just 2.2% of individuals here; suggesting that *B. macropus* is not the primary cause of this morbidity/mortality event, and that other stimuli may be responsible. Nevertheless, *B. macropus* may be responsible for some of the health signs observed in the two infected individuals, particularly pale gums which is indicative of anaemia and not associated with *Phalaris* toxicity (Bacci et al., 2014).

Previous work also indicated that *B. macropus* may be rare in this region by failing to detect any *B. macropus* in >3500 questing *I. holocyclus* or *H. bancrofti* ticks from the same regions as this study

Fig. 3. Maximum likelihood phylogenetic tree of *Babesia* and *Theileria* 18S rRNA sequences (751 bp) produced with IQTREE v2.2.0 with model selection and 1000 bootstrap approximations. Sequences from this study are in bold text, black text indicates piroplasms from Australian wildlife. Square brackets indicate GenBank accession, parenthesis specify host and vector associations.

(Gofton et al., 2022). *Ixodes holocyclus* and *H. bancrofti* are common parasites of EGKs and putative vectors for *B. macropus*, although it is probable that other tick vectors also occur (Dawood et al., 2013). *Babesia macropus* is also known to sequester in large numbers in the small vessels of the visceral organs and parasitised erythrocytes can be rare in peripheral blood samples, which could have contributed to low detection rate in this work (Donahoe et al., 2015). Future analysis of brain or kidney samples may provide a more accurate estimate of *B. macropus* prevalence.

The novel *Theileria* sp. (previously designated *Theileria* sp. nov AU1048 (Storey-Lewis et al., 2018)) was identified in 15.7% of EGKs and also previously in approximately 13.1% of questing *I. holocyclus* ticks in the same region, although not in any *H. bancrofti* ticks (Gofton et al., 2022). Collectively, this data indicates that this *Theileria* sp. nov AU1048 is likely maintained in a sylvatic cycle involving at least *I. holocyclus* as a primary vector (and perhaps other tick species, but not *H. bancrofti*) and at least EGKs and perhaps also red-necked wallabies as vertebrate hosts (Gofton et al., 2022; Storey-Lewis et al., 2018). The

