MORPHOMETRIC ANALYSIS OF MONOGENEAN PARASITES OF CICHLID AND CYPRINID FISH IN LAKE NAIVASHA, KENYA

RINDORIA, NEHEMIAH MOGOI

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted or presented for examination in any institution

Signature: _____ Date: _____

Mr. Rindoria Nehemiah Mogoi

SM18/3673/13

RECOMMENDATION

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations

Signature: _____ Date:_____

Dr. Elick O. Otachi

Department of Biological Sciences

Egerton University

Signature: _____ Date: _____

Prof. Andrew W. Yasindi

Department of Biological Sciences

Egerton University

Signature: _____ Date: _____

Prof. Annemarie Avenant-Oldewage

Department of Zoology

University of Johannesburg

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DEDICATION

To wife Gladys, daughters Egrah, Elsie and my late dad (Rindoria) may his soul rest in eternal peace.

If I were to attempt composition of an ode describing my love and appreciation, any amount of words would miserably fail at conveying its true extent…..

Thank you!

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ABSTRACT

Fish diseases and parasitic infections have been recognized as detrimental and limiting factors in the development of capture fisheries and aquaculture. Research on monogeneans parasitizing fish from Lake Naivasha, Kenya is scanty. This study aimed to identify the species of monogenean parasites of some cichlid and cyprinid fish in Lake Naivasha, Kenya. Fifty specimens of each fish species comprising: *Oreochromis niloticus*, *O. leucostictus*, *Tilapia zillii* and *Barbus paludinosus* were captured using a fleet of gill nets with mesh sizes 2, 2.5, 3 and 4 inches from December 2014 to January 2016. The fish were transported alive to the laboratory in the Department of Biological Sciences, Egerton University, Njoro, where they were killed by severing the spinal cord followed by a dissection. Using parasitological examination procedures, the fish gills were examined for the presence of monogeneans. The parasites were identified using morphometric analysis with the help of identification keys. From this study the following parasites were recovered: *O. leucostictus* had *Scutogyrus gravivaginus* with a prevalence (P) = 2%, *Cichlidogyrus sclerosus* (P = 100 %), *C. tilapiae* (P = 6%), *C. halli* (P = 4 %). From *O. niloticus*, *C. sclerosus* (P = 8 %) and *C. tilapiae* (P = 12%) were identified. From *T. zillii*, *C. digitatus* (P = 42%), *C. yanni* (P = 2 %), *C. aegypticus* (P = 2 %), *C. arthracanthus* (P = 2 %), *C. sclerosus* (P = 2 %), *C. tilapiae* (P = 2%); and *C. vexus* (P = 4%) were identified. From the cyprinid *B. paludinosus* a new species of *Dactylogyrus* (P = 22%) was found. All these species are reported for the first time from Lake Naivasha, and form the first monogenean biogeographical records from the Republic of Kenya and therefore adds to our knowledge of the biodiversity. It was also concluded that Lake Naivasha harbours the ancyrocephalids and dactylogyrids on the gill filaments of the cichlids and cyprinid fishes and recommend that further studies on their ecology, seasonality and water quality should be carried out to determine whether they facilitate the spread of these monogenean parasites. Additionally, more studies should be done to identify monogeneans infecting the other fish species which were not part of the present study.

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LIST OF ABBREVIATIONS AND ACRONYMS

ESP	Economic Stimulus Program
FAO	Food and Agricultural Organization
GDP	Gross Domestic Product
ICOPA IV	4th International Congress of Parasitology
KNBS	Kenya National Bureau of Statistics
NACOSTI	National Commission of Science Technology and Innovation
MI	Mean intensity
P	Prevalence
SDOFK	State Department of Fisheries of Kenya
WWF	World Wildlife Fund

CHAPTER ONE
INTRODUCTION

1.1 Background information

The Kenya fisheries sector contributes 0.5% to the GDP and thus plays an important role in the national economy (KNBS, 2012). This figure could be much higher if value addition was considered and the efforts to reduce post-harvest losses are increased (Mwangi, 2008). Fishing as an economic activity earns people a living, provides proteins, industrial by-products and supports other industries and is therefore important to the fishing communities, fish traders, fish processors and fish farmers (Mwangi, 2008). However, there has been a consistent and persistent decline in world fisheries with the Republic of Kenya being no exception (FAO, 2012). Kenya has experienced a significant decline in fish caught due to various reasons such as destructive fishing, overfishing, biodiversity reduction, climate change, receding water levels, pollution, eutrophication, variability in macrophyte densities and general environmental degradation especially in the catchments (Cowx *et al*., 2003; Matsuishi *et al*., 2006). It is not surprising that in the recent years; efforts by the Government of Kenya to intensify fish production have been increasing. For example, the Government of Kenya through the economic stimulus program (ESP) in a supplementary budget of the year 2009/2010 allocated Kshs 1.12 Billion for the establishment of 200 fish ponds in each of the 140 potential constituencies in an effort aimed at enhancing food security by intensifying aquaculture (Otachi *et al*., 2011). With this expected growth in fish production, the quality and biosafety of fish need to be guaranteed (State Department of Fisheries of Kenya SDOFK, 2013).

Parasites are an important component of host (fish) biology, survival, population structure and indeed, ecosystem functioning (Marcogliese, 2004). They can be found in any fish species and in any type of aquatic system. They range from protozoans including flagellates, ciliates, and apicomplexans to metazoans such as myxozoans, trematodes, cestodes, acanthocephalans, nematodes and crustaceans (Lewis, 1991). Dobson *et al*., (2008) reported that over 40% of all known species on earth are parasitic with parasitism being ubiquitous in some taxa and either absent or rare in others. The rate of discovery of new parasite species has grown linearly or exponentially in some well-studied helminth taxa and in contrast, sampling of parasite diversity from the most diverse parts of the world is thin at best. The knowledge of the status of parasite

diversity in the tropics is still minimal (Dobson *et al*., 2008). For example, a literature search in scopus (www.scopus.com), with the combination of the key words: fish+parasites+Kenya, returns a paltry less than 30 research articles of which a few deal with parasites of fish (e.g., Aloo and Dezfuli, 1997; Aloo, 1999; 2002; Aloo *et al*., 2004; Amin and Dezfuli, 1995; Cowx *et al*., 2003). This indicates a slow progress in research in this field in Kenya, considering that this combines marine and freshwater fish parasites. The continued discovery of new species from Kenyan lakes e.g. Lake Turkana (Moravec *et al*., 2009a, b; Přikrylová *et al*., 2012a, b) is not only encouraging but also evidence of the understudied community of fish parasites.

Monogenean trematodes are small worms of about 0.3-2mm long (Paperna, 1996), characterized by the presence of an oral sucker at the anterior end and a complex chitinous attachment structure known as the opisthaptor at the posterior end (Bychowsky, 1961). They are considered serious ectoparasites with some causing mortalities in fish. They are highly host specific and each monogenean species infects only one or very few host species (Poulin, 1992; Sasal *et al*., 1999) with only a few species occurring in closely related hosts. The high host specificity shown by most monogeneans, however, makes it easier to search for a link between the ecological characteristics of the hosts and the diversity of their monogenean parasites. Most monogeneans parasitize gills of fish (Kearn, 1994; Whittington *et al*., 2000) where they occur on gill filaments, although some are located on gill rakers or the lateral surfaces of gill arches (Bychowsky, 1961). The general impression in the literature is that little effect is produced upon the host when monogeneans occur in small numbers. However, when they attain high infection levels, a condition characteristic of overcrowded host populations (for example fish ponds), they are usually pathogenic. Some species such as *Dactylogyrus vastator* are highly virulent. Damages include haemorrhages and ulceration of host epithelium, development of epithelial outgrowths, and production of excessive amounts of mucus, which can disturb the respiratory function of the gills and ionic exchange (Erasmus and Chapman, 1972; Ukoli, 1984). Heavy infection can also lead to anaemia in the case of those that feed primarily on host blood (Erasmus and Chapman, 1972).

