CHAPTER 1:

INTRODUCTION AND LITERATURE REVIEW

1.1. Parasitism

An ecosystem is a functional ecological unit in which there are various interactions among species, particularly where different species exploit one another for a number of reasons, including transportation (phoresis) such as the interaction between mites and dung beetles, shelter (inquilinism) such as birds building their nests in trees, or nutrition such as in parasitic relationships (Begon *et al*., 2006; Hickman *et al*., 2004).

Parasitism is an important phenomenon in life and involves nearly all organisms. Those that are not parasitic themselves most likely acts as host for several parasite species (Begon *et al*., 2006). Parasitism can be defined as "*a form of symbiosis in which one organism (parasite) benefits at the expense of another organism usually of a different species (host). The association may also lead to the injury of the host*" (Biology-online, 2009). However, the parasite usually does not intentionally kill its host as that would also harm the parasite itself as it relies on its host for its own survival. In certain cases parasites could even provide an indirect advantage to the survival of their host species (van As *et al*., 2012).

Evolution can be defined as "*all the changes in the characteristics and diversity of life on earth throughout its history*" (Hickman *et al*., 2004). Species have evolved over thousands of years to adapt to their environment, resulting in a great diversity of species, with some morphologically similar and others morphologically distinct (Hickman *et al*., 2004). Certain species are more closely related than others. Due to this great diversity as well as the fact that new species are continuously being discovered, it is becoming increasingly difficult to classify species into groups. Thus taxonomy is an important tool used to classify species based on similar characteristics (Roberts & Janovy, 2009; van As *et al*., 2012). Taxonomy can be defined as "*study of the principles of scientific classification, systematic ordering and naming of organisms*" (Hickman *et al*., 2004).

Aristotle (Greek philosopher and biologist) was the first person to classify organisms based on morphological similarities. Carolus Linnaeus (Swedish botanist) proposed a system for classification based on a hierarchy of taxa, in which closely related species are placed into the same genus, a group of genera are then placed into the same family. Closely related families are group into orders, with related orders grouped into classes. Together these make up the different kingdoms (Hickman *et al*., 2004; van As *et al*., 2012).

Parasites are often host-specific and can be endoparasites where these organisms live inside their hosts or ectoparasites where they live on the surface of their hosts. Parasites can also be classified as facultative or obligate. Facultative parasites only become parasitic when they come into contact

with the appropriate host, such as a leech. Obligate parasites, which require a host to survive, can be subdivided into permanent parasites where the organism spends its entire adult life-cycle in or on a host such as monogenean flatworms or temporary parasites that only parasitises a host long enough to secure enough food to continue their life-cycle such as mosquitoes (Begon *et al*., 2006; Hickman *et al*., 2004; van As *et al*., 2012).

Parasites are extremely important to humans. Ecological they can provide information on the historical dispersal and migration patterns of their host species, as they can be used as biological tags. Medically they play an important role in the health of humans and animals, they can transmit diseases such as malaria and bilhazia. They can however also be to our advantage. Leeches are being used to stimulate blood flow and certain ailments can be treated such as blood clots from small body parts, headaches and haemorrhoids (van As *et al*., 2012).

1.2. Polystomes

The phylum Platyhelminthes, known as flatworms, is subdivided into four classes namely Cestoda, Monogenea, Trematoda and Tubellaria. Members of Cestoda, Monogenea and Trematoda are entirely parasitic organisms while Tubellaria is mostly free-living. Monogenea, Trematoda and Tubellaria all contain an incomplete gut that may be branched, while in Cestoda a gut is absent (Hickman *et al*., 2004; Roberts & Janovy, 2009). Platyhelminths are characterised by its bilateral symmetry and the presence of a dorsoventrally flattened body, triploblastic, acoelomate body and asexual reproduction (Roberts & Janovy, 2009).

The parasites focussed on in this study are from the class Monogenea, commonly referred to as monogenetic flukes (Hickman *et al*., 2004). In particular this study focussed on the family Polystomatidae. Monogenea are generally characterised by a leaflike or cylindrical body shape, epidermis is a syncytial tegument without cilia, monoecious, direct development with a single host as well as by the presence of an attachment organ (haptor) containing clamps, suckers and/or hooks. Monogeneans have a cosmopolitan distribution and occur in a number of organisms (Hickman *et al*., 2004; Roberts & Janovy, 2009).

Monogeneans are mainly parasitic on fish but the family Polystomatidae radiated onto the tetrapods and are known from the skin and gills of the Australian lungfish, kidneys and urinary bladders of frogs, gills and skin of salamanders, cloaca and phalodeum of caecileans, on the eye, in the nose, mouth or urinary bladder of freshwater turtles and on the eye of the hippopotamus (Badets & Verneau, 2009; Bakke, 2009; Bentz *et al.,* 2006; Du Preez *et al*., 2010; Hickman *et al*., 2004; Littlewood *et al*., 1997; Morrison & Du Preez, 2012; Roberts & Janovy, 2009; Verneau *et al*., 2009; Whittington, 1998).

