








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

AHMAD MURSYID¹, ANANG SETIAWAN ACHMADI², WILSON NOVARINO³,
HERU HANDIKA⁴, HERJUNO ARI NUGROHO², SYAHFITRI ANITA², AMY LOUISE ADAMS⁵,
KAREN MARIE CAVEY ROWE^{5,6}, AND KEVIN CHRISTOPHER ROWE^{5,6}

¹ Masters in Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Limau Manis, Padang 25163, Indonesia

² Bogor Zoological Museum, Research Center for Biology, Cibinong, West Java, Indonesia

³ Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Limau Manis, Padang 25163, Indonesia

⁴ Museum of Natural Science and Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, United States of America

⁵ Sciences Department, Museums Victoria, Melbourne, Australia

⁶ School of BioSciences, The University of Melbourne, Melbourne, Australia

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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ORCID iD: A. Mursyid 0000-0003-2358-0151, A. S. Achmadi 0000-0002-0784-9870, W. Novarino 0000-0003-4189-7750, H. Handika 0000-0002-2834-7175, A. L. Adams 0009-0005-4409-7953, Karen Marie Cavey Rowe 0000-0002-6131-6418, Kevin Christopher Rowe 0000-0002-1215-9153

Corresponding author: Kevin Christopher Rowe krowe@museum.vic.gov.au

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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 dengan urutan tingkat infeksi paling umum kedua teridentifikasi sebagai spesies cosmopolitan dan kemungkinan spesies *Trypanosoma* 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 kemungkinan 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 spesies *Trypanosoma* dari klade 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