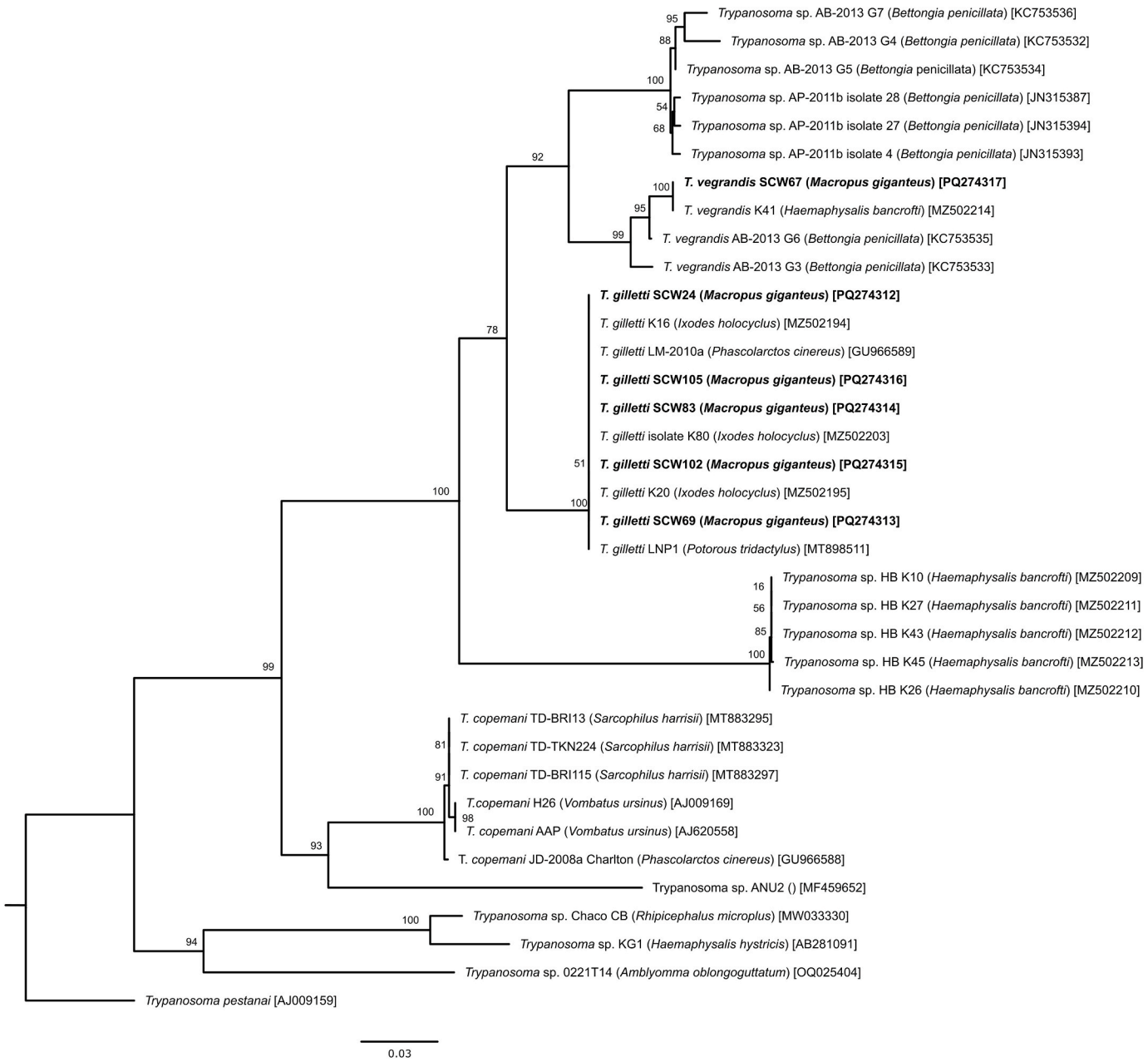

Fig. 4. Maximum likelihood phylogenetic tree of Australian tick-borne trypanosomes based on 18S rRNA sequences (1536 bp) produced with IQTREE v2.2.0 with model selection and 1000 bootstrap approximations. Sequences from this study are in bold text, square brackets indicate GenBank accession, parenthesis specify host and vector associations.

phylogenetic distinctness of *Theileria* sp. nov AU1048 indicates that it is a unique and discrete species, and future work should generate additional molecular, morphological, ecological, and epidemiological data to support its formal classification (Fig. 3).

While this novel piroplasm was detected in individuals affected by an anecdotally reported morbidity/mortality event, we do not have the associated clinical or pathological data to draw conclusions regarding its pathogenicity to EGKs, as the brief observations on animal health provided by wildlife carers are not sufficient to form a definitive causal association between the parasite and clinical signs. Nevertheless, many of the reported health observations such as, blindness, lethargy, ataxia, astasia, and pale gums are consistent with the clinical signs associated with other piroplasm species (e.g. *B. macropus*) (Donahoe et al., 2015). Future work should focus on obtaining and incorporating clinical and pathological data to determine whether *Theileria* sp. nov AU1048 is associated with clinical disease in EGKs.

This work also identified two trypanosomes in EGK blood samples: *T. gilletti* and *T. vegrandis*. Eastern grey kangaroos are known to carry another trypanosome species, *Trypanosoma* sp. H25, but this is the first report of *T. gilletti* and *T. vegrandis* in this host (Cooper et al., 2017; Thompson et al., 2014). *Trypanosoma gilletti* is most commonly found in koalas (*Phascolarctos cinereus*) throughout eastern Australia where is has been implicated in the poor health of individual animals (McInnes et al., 2011). However, more recent molecular surveillance has also found *T. gilletti* infecting swamp wallabies (*Wallabia bicolor*), southern brown bandicoots (*Isoodon obesulus*), long-nosed potoroo, and brush-tailed bettongs indicating it has a wider host range than previously known (Cooper et al., 2018; Ortiz-Baez et al., 2020; Hall et al., 2021). The finding of *T. vegrandis* in EGKs is also novel, but less surprising, as *T. vegrandis* is known to have a wide geographic and host distribution

