Contributions to Mammalogy and Zooarchaeology of Wallacea

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Trypanosoma (Euglenozoa: Kinetoplastea) Infections in Rodents, Bats, and Shrews along an Elevation and Disturbance Gradient in Central Sulawesi, Indonesia

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Abstract. Surveillance of wildlife pathogens is critically important to the conservation of species and human health. However, few species of wildlife in biodiverse countries like Indonesia, especially endemic species in intact ecosystems, have been screened for most wildlife pathogens, including the abundant and diverse blood parasites in the family Trypanosomatidae. We used PCR and sequencing to screen for the presence of *Trypanosoma* infections in 616 native mammalian specimens (355 samples from 15 rodent species, 155 samples from 7 shrew species, and 96 samples from 12 bat species) collected in 2013 and 2018 along an elevation and disturbance gradient in and adjacent to Cagar Alam Gunung Dako, Toli-Toli, Central Sulawesi. We identified *Trypanosoma* infections with an average prevalence of 22.1% across all species, 21.7% in rodents, 30.3% in shrews, and 10.4% in bats. Infections were dominated by sequences similar to *T. cyclops* in the Theileri clade, which accounted for 86.6% of infections and are most likely native trypanosomes to Sulawesi. The second most common trypanosome sequences matched cosmopolitan and probably introduced trypanosomes in the Lewisi clade. They accounted for 9.7% of infections in all mammals but were only detected in rodents of the family Muridae where they accounted for 16.9% of infections. We also detected five infections in bats (50% of bat infections) by two trypanosomes from the

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Cruzi clade, one matching *T. dionisii* and the other unassignable to a named species but with sequence similarity to a diverse clade of trypanosomes found in Neotropical bats, Australian marsupials and rodents, and Malagasy lemurs. We found significant differences in prevalence of the Theileri clade (*T. cyclops*) among elevations with higher infection rates in more intact and healthier rainforest. While no health impacts are evident from infections by these Theileri clade (*T. cyclops*) trypanosomes, their infections across mammalian orders including rodents, bats, shrews, primates and marsupials suggest that they may infect humans and domestic livestock. Our discovery of infections of rodents on Mt. Dako by introduced trypanosomes from the Lewisi clade and infections of bats by *T. dionisii* and an unnamed trypanosome from the Cruzi clade warrant further surveillance of trypanosome infections in wildlife of Sulawesi.

Abstrak [Bahasa Indonesia]. Pengamatan tentang patogen sangat penting dalam dunia konservasi dan kesehatan manusia. Namun, hingga saat ini penelitian tentang keberadaan kebanyakan parasit satwa liar masih sangat sedikit sekali, terutama pada spesies endemik di ekosistem yang utuh di negara dengan tingkat keanekaragaman biodiversitas yang tinggi seperti Indonesia. Ini termasuk pada kelompok parasit darah famili Trypanosomatidae yang memiliki tingkat keberagaman yang tinggi dan melimpah. Kami menggunakan teknik PCR untuk mengidentifikasi keberadaan infeksi famili Trypanosomatidae pada 616 spesimen spesies mamalia asli (355 sampel dari 15 spesies Rodentia, 155 sampel dari 7 spesies celurut, dan 96 sampel dari 12 spesies kelelawar) yang dikoleksi pada tahun 2013 dan 2018 disepanjang lereng elevasi dan tingkat gangguan habitat di Cagar Alam Gunung Dako, Toli-Toli, Sulawesi Tengah dan daerah terdekat. Kami mengidentifikasi rata-rata tingkat prevalensi infeksi *Trypanosoma* sebesar 22,1% untuk semua spesies, 21,7% pada hewan pengerat, 30,3% pada celurut, dan 10,4% pada kelelawar. Infeksi *Trypanosoma* didominasi oleh sekuen yang mirip dengan *T. cyclops* di klade Theileri yang menyumbang 86,6% dari total infeksi dan diduga sebagai *Trypanosoma* asli Sulawesi. Sekuen denganurutan tingkat infeksi paling umum kedua teridentifikasi sebagai spesies cosmopolitan dan kemungkinan spesies *Tryponosoma* introduksi di klade Lewisi. Kelompok ini menyumbang 9,7% di semua mamalia dan terbatas pada kelompok hewan pengerat dari famili Muridae dimana mereka menyumbang 16,9% dari total infeksi. Kami juga mendeteksi lima infeksi *Trypanosoma* dari klade Cruzi pada kelelawar (50% dari total infeksi pada kelelawar), dimana satu sampel teridentifikasi sebagai *T. dionisii* dan sampel lainnya belum diberikan nama, tetapi hasil sekuen memiliki kesamaan dengan sub-klade Australia dan *Neobats*. Kami menemukan perbedaan tingkat prevalensi yang signifikan dari klade Theileri (*T. cyclops*) pada berbagai ketinggian habitat dengan tingkat infeksi tertinggi pada hutan hujan tropis yang lebih utuh dan sehat. Meskipun tidak ada dampak kesehatan yang terbukti dari infeksi oleh trypanosoma klade Theileri (*T. cyclops*), ditemukannya infeksi jenis tersebut pada beberapa Ordo Mamalia, termasuk pada kelompok hewan pengerat, kelelawar, celurut, primata, dan hewan berkantung mengindikasikan bahwa parasit tersebut kemungkingkan dapat menginfeksi manusia dan hewan ternak. Ditemukannya hewan pengerat yang terinfeksi *Trypanosoma* introduksi di Gunung Dako dari klade Lewisi dan infeksi kelelawar oleh *T. dionisii* serta beberapa species Trypanosoma dari *clade* Cruzi yang belum dinamai, menunjukkan bahwa pengamatan lebih lanjut terhadap infeksi trypanosoma pada satwa liar Sulawesim masih perlu dilakukan.