Several studies on parasites of fish have been undertaken in Lake Naivasha in the past (Malvestuto and Ogambo-Ongoma, 1978; Aloo, 1999, 2002; Aloo and Dezfuli, 1997; Amin and Dezfuli, 1995; Otachi *et al*., 2014) with the presence of parasites such as *Contracaecum* sp., *Amirthalingamia* sp., *Polyacanthorhynchus kenyensis*, *Clinostomum complanatum*, *Cyclustera* sp. being reported.

However, there is a conspicuous absence of reports of ectoparasites from fish of this lake, including monogeneans from these earlier studies, except in the study by Otachi *et al*., (2014). In the study by Aloo (2002) the absence of ectoparasites of fish in which 1100 fish were examined (652 *Oreochromis leucostictus* Trewavas, 1933 and 448 *Tilapia zillii* Gervais, 1848) was unique and was attributed to a probable water quality problem in the lake. Contrastingly, Otachi *et al*., (2014) has shown that monogenean trematodes form the bulk of fish parasites in this lake with a prevalence of between 25.5% and 99.3% in common carp *Cyprinus carpio* (Linnaeus, 1758), 64.5% in Redbelly tilapia *T. zillii* (Gervais, 1848), 91.1% in Blue spotted tilapia *O. leucostictus* (Trewavas, 1933) and 83.6% in Straightfin barb *Barbus paludinosus* (Peters, 1852). However, the putative identity of the monogeneans observed thus far remains a challenge. A morphometric analysis is the best approach in their identification. This is because, in the absence of records and studies on monogeneans of fishes in Kenya, the available identification keys are not adequate. Therefore, the aim of this study was to determine the morphometric analysis of monogenean parasites of cichlid and cyprinid fish in Lake Naivasha.

1.2 Statement of the problem

Monogeneans are serious ectoparasites of fish which can induce morbidities and mortalities in fish. This is because they attack the gills which are the chief organs for respiration. There is lack of knowledge and prior records of the species of monogenean parasites of cichlid and cyprinid fish in Lake Naivasha which makes their putative identification difficult. In this, study morphometric description and identification is envisioned to overcome the problem of identification.

1.3 Objectives

1.3.1 General objective

To carry out a morphometric analysis of monogenean parasites of cichlid and cyprinid fish in Lake Naivasha, Kenya

1.3.2 Specific objectives

1. To identify the monogeneans parasitizing the cichlid and cyprinid fish from Lake Naivasha using morphometric analysis

2. To determine the infection parameters: prevalence and mean intensities of the monogeneans parasitizing the cichlid and cyprinid fish from Lake Naivasha

1.4 Research questions

1. Which monogeneans parasitizize the cichlid and cyprinid fish in L. Naivasha Kenya?
2. What are the prevalences' and mean intensities of monogeneans parasitizing cichlid and cyprinid fish in L. Naivasha Kenya?

1.5 Justification

The Government of Kenya has in the recent past put emphasis on fisheries and aquaculture as an important sub-sector with potential to contribute to food security, employment creation and poverty reduction. For example, aquaculture was identified as one of the key sectors for funding during the midterm expenditure budget of 2009/10 with an estimated Kshs 1.12 Billion put into establishing more than 200 fishponds in more than 140 high potential constituencies. With this expected growth in fish production, it wass important that threats are identified for prevention and control. Monogeneans are particularly easily transmitted from the wild stocks to cultured stocks of fish due to their direct nature of transmission and life cycle. They are known pests in aquaculture and are monotonous killers of their hosts (fish). In studying their morphometry, their identification will be possible and will lead to devising prevention and control mechanisms.

1.6 Scope of thesis and limitations

1.6.1 Scope

The main research themes investigated are presented as objectives in separate chapters, however cross-referencing of different chapters will facilitate understanding of the overall objective.

Chapter One: Introduction

This chapter provides the background information of the monogenean parasites and their study in Lake Naivasha, Kenya. The statement of the problem, objectives, hypotheses and justification of the study are stated therein.

Chapter Two: Literature review

This chapter provides a literature review on monogenean parasites and their taxonomy (classification) is discussed. The major sites of attachment of monogeneans in/on fish, their feeding habits and what they feed are elaborated on. The monogenean mating process is discussed

with mechanisms on host (fish) location. The clinical signs of fish infected by the monogeneans and their effects on fish are explained. The current research on monogeneans in the world, Africa, Kenya and specifically in Lake Naivasha is discussed.

Chapter Three: Gill monogeneans of *Oreochromis niloticus* (Linnaeus, 1758) and *Oreochromis leucostictus* (Trewavas, 1933) in Lake Naivasha, Kenya

In this chapter, the identification of the gill monogeneans of two cichlid fish: *O. niloticus* and *O. leucostictus* using the morphometric analysis is discussed.

Chapter Four: A morphometric analysis of gill monogeneans infecting the Redbelly tilapia, *Tilapia zillii* (Gervais, 1848) from Lake Naivasha, Kenya: New biogeographical records

In this chapter, morphometric analysis is used to identify gill monogeneans from the cichlid *T. zillii*. The methods used are also discussed.

Chapter Five: Description of *Dactylogyrus hansi* sp. nov. from straightfin barb *Barbus paludinosus* (Peters, 1852) (Pisces: Cyprinidae) in Lake Naivasha, Kenya

This chapter attempts a detailed description of the monogenean gill parasite from the cyprinid fish *B. paludinosus*. The description provides the identity of the parasite through morphometric analysis and details the methods used.

Chapter Six: Conclusions and recommendations

This chapter gives chapter by chapter conclusions and discusses recommendations for the monogeneans of three cichlid and one cyprinid fish in Lake Naivasha, Kenya.

1.6.2 Limitations

The lack of proper equipment and materials (primers) for molecular analysis in our laboratory posed a big challenge. This took a lot of time to get a collaborator in South Africa and Belgium to assist in the molecular analysis from which we expect the data soon.

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CHAPTER TWO
LITERATURE REVIEW

2.1 Taxonomy of monogenean parasites

Monogeneans belong to the Kingdom Animalia, phylum Platyhelminthes, class Trematoda and sub-class Monogenea (Boeger and Kristky, 1997; Mollaret *et al*., 2000). They can be divided into two major groups, the monopisthocotyleans (epithelium feeding), which have hook-like organs on their haptors and the polyopisthocotyleans (blood feeding), which use clamp-like structures for attachment (Whittington, 2004; Perkins *et al*., 2010). There are more than 13 families of monogeneans, of which, four are frequently diagnosed on cultured fishes. The four families are Gyrodactylidae, Dactylogyridae, Ancyrocephalidae, and Capsalidae, whose members are commonly called gyrodactylids, dactylogyrids, ancyrocephalids, and capsalids, respectively (Reed *et al*., 2012). The distinguishing characteristics include the presence or absence of eye spots, and the number of pairs of anchors, transverse bars, and marginal hooks on their haptors (Cribb *et al*., 2002). These features can only be seen with a compound microscope. The most common groups of monogeneans on freshwater fishes are the gyrodactylids and the ancyrocephalids, which differ markedly in their reproductive strategies as well as their preferred attachment sites on host fish. The distinguishing features of the four monogenean families are summarized in Table 1.