Polystomes vary from 1 mm to more than 30 mm in length. The body is elongated or somewhat pyriform, tapering anteriorly, with a posterior haptor that is either bilobed with two suckers as in Sphyranura or discoid with six suckers, with or without hamuli (large haptoral hooks) and with 16 marginal hooklets (Bakke, 2009; Price 1939). Surrounding the terminal or subterminal mouth is an

oral sucker. The crura of the bifurcate intestine are with or without diverticula, united posteriorly or not. One, two or multiple testes are present (Price 1939; Roberts & Janovy, 2009).

The median genital pore is situated in the region of the intestinal bifurcation and the cirrus is with or without genital spines arranged in a single or a double ring. A compact ovary is anterior to the testis. Vitellaria are follicular diffuse throughout body, restricted in two lateral fields or densely compacted. Vaginae, when present, are in an antero-lateral position (Bakke, 2009; Price 1939; Roberts & Janovy, 2009).

1.3. Polystome classification

Van Beneden (1858) was the first to distinguish Monogenea from Digenea, while Fuhrmann (1928) helped separate Monogenea into a distinct category, but still closely allied to Digenea. Bychowsky (1937) was the fisrt to place Monogenea as a distinct class. Braun (1889 & 1893) was the first to give a comprehensive overview of the monogeneans. Several modern literature sources still consider Monogenea as a subclass of Trematoda, however cladistic analysis has place Monogenea closer to Cestoda (tapeworms), with some authorities arguing that they are sister taxa as they have a number of characteristics in common. Thus Monogenea is now considered as the sister group to the infraclass Cestodaria. Boeger and Kritsky (1993) use the name Monogenoidea to denote the class, this view is supported by several authors including Roberts and Janovy (Hickman *et al*., 2004; Roberts & Janovy, 2009). However, most other taxonomists refer to Monogenea. For the purpose of this document we will refer to Monogenea.

The general classification of polystomes, followed in this study is as follows:

Kingdom:	Animalia	Linnaeus, 1753
Phylum:	Platyhelminthes	Gegenbaur, 1859
Class:	Monogenea	Carus, 1863
Subclass:	Polystomationea	Lebedev, 1986
Order:	Polystomatidea	Lebedev, 1988
Superfamily:	Polystomatoidea	Price, 1936
Family:	Polystomatidae	Carus, 1863

Polystomatid flatworms are classified in 24 genera and are known from a large range of hosts including the Australian lungfish (1 genus), amphibians (19 genera), freshwater turtles (3 genera) and the hippopotamus (1 genus).

The three genera that are found in freshwater turtles are *Neopolystoma* Price, 1939, *Polystomoidella* Price, 1939 and *Polystomoides* Ward, 1917 (Morrison & Du Preez, 2012). *Oculotrema* Stunkard, 1924 is the only polystome genus parasitising a mammal, namely the hippopotamus (Du Preez & Moeng,

2004). The sole polystome genus found in the Australian lungfish is *Concinnocotyla* Pichelin Whittington & Pearson, 1990 (Pichelin *et al*., 1990).

The 19 polystome genera known to parasitize amphibians, include *Diplorchis* Ozaki, 1931, *Eupolystoma* Kaw, 1950, *Kankana* Raharivolololniaina, Verneau, Berthier, Vences & Du Preez, 2011, *Madapolystoma* Du Preez, Raharivololoniaina, Verneau & Vences, 2010, *Mesopolystoma* Vaucher, 1981, *Metapolystoma* Combes, 1976, *Neodiplorchis* Yamaguti, 1963, *Neoriojatrema* Imkongwapang & Tandon, 2010, *Parapolystoma* Ozaki, 1935, *Parapseudopolystoma* Nasir & Zambrano, 1983, *Polystoma* Zeder, 1800, *Protopolystoma* Bychowsky, 1957, *Pseudodiplorchis* Yamaguti, 1963, *Riojatrema* Lamothe-Argumedo, 1964, *Sundapolystoma* Lim & Du Preez, 2001 and *Wetapolystoma* Gray, 1983 that infect anurans. Polystome genera parasitising salamanders, include *Pseudopolystoma* Yamaguti, 1963 and *Sphyranura* Wright, 1879. A single genus *Nanopolystoma* Du Preez, Wilkinson & Huyse, 2008 is known from caecileans (Du Preez *et al*., 2008).

1.4. Polystome hosts

Polystomes co-evolved and speciated with their hosts. They are usually very host-specific (Combes, 1966, 1968; Combes & Channing, 1979; Du Preez & Kok, 1992b, 1993, 1997; Euzet *et al*., 1974a, b; Kok & Du Preez, 1987; Kok & Van Wyk, 1986; Murith, 1981a, b, 1982; Tinsley, 1973, 1974a) and by studying them morphologically and at the molecular level new insights on the early evolution of amphibians in the Mesozoic era came to light. As fossil records of amphibians are relatively rare, polystome parasites can provide valuable information on its host phylogeny. They provide us with biological prints assisting us to unravel the secrets of amphibian evolution over close and distant geological periods (Badets & Verneau, 2009; Bentz *et al*., 2006; Saeed *et al*., 2007; Tinsley, 1981; Tinsley *et al*., 2012; Verneau *et al*., 2009). However, the information regarding polystomes is incomplete and scattered in old literature.