Introduction

Species in the genus *Trypanosoma* are protists in the family Trypanosomatidae (Euglenozoa: Kinetoplastea) (Kostygov *et al.*, 2021). All members of this family are known to be parasitic in vertebrates. *Trypanosoma*, in particular, are known to infect a wide range of vertebrates across almost all classes (Hamilton *et al.*, 2007; Botero *et al.*, 2013; Thompson *et al.*, 2014; Cooper *et al.*, 2017; Calzolari *et al.*, 2018). While trypanosome infections have been detected in fewer than 150 mammalian species, they probably infect all mammalian species, of which there are over 6000 (Thompson *et al.*, 2014; Winterhoff *et al.*, 2020). Within mammals, some *Trypanosoma* are exclusive to certain orders, such as *T. lewisi* which infects only rodents (Rodentia) and is associated with the spread of invasive rodents such as black rats, *Rattus rattus* (Pumhom *et al.*, 2014). Other species infect a wide range of mammals, such as *T. cruzi* which is most common in bats but also known to infect other mammals (Cooper *et al.*, 2017). However, most mammals have not been screened for trypanosomes, including widespread groups such as shrews. Many species of *Trypanosoma* are yet to be formally described and many infections are detected by DNA methods that cannot always assign samples to species. Thus, *Trypanosoma* species are routinely organized into major phylogenetic clades including the Theileri, Lewisi, Cruzi, and Brucei clades (Cooper *et al.*, 2017). The names of these clades are based on some of the most common and significant *Trypanosoma* species found in mammals (i.e., *T. theileri*, *T. lewisi*, *T. cruzi*, and *T. brucei*) but they include numerous other species many of which have not been formally described (Cooper *et al.*, 2017).

Some species of *Trypanosoma* cause clinical symptoms in humans such as *T. brucei*, which causes sleeping sickness and Chagas disease (Cooper *et al.*, 2017). In Indonesia, the disease trypanosomiasis, caused by the introduced species *T. evansi*, that originated in Africa, inflicts considerable losses to livestock such as horses, cows, and buffaloes (Wardhana & Savitri, 2018; Setiawan *et al.*, 2021). *Trypanosoma evansi* infects livestock around the world and has spread to almost all major islands in Indonesia including Sulawesi (Dieleman, 1986; Luckins, 1998; Setiawan *et al.*, 2021). *Trypanosoma* species in the Theileri clade and closely related to *T. cyclops* (Weinman, 1972), were detected recently on Sulawesi infecting endemic rodents with high prevalence (Winterhoff *et al.*, 2020). Given that the island of Sulawesi is located between the Asian and Australian continental shelves, it is particularly relevant to the biogeography

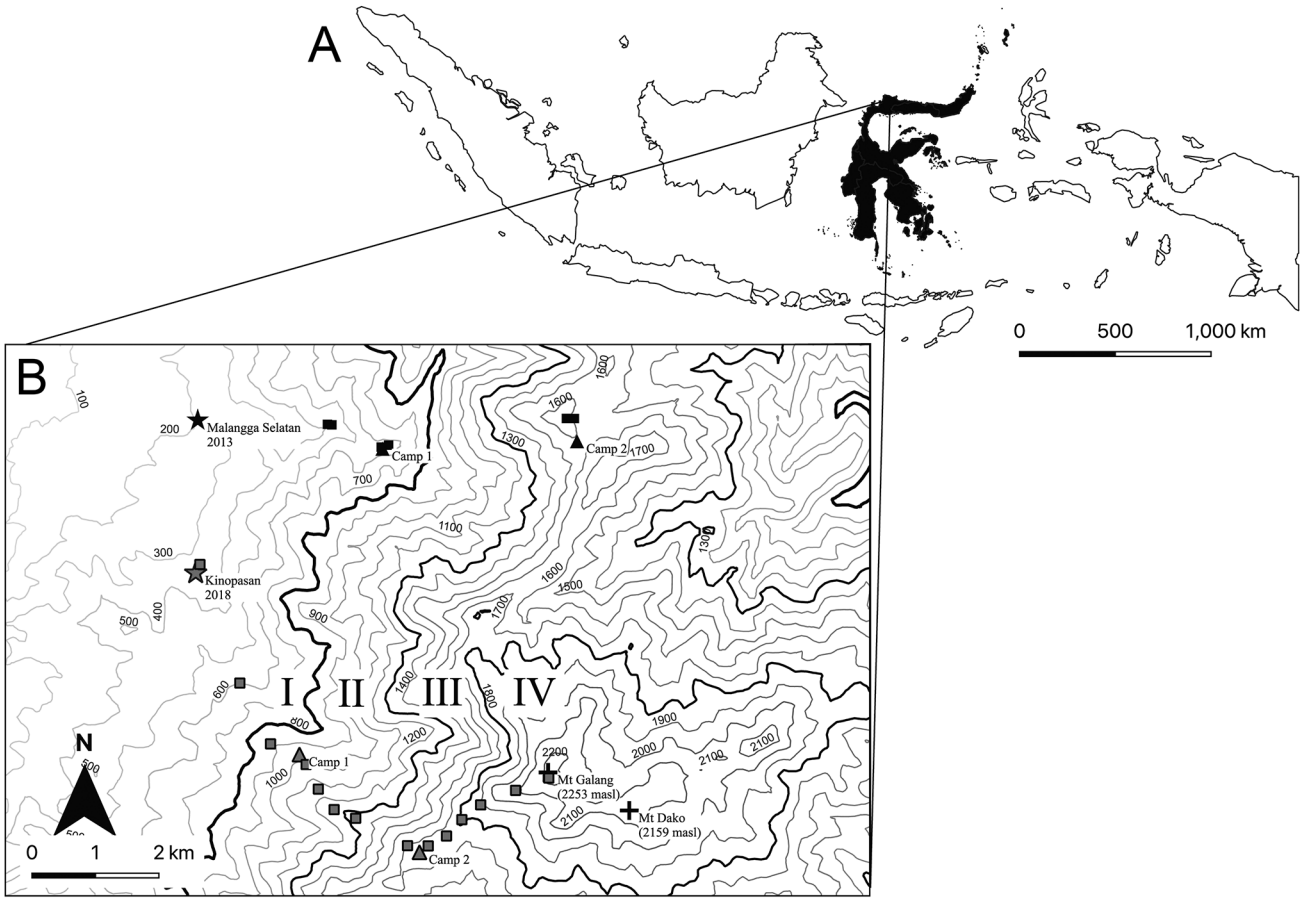

Figure 1. (*A*) Location of Gunung Dako Cagar Alam on Sulawesi Island Indonesia. (*B*) Detail of elevational transects surveyed in 2013 (black symbols) and 2018 (grey symbols). The villages of Malangga Selatan and Kinopasan are indicated with stars. Camps are indicated with triangles. The approximate centroids of traplines are indicated with squares. Topographic lines are marked at 100 m intervals. Elevational bins used in this study are labelled with roman numerals with topographic lines at 800, 1300, and 1800 m in bold.

and spread of pathogens between the continents, with *T. cyclops* an example. *Trypanosoma cyclops* was originally described from an infection in a Malaysian primate, *Macaca nemestrina* (Weinman, 1972) and has been detected with genetic methods from rodents and marsupials from Sri Lanka to Australia (Thompson *et al.*, 2014; Cooper *et al.*, 2017; Winterhoff *et al.*, 2020). They are most likely endemic to these areas, including across Indonesia, and are likely to infect a wide range of mammalian hosts. Species of trypanosomes that are spread by introduced rodents (e.g., *Rattus* spp.) in the Lewisi clade were also detected on Sulawesi and infecting endemic rodents, albeit at much lower prevalence than the endemic *T. cyclops* (Winterhoff *et al.*, 2020). Given the widespread distribution of invasive rodents across Indonesia and on the island of Sulawesi (e.g., *Rattus exulans*, *R. tanezumi*, *R. norvegicus*, and *Mus musculus*), *T. lewisi* is likely to be distributed throughout Indonesia.