2.2 Mode of attachment of gill monogeneans

Gill parasites are continually exposed to strong gill ventilating currents generated by their fish hosts (Bakke *et al*., 2007). On occasions when particulate materials or pollutants enter the gill chamber, parasites may also be subjected by their hosts to 'coughing' pulses, in which the strength of the water flow may be suddenly and briefly increased and its direction temporarily changed. Thus, a parasite must at all times attach itself securely to the gills and be capable of resisting the extra stresses generated during 'coughing' episode. Hooks are the 'hallmark' of monogeneans. These elegantly shaped structures occur at the posterior end of the body on a muscular, often disc-shaped attachment organ called the haptor (Arafa *et al*., 2009). Hooks are of two sizes: up to 16 tiny hooklets, ranging in length from about 7 to 40 µm, providing anchorage for the much larger adult parasites (Bush *et al*., 2001)

Table 1: The distinguishing characteristics of the different families of monogeneans parasitizing fish (Source: Reed *et al*., 2012)

	Features				
Family	Anchors	Number of marginal hooks	Eye spots	Oviparous/ Viviparous	Environment of fish
Ancyrocephalidae (ancyrocephalids)	two pairs of anchors one pair connected to dorsal and transverse bar, and one pair connected to ventral bar	12-14; sometimes absent	present	oviparous	fresh water and marine
Capsalidae (capsalids)	two pairs of anchors, a pair of sensory sclerites	14 small hooklets along periphery	present but less obvious in adults	oviparous	mainly marine and some brackish
Dactylogyridae (dactylogyrids)	one pair of anchors and dorsal transverse bar, may have vestigial and ventral transverse bar	12-14 sometimes absent	present	oviparous	most found on freshwater cyprinids
Gyrodactylidae (gyrodactylids)	one pair of anchors with dorsal & ventral transverse bars ventral bar has shield	16	absent	viviparous	freshwater and marine

2.3 Feeding in monogeneans

Skin parasitic monogeneans feed on host epidermal cells. A large protrusible glandular pharynx is used to erode and ingest host epidermis. Monogeneans have no blood system and intestinal branches transport nutrients to remote parts of the body (Reed *et al*., 2012). Gill parasites also feed on epidermis, but the larger clamp-bearing gill parasites ingest blood, as indicated by intestinal deposits of the indigestible brown or black pigment haematin derived from the host's haemoglobin (Kearn, 1998).

2.4 Mating in monogeneans

They employ mechanisms of impregnation which ranges from insertion of the penis into a vagina to hypodermic impregnation, where the penis injects sperm into the body tissues and sperm migrates to the female reproductive tract (Gussev *et al*., 1993). Adult clamp-bearing monogeneans have become sedentary, posing a challenge in sperm exchange (Kearn, 1998). Monogeneans are monoecious but cross fertilization appear to dominate in most cases as the reproductive systems are not connected, necessitating cross- fertilization in majority of cases (Bush *et al*., 2001)

2.5 Location of suitable hosts

With the exception of gyrodactylids, which give birth to live young ones, monogeneans lay eggs. These eggs have shells made of a tough protein called sclerotin and are released into the waters. The larva, which is usually ciliated, embryonates inside the egg. A detachable lid or operculum permits the larva to escape into the water where it is propelled by beating cilia. This tiny larva is only 0.25 mm long and is just visible to the naked eye. It has the task of locating and attaching itself to the host (Buchmann and Bresciani, 2006). Since most monogeneans are specific to only one or two closely related hosts, this is a very demanding task (Harris, 1993). The larvae of *Acanthocotyle* sp. are unable to swim (they have no cilia) and fail to hatch spontaneously instead, a host ray settles on top of the eggs on the sea bottom, ray mucus induces hatching in seconds. The larva does not need to swim since by greatly elongating its body the lower skin of the resting host can usually be reached by its anterior adhesive pads. The viviparous gyrodactylids have no cilia and parasites transfer either directly to new hosts when fish make contact (Reed *et al*., 2012).

2.6 Clinical signs and effects of monogeneans

Fish infested with monogeneans may become lethargic, swim near the surface, may have clamped fins, seek the corners of aquaria or the sides of the pond, and have a diminished appetite. They

may be seen flashing. Scale loss may occur at the point where the monogeneans are attached, and the skin may vary in colour where the parasites have fed. Heavy gill infestations result in respiratory disease. Gills may be swollen and pale, respiration rate may be increased, and fish will be less tolerant of low-oxygen conditions. Piping (gulping air at the water surface) may be observed in fish in severe respiratory distress. Large numbers of monogeneans on either the skin or gills may result in significant damage and mortality (Paperna, 1996). Secondary infections with bacteria and water moulds are common on tissue that has been damaged by monogeneans. Gray patches and open wounds may appear on the skin and, the eyes may be swollen and appear cloudy (Hoffman, 1999).

2.7 Current research on monogeneans

Monogeneans have long been recognized as serious parasites of fish both in aquaculture and fisheries (Paperna and Thurston, 1969a, b). Because of this recognition, there has been an increasing interest and explosion of knowledge, reports and description of new species of monogeneans from the African continent (e.g Guegan and Lambert, 1990; 1991; Pariselle and Euzet, 1995; 1998; 2003; N'Douba *et al*., 1999; Pouyaud *et al*., 2006; Přikrylová *et al*., 2009a, b; 2012a, b; Musilová *et al*., 2009; Mendlová *et al*., 2010; Vanhove *et al*., 2011a, b; García-Vásquez *et al*., 2011; Gillardin *et al*., 2012; Bukinga *et al*., 2012; Crafford *et al*., 2012; and Řehulková *et al*., 2013). However, much of the research has mainly been concentrated in Western and Southern African countries with very little coming from East Africa (for example, Barson *et al*., 2010; Vanhove *et al*., 2011a; Akoll *et al*., 2012; Bukinga *et al*., 2012; Gillardin *et al*., 2012, Přikrylová *et al*., 2012a, b). The earliest studies on monogeneans from East African water bodies which included Lakes Victoria, Edward and Albert were in the years 1965-1969 (Paperna and Thurston 1968; 1969a, b; Thurston 1970). In a book by Paperna (1979), only data from River Nzoia in Kenya are available and from Lake Victoria which is a shared lake between three East African countries. Therefore, it seems that these sampling efforts were not replicated and momenta sustained in other water bodies in Kenya. Indeed the lack of records of monogeneans from fish in Kenya has been recognized (Florio *et al*., 2009; Otachi *et al*., 2014). For example, Pariselle and Euzet (2009) have provided a systematic revision of dactylogyridean parasites from cichlid fishes in Africa, Levant and Madagascar consisting of ninety-seven (97) species belonging to six (6) dactylogyridean genera and interestingly, in this detailed revision there is not a single mention of records and/or locality of any species from Kenya. Furthermore, Le Roux and Avenant-Oldewage

(2010) have also provided a checklist of the genus *Cichlidogyrus* and its hosts including geographical records, and no such host-parasite records have been mentioned for Kenya in this checklist. Due to monogeneans strict host specificity, it is expected that examination of new hosts is likely to lead to discovery of new species (Gussev *et al*., 1993; Crafford *et al*., 2012; Rindoria *et al*., 2015).

2.8 Summary of literature review

Monogeneans are largely ectoparasitic, thin, flattened, host- and site specific parasites with a simple life cycle involving a single host (often fish). They range in size between 0.3 mm and 20 mm and are mostly bilaterally symmetrical with the body sub-divided into a number of organs. They possess attachment organs both anteriorly and posteriorly (opisthaptor) which is highly variable among genera. It may contain suckers, clamps or large hooks (anchors) as well as marginal hooks. In some genera anchors (one or two pairs) are associated with spikes or accessory sclerites and are supported by a connecting bar. Monogeneans feed on the epidermis (epithelium feeding) but the larger clamp-bearing gill parasites ingest blood (blood feeding). Most monogeneans lay eggs with the exception of gyrodactylids, which give birth to live young ones. During mating they employ mechanisms of impregnation. Heavy gill infestations result in respiratory disease and even lead to mortality.

Despite the large number of internationally published papers on the monogeneans, little is known about monogenean fauna of Kenya's cichlid and cyprinid fishes.