including the western grey kangaroo (*M. fuliginosus*),Tammar wallaby (*M. eugenii*), brush-tailed bettong, long-nosed potoroo, Quolls (*Dasyurus* spp.), southern brown bandicoot, western brown bandicoot (*I. obesulus fusciventer*), brush-tailed possum (*Trichosurus vulpecula*), koala, Gould's wattled bat (*Chalinolobus gouldii*), lesser long-eared bat (*Nyctophilus geoffroyi*), and little red flying fox (*Pteropus scapulatus*) (Smith et al., 2008; Paparini et al., 2011; Austen et al., 2015; Barbosa et al., 2016; Cooper et al., 2018; Hall et al., 2021). Like other members of the *T. pestanai* clade, *T. gilletti* and *T. vegrandis* are tick-borne trypanosomes (Koual et al., 2023) and previous work identified *T. gilletti* and *T. vegrandis* in questing *I. holocyclus* and *H. bancrofti* ticks, respectively, in the same region as samples from this study, indicating these ticks may act as vectors for these trypanosome species (Gofton et al., 2022). Therefore, while *T. vegrandis* and *T. gilletti* share the EGKs as a host, there appears to be a high degree of specificity in their compatibility with different vector ticks.

Despite our results indicating that EGKs were infected with tick-borne haemoparasites, we cannot conclude that these parasites were associated with this morbidity/mortality event, and it is likely that other stimuli contributed to the health observations reported in affected animals. For example, chronic phalaris toxicity caused by ingestion of *Phalaris* spp. grasses can result in similar signs as observed here including ataxia, head tremors, lethargy, wasting, and blindness (Bacci et al., 2014; Fowler, 1983; Rogers, 2022). *Phalaris* spp. are widespread in eastern and southern Australia and chronic phalaris toxicity was recently shown to be widespread in macropods throughout Victoria, Australia (Chen et al., 2024). It is therefore probable that chronic phalaris toxicity contributed to this morbidity/mortality event, and to the signs observed in many individuals, including those infected by tick-borne piroplasms or trypanosomes.

We identified haemoparasites in 20 out of 89 (22.5%) EGK blood samples, with a prevalence of individual haemoparasites ranging between 1.1 and 15.7%. However, these levels are comparable or lower than haemoparasite prevalences in other Australian wildlife populations. For example, *T. vegrandis* has been found in >24% of brush-tailed possums and brush-tailed bettongs (Northover et al., 2024) and in >11% of Koalas (Barbosa et al., 2017), *T. copemani* in >60% of brush-tailed bettongs (Botero et al., 2013) and piroplasm such as *Theileria* cf. *peramelis* and *Theileria penicillata* have been identified in 75% of long-nosed bandicoots (*Perameles nasuta*) and >80% of brush-tailed bettongs, respectively (Egan et al., 2021; Rong et al., 2012). Therefore, the relativley low haemoparasite prevalence detected here in EGKs, may represents a relatively normal baseline level of parasite-infection, and their detection during this anecdotally reported morbidity/mortality event may be incidental.