Introduced parasites are threats to native species worldwide, especially island endemics. Some species of trypanosome cause diseases in wildlife leading to population declines. For example, *T. copemanii* infections are linked to the rapid decline of populations of an Australian marsupial, the woylie (*Bettongia penicillata*) (Thompson *et al.*, 2014). Zoonotic diseases threatening wildlife can emerge through "spill-over" or "spill-back" from invasive species and domesticated animals, especially when an infected population with a high pathogen prevalence comes into contact with a novel host population (Thompson, 2013). Transmission of diseases from introduced species to novel wildlife hosts also pose risks of emerging diseases infecting domestic animals and/or humans (Cleaveland *et al.*, 2001; Gortázar *et al.*, 2007; Martin *et al.*, 2011).

In this study, we used PCR and sequencing to identify trypanosome infections in native rodents, bats, and shrews on a protected mountain of Sulawesi where trypanosome communities have not been assessed. Notably, no *Trypanosoma* infection has ever been reported from shrews in Indonesia, and this is the first study to include these host species from Indonesia. To test if infection rates are correlated with forest disturbance or with proximity to humans and their commensal species, we sampled from village to peak along an elevation gradient spanning nearly 2000 m.

Material and methods

Sampling of small mammals

In this study we surveyed small mammals (rodents, bats, and shrews) in and adjacent to Cagar Alam Gunung Dako (Mount Dako Nature Reserve), Sulawesi, Indonesia (Fig. 1). The reserve is located in the Galang District of the Toli-Toli Regency at the northern end of the Central Sulawesi province. It has an area of 197 km^2 and surrounds the peaks of Mount Dako (2,159 m asl) and the slightly taller Mount Galang (2,253 m asl). Surveys were conducted in March 2013 and July 2018 along two elevational transects starting from the villages of Malangga Selatan and Kinopasan, respectively. Surveys were conducted using a combination of Sherman traps, snap traps, mistnets, and pitfall traps. We merged trap lines into four elevational bins (Fig. 1), reflecting a gradient of human impacts from village edge to the peak of Mt. Galang. The lowest elevational bin (300–800 m asl) was adjacent to villages, farms, and plantations. The second elevational bin was in secondary forest above active plantations (801–1300 m asl). The third (1301–1800 m asl) and fourth (1801–2225 m asl) elevational bins were in largely intact forest well inside the reserve. While preparing specimens, liver and other tissues were perfused with RNA later or ethanol and stored in liquid nitrogen until returning from the field (Table S1). Sampling was led by the Research Center for Biology, Indonesian Institute of Sciences (LIPI) with permits from the Indonesian Ministry of Technology and Higher Education (RISTEK), along with authorization from the Ministry of Environment and Forestry Indonesia (Central Sulawesi BKSDA). Procedures followed animal ethics permit MVAEC-15002.

Molecular detection and sequencing

To identify samples infected with *Trypanosoma*, we extracted genomic DNA from liver tissue using QIAextractor (DX reagents and plasticware), QIAGEN DNeasy blood and tissue kits, or Wizard SV 96 Genomic DNA Purification Systems following manufacturer's guidelines (QIAGEN Inc., Valencia, CA, USA; Promega, Madison, WI, USA). We used a universal set of trypanosome primers targeting *ca.* 906 bp fragments from the 18S gene region following the PCR protocol previously described (Winterhoff *et al.*, 2020). PCR reactions were screened using electrophoretic gels and those with visible bands in the correct size range were considered a positive infection. Each of these was purified using ExoSAP (USB Corporation, Cleveland, Ohio, USA) and sequenced on an Applied Biosystems 3730 Automatic DNA Sequencer (Applied Biosystems, Foster City, California, USA) using PCR primers. Successful sequences were identified to genus and species where possible using the nucleotide BLAST tool within the NCBI GenBank database. DNA sequences are available in GenBank under accessions OR036096–OR036228.

Statistical analysis

To examine how trypanosome infections were related to ecological factors, we tested the relationship between prevalence and elevation. We estimated trypanosome prevalence (number infected vs. non-infected) for each trypanosome clade with more than 10 infections (i.e., Theileri and Lewisi) in each elevational bin and tested for significant differences among bins using a Chi-square test of independence. In the case where significant differences were found among elevation bins, we used a Chi-square goodness of fit test to determine whether the number of infections within a bin was significantly different than random. For both tests, we used the "chisq.test" function from the "stats" package in R (version 3.6.3, R Core Team, 2020).

Results

We collected and screened 616 specimens from three mammalian orders for trypanosome infections (Table 1; Table S1). Ten samples with positive PCR bands (eight shrews, two rodents) failed to produce reliable sequences that could be assigned to a trypanosome clade. We excluded these from our sample sizes leaving 606 samples comprising 355 rodents (16 species), 155 shrews (7 species), and 96 bats (11 species). We detected identifiable trypanosome infections by sequencing in 134 samples including in seven species of rodents (*Bunomys chrysocomus, Frateromys fratrorum, Haeromys minahassae, Maxomys musschenbroekii, Rattus hoffmanni, Taeromys dominator,* and *T. taerae*), five species of shrews (*Crocidura balete, C. elongata, C. lea, C. nigripes, C. pseudorhoditis*), and six species of bats (*Cynopterus brachyotis, Macroglossus minimus, Rhinolophus celebensis, Rousettus celebensis, Thoopterus nigrescens,* and *Tadarida sarasinorum*). Excluding samples that failed at sequencing, the average prevalence across all samples was 21.9%. Shrews had the highest prevalence with 30.3% of specimens infected by *Trypanosoma* compared to 21.4% in rodents and 10.4% in bats.