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CHAPTER THREE

3.0 GILL MONOGENEANS OF *Oreochromis niloticus* (Linnaeus, 1758) AND *Oreochromis leucostictus* (Trewavas, 1933) IN LAKE NAIVASHA, KENYA

3.1 Abstract

An investigation of gill monogeneans from the Nile tilapia *Oreochromis niloticus* and the blue spotted tilapia *Oreochromis leucostictus* (50 individuals per species) was done between the months of November 2014 to February 2015 in Lake Naivasha, Kenya. Standard parasitological procedures were used to examine fish gills for the presence of monogeneans. The observed monogeneans were collected, preliminarily identified using identification keys, quantified and fixed in 4 % formalin for morphological studies and in absolute ethanol for molecular studies. Four parasite species comprising of three species of the genus *Cichlidogyrus* and one species of the genus *Scutogyrus* were recovered. *Cichlidogyrus sclerosus* and *Cichlidogyrus tilapiae* infested both fish species but the *C. sclerosus* was most prevalent in *O. leucostictus* (Prevalence (P) = 100 %, Mean intensity (MI) = 3.4) and *C. tilapiae* in *O. niloticus* (P = 8 %, MI = 4). *Cichlidogyrus tilapiae* had a P = 12 % and MI = 5.0 and a P = 6 % and MI = 3.0 in *O. niloticus* and *O. leucostictus*, respectively. *Cichlidogyrus halli* (P = 4 %, MI = 15.5) and *Scutogyrus gravivaginus* (P = 2 %, MI = 1.0) were only found in *O. leucostictus*. This is the first time that these monogeneans have been identified from Lake Naivasha, Kenya, presenting new geographical records. It was concluded that ancyrocephalids (*Cichlidogyrus* spp.) dominated the two cichlid fish species in Lake Naivasha, Kenya.

3.2 Introduction

Fisheries contribute 0.5 % to the GDP of Kenya (KNBS 2012) and thus play an important role in the national economy. The fisheries of Lake Naivasha depend on several introduced fish species (Gherardi *et al*., 2011). Fish parasites have been recognized as one of the detrimental and limiting factors in the development of capture fisheries and aquaculture. Several studies on parasites of fish have been undertaken in Lake Naivasha (Malvestuto and Ogambo-Ongoma 1978; Aloo 1999, 2002; Aloo and Dezfuli 1997; Amin and Dezfuli 1995; Otachi *et al*., 2014). However, in all the earlier studies, no ectoparasites were reported with the exception of the study by Otachi *et al*., (2014). Therefore, research on monogeneans parasitizing fish from Lake Naivasha, Kenya is scanty. Otachi *et al*., (2014) showed that monogenean trematodes form the bulk of fish parasites

in this lake with prevalences of between 25.5 and 99.3 % in common carp *Cyprinus carpio* (Linnaeus, 1758), 64.5 % in Red belly tilapia *Tilapia zillii* (Gervais, 1848), 91.1 % in Blue spotted tilapia *Oreochromis leucostictus* (Trewavas, 1933), and 83.6 % in straightfin barb *Barbus paludinosus* (Peters, 1852). However, as noted by Otachi *et al*., (2014), the putative identity of the monogeneans observed thus far remains a challenge. The aim of this study was to identify the monogeneans infecting cichlid hosts in Lake Naivasha, Kenya.

3.3 Materials and methods

3.3.1 Study site

Fish were caught from the main Lake Naivasha (Fig. 1). This is the second largest freshwater lake in Kenya after the Kenyan portion of Lake Victoria (Mavuti and Harper 2005). The lake lies at 00°45′ S and 36°20′ E in a closed basin at an altitude 1890 m above sea level, within the Eastern Rift Valley of Kenya. It is in the Rift Valley without a surface outlet but with a substantial exchange with groundwater (Gaudet and Melack 1981). It has an approximate surface area of 160 km^2, a volume of 4.6 km^3 (Campbell *et al*., 2003), and an average depth of 6 m with the deepest area being 7 m (Hickley *et al*., 2008). These values vary with extreme weather conditions.

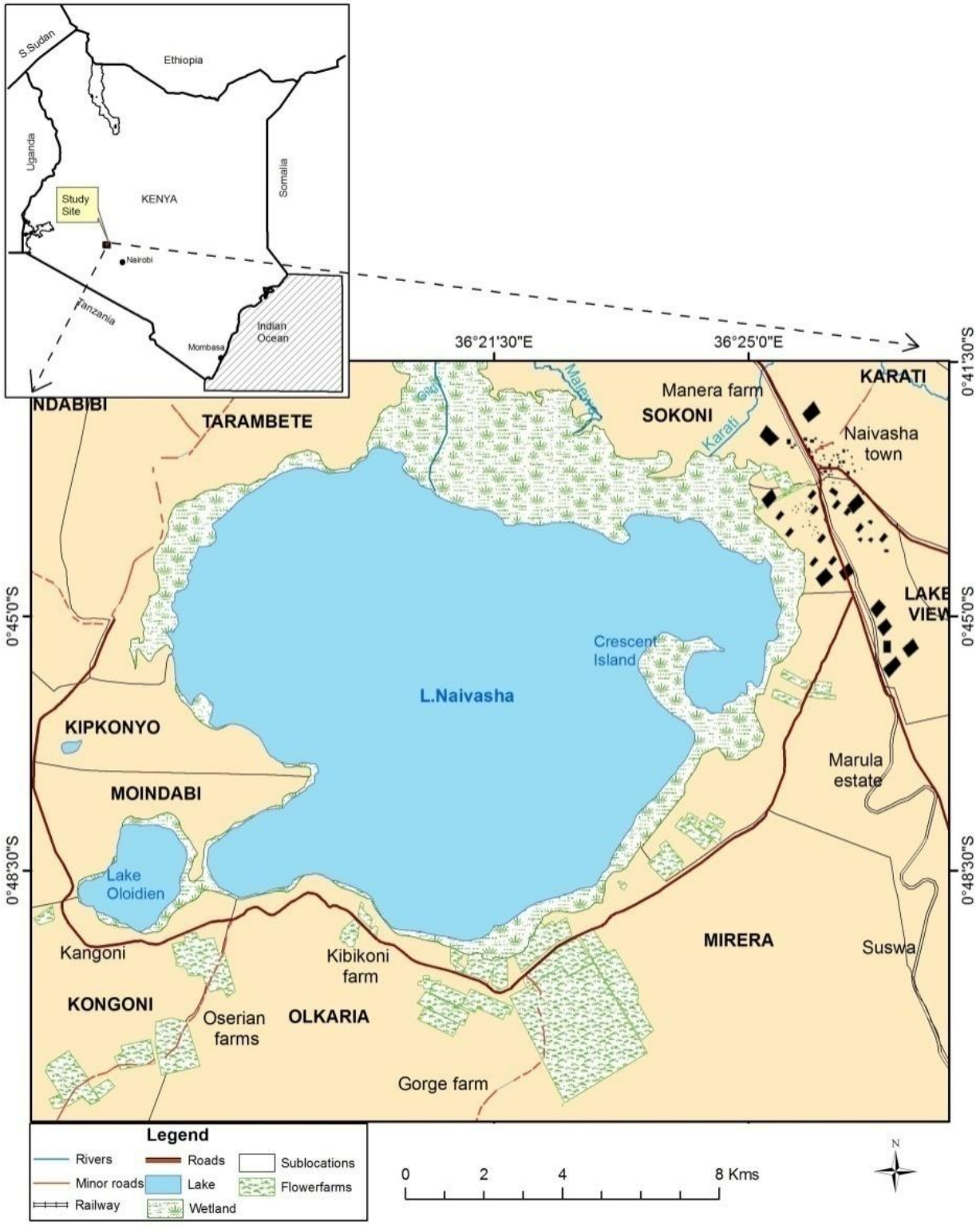

Figure 1: A map of Kenya showing the location of study site (source: Rindoria *et al*., 2015)

