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The trematode genera *Paragonimus* and *Schistosoma* in East Asia : molecular evolution, phylogeny and biogeography.

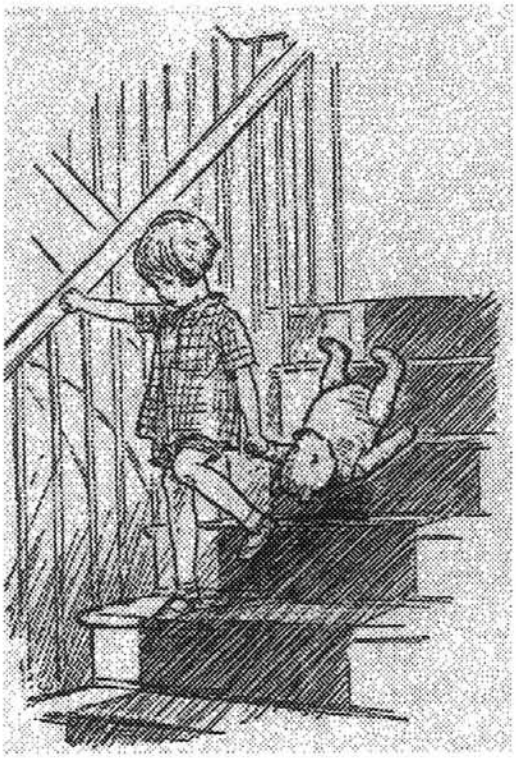
Thesis submitted by

Lynne van Herwerden BSc., BSc. (Med.) Hons., MSc. University of Cape Town.

for the degree of Doctor of Philosophy
in the School of Biological Sciences (Zoology)
James Cook University of North Queensland

Dedication -

For my family



"... bump, bump, bump ... . It is as far as he knows, the only way of coming downstairs, but sometimes he feels that there really is another way, if only he could stop bumping for a moment and think of it. And then he feels that perhaps there isn't."

taken from *Winnie - the - Pooh*,

- A.A. Milne, illustration by E.H. Shepard

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ABSTRACT

Trematode infections affect one fifth of the worlds' population, with more than 200 million Chinese alone being at risk (World Health Organisation 1997, Hotez *et al.* 1997). Of particular interest to this study are the Asian species of the trematode genera *Paragonimus* and *Schistosoma*, which infect many mammals, including humans. It is becoming clear that within both genera species complexes exist that require further characterisation. Molecular markers have given some insight into the nature of these complexes. In this thesis additional molecular markers are investigated as sources of information about the nature and distribution of the *S. japonicum*, *P. westermani* and *P. ohirai* species complexes. These two genera are of additional interest because they are thought to have shared a similar biogeographic history in Asia, which should be reflected in their molecular evolution.

I have obtained DNA sequences from one nuclear (ITS1) and one mitochondrial (ND1) marker of five *Paragonimus* species (*P. westermani*, *P. miyazakii*, *P. macrorchis*, *P. ohirai* and *P. iloktsuenensis*) present in East Asia, including numerous geographic isolates of the *P. westermani* and *P. ohirai* species complexes. In addition, I have obtained ITS1 sequences of three schistosome species (*S. japonicum*, *S. malayensis* and *S. mekongi*) present in East Asia, which comprise the *S. japonicum* species complex and an African schistosome species (*S. mansoni*) for comparative purposes. I could differentiate all species using ITS1 and ND1 sequence data.

ITS1 is contained within the ribosomal DNA gene cluster, which is repeated in tandem many times. ITS1 does not experience the constraints of coding regions and theoretically undergoes sequence homogenisation within individuals and species. Despite this, intra-individual sequence variation was sometimes greater than inter-species variation, particularly among northeast Asian *P. westermani* isolates. Such variation confounded phylogenetic inferences for this group. There are some interesting aspects relating to processes of concerted evolution generally, when considering the differences within and between the two trematode genera, *Paragonimus* and *Schistosoma*, in this study. Additionally, a transcriptional enhancer motif (TATAAT) is embedded within the repeats of the ITS1 of the Asian schistosomes. The repeats are the cause of size variation and given the improbability of such motifs occurring by chance, I propose that the abundant, large variants containing multiple copies of repeats may have a role in the stage- or tissue-specific regulation of transcription of the ribosomal genes.

In contrast, the ND1 gene codes for a mitochondrial protein and is therefore functionally constrained. Phylogenetic inferences could be made from ND1 sequence data obtained from *Paragonimus*. However, many clones had to be sequenced per individual to achieve this, as multiple ND1 lineages occurred within individuals of all *Paragonimus* species and strains investigated. Presumed pseudogenes were identified, which may be present in either the nuclear genome or in different types of mitochondria, as *Paragonimus* species have two types of mitochondria which differ structurally. It seems likely that both nuclear pseudogenes and heteroplasmic mitochondrial genes occur, though this hypothesis remains to be tested.

The *Paragonimus westermani* species complex has been further studied, to determine the origins of parthenogenetic triploid forms. Ribosomal DNA-ITS restriction fragments, ND1 sequences and simple sequence repeat (SSRs) "fingerprints" of triploids from China, Korea and Japan all indicate that the triploids are genetically different from one another. I conclude from this that triploids may have arisen more than once independently, possibly by relatively rare matings between diploid and tetraploid *P. westermani* individuals, which occur in sympatry with the triploids in NE China. Alternatively, triploid lineages may have arisen once and diverged subsequently by mutation alone.