Based on sequences of the 18s rDNA gene we identified infections by trypanosomes from three major clades (Cooper *et al.*, 2017); the Theileri, Lewisi and Cruzi clades (Table 2). Sequences of the Theileri clade matched closely (> 99% sequence similarity) to *T. cyclops*, originally described from Malaysian macaques, and previously detected in terrestrial leeches, frogs, marsupials and rodents from mainland Asia, Sulawesi, and Australia (Cooper *et al.*, 2017; Winterhoff *et al.*, 2020). These Theileri clade (*T. cyclops*) infections were the most common with a prevalence of 19.1% (116 infected individuals) across all samples we screened accounting for 87.2% of all infections. They were also the most common infection in each of the three orders infecting 30.3% of shrews, 18.0% of rodents, and 5.2% of bats.

Lewisi clade trypanosomes were the next most commonly detected infection accounting for 9.0% of infections and detected in 2.0% of samples. All infections were detected in rats and mice (family Muridae) where they comprised 15.8% of infections with 3.4% of individuals infected. We did not detect Lewisi clade infections in squirrels (family Sciuridae, n = 14). Lewisi clade sequences were nearly all identical and indistinguishable from several named species of *Trypanosoma* in the Lewisi clade with > 99% sequence similarity and which cannot be differentiated by 18s rDNA sequences alone. These included *T. lewisi*, *T. kuseli*, *T. otospermophili*, *T. musculi*, *T. microti* and *T. rabinowitschae* (see phylogeny in Winterhoff *et al.*, 2020).

Two Cruzi clade trypanosomes were detected in five bat samples with a prevalence of 5.2% among bats. One sample, infecting *Tadarida sarasinorum*, had 99% sequence similarity to *T. dionisii*, a close relative of *T. cruzi* and *T.*

Table 1. Sample sizes of mammalian species screened in this study, elevation range of samples, and number of samples where infections were detected. Samples with "sp." were not identified to species for this study. Generic taxonomy for murines follows Handika *et al*. (2021). Taxonomy for *Crocidura* follows Esselstyn *et al.* (2021).

					Infections	
Order	Family	Species	Elevation range (m asl)	Sample size	Theileri clade	Lewisi clade	Cruzi clade
Chiroptera	Hipposideridae	*Hipposideros* sp.	560–700	1	0	0	0
Chiroptera	Megadermatidae	*Megaderma spasma*	560–700	1	0	0	0
Chiroptera	Molossidae	*Tadarida sarasinorum*	1740–1750	3	0	0	1
Chiroptera	Pteropodidae	*Chironax melanocephalus*	560–1240	5	0	0	0
Chiroptera	Pteropodidae	*Cynopterus brachyotis*	939–1750	33	4	0	0
Chiroptera	Pteropodidae	*Macroglossus minimus*	939–965	3	1	0	0
Chiroptera	Pteropodidae	*Rousettus celebensis*	310–965	38	0	0	1
Chiroptera	Pteropodidae	*Styloctenium wallacei*	1560–1630	1	0	0	0
Chiroptera	Pteropodidae	*Thoopterus nigrescens*	310–330	5	0	0	1
Chiroptera	Rhinolophidae	*Rhinolophus celebensis*	310–1750	5	0	0	2
Chiroptera	Vespertilionidae	*Myotis* sp.	750–975	1	0	0	0
Chiroptera	**All families**	**all species**	**310–1750**	**96**	**5**	**0**	**5**
Eulipotyphla	Soricidae	*Crocidura balete*	1560–2170	4*	1	0	0
Eulipotyphla	Soricidae	*Crocidura caudipilosa*	560–1965	12	0	0	0
Eulipotyphla	Soricidae	*Crocidura elongata*	560–2170	30*	9	0	0
Eulipotyphla	Soricidae	*Crocidura lea*	750–1630	13*	8	0	0
Eulipotyphla	Soricidae	*Crocidura nigripes*	310–1850	35	8	0	0
Eulipotyphla	Soricidae	*Crocidura pseudorhoditis*	560–2170	60*	21	0	0
Eulipotyphla	Soricidae	*Crocidura quasielongata*	310–330	1	0	0	0
Eulipotyphla	**Soricidae**	**all species**	**310–2170**	**155**	**47**	**0**	**0**
Rodentia	Muridae	*Bunomys chrysocomus*	310–1390	31	2	0	0
Rodentia	Muridae	*Frateromys fratrorum*	750–1750	58*	17	1	0
Rodentia	Muridae	*Haeromys minahassae*	410–1630	2	0	1	0
Rodentia	Muridae	*Hyorhinomys stuempkei*	1560–1965	3	0	0	0
Rodentia	Muridae	*Margaretamys* sp.	2200–2230	1	0	0	0
Rodentia	Muridae	*Maxomys dollmani*	1240–1965	4	0	0	0
Rodentia	Muridae	*Maxomys hellwaldii*	410–450	1	0	0	0
Rodentia	Muridae	*Maxomys musschenbroekii*	550–2170	113	24	2	0
Rodentia	Muridae	*Rattus facetus*	550–2170	19	0	0	0
Rodentia	Muridae	*Rattus hoffmanni*	310–1750	45	2	8	0
Rodentia	Muridae	*Taeromys callitrichus*	560–700	2	0	0	0
Rodentia	Muridae	*Taeromys celebensis*	1740–1765	1	0	0	0
Rodentia	Muridae	*Taeromys dominator*	310–2230	27	4	0	0
Rodentia	Muridae	*Taeromys taerae*	975–2170	34*	15	0	0
Rodentia	Sciuridae	*Prosciurillus murinus*	310–1755	14	0	0	0
Rodentia	**all families**	**all species**	**310–2170**	**355**	**64**	**12**	**0**
all **orders**	**all families**	**all species**	**310–2170**	**606**	**116**	**12**	**5**

* Sample sizes exclude 10 samples with positive PCR bands that failed at sequencing.
Crocidura balete (n = 1), *C. elongata* (n = 4), *C. lea* (n = 2), *C. pseudorhoditis* (n = 1), *Frateromys fratrorum* (n = 1), and *Taeromys taerae* (n = 1).

erneyi (Schizotrypanum subclade of Espinosa-Álvarez *et al.*, 2018). The remaining four infections, detected in *Rhinolophus celebensis, Rousettus celebensis,* and *Thoopterus nigrescens*, had identical sequences but were more distantly related in the Cruzi clade (near Australian and Neobats subclades of Espinosa-Álvarez *et al.*, 2018) with 98% sequence similarity to *T. livingstonei*, *T. ralphi*, *T. grayi*, and *T. terrestris*, but not clearly assignable to any named species.