3.3.2 Fish collection, parasite recovery, and measurement of sclerotized parts

One hundred specimens of the two cichlids (50 each of, Nile tilapia *Oreochromis niloticus* and blue spotted tilapia *O. leucostictus*) were collected using a fleet of gill nets with mesh sizes 2, 2.5, 3, 3.5, 4, and 4.5 in. between November 2014 and February 2015. The fish were transported alive in a fish tank with lake water to the laboratory in the Department of Biological Sciences, Egerton University, Njoro, where they were killed by cervical dislocation (Schäperclaus 1990). In the laboratory the fish were separated according to species to avoid cross contamination, they were then dissected and the gills removed and examined with a dissecting microscope and a motic BA210 compound microscope. The monogeneans were detached from the gills using a pair of fine forceps. Some of the monogeneans were individually transferred to a drop of ammonium picrate-glycerine (Malmberg, 1957) in a glass slide for observations of the sclerotized structures (Ergens 1969). The preparation was covered with a cover slip and sealed with a transparent nail varnish for examination of their internal anatomy. Some monogeneans were flattened and fixed in 4% formalin prepared from folmaldehyde solution while others were preserved in absolute ethanol. The sclerotized structures such as the haptor and the copulatory complex were drawn using corelDRAW Graphics Suite 12 software (Corel Corporation, 2003). Measurements were made with Motic software in which a motic camera (moticam 2300, 3.0 pixel USB 2.0) was attached to a Motic BA210 compound microscope. All measurements are given in micrometers as the mean ± the standard deviation followed by the range in parentheses, as proposed by Gussev (1962). Monogenean identification was done using the identification keys by Pariselle and Euzet (1995, 2009). The method of numbering of the haptorial pieces followed that adopted at ICOPA IV (Euzet and Prost 1981). The terminology used was those proposed by Pariselle and Euzet (1995): uncinulus for the marginal hooklets; gripus for the large median hooks. The measurements made in this study were as follows: gripus (G): a = total length, b = blade length, c = root length, d = shaft length, and e = point length; male apparatus (MA): penis total length (Pe), heel (He), and accessory piece length (AP); auxiliary plate (Pl); dorsal transverse bar (DB): h = length of auricle, w = maximum width, x = total length and y = distance between auricles; uncinuli length (U); ventral transverse bar (VB): w = maximum width and x = length of one branch; vagina (Vg): L = total length and l = maximum width according to Pariselle and Euzet (1997). The prevalence (P) and mean intensities (MI) were determined following the definition by Bush *et al*., (1997). The measures of monogeneans community structure such as the Shannon–Wiener index, Margalef

richness index and Berger–Parker dominance index as proposed by Magurran (1988) were determined using the online Biodiversity calculator (Danoff-Burg and Xu, 2005).

3.4 Results

One species of the genus *Scutogyrus* (*Scutogyrus gravivaginus* Paperna & Thurston, 1969) and three species of *Cichlidogyrus* (*C. halli* Price & Kirk, 1967; *C. tilapiae* Paperna, 1960; *C. sclerosus* Paperna & Thurston, 1969) were found on the gills of *O. leucostictus* (Table 2). The gills of *O. niloticus* were infested with two species of the genus *Cichlidogyrus* (*C. tilapiae* and *C. sclerosus*). The most dominant taxa were *C. Sclerosus* and *C. tilapiae* in *O. leucostictus* and *O. niloticus* with Berger–Parker index of dominance of 0.7296 and 0.5714, respectively (Table 3).

Table 2: Prevalence (P) and Mean intensity (MI) of the three species of *Cichlidogyrus* and one species of *Scutogyrus* on the two Cichlids: *O. leucostictus* and *O. niloticus* from Lake Naivasha between November 2014 to February 2015.

Monogenean species	***O. leucostictus* (n=50)**		***O. niloticus* (n=50)**	
	P (%)	MI	P (%)	MI
C. sclerosus	100	3.4	8	4.0
C. tilapiae	6	3.0	12	5.0
C.halli	4	15.5	ND	ND
S. scutogyrus	2	1.0	ND	ND

ND Not detected

Table 3: Comparison of the diversity characteristics of the monogenean communities of the two cichlids: *O. leucostictus* and *O. niloticus* from Lake Naivasha between November 2014 to February 2015.

Total component communities	***O. leucostictus* (n = 50)**	***O. niloticus* (n = 50)**
Total number of species	4	2
Shannon–Wiener index	0.7901	0.2966
Berger–Parker index	0.7296	0.5714
Margalef richness	0.5504	0.5139

Total component communities	***O. leucostictus* (n = 50)**	***O. niloticus* (n = 50)**
Dominant species	*C. sclerosus*	*C. tilapiae*

3.4.1 *Scutogyrus gravivaginus* Paperna & Thurston, 1969

Description: (Fig. 2) Only 1 specimen was recovered and measured (µm): Adult: 576 long, 150 wide. Two pairs of eyes with lens on the first pair. Haptor with two pairs of hamuli and seven pairs of uncinuli. Gripus: a = 27, b = 19, c = 8, d = 9, e = 5. Dorsal transverse bar: x = 33, y = 12, h = 57, w = 7. Ventral transverse bar: x = 37, w = 4. Uncinuli: u = 32. Copulatory organ of this parasite is larger with a basal portion: AP = 56, Pe = 70, He = 27. Vagina is highly sclerotized L = 37, l = 9. The measurement in this parasite conforms to descriptions in Paperna and Thurston (1969), Douëllou (1993) and Matla (2012) which confirms its identification.

Type-host: *O. leucostictus* Trewavas, 1933, (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45′ S, 36°20′ E

Site of infection: Gills.

Material studied: 50 individuals.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro, Kenya.

Remarks

This is the first time *S. gravivaginus* is reported from Lake Naivasha, Kenya. The parasite was first described as *Cichlidogyrus longicornis gravivaginus* by Paperna and Thurston (1969) from the gills of *O. leucostictus* in Lake Albert, Uganda. It was described with other subspecies *Cichlidogyrus longicornis longicornis* from the gills of *O. niloticus*. The parasite was elevated to the species level as *C. gravivaginus* following Douëllou (1993) in her redescription of specimens from the gills of *Oreochromis mortimeri* in Lake Kariba, Zimbabwe. The copulatory organ of this parasite is larger with a basal portion of a heavily sclerotized vagina with a rounded part and an elongated part ending in three finger-like extensions. The measurements in this parasite conform to descriptions in Paperna and Thurston (1969); Douëllou (1993) and Matla (2012) which confirms its identification.