A previous comprehensive study of tick-borne microorganisms in the south-coast of NSW identified several haemoparasites in questing ticks which were not found infecting EGKs in this study. *Babesia mackerrasorum* and *Trypanosoma* sp. nov. HB were both identified at high prevalence in questing *H. bancrofti*, and *Theileria* sp. nov. IH was identified in a small number of *I. holocyclus* ticks (Gofton et al., 2022). It is therefore likely these novel haemoparasites utilise other host species, such as other Macropdidae species which are common hosts of these ticks (Barker and Barker, 2023; Laan et al., 2011). Alternatively, it is also possible that nested-PCR failed to detect haemoparasite polyparasitism which is widespread in many Australian marsupials (Barbosa et al., 2017; Cooper et al., 2018). In cases of trypanosome polyparasitism nested PCR has been shown to identify only the most abundant trypanosome species and underestimate the diversity and prevalence of trypanosome co-infections. It is therefore possible that *Trypanosome* sp. HB is a subdominant parasite in EGKs and therefore not detected here by nested-PCR in the presence of *T. gilletti* or *T. vegrandis* (Barbosa et al., 2017). Studies utilising trypanosome 18S rRNA metabarcoding could be used in future studies to overcome this limitation and estimate more accurately the presence and level of trypanosome polyparasitism in EGKs.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Makenna Short: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Kira Lowe:** Writing – review & editing, Investigation, Data curation. **Michelle Michie:** Writing – review & editing, Supervision, Project administration, Investigation, Data curation. **Ina Smith:** Writing – review & editing, Project administration, Investigation. **Kim Blasdell:** Writing – review & editing, Project administration, Investigation. **Alexander G. Maier:** Writing – review & editing, Supervision, Resources, Project administration. **Alexander W. Gofton:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors' declare that no conflict of interest exists.

Acknowledgments

The authors would like the acknowledge the dedicated work of wildlife carers from the WIRES Mid-South Coast Branch for providing samples and health observations on effected animals.

References

Austen, J.M., O'Dea, M., Jackson, B., Ryan, U., 2015. High prevalence of *Trypanosoma vegrandis* in bats from Western Australia. Vet. Parasitol. 214, 342–347. https://doi.org/10.1016/j.vetpar.2015.10.016.

Bacci, B., Whiteley, P.L., Barrow, M., Phillips, P.H., Dalziel, J., El-Hage, C.M., 2014. Chronic phalaris toxicity in eastern grey kangaroos (*Macropus giganteus*). Aust. Vet. J. 92, 504–508. https://doi.org/10.1111/avj.12272.

Barbosa, A., Austen, J., Gillett, A., Warren, K., Paparini, A., Irwin, P., Ryan, U., 2016. First report of *Trypanosoma vegrandis* in koalas (*Phascolarctos cinereus*). Parasitol. Int. 65, 316–318. https://doi.org/10.1016/j.parint.2016.03.004.

Barbosa, A., Irwin, P., 2019. Haemoprotozoan parasites. In: Current Therapy in Medicine of Australian Mammals. CSIRO, Collingwood.

Barbosa, A.D., Austen, J., Portas, T.J., Friend, J.A., Ahlstrom, L.A., Oskam, C.L., Ryan, U. M., Irwin, P.J., 2019. Sequence analyses at mitochondrial and nuclear loci reveal a novel *Theileria* sp. and aid in the phylogenetic resolution of piroplasms from Australian marsupials and ticks. PLoS One 14, e0225822. https://doi.org/10.1371/journal.pone.0225822.

Barbosa, A.D., Gofton, A.W., Paparini, A., Codello, A., Greay, T., Gillett, A., Warren, K., Irwin, P., Ryan, U., 2017. Increased genetic diversity and prevalence of co-infection with *Trypanosoma* spp. in koalas (*Phascolarctos cinereus*) and their ticks identified using next-generation sequencing (NGS). PLoS One 12, e0181279. https://doi.org/10.1371/journal.pone.0181279.

Barker, S. c, Barker, D., 2023. Ticks of Australasia: 125 species of ticks in and around Australia. Zootaxa 5253, 1–670. https://doi.org/10.11646/zootaxa.5253.1.1.

Belaganahalli, M.N., Maan, S., Maan, N.S., Pritchard, I., Kirkland, P.D., Brownlie, J., Attoui, H., Mertens, P.P.C., 2014. Full genome characterization of the *culicoides*-borne marsupial orbiviruses: wallal virus, mudjinbarry virus and Warrego viruses. PLoS One 9, e108379. https://doi.org/10.1371/journal.pone.0108379.