The average prevalence of any trypanosome infection varied across elevational bins with the highest prevalence at middle elevations (range 10.8–26.2%; Table 3). However, this pattern was driven primarily by the prevalence of Theileri clade (*T. cyclops*) infections (range 3.3–24.1%; Table 3). A chi-square test for independence showed that Theileri clade (*T. cyclops*) infections were not randomly distributed among elevational bins ($\chi^2 = 25.124$, $p < 0.0001$) and largely because infections in the lowest elevational bin (4 of 120 specimens) were significantly less than expected (chi-square goodness of fit, $\chi^2 = 18.97$, $p < 0.0001$). Infections were slightly but not significantly higher than expected at middle elevational bins and exactly as expected at the highest elevational bin.

Table 2. Sample sizes of hosts and prevalence of each trypanosome clade for each mammalian order. Sample sizes are counts whereas prevalences are percentages. Sample sizes exclude failed sequences noted in Table 1.

	Sample size			Theileri clade (*T. cyclops*)				Lewisi clade			Cruzi clade		
	♂	♀	na	♂	♀	na	all	♂	♀	all	♂	♀	%
Rodentia	175	175	5	28	35	1	18.0%	5	7	3.4%	0	0	0
Eulipotyphla	71	80	4	25	21	1	30.3%	0	0	0	0	0	0
Chiroptera	34	61	1	2	3	0	5.2%	0	0	0	4	1	5.2%
all orders							19.1%			2.0%			0.8%

For Lewisi clade infections, we calculated prevalence based on murid rodents alone as these trypanosomes only infected rodents in this family. While the highest rates of infections occurred at the lowest elevation (6 of 63 specimens; Table 3) and no infections occurred at the highest elevation, a chi-squared test for independence found that infections were only marginally significantly different from randomly distributed among elevational bins ($\chi^2 = 4.864$, $p = 0.182$). For the Cruzi clade, we calculated prevalence based only on bat specimens as these trypanosomes only infected bats. The small sample size of Cruzi infections precluded any statistical analysis. Of the five detections of Cruzi clade trypanosomes, three were at the lowest elevational bin and one each at the middle elevational bins but percent infections were consistently low, ranging from 3.7–6.5% of specimens. No bats were collected from the highest elevational bin hence prevalence could not be calculated.

Discussion

Our study demonstrates the breadth of *Trypanosoma* infections in native bats, shrews, and rodents on Sulawesi, Indonesia (Winterhoff *et al.*, 2020). Our sampling from Mount Dako, detected *Trypanosoma* infecting 17 mammalian species native to Sulawesi, including seven murid rodent species (50% of species), five shrew species (71% of species), and five bat species (45% of species). The trypanosome infections we detected fell within three of the four major *Trypanosoma* clades known to infect mammals: Theileri clade (*T. cyclops*) which contains trypanosomes endemic to placental mammals and marsupials in Malaysia, Sri Lanka and Australia (Hamilton *et al.*, 2005; Pumhom *et al.*, 2014); Lewisi clade which contains the invasive and globally distributed *T. lewisi*; and Cruzi clade which contains trypanosomes from Old and New World bats, South American mammals and Australian marsupials (Hamilton *et al.*, 2012). Consistent with Winterhoff *et al*. (2020), infections were dominated by the Theileri clade (*T. cyclops*), which accounted for > 86% of infections. Notably, we did not detect any trypanosomes from the Brucei clade, which contains the introduced *Trypanosoma evansi* known to infect cattle on Sulawesi (Setiawan *et al.*, 2021).

Theileri clade (*T. cyclops*) infections were present in all three host orders sampled indicating that all three are reservoirs for infection. Shrews exhibited the highest prevalence of Theileri clade (*T. cyclops*) infections being nearly two times higher than in rodents and nearly six times higher than in bats suggesting that native shrews are an important and unrecognized reservoir for infection. The occurrence of Theileri clade (*T. cyclops*) infections in three distantly related mammalian orders suggests that these trypanosomes infect a broad range of other mammalian species on Sulawesi. Prevalence of Theileri clade (*T. cyclops*) trypanosomes was highest in intact forest at mid-to-upper elevations lending further support to the notion that they are widespread parasites of endemic mammalian communities on Sulawesi (Winterhoff *et al.*, 2020). Documentation of widespread infection by Theileri clade (*T. cyclops*) trypanosomes across rodents, bats, shrews, primates and marsupials suggests that these trypanosomes can infect most other mammalian species including humans and domesticated animals. While Theileri clade (*T. cyclops*) trypanosomes were more prevalent at higher elevations on Mount Dako, they also were present at the lowest elevations where endemic host species overlap with humans and domesticated animals. Theileri clade (*T. cyclops*) trypanosomes have not been recorded in humans, domesticated animals or other introduced species on Sulawesi. However, atypical human cases of other *Trypanosoma* (e.g., *T. lewisi*) occur elsewhere in Southeast Asia (Pumhom *et al.*, 2015) and few relevant samples on Sulawesi have been screened for *Trypanosoma* with PCR methods that could detect the Theileri clade. A recent study screening 100 cattle on Sulawesi did not detect any Theileri clade sequences, while detecting three sequences of the introduced *T. evansi* (Setiawan *et al.*, 2021). However, these cattle were sampled in communities near the large urban centre of Makassar and far from native mammalian communities where Theileri clade (*T. cyclops*) is likely to be a reservoir. Cattle or other domesticated animals may be at greater risk of disease spillover where they are closer to intact and more diverse mammalian communities. Disease spillover from reservoir host species to naïve hosts can lead to higher virulence in naïve hosts compared to reservoir hosts, including diseases caused by *Trypanosoma* (Wyatt *et al.*, 2008; Truc *et al.*, 2013; Pumhom *et al.*, 2014). However, to our knowledge, no illness in humans, domesticated animals or wildlife has been associated with infection by the Theileri clade (*T. cyclops*). Consequently, further research is needed into the potential for disease transmission and associated health impacts to humans, domesticated animals, and wildlife from Theileri clade (*T. cyclops*) trypanosomes.

Consistent with previous sampling on Sulawesi, Lewisi clade trypanosomes were only detected in murid rodent species (Winterhoff *et al.*, 2020). Among the Lewisi clade, *T. lewisi* is a cosmopolitan rat-specific trypanosome, whereas

Table 3. Mammalian host sample sizes and prevalence of *Trypanosoma* and *Trypanosoma* clades in each elevational bin. Prevalence of any *Trypanosoma* and of the Theileri clade (*T. cyclops*) were calculated using sample sizes of all mammalian species. For the Lewisi clade, prevalence was calculated considering only sample sizes of murid species. For the Cruzi clade, prevalence was calculated considering only sample sizes of bats. Sample sizes exclude failed sequences noted in Table 1.