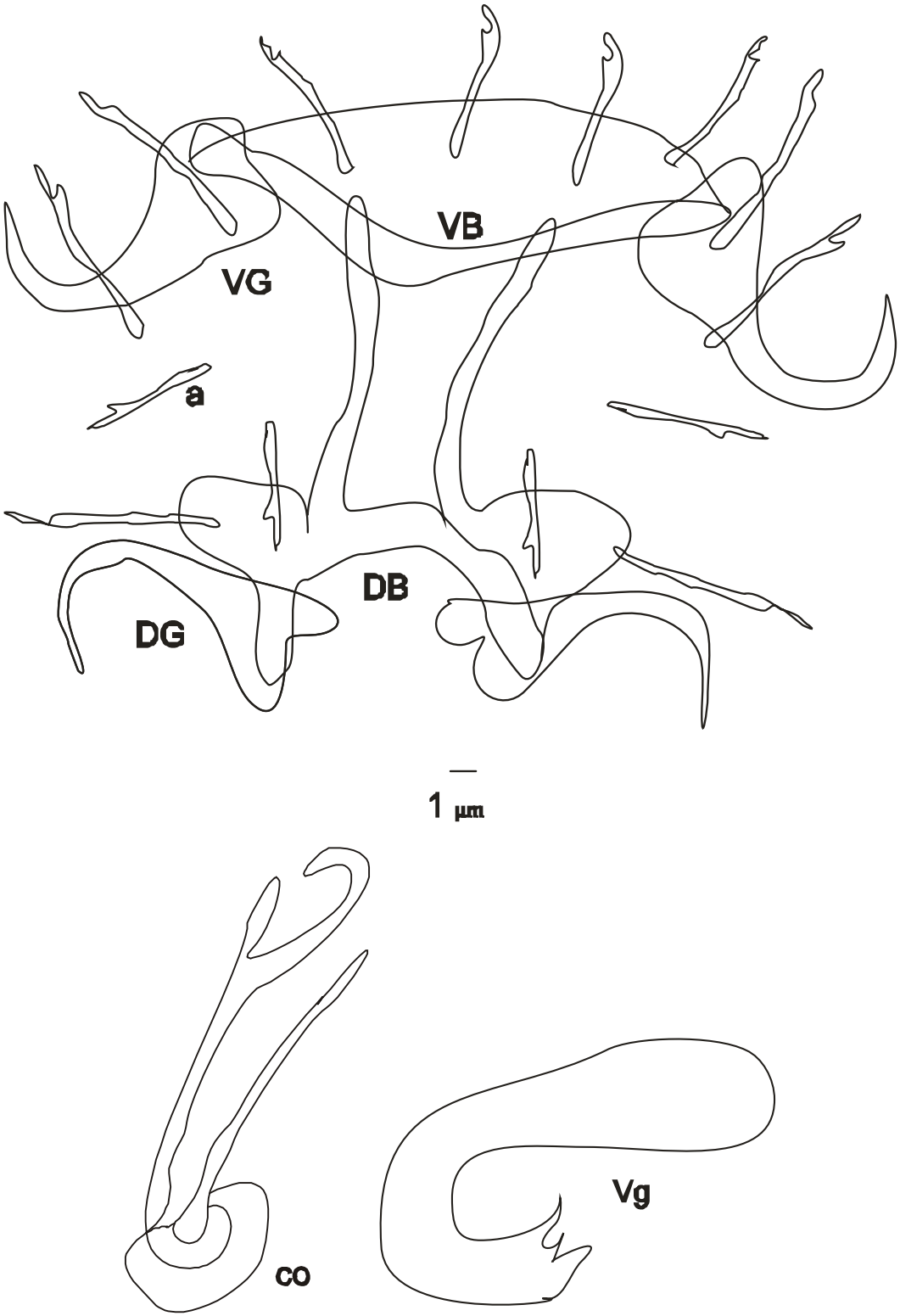

Figure 2: *Scutogyrus gravivaginus* Paperna and Thurston, 1969: Haptorial features and copulatory organ. a uncinuli (marginal hooks), CO copulatory organ, DB dorsal bar, DG dorsal gripus, Vg Vagina, VG ventral gripus.

3.4.2 *Cichlidogyrus sclerosus* Paperna & Thurston, 1969

Description (Fig. 3) (10 specimens measured (µm): The body is elongate, Adult: 445 ± 76.1 (368–546) long, 215 ± 43.5 (147–286) wide. Two pairs of eyes with lens on the first pair. Haptor is rounded with two pairs of hamuli and seven pairs of uncinuli. Gripus: a = 27 ± 4.1 (21–34), b = 22 ± 1.9 (19–25), c = 7 ± 2.2 (5–11), d = 12 ± 1.0 (10–13), e = 12 ± 1.6 (11–16). Dorsal transverse bar massive, X-shaped, branches wide, appendages pyriform with rounded ends: x = 36 ± 6.3 (24–43), y = 11 ± 1.0 (10–12), h = 17 ± 2.4 (13–22), w = 8 ± 1.2 (6–9). Ventral transverse bar V-shaped: x = 29 ± 3.6 (24–35), w = 6 ± 1.0 (4–7), with rounded extremities. The dorsal and ventral hamuli are of same shape and of similar size. Uncinuli: u = 16 ± 4.3 (11–27). Male copulatory complex is large, with serrated plate, thin copulatory tube arched, with tapered end: Pe = 61 ± 9.9 (43–71), He = 9 ± 1.5 (7–12).

Type-host: *O. leucostictus* Trewavas, 1933, and *O. niloticus* Linnaeus, 1758 (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45′ S, 36°20′ E

Site of infection: Gills.

Material studied: 50 individuals.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro Kenya.

Remarks

The finding of *C. sclerosus* in this study represents the first record from Lake Naivasha, Kenya. This parasite was originally described by Paperna and Thurston (1969) based on the specimens from the gills of *O. niloticus niloticus* (as *Tilapia nilotica*), *Oreochromis mossambicus* (as *Tilapia mossambica*), *O. leucostictus* (as *Tilapia leucosticta*), *Tilapia zillii*, and *Haplochromis* sp. in Uganda, Africa. The species has so far been reported from various cichlid fishes from Israel in the Middle East, from Uganda, Egypt, South Africa, Botswana, and Zimbabwe in Africa; from Thailand, Singapore, Hong Kong, Philippines, and Japan in Asia; and from the American countries of Mexico, Cuba, and Colombia (Douëllou 1993; Jiménez *et al*., 2001; Pouyaud *et al*., 2006; Kohn *et al*., 2006; Mendora-Franco *et al*., 2006; Lerssutthichawal 2008; Boungou *et al*., 2008; Pariselle and Euzet 2009; Le Roux and Avenant-Oldewage 2010; Madanire-Moyo *et al*., 2011; Akoll *et al*., 2012; Maneepitaksanti and Nagasawa 2012; Maneepitaksanti *et al*., 2014).

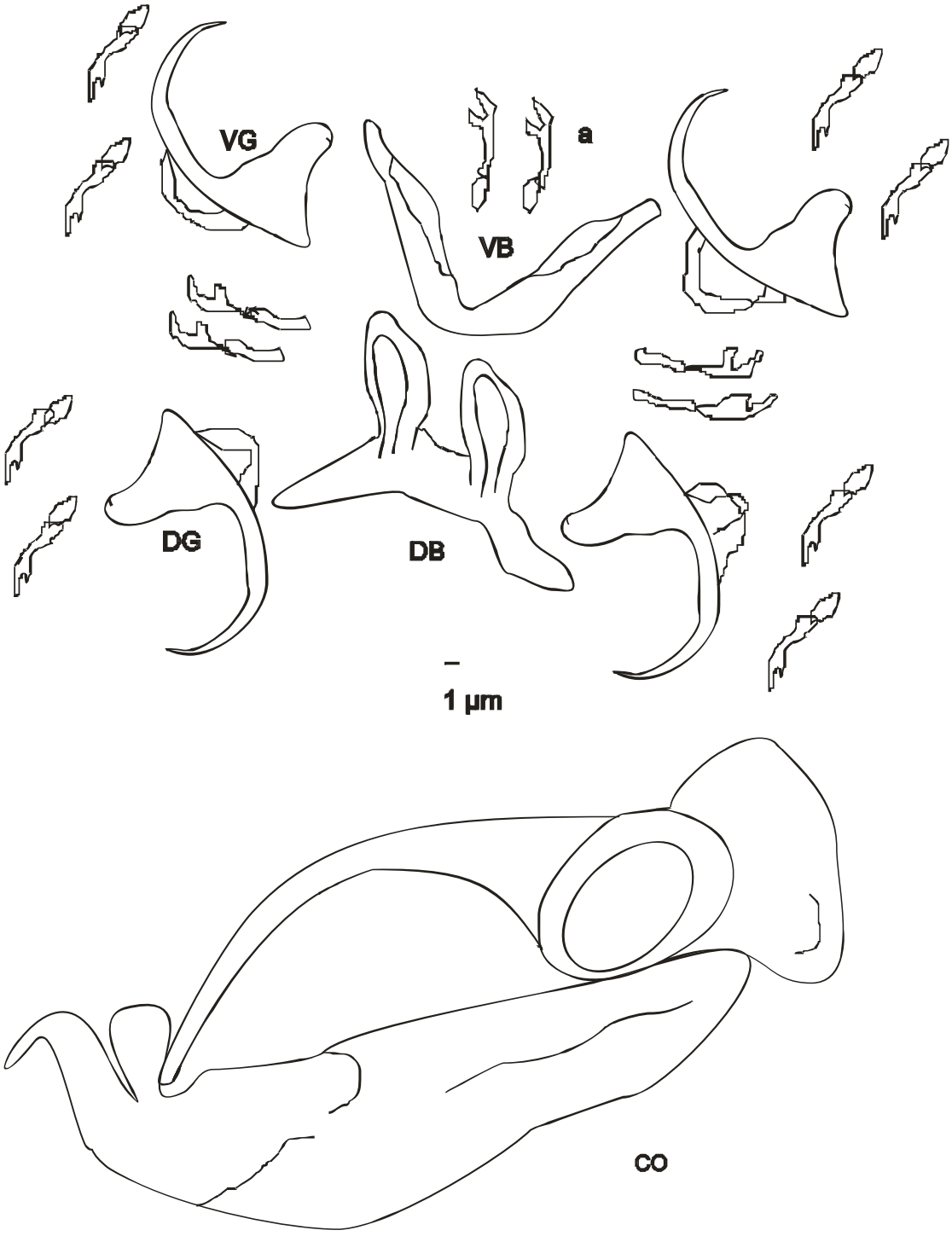

Figure 3: *Cichlidogyrus sclerosus* Paperna and Thurston, 1969: Haptoral structures and copulatory organ. a uncinuli (marginal hooks), CO copulatory organ, DB dorsal bar, DG dorsal gripus, VG ventral gripus.