The lungfish is the only known survivor of ceratodont lungfish-like ancestors and is cleidographically positioned between fish and tetrapods. They are present on the South-American, African and Australian continents. Some are capable of estivation. The only lungfish currently known to host a polystomatid parasite (*Concinnocotyla*) is the Australian lungfish, *Neoceratodus forsteri* Kreft, 1870 (Kemp & Molnar, 1981; Satchell *et al*., 2000).

Salamanders and newts are aquatic or semi-aquatic amphibians, and belong to the Order Caudata. They have a lizard like appearance with a long tail attached to a slender body, small eyes and a flattened head (Bond, 2013; San Diego Zoo Global, 2014). They are commonly found in temperate climates, particularly North America, in habitats such as wetlands, rainforests or streams. There are three salamanders that are known to host polystomes namely *Necturus maculosus* Rafinesque, 1818 (mudpuppy), *Eurycea tynerensis* Moore and Hughes, 1939 (Oklahoma salamander) and *Onychodactylus japonica* Houttuyn, 1782 (Japanese clawed salamander) (Hickman *et al*., 2004; San Diego Zoo Global, 2014).

The most common order of amphibians is Anura, containing frogs and toads that inhabit various habitats. The main characteristic of these species include the absence of a tail in the adults and powerfull hindlimbs that enable them to jump. Other characteristics include an absence of scales, large mouth, two pairs of limbs, trunk and head are fused and 6–10 vertebrae with an urostyle. Anuran species have a cosmopolitan distribution and are divided into 44 families. Amphibians are the only living vertebrates that require a transition from water to land in their phylogeny as well as in their ontogeny (Hickman *et al*., 2004).

Caecilians comprise one of the three orders of amphibians, namely Gymnophiona, which is the least known and least spaceous of the three (Du Preez *et al*., 2008). Caecilians are elongate tetrapod vertebrates that are commonly found in terrestrial, semi-aquatic or some even in aquatic habitats. These limbless amphibians are adapted to burrowing (soil-dwelling) and only occur in tropical environments (Hickman *et al*., 2004). They are commonly found in India, central Africa and Southeast Asia. The generic characteristics include a long, slender body, long ribs, terminal anus, rudimentary eyes, snout containing a pair of sensory tentacles, a terminal (primitive species) or subterminal (advanced species) mouth, no tail (advanced species) or a short tail (primitive species) (Hickman *et al*., 2004; Nussbaum & Wilkinson, 1989).

Chelonians comprise turtles, terrapins and tortoises from the order Testudines/Chelonia. Chelonians are oviparous reptilians with a diverse distribution range and appear capable of adjusting to the presence of humans (Morrison & Du Preez, 2012). The characteristics of chelonians include a shell, comprising a ventral plastron and a dorsal carapace, absence of teeth (instead the jaw contains keratinized plates) and the limbs and limb girdles are found inside the ribs (Hickman *et al*., 2004).

The hipppopotamus (*Hippopotamus amphibious* Linnaeus, 1758) is a semi-aquatic mammal commonly found in sub-Saharan Africa and the only mammal known to host a polystome species, namely *Oculotrema hippopotami* Stunkard, 1924b (Stunkard, 1924b). They are territorial animals that reach maturity at approximately seven to eight years old for the males and approximately nine to ten years old for the females. The geographical distribution and population of hippo species has declined over the years due to anthropogenic activities, such as poaching for ivory and meat as well as habitat fragmentation. However some hippo populations occur in protected regions in southern, eastern and western Africa and the conservation status is Least Concerned (Okello *et al*., 2005).

1.5. Aims and objectives

This study will focus on three aspects namely:

1. *To produce a reference document for all the known polystome species.*

Information on the known polystomes are scattered and in various languages in sometimes poorly known journals or journals that have been terminated. The primary objective of the present study was to compile a single reference document to be used in future as a reference to all known polystomes. This could aid in future research on polystome species as general information as well as citations of

important articles on all known polystome species will be available in a single source. Several synonyms do exist and for some species great uncertainty exist regarding the validity of taxonomic names. This proposed reference document will aid significantly to clear up any uncertainties regarding the identification of polystome species.

2. *To evaluate the shape of the intestinal tract as a taxonomic characteristic.*

The development of a classification tool is proposed to classify intestinal tracts based on shape. This section could assist other researchers in the identification and classification of polystome species, particularly if the shape of the digestive tract is specific to certain polystomes.

3. *To describe a new polystome species collected in North America.*

Describe a new *Polystomoides* sp. collected from *Pseudemys nelsoni* in Gainesville, Florida, USA.

Following the **Introduction and literature review** as **Chapter 1**, **Chapter 2** provides a reference document for all the known polystome species, while **Chapter 3** provides a classification tool based on the different shapes of intestinal tracts observed among polystomes. **Chapter 4** contains the description and classification of a new polystome species. **Chapter 5** is the conclusion that serves to combine the different aspects of the research project.