Despite their suggested shared biogeographic history, members of the two genera exhibit rather different properties with respect to their ND1 and ITS1 genes. This is likely to be a reflection of their different phylogenetic histories. Molecular techniques have been used successfully to infer phylogenies which could be used to evaluate a previously published biogeographic hypothesis, but only to a limited extent, using the markers. It is clear that the situation for *P. westermani* in particular is complex and requires further investigation. Molecular evolution of these markers has proved to be an interesting component of the study that has brought to light some novel ideas and applications of these markers, not as phylogenetic tools, but as tools to study processes of concerted evolution in the nuclear genome and mitochondrial evolution.

CONTENTS

Chapter 3. General materials and methods

Chapter 4. Intra- and inter-specific variation in nuclear ribosomal internal transcribed spacer 1 of the *Schistosoma japonicum* species complex

Chapter 5. Intra - and inter- individual variation in ITS1 of *Paragonimus westermani* (Trematoda: Digenea) and related species

Chapter 6. Multiple repeats in ITS1 variants of some trematodes (Digenea) contain a putative enhancer.

Chapter 7. Intra - and inter- individual variation in ITS1 of *Paragonimus* and *Schistosoma*: phylogenetic inferences

Chapter 8 Multiple variants of NADH Dehydrogenase subunit 1 (ND1) genes in some trematodes.

Chapter 9 Clonal diversity in populations of a parthenogenetic trematode, *Paragonimus westermani.*

Chapter 10 General Discussion

LIST OF TABLES

Chapter 1. General Introduction.

Chapter 2. Literature Review.

Chapter 3. General Materials and Methods

Chapter 4. Intra- and inter-specific variation in nuclear ribosomal Internal transcribed spacer 1 of the *Schistosoma japonicum* species complex.

Chapter 5. Intra - and inter- individual variation in ITS1 of *Paragonimus westermani* (Trematoda: Digenea) and related species.

Chapter 6. Multiple ITS1 variants and putative transcriptional enhancers in Trematodes (Digenea).

Chapter 7 Intra - and inter- individual variation in ITS1 of two trematode (Digenea) genera, *Paragonimus* and *Schistosoma*: phylogenetic inferences.

Chapter 8 Multiple lineages of the mitochondrial gene NADH Dehydrogenase subunit 1 (ND1) in some trematodes.

Chapter 9 Clonal diversity in parthenogenetic triploid *Paragonimus westermani*.

Chapter 10 General Discussion

LIST OF FIGURES

Chapter 1. General Introduction.

Chapter 2. Literature Review.

Chapter 3. General Materials and Methods

Chapter 4. Intra- and inter-specific variation in nuclear ribosomal Internal transcribed spacer 1 of the *Schistosoma japonicum* species complex.

Chapter 5 Intra - and inter- individual variation in ITS1 of *Paragonimus westermani* (Trematoda: Digenea) and related species

Chapter 6. Multiple ITS1 variants and putative transcriptional enhancers in Trematodes (Digenea).

Chapter 7. Intra- and inter-individual variation in ITS1 of two trematode (Digenea) genera, *Paragonimus* and *Schistosoma*: implications for phylogenetic studies.

Chapter 8. Multiple lineages of NADH Dehydrogenase subunit 1 (ND1) genes in some trematodes.

Chapter 9 Clonal diversity in parthenogenetic triploid *Paragonimus westermani*.

LIST OF PUBLICATIONS

The following papers have been produced from material within this thesis.

1) D. Blair, L. van Herwerden, H. Hirai, S. Habe, M. Hirata, K. Lai, S. Upatham, T. Agatsuma (1997) Relationships between *Schistosoma malayensis* and other Asian schistosomes deduced from DNA sequences. *Molecular & Biochemical Parasitology* **85**: 259 - 263.

2) L. van Herwerden, D. Blair & T. Agatsuma (1998a) Intra- and inter-specific variation in nuclear ribosomal internal transcribed spacer 1 of the *Schistosoma japonicum* species complex. *Parasitology* **116:** 311-317.

3) L. van Herwerden, D. Blair & T. Agatsuma (1998b) Intra - and inter-individual variation in ITS1 of *Paragonimus westermani* (Trematoda: Digenea) and related species: implications for phylogenetic studies. (In press) *Molecular Phylogenetics and Evolution.*

4) L. van Herwerden, D. Blair & T. Agatsuma (1998c) Clonal diversity in parthenogenetic triploid *Paragonimus westermani*. (submitted) *International Journal of Parasitology*

5) L. van Herwerden, J. Caley and D. Blair (1998) Multiple ITS1 variants and putative transcriptional enhancers in trematodes (Digenea). *Nucleic Acids Research* (in preparation)

6) L. van Herwerden & D. Blair. Multiple lineages of NADH Dehydrogenase subunit 1 (ND1) genes in some trematodes. (submitted) *Journal of Molecular Evolution.*

CONFERENCE PROCEEDINGS

1. L. van Herwerden, D. Blair, T. Watanobe & T. Agatsuma. (1996). Molecular phylogenetic studies of the *Paragonimus westermani* species complex. *PASEAN: [Parasites in South East Asia Now.*]The Australian Society for Parasitology and The Parasite Control Association of Indonesia. Bali, Indonesia, September 1996.

2. L. van Herwerden and D.Blair, (1997). Comparison of nuclear and mitochondrial sequences for phylogenetic studies of human lung flukes. *Genetics Society of Australia,* (GSA) Perth, Western Australia, September 1997.

3. L. van Herwerden and D. Blair, (1998) Multiple lineages of NADH Dehydrogenase subunit 1 (ND1) genes in some trematodes. *Genetics Society of Australia,* Sydney, New South Wales, July 1998.

DEPARTMENTAL CONFERENCES

1. L. van Herwerden (1996). ITS sequences as a tool in phylogenetic studies of some trematodes. *ZONQ: Zoology in North Queensland,* James Cook University, Townsville, North Queensland, April 1996.