Botero, A., Thompson, C.K., Peacock, C.S., 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 brush tailed bettong or woylie (*Bettongia penicillata*). Int. J. Parasitol. Parasites Wildl. 2, 77–89. https://doi.org/10.1016/j.ijppaw.2013.03.001.

Chen, T., Hufschmid, J., Whiteley, P., El-Hage, C., Davis, N., Skerratt, L., 2024. Chronic phalaris toxicity in macropods is widespread and peaks in July in Victoria, Australia. Aust. Vet. J. 102, 331–338. https://doi.org/10.1111/avj.13327.

Cooper, C., Clode, P.L., Peacock, C., Thompson, R.C.A., 2017. Host–parasite relationships and life histories of trypanosomes in Australia. In: Rollinson, D., Stothard, J.R. (Eds.), Advances in Parasitology. Academic Press, pp. 47–109. https://doi.org/10.1016/bs.apar.2016.06.001.

Cooper, C., Keatley, S., Northover, A., Gofton, A.W., Brigg, F., Lymbery, A.J., Pallant, L., Clode, P.L., Thompson, R.C.A., 2018. Next generation sequencing reveals widespread trypanosome diversity and polyparasitism in marsupials from Western Australia. Int. J. Parasitol. Parasites Wildl. 7, 58–67. https://doi.org/10.1016/j.ijppaw.2018.01.005.

Dawood, K.E., Morgan, J.A.T., Busfield, F., Srivastava, M., Fletcher, T.I., Sambono, J., Jackson, L.A., Venus, B., Philbey, A.W., Lew-Tabor, A.E., 2013. Observation of a novel *Babesia* spp. in eastern grey kangaroos (*Macropus giganteus*) in Australia. Int. J. Parasitol. Parasites Wildl. 2, 54–61. https://doi.org/10.1016/j.ijppaw.2012.12.001.

Donahoe, S.L., Peacock, C.S., Choo, A.Y.L., Cook, R.W., O'Donoghue, P., Crameri, S., Vogelnest, L., Gordon, A.N., Scott, J.L., Rose, K., 2015. A retrospective study of *Babesia macropus* associated with morbidity and mortality in eastern grey kangaroos (*Macropus giganteus*) and agile wallabies (*Macropus agilis*). Int. J. Parasitol. Parasites Wildl. 4, 268–276. https://doi.org/10.1016/j.ijppaw.2015.02.002.

Egan, S.L., Taylor, C.L., Austen, J.M., Banks, P.B., Northover, A.S., Ahlstrom, L.A., Ryan, U.M., Irwin, P.J., Oskam, C.L., 2021. Haemoprotozoan surveillance in peri-urban native and introduced wildlife from Australia. Curr. Res. Parasitol. Vector-Borne Dis. 1, 100052 https://doi.org/10.1016/j.crpvbd.2021.100052.

Fowler, M.E., 1983. Plant poisoning in free-living wild animals: a review. J. Wildl. Dis. 19, 34–43. https://doi.org/10.7589/0090-3558-19.1.34.

Gofton, A.W., Blasdell, K.R., Taylor, C., Banks, P.B., Michie, M., Roy-Dufresne, E., Poldy, J., Wang, J., Dunn, M., Tachedjian, M., Smith, I., 2022. Metatranscriptomic profiling reveals diverse tick-borne bacteria, protozoans and viruses in ticks and wildlife from Australia. Transbound. Emerg. Dis. 69, e2389–e2407. https://doi.org/10.1111/tbed.14581.

Hall, J., Rose, K., Austen, J., Egan, S., Bilney, R., Kambouris, P., MacGregor, C., Dexter, N., 2021. Baseline health parameters for A newly established population of long-nosed potoroo (*Potorous tridactylus*) at booderee national park, Australia. J. Wildl. Dis. https://doi.org/10.7589/JWD-D-20-00168.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol. Biol. Evol. 35, 518–522. https://doi.org/10.1093/molbev/msx281.