	Elevational bins (m asl)			
	I (300–800)	II (801–1300)	III (1301–1800)	IV (1801–2225)
sample size (all mammals)	120	97	336	53
prevalence *Trypanosoma* (all mammals)	10.8%	23.7%	26.2%	18.9%
prevalence Theileri clade (all mammals)	3.3%	21.6%	24.1%	18.9%
sample size (Muridae)	63	50	211	17
prevalence Lewisi clade (Muridae)	9.5%	2.0%	2.8%	0
sample size (Chiroptera)	46	23	27	0
prevalence Cruzi clade (Chiroptera)	6.5%	4.3%	3.7%	na

other Lewisi clade species are known to infect other rodent species (Hamilton *et al.*, 2005, 2007). Thus, it is likely that the Lewisi clade trypanosomes infecting endemic murid rodents on Sulawesi were introduced with introduced murid rodents (e.g., *Mus musculus, Rattus exulans, R. norvegicus* or *R. rattus;* Winterhoff *et al*., 2020). Prevalence of Lewisi clade trypanosomes was highest at the lowest elevations sampled corresponding to areas of greatest human habitat disturbance. This also is consistent with it being introduced through the spread of introduced and commensal murid host species which are most common around human disturbance (Pumhom *et al.*, 2014; Salzer *et al.*, 2016). While none of these introduced rodents were sampled in this study they were observed in the village where limited trapping was conducted. Nearly 70% of Lewisi clade infections occurred in an endemic *Rattus* species (*R. hoffmanni*) including all infections at the lowest elevation. However, Lewisi clade infections were also detected in three other native murid host genera (i.e., *Frateromys*, *Haeromys* and *Maxomys*), whereas surveys on Mts Latimojong and Bawakaraeng detected Lewisi clade infections from endemic species of *Rattus* and *Bunomys* (Winterhoff *et al*., 2020). In addition, Lewisi infections were detected in specimens collected in relatively intact forest within Mount Dako Cagar Alam (nature reserve) at elevations up 1750 m asl, where introduced murids were not detected, suggesting that these introduced trypanosomes are penetrating protected areas of Sulawesi. On Mount Bawakaraeng, Lewisi clade infections were also detected at the highest elevations (> 2800 m asl), but where human disturbance was also substantial and introduced *R. exulans* were present (Winterhoff *et al.*, 2020). Spillover of *T. lewisi* from introduced *Rattus* species to endemic murid rodents has been reported in other forest habitats including neighbouring landmasses in the Indo-Australian region (Dobigny *et al.*, 2011; Milocco *et al.*, 2013; Pumhom *et al.*, 2014; Salzer *et al.*, 2016). This transmission risk may have implications for native wildlife health, as virulence of *T. lewisi* may increase in naïve hosts or affect host susceptibility to other infections (Brown, 1915; Hoare, 1972; Averis *et al.*, 2009; Milocco *et al.*, 2013). Where introduced, the prevalence of *T. lewisi* in native rodent hosts can exceed the prevalence of native trypanosomes (Salzer *et al.*, 2016). However, the prevalence of Lewisi clade infections in endemic rodent host species of Mount Dako (this study) and two other mountains of Sulawesi (Winterhoff *et al.*, 2020) is much lower than for the Theileri clade (*T. cyclops*). Further research into the distribution patterns of Lewisi clade trypanosomes including penetration into intact native ecosystems and their potential epidemiological effects on native wildlife is required.

Our limited sampling of bats on Sulawesi suggest that bats are hosts to numerous undocumented species of Cruzi clade trypanosomes that occur at low prevalence and will require much greater sampling to detect. While most species of Cruzi clade trypanosomes infect bats exclusively, others infect a wide range of mammalian hosts (e.g., *T. cruzi* and *T. rangeli*; Espinosa-Álvarez *et al.*, 2018), and spillover effects to wildlife, humans or domesticated animals are possible (Maeda *et al.*, 2012; Dario *et al.*, 2016, 2017). Like Lewisi clade trypanosomes, we detected Cruzi clade trypanosomes in the most samples at the lowest elevations (n = 3), but they occurred across all elevations where bats were sampled with no statistical differences detected among elevations. We detected one sequence of the Cruzi clade that was nearly identical to *T. dionisii*, which is the first record of this cosmopolitan bat-infecting trypanosome from Indonesia. *Trypanosoma dionisii* has previously been detected in a broad range of bat species from North and South America, Africa, Europe, China, Japan and Australia, so its presence on Sulawesi is not surprising (Hamilton *et al.*, 2012; Espinosa-Álvarez *et al.*, 2018; Mafie *et al.*, 2019; Wang *et al.*, 2019; Austen *et al.*, 2020; Clément *et al.*, 2020). While *T. dionisii* is generally considered non-pathogenic in bats, it has the potential to infect other mammalian species including humans with unknown epidemiological effects (Maeda *et al.*, 2012; Dario *et al.*, 2016, 2017). The four other Cruzi clade sequences detected in this study were identical to each other but not clearly related to any known Cruzi clade species. They are closest to several species in the "Australian" and "Neobats" subclades (as defined by Espinosa-Álvarez *et al.*, 2018) that include trypanosomes found in Neotropical bats, Australian marsupials and rodents, and Malagasy lemurs. Further sampling is required to determine the taxonomy, prevalence, transmissibility, and implications of Cruzi clade trypanosomes in wildlife, in particular in bat hosts where their ecological traits, behaviours and global distribution increase the chances of parasitic spill-over to new host species (Melaun *et al.*, 2014; Lima *et al.*, 2015; Clément *et al.*, 2020).

