Retracing the evolutionary history of the Trypanosomatidae: the use of kinetoplast DNA in molecular systematics, species identification and diagnostics

By

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Certificate of Original Authorship

I, **Alexa Kaufer** declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy in the School of Life Sciences/Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Format of Thesis

I, **Alexa Kaufer** declare the format of this manuscript is ‘Thesis by compilation’.

Publications Arising from Thesis

KAUFER, A., STARK, D. & ELLIS, J. 2019. Evolutionary Insight into the Trypanosomatidae Using Alignment-Free Phylogenomics of the Kinetoplast. *Pathogens*, 8, 157.

KAUFER, A., ELLIS, J. & STARK, D. 2019. Identification of Clinical Infections of *Leishmania* imported into Australia: Revising Speciation with Polymerase Chain Reaction-RFLP of the Kinetoplast Maxicircle. *The American Journal of Tropical Medicine and Hygiene*, 101, 599.

KAUFER, A., BARRATT, J., STARK, D. & ELLIS, J. 2019. The complete coding region of the maxicircle as a superior phylogenetic marker for exploring evolutionary relationships between members of the Leishmaniinae. *Infection, Genetics and Evolution,* 70, 90-100.

KAUFER, A., ELLIS, J., STARK, D. & BARRATT, J. 2017. The evolution of trypanosomatid taxonomy. *Parasites & Vectors*, 10, 287.

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Supplementary Information

The thesis chapters (2 – 4) make reference to supplementary, supporting or additional information. This information is contained in files that can be found at the URL specified in the chapter and on the USB provided.

Thesis Abstract

The Trypanosomatidae (class Kinetoplastida) are a diverse and widespread group of protists characterised by their possession of a unique and highly specialised mitochondrial homologue known as the kinetoplast. All trypanosomatid parasites are exclusively parasitic, with the majority of genera being restricted to a single invertebrate host (i.e. monoxenous lifecycle). However, they are primarily known for their pathogenic dixenous members (i.e. having a two-host life cycle), that serve as the aetiologic agents of several important neglected tropical diseases (NTDs) including leishmaniasis, Chagas disease and human African Trypanosomiasis. Recent advancements in molecular biology have improved our knowledge of evolutionary relationships between trypanosomatid species by revolutionising the genetic approach to trypanosomatid systematics. The kinetoplast DNA, and more specifically, the maxicircle genome represents a valuable taxonomic marker given its unique presence across all Kinetoplastids. The research described in this thesis was performed to explore the suitability of the kinetoplast DNA as a much-needed standardised framework for the taxonomic classification and species identification of protozoans falling within the Trypanosomatidae family.

The main research outcome provides the most in-depth analysis of the trypanosomatid family to date, demonstrating extensive evidence for the superiority of the maxicircle for the taxonomic resolution of the Trypanosomatidae. Chapter 1 presents a comprehensive review outlining the important developments that have been made in the field of trypanosomatid taxonomy, advancing our current knowledge over the relationships between members of the trypanosomatid family. Chapter 2 demonstrates the superiority of the maxicircle genome for the phylogenetic inference of

the Leishmaniinae. Phylogenetic analyses provided support imperative towards the Supercontinents hypothesis of dixenous parasitism within the Trypanosomatidae. The comprehensive analysis of the entire family of trypanosomatid parasites in Chapter 3 revealed strong support for the multiple origin and independent evolution of dixenous parasitism within the trypanosomatid family. Kinetoplast DNA is an organelle exclusive to the Kinetoplastids, unique in its structure, function and mode of replication and thus represents a unique diagnostic target, with the potential to differentiate different species and strains of *Leishmania.* The implementation of novel diagnostic procedures for leishmaniasis such as the one reported in Chapter 4 is intended to establish a gold standard practice for the diagnosis and treatment of leishmaniasis. Ultimately, Chapter 5 reviews the completion of this thesis, demonstrating that use of the maxicircle genomes provide an excellent benchmark for future studies involving the phylogenetic analyses, taxonomic classification and species identification of the Trypanosomatidae.