**Declaration:**

I declare that the thesis hereby submitted to the University of Limpopo, for the degree of PhD in Biology has not previously been submitted by me for a degree at this or any other university; that is my work in design and in execution, and all my material contained herein has been duly acknowledged.

……………………… …………………………..

**E.B.E. Moema (Ms) Date**

**Student Number: 19716955**

**ABSTRACT**



**ABSTRACT:**

Digenean parasites are known to be a large and diverse group of parasites. Some of these parasites are free-living, a few are ecto-parasitic, while the majority are endo-parasitic in most invertebrates and vertebrates. Digenean parasites have always been known to be host specific. However, the degree to which these parasites are host specific, is determined by the number of hosts they are able to utilise and the parasitic stages they would currently be at.

Morphologically the link between the cercarial and other parasitic stages such as metacercarial and adult stages, were found to be very difficult to establish, since different developmental stages utilise different types of hosts. For instance, cercariae may use the same or different hosts for their metacercarial stages. An example of this is in the case of freshwater snails where the cercariae re-penetrate the same snail and encyst as metacercariae, and then the snail hosts serve also as second intermediate hosts. Adult digenean parasites on the other hand utilise vertebrate hosts different from those serving as second intermediate hosts, as final or definitive hosts.

Digenean trematodes like any other helminth parasites have been well researched for decades due to their widespread health-related diseases that they cause and their economic impact globally, especially in third world countries. Research in this field included aspects of species diversity, morphology, distribution, epidemiology and immunology. Despite all of these aspects, these parasites continue to thrive in the face of numerous strategies aimed at their control. Lately polymerase chain reaction (PCR) techniques have been employed to assist with parasite biology and identification, especially with regards to round- and flatworms. Several genome projects like the *Schistosoma* Genome Project (SGP) initiated in 1992, was established in an attempt to create gene banks and to allow researchers to utilise technology for genomic analysis in the study of organisms relevant to public health in developing countries.

**ABSTRACT**



The methods of gene discovery and their functional discovery have been accelerated significantly and are being progressively applied in numerous organisms of medical and veterinary importance. On the other hand parasitic helminths lag behind parasitic protozoa in the sense that *in vitro* cultivation systems have not been developed to support the entire life cycle of these helminth parasites, genomic databases are far from being complete and lastly there are no established methods for the highly efficient manipulation of endogenous genes within living worms. The present study was aimed at supplying morphological descriptions and additional information through PCR techniques to enable us later to complete the life cycles of the lesser known parasites experimentally.

The study was achieved by collecting materials from six localities, namely Boekenhoutskloof farm dam, Supersand dam, Rietvlei dam, Kiewiet farm dam and Northern farm dam. All these five localities were located in the Gauteng Province, proximal to Tshwane. The sixth locality was Metsi-pepa in the North-West Province that was selected due to the unique eye source that feeds the Mooi River. The collected materials were then studied employing standard light and scanning electron microscopy techniques, as well as applying PCR techniques in order to identify and classify the digenean parasites collected during the study. Life cycle studies were also attempted through experimental infections of potential definitive hosts.

Seven different types of snail species were collected during the research study, namely *Bulinus africanus, Bulinus tropicus, Lymnaea natalensis, Gyraulus connollyi, Burnupia mooiensis, Biomphalaria pfeifferi* and *Ferrissia fontinalis*. Of these, *Lymnaea natalensis* was found to be the most abundant snail of the entire snail species collected over a period of four years. Of the above-mentioned snail species only four types were found to be infected with various types of cercariae.

**ABSTRACT**



*Lymnaea natalensis* produced three different cercarial types: a) strigeid cercaria B with its characteristic three pairs of linear penetration glands and a very large sinous intestinal caeca, b) a xiphidio cercaria with three pairs of penetration glands, and c) an avian schistosome cercaria. *Bulinus tropicus* was found to produce two cercarial types: a) an echinostome cercaria with a collar consisting of 27-spines and b) strigeid cercaria A. The third infected snail species was *B. africanus*, found to be infected with only one type of cercaria, namely xiphidio cercaria B with its characteristic clustered penetration glands. The fourth infected snail species, *G. connollyi*, housed two types of monostome cercariae: a) monostome cercaria A possessing 3 pairs of linear penetration glands and b) a clinostomatid cercaria with its characteristic head membrane.

Nine metacercarial types were collected from various second intermediate hosts. 27-spined echinostomatid metacercaria A and 43-spined metacercaria B were found encysted on the gills of the following fish hosts: *Pseudocrenilabrus philander* and *Tilapia sparrmanii*, as well as in the mantle of the snail hosts, *L. natalensis* and *B. africanus*. Two strigeid metacercarial types, a) strigeid metacercaria A encysted within a green cyst and b) strigeid metacercaria B with distinct fore- and hindbodies, were collected from the fish hosts, *P. philander*. Strigeid metacercaria A was sporadically also found in *T. sparrmanii*. Two diplostomatid metacercariae were collected from their fish hosts, a) diplostomatid metacercaria A from the cranial cavity of *Clarias gariepinus* and b) diplostomatid metacercaria B from the vitreous chamber of *T. sparrmanii and P. philander*. Two metacercarial types of the family Clinostomatidae, a) a clinostomatid metacercaria and b) an Euclinostomatid metacercaria, were collected from the buccal cavity and the muscle tissue of *T. sparrmanii*, respectively. The last metacercarial stages, namely xiphidio metacercariae, were collected from various hosts such as freshwater shrimps (branchial region), *T. sparrmanii* (gill filaments) and *L. natalensis* (mantle). This parasite was found to have developed sexually more in freshwater shrimps compared to the same stage in other infected second intermediate hosts.

**ABSTRACT**



Three adult parasites were also identified and described. They include an amphistome fluke of the genus *Cotylophoron* and the liver fluke, *Fasciola gigantica*. These two parasites were collected from the rumen and the hepatic ducts of a heifer at the Northern farm, respectively. The third fluke, *Echinoparyphium elegans*, was obtained from experimentally infected kittens.

It was, however, difficult to link the different stages within the same family using only morphological characteristics. The morphological characterization of digenean parasites, especially the adult stages, has been well-documented over the past few decades worldwide. This has, however, not been the case with larval stages. Recent studies have shown that there were many attempts by researchers pertaining to molecular studies using PCR techniques. In most cases the studies were achieved by using matured (adult) stages of digenean parasites. These include the studies done on digenean parasites at species level, family level, superfamily level, suborder level and on general digeneans. Most of these phylogenetic studies were only conducted on medically and veterinary important digeneans.

The present study focused more on the amplification of parasites at family level. The universal primers were used to target ITS-1, ITS-2 and LSU regions. Not all the specimens yielded desired amplicons. Only certain stages of the following four families; a) Clinostomatidae, b) Schistosomatidae, c) Echinostomatidae and d) Strigeidae were able to be amplified and sequenced. From this study, it is evident that in future, specific primers for specific digenean parasites need to be designed and used in order for us to achieve our desired goals (i.e. being able to amplify as many digenean specimens as possible including the lesser known trematodes).

**ABSTRACT**



The recent study also demonstrated that much more work needs to be done in order for us to understand parasite-host relationships in the localities studied. Experimental life cycle studies are therefore imperative in order to solve most of our cercaria, metacercaria and adult trematode questions raised during the present study.

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