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Supplementary information

Table S1. Mammalian specimens screened for *Trypanosoma*. All registration numbers from Museums Victoria (NMV)—published separately as a *figshare* dataset

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References

Austen, J. M., E. Van Kampen, S. L. Egan, M. A. O'Dea, B. Jackson, U. M. Ryan, P. J. Irwin, and D. Prada. 2020. First report of *Trypanosoma dionisii* (Trypanosomatidae) identified in Australia. *Parasitology* 147: 1801–1809.
https://doi.org/10.1017/S0031182020001845

Averis, S., R. C. A. Thompson, A. J. Lymbery, A. F. Wayne, K. D. Morris, and A. Smith. 2009. The diversity, distribution and host-parasite associations of trypanosomes in Western Australian wildlife. *Parasitology* 136: 1269–1279.
https://doi.org/10.1017/S0031182009990801

Botero, A., C. K. Thompson, C. S. Peacock, P. L. Clode, P. K. Nicholls, A. F. Wayne, A. J. Lymbery, and R. C. A. Thompson. 2013. Trypanosomes genetic diversity, polyparasitism and the population decline of the critically endangered Australian marsupial, the brush tailed bettong or woylie (*Bettongia penicillata*). *International Journal for Parasitology: Parasites and Wildlife* 2: 77–89.
https://doi.org/10.1016/j.ijppaw.2013.03.001

Brown, W. H. 1915. Concerning changes in the biological properties of *Trypanosoma lewisi* produced by experimental means, with especial reference to virulence. *The Journal of Experimental Medicine* 21: 345–364.
https://doi.org/10.1084/jem.21.4.345

Calzolari, M., G. Rugna, E. Clementi, E. Carra, M. Pinna, F. Bergamini, M. Fabbi, M. Dottori, L. Sacchi, and J. Votýpka. 2018. Isolation of a trypanosome related to *Trypanosoma theileri* (Kinetoplastea: Trypanosomatidae) from *Phlebotomus perfiliewi* (Diptera: Psychodidae). *BioMed Research International* 2018: 1–8.
https://doi.org/10.1155/2018/2597074

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: Pathogen characteristics, host range and the risk of emergence. *Philosophical Transactions of the Royal Society B: Biological Sciences* 356: 991–999.
https://doi.org/10.1098/rstb.2001.0889

Clément, L., M. Dietrich, W. Markotter, N. J. Fasel, A. Monadjem, A. López-Baucells, D. Scaravelli, P. Théou, R. Pigeault, M. Ruedi, and P. Christe. 2020. Out of Africa: the origins of the protozoan blood parasites of the *Trypanosoma cruzi* clade found in bats from Africa. *Molecular Phylogenetics and Evolution* 145: 106705.
https://doi.org/10.1016/j.ympev.2019.106705

Cooper, C., P. L. Clode, C. Peacock, and R. C. A. Thompson. 2017. Host–parasite relationships and life histories of trypanosomes in Australia. *Advances in Parasitology* 97: 47–109.
https://doi.org/10.1016/bs.apar.2016.06.001

Dario, M. A., R. M. M. da Rocha, P. Schwabl, A. M. Jansen, and M. S. Llewellyn. 2017. Small subunit ribosomal metabarcoding reveals extraordinary trypanosomatid diversity in Brazilian bats. *PLoS Neglected Tropical Diseases* 11: 1–15.
https://doi.org/10.1371/journal.pntd.0005790

Dario, M. A., M. S. Rodrigues, J. H. D. S. Barros, S. C. D. C. Xavier, P. S. D'Andrea, A. L. R. Roque, and A. M. Jansen. 2016. Ecological scenario and *Trypanosoma cruzi* DTU characterization of a fatal acute Chagas disease case transmitted orally (Espírito Santo state, Brazil). *Parasites and Vectors* 9: 1–14.
https://doi.org/10.1186/s13071-016-1754-4

Dieleman, E. F. 1986. Trypanosomiasis in Indonesia: a review of research, 1900–1983. *The Veterinary Quarterly* 8: 250–256.
https://doi.org/10.1080/01652176.1986.9694049

Dobigny, G., P. Poirier, K. Hima, O. Cabaret, P. Gauthier, C. Tatard, J. M. Costa, and S. Bretagne. 2011. Molecular survey of rodent-borne *Trypanosoma* in Niger with special emphasis on *T. lewisi* imported by invasive black rats. *Acta Tropica* 117: 183–188.
https://doi.org/10.1016/j.actatropica.2010.11.004

Espinosa-Álvarez, O., P. A. Ortiz, L. Lima, A. G. Costa-Martins, M. G. Serrano, S. Herder, G. A. Buck, E. P. Camargo, P. B. Hamilton, J. R. Stevens, and M. M. G. Teixeira. 2018. *Trypanosoma rangeli* is phylogenetically closer to Old World trypanosomes than to *Trypanosoma cruzi*. *International Journal for Parasitology* 48: 569–584.
https://doi.org/10.1016/j.ijpara.2017.12.008

Esselstyn, J. A., A. S. Achmadi, H. Handika, M. T. Swanson, T. C. Giarla, and K. C. Rowe. 2021. Fourteen new, endemic species of shrew (genus *Crocidura*) from Sulawesi reveal a spectacular island radiation. *Bulletin of the American Museum of Natural History*, 454: 1–108.
https://doi.org/10.1206/0003-0090.454.1.1

Gortázar, C., E. Ferroglio, U. Höfle, K. Frölich, and J. Vicente. 2007. Diseases shared between wildlife and livestock: a European perspective. *European Journal of Wildlife Research* 53: 241–256.
https://doi.org/10.1007/s10344-007-0098-y

Hamilton, P. B., W. C. Gibson, and J. R. Stevens. 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.
https://doi.org/10.1016/j.ympev.2007.03.023

Hamilton, P. B., J. R. Stevens, J. Gidley, P. Holz, and W. C. Gibson. 2005. A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (Haemadipsidae). *International Journal for Parasitology* 35: 431–443.
https://doi.org/10.1016/j.ijpara.2004.12.005

Hamilton, P. B., M. M. G. Teixeira, and J. R. Stevens. 2012. The evolution of *Trypanosoma cruzi*: the "bat seeding" hypothesis. *Trends in Parasitology* 28: 136–141.
https://doi.org/10.1016/j.pt.2012.01.006

Handika, H., A. S. Achmadi, J. A. Esselstyn, and K. C. Rowe. 2021. Molecular and morphological systematics of the Bunomys division (Rodentia: Muridae), an endemic radiation on Sulawesi. *Zoologica Scripta* 50: 141–154.
https://doi.org/10.1111/zsc.12460

Hoare, C. A. 1972. *The Trypanosomes of Mammals. A Zoological Monograph.* Oxford: Blackwell Scientific Publications.

Kostygov, A. Y., A. Karnkowska, J. Votýpka, D. Tashyreva, K. Maciszewski, V. Yurchenko, and J. Lukeš. 2021. Euglenozoa: taxonomy, diversity and ecology, symbioses and viruses. *Open Biology* 11: 200407.
https://doi.org/10.1098/rsob.200407

Lima, L., O. Espinosa-Álvarez, C. M. Pinto, M. Cavazzana, A. C. Pavan, J. C. Carranza, B. K. Lim, M. Campaner, C. S. A. Takata, E. P. Camargo, P. B. Hamilton, and M. M. G. Teixeira. 2015. New insights into the evolution of the *Trypanosoma cruzi* clade provided by a new trypanosome species tightly linked to Neotropical *Pteronotus* bats and related to an Australian lineage of trypanosomes. *Parasites and Vectors* 8: 1–19.
https://doi.org/10.1186/s13071-015-1255-x

Luckins, A. G. 1998. Trypanosomiasis caused by *Trypanosoma evansi* in Indonesia. *The Journal of Protozoology Research* 8: 144–152.