Hooper, P.T., Lunt, R.A., Gould, A.R., Hyatt, A.D., Russell, G.M., Kattenbelt, J.A., Blacksell, S.D., Reddacliff, L.A., Kirkland, P.D., Davis, R.J., Durham, P.J., Bishop, A. L., Waddington, J., 1999. Epidemic of blindness in kangaroos-evidence of a viral aetiology. Aust. Vet. J. 77, 529–536. https://doi.org/10.1111/j.1751-0813.1999.tb12127.x.

Jefferies, R., Ryan, U.M., Irwin, P.J., 2007. PCR-RFLP for the detection and differentiation of the canine piroplasm species and its use with filter paper-based technologies. Vet. Parasitol. 144, 20–27. https://doi.org/10.1016/j.vetpar.2006.09.022.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066. https://doi.org/10.1093/nar/gkf436.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Koual, R., Buysse, M., Grillet, J., Binetruy, F., Ouass, S., Sprong, H., Duhayon, M., Boulanger, N., Jourdain, F., Alafaci, A., Verdon, J., Verheyden, H., Rispe, C., Plantard, O., Duron, O., 2023. Phylogenetic evidence for a clade of tick-associated trypanosomes. Parasites Vectors 16, 3. https://doi.org/10.1186/s13071-022-05622-y.

Laan, B., Handasyde, K., Beveridge, I., 2011. Occurrence of the tick *Haemaphysalis bancrofti*, Nuttal & Warburton, 1915, in Victoria with additional data on its distribution and with scanning electron micrographs of life cycle stages. Proc. Roy. Soc. Vic. 123, 189–1999.

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. https://doi.org/10.1017/S0031182009006313.

McInnes, L.M., Hanger, J., Simmons, G., Reid, S.A., Ryan, U.M., 2011. Novel trypanosome *Trypanosoma gilletti* sp. (Euglenozoa: trypanosomatidae) and the extension of the host range of *Trypanosoma copemani* to include the koala (*Phascolarctos cinereus*). Parasitology 138, 59–70. https://doi.org/10.1017/S0031182010000971.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol. Biol. Evol. 37, 1530–1534. https://doi.org/10.1093/molbev/msaa015.

Northover, A., Godfrey, S., Lymbery, A.J., Wayne, A.F., Keatley, S., Ash, A., Badsha, D., Egan, S., Barr, J., Thompson, R., 2024. The parasites of free-ranging terrestrial wildlife from Australia's south-west. Aust. J. Zool. 71 https://doi.org/10.1071/ZO23048.

Ortiz-Baez, A.S., Cousins, K., Eden, J.-S., Chang, W.-S., Harvey, E., Pettersson, J.H.-O., Carver, S., Polkinghorne, A., Šlapeta, J., Rose, K., Holmes, E.C., 2020. Meta-transcriptomic identification of *Trypanosoma* spp. in native wildlife species from Australia. Parasites Vectors 13, 447. https://doi.org/10.1186/s13071-020-04325-6.

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. https://doi.org/10.1016/j.vetpar.2011.07.009.

Rogers, J., 2022. Blindness in some eastern grey kangaroos. Control Ther. Ser.

Rong, J., Bunce, M., Wayne, A., Pacioni, C., Ryan, U., Irwin, P., 2012. A high prevalence of *Theileria penicillata* in woylies (*Bettongia penicillata*). Exp. Parasitol. 131, 157–161. https://doi.org/10.1016/j.exppara.2012.03.013.

Smith, A., Clark, P., Averis, S., Lymbery, A.J., Wayne, A.F., Morris, K.D., 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. https://doi.org/10.1017/S0031182008004824.

Storey-Lewis, B., Mitrovic, A., McParland, B., 2018. Molecular detection and characterisation of *Babesia* and *Theileria* in Australian hard ticks. Ticks Tick-Borne Dis. 9, 471–478. https://doi.org/10.1016/j.ttbdis.2017.12.012.

Thompson, C.K., Godfrey, S.S., Thompson, R.C.A., 2014. Trypanosomes of Australian mammals: a review. Int. J. Parasitol. Parasites Wildl. 3, 57–66. https://doi.org/10.1016/j.ijppaw.2014.02.002.