3.4.3 *Cichlidogyrus halli* Price & Kirk, 1967

Description (Fig. 4) (Only two specimens were recovered). Elongated body, 405 ± 34.7 (405–454) long and 223 ± 14.1 (203–223) wide. One pair of eyes with lens, haptor ellipsoid, with two pair of hamuli, dorsal hamuli is smaller a = 23 ± 3.7 (23–29) b = 18 ± 5.7 (18–26), c = 3 ± 0.6 (3–4), d = 13 ± 2.6 (9–13), e = 16 ± 3 (12–16). Ventral transverse bar V-shaped, x=30 ± 1.8 (28–30), w = 9 ± 0.1 (8.3-8.5). Dorsal transverse bar large and massive, x = 19 ± 0.2 (18.6-18.9), y =11 ± 0.4 (10–11), w = 9 ± 1.4 (9–11), auricles wide apart, h = 22 ± 1.4 (20–22), seven pairs of uncinuli are long U = 12 ± 0.7 (12–13). Copulatory tube is large and S-shaped, Pe = 40 ± 2 (37–40) long with irregular shape, accessory piece is lancet-shaped and shorter, He = 12 ± 3.4 (12–17)

Type-host: *O. leucostictus* Trewavas, 1933, (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45'S, 36°20'E

Site of infection: Gills.

Material studied: 50 individuals.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro Kenya.

Remarks

Cichlidogyrus halli is reported in this study for the first time in Lake Naivasha, Kenya. This species was first described as *Cleidodiscus halli* Price and Kirk (1967) from the gills of *Oreochromis shiranus shiranus* (as *Tilapia s. shirana*) in Malawi, Africa. It has since been recorded from various cichlid fishes in African countries such as Ghana, Egypt Malawi, Guinea, Senegal, Ivory Coast, Burkina Faso, Uganda, South Africa, Sierra Leone, Benin, and Zimbabwe. It has been also recorded from cichlid fish from Japan in Asia (Douëllou 1993; Jiménez *et al*., 2001; Pouyaud *et al*., 2006; Kohn *et al*., 2006; Mendora-Franco *et al*., 2006; Lerssutthichawal 2008; Boungou *et al*., 2008; Pariselle and Euzet 2009; Le Roux and Avenant-Oldewage 2010; Madanire-Moyo *et al*., 2011; Akoll *et al*., 2012; Maneepitaksanti and Nagasawa 2012; Maneepitaksanti *et al*., 2014). The species is relatively large compared other *Cichlodogyrus* spp. found in the lake. It has two eyes. The copulatory organ is simple and long with S-shaped copulatory tube having an irregular basal portion. The accessory piece ends with a triangular extremity. It has seven pairs of uncinuli. The sclerotized parts and their measurements agree with that provided by Price and Kirk (1967); Douëllou (1993) and Matla (2012) which confirms the species' identification.

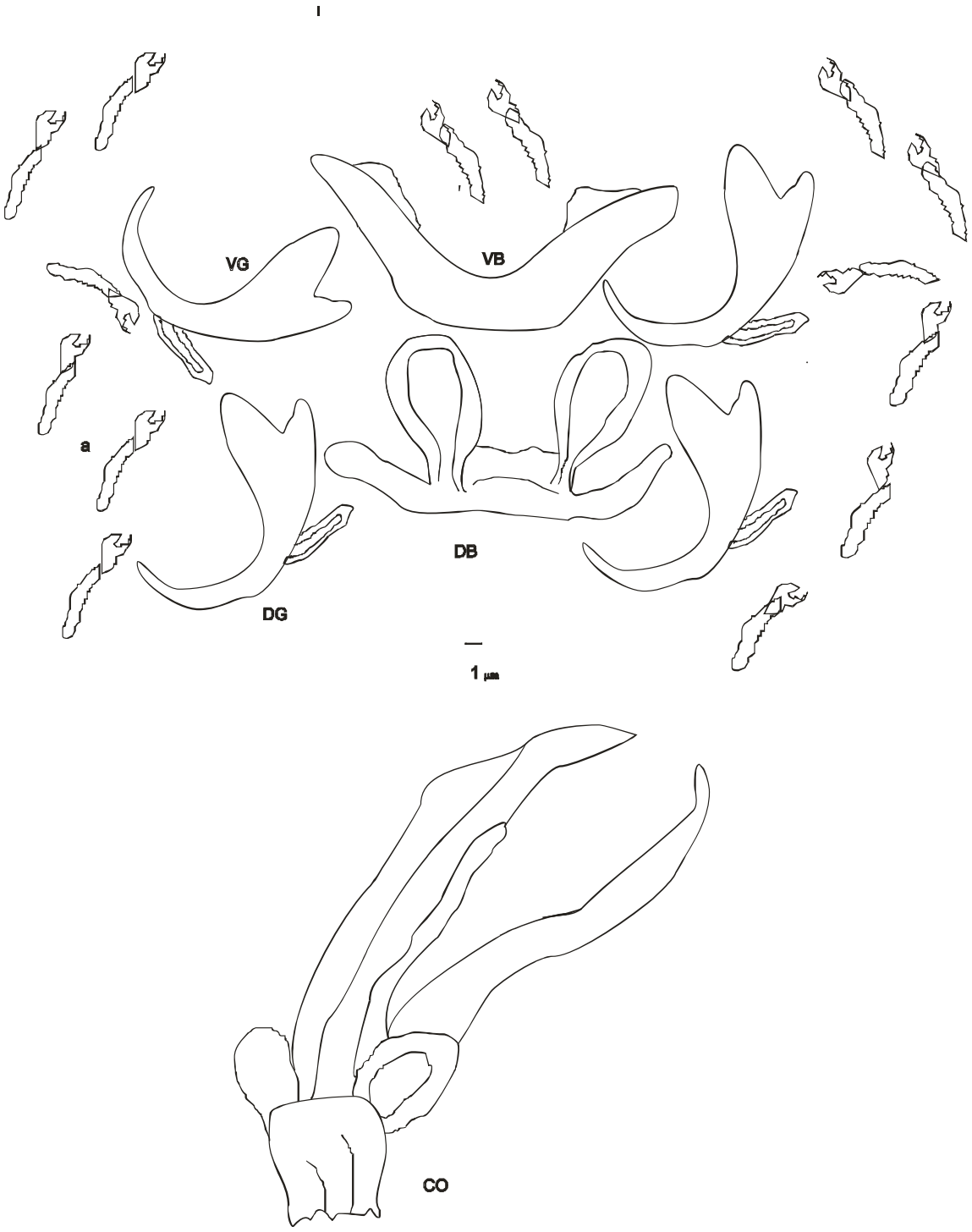

Figure 4: *Cichlidogyrus halli* Price and Kirk 1967: Haptoral structures and copulatory organ. a uncinuli (marginal hooks), CO copulatory organ, DB dorsal bar, DG dorsal gripus, VG ventral gripus

3.4.4 *Cichlidogyrus tilapiae* Paperna, 1960

Description (Fig. 5) (10 specimens measured (µm): The body is slender tapering at the posterior end. Adult: 392 ± 62.8 (353–538) long, 126 ± 28.6 (82–187) wide. Two pairs of eyes with lens on the first pair. Haptor ellipsoid with two pairs of hamuli and seven pairs of uncinuli. Gripus: a = 34 ± 5.9 (27–42), b = 25 ± 2.7 (22–30), c = 5 ± 1.5 (3–8), d = 14 ± 5.5 (7–23), e = 8 ± 1.5 (5–9). Dorsal transverse bar: x = 22 ± 5.1 (13–27), y = 9 ± 1.3 (7–12), h = 28 ± 3.6 (23–34), w = 7 ± 1.0 (6–9). Ventral transverse bar: x = 28 ± 3.6 (23–34), w = 5 ± 0.9 (4–6). Uncinuli: u = 15 ± 1.8 (14–18). Male copulatory complex with a short, simple, straight copulatory tube that is wider at the base: Pe = 29 ± 3.0 (25–34), He = 6 ± 1.2 (4–7). Accessory piece is straight with a sharp hook at the terminal end: AP = 29 ± 3.5 (25–35). Vagina was not observed.