Maeda, F. Y., C. Cortez, R. M. Alves, and N. Yoshida. 2012. Mammalian cell invasion by closely related *Trypanosoma* species *T. dionisii* and *T. cruzi*. *Acta Tropica* 121: 141–147.
https://doi.org/10.1016/j.actatropica.2011.10.017

Mafie, E., A. Saito-Ito, M. Kasai, M. Hatta, P. T. Rivera, X. H. Ma, E. R. Chen, H. Sato, and N. Takada. 2019. Integrative taxonomic approach of trypanosomes in the blood of rodents and soricids in Asian countries, with the description of three new species. *Parasitology Research* 118: 97–109.
https://doi.org/10.1007/s00436-018-6120-3

Martin, C., P. P. Pastoret, B. Brochier, M. F. Humblet, and C. Saegerman. 2011. A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. *Veterinary Research* 42: 1–16.
https://doi.org/10.1186/1297-9716-42-70

Melaun, C., A. Werblow, M. Busch, A. Liston, and S. Klimpel. 2014. Bats as potential reservoir hosts for vector-borne diseases. In *Bats (Chiroptera) as Vectors of Diseases and Parasites*, ed. S. Klimpel and H. Mehlhorn, pp. 25–61. Heidelberg: Springer.
https://doi.org/10.1007/978-3-642-39333-4_3

Milocco, C., K. Kamyingkird, M. Desquesnes, S. Jittapalapong, V. Herbreteau, Y. Chaval, B. Douangboupha, and S. Morand. 2013. Molecular demonstration of *Trypanosoma evansi* and *Trypanosoma lewisi* DNA in wild rodents from Cambodia, Lao PDR and Thailand. *Transboundary and Emerging Diseases* 60: 17–26.
https://doi.org/10.1111/j.1865-1682.2012.01314.x

Pumhom, P., S. Morand, A. Tran, S. Jittapalapong, and M. Desquesnes. 2015. *Trypanosoma* from rodents as potential source of infection in human-shaped landscapes of South-East Asia. *Veterinary Parasitology* 208: 174–180.
https://doi.org/10.1016/j.vetpar.2014.12.027

Pumhom, P., D. Pognon, S. Yangtara, N. Thaprathorn, C. Milocco, B. Douangboupha, S. Herder, Y. Chaval, S. Morand, S. Jittapalapong, and M. Desquesnes. 2014. Molecular prevalence of *Trypanosoma* spp. in wild rodents of Southeast Asia: influence of human settlement habitat. *Epidemiology and Infection* 142: 1221–1230.
https://doi.org/10.1017/S0950268813002161

R Core Team. 2020. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Salzer, J. S., C. M. Pinto, D. C. Grippi, A. J. Williams-Newkirk, J. K. Peterhans, I. B. Rwego, D. S. Carroll, and T. R. Gillespie. 2016. Impact of anthropogenic disturbance on native and invasive trypanosomes of rodents in forested Uganda. *EcoHealth* 13: 698–707.
https://doi.org/10.1007/s10393-016-1160-6

Setiawan, A., W. Nurcahyo, D. Priyowidodo, R. T. Budiati, and D. S. R. Susanti. 2021. Genetic and parasitological identification of *Trypanosoma evansi* infecting cattle in South Sulawesi, Indonesia. *Veterinary World* 14: 113–119.
https://doi.org/10.14202/vetworld.2021.113-119

Thompson, C. K., S. S. Godfrey, and R. C. A. Thompson. 2014. Trypanosomes of Australian mammals: a review. *International Journal for Parasitology: Parasites and Wildlife* 3: 57–66.
https://doi.org/10.1016/j.ijppaw.2014.02.002

Thompson, R. C. A. 2013. Parasite zoonoses and wildlife: One Health, spillover and human activity. *International Journal for Parasitology* 43: 1079–1088.
https://doi.org/10.1016/j.ijpara.2013.06.007

Truc, P., P. Büscher, G. Cuny, M. I. Gonzatti, J. Jannin, P. Joshi, P. Juyal, Z. R. Lun, R. Mattioli, E. Pays, P. P. Simarro, M. M. G. Teixeira, L. Touratier, P. Vincendeau, and M. Desquesnes. 2013. Atypical human infections by animal trypanosomes. *PLoS Neglected Tropical Diseases* 7: e2256.
https://doi.org/10.1371/journal.pntd.0002256

Wang, L. J., H. J. Han, M. Zhao, J. W. Liu, L. M. Luo, H. L. Wen, X. R. Qin, C. M. Zhou, R. Qi, H. Yu, and X. J. Yu. 2019. *Trypanosoma dionisii* in insectivorous bats from northern China. *Acta Tropica* 193: 124–128.
https://doi.org/10.1016/j.actatropica.2019.02.028

Wardhana, A. H., and D. H. Savitri. 2018. Surra: trypanosomiasis in livestock is potential as zoonotic disease. *Indonesian Bulletin of Animal and Veterinary Sciences* 28: 139–151.
https://doi.org/10.14334/wartazoa.v28i3.1835

Weinman, D. 1972. *Trypanosoma cyclops* n. sp.: a pigmented trypanosome from the Malaysian primates *Macacca nemestrina* and *M. ira*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 66: 628–633.
https://doi.org/10.1016/0035-9203(72)90309-4

Winterhoff, M., A. S. Achmadi, E. J. Roycroft, H. Handika, R. T. J. Putra, K. M. C. Rowe, S. L. Perkins, and K. C. Rowe. 2020. Native and introduced trypanosome parasites in endemic and introduced murine rodents of Sulawesi. *Journal of Parasitology* 106: 523–536.
https://doi.org/10.1645/19-136

Wyatt, K. B., P. F. Cisiampos, M. T. P. Gilbert, S. O. Kolokotronis, W. H. Hynes, R. DeSalle, P. Daszak, R. D. E. MacPhee, and A. D. Greenwood. 2008. Historical mammal extinction on Christmas Island (Indian Ocean) correlates with introduced infectious disease. *PLoS One* 3: e3602.
https://doi.org/10.1371/journal.pone.0003602