Type-host: *O. leucostictus* Trewavas, 1933, and *O. niloticus* Linnaeus, 1758 (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45′ S, 36°20′ E

Site of infection: Gills.

Material studied: 50 individuals.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro Kenya.

Remarks

The collection of *C. tilapiae* in this study constitutes the first record from Lake Naivasha, Kenya. This parasite was first described by Paperna, (1960) using specimens from the gills of *O. niloticusniloticus* (as *T. nilotica*), *Sarotheroderon galilaeus galilaeus* (as *Tilapia galilaea*), *Tristramella sacra*, and *Trastramella simonis simonis* (as *Tilapia simonis*) in Israel. The parasite has been reported from various cichlid fishes from Israel in Middle East; from Uganda, Tanzania, Egypt, Ghana, South Africa, Burkina Faso, Ivory Coast, and Zimbabwe in Africa; from Bangladesh, Thailand, Philippines, and Japan in Asia; and from American coutries of Mexico, Cuba, and Colombia. (Douëllou, 1993; Jiménez *et al*., 2001; Pouyaud *et al*., 2006; Kohn *et al*., 2006; Mendora-Franco *et al*., 2006; Lerssutthichawal, 2008; Boungou *et al*., 2008; Pariselle and Euzet, 2009; Le Roux and Avenant-Oldewage, 2010; Madanire-Moyo *et al*., 2011; Akoll *et al*., 2012; Maneepitaksanti and Nagasawa 2012; Maneepitaksanti *et al*., 2014).

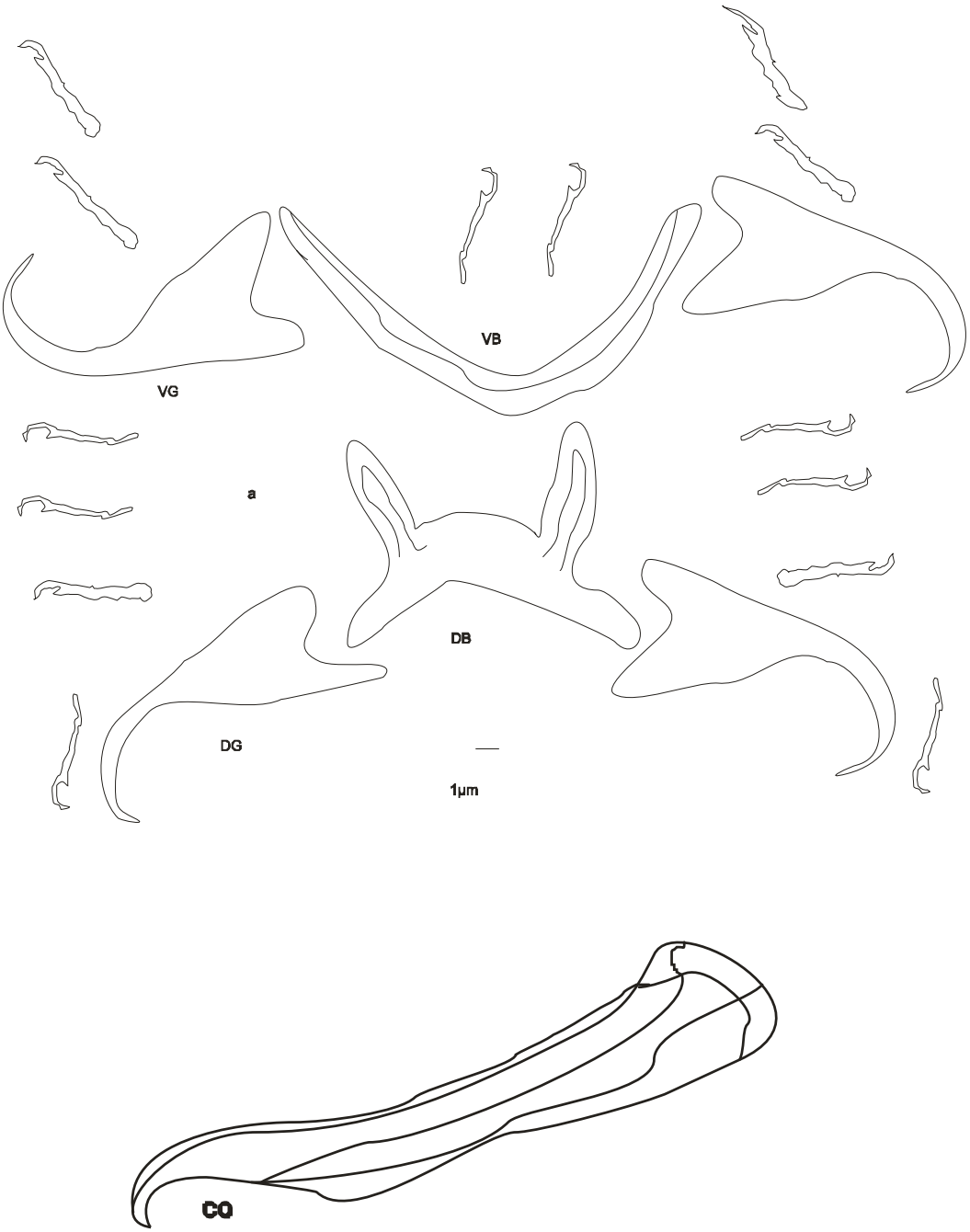

Figure 5: *Cichlidogyrus tilapiae* Paperna, 1960. a uncinuli (marginal hooks), CO copulatory organ, DB dorsal bar, DG dorsal gripus, VG ventral gripus

3.5 Discussion

The morphology and measurements of the four monogeneans from the gills of the two cichlid fishes from L. Naivasha, Kenya, corresponded to those of *C. gravivaginus* Paperna and Thurston (1969), *C. halli* Price and Kirk, (1967), *C. tilapiae* Paperna, (1960) and *C. sclerosus* Paperna and Thurston, (1969). The findings of this study of *C. tilapiae* (P = 12 %, MI = 5.0) and *C. sclerosus* in *O. niloticus* (P = 8 %, MI = 4.0) are comparable to other studies on *O. niloticus* in other parts of the world (Boungou *et al*., 2008; Akoll *et al*., 2012; Maneepitaksanti and Nagasawa, 2012 Maneepitaksanti *et al*., 2014). For example, Akoll *et al*., (2012) study in Uganda, found the same *Cichlidogyrus* species as in this study, with an almost equal mean intensities but not the prevalence (P = 50 %, MI = 6.6). However, this study found lower prevalence than in the study by Akoll *et al*., (2012). This could be due to the fact that in this study we separated the data for the two species, while it was presented as combined for the two species in the Akoll *et al*., (2012) study. Differences could also have resulted from the different sample sizes studied: (n = 140) as compared to this study (n = 50), and the sampling environments. For example, in this study we obtained fish from the wild, while in the study by Akoll *et al*., (2012) several water bodies were sampled such as a stream, reservoir, dam, and including aqua cultured-caged fish and all data were pooled together. The low mean intensity (4.0 and 5.0) recorded in this study can also be explained by the fact that *O. niloticus* has been found to have a strong immune resistance against further invasion of ectoparasites (Sandoval-Gio *et al*., 2008).

This study recorded higher mean intensities of *C. tilapiae* compared to the study of Tombi *et al*., (2014) from Melen fish station in Yaounde, Cameroon who found it on the gills of *O. niloticus* (MI = 1.38 left side, 1.35 right side). Contrastingly, other studies have found *C. halli* and *Scutogyrus* spp. in *O. niloticus* but this study only found them in *O. leucostictus* (Boungou *et al*., 2008; Maneepitaksanti and Nagasawa, 2012; Tombi *et al*., 2014). Lambert (1997) hypothesized that the introduction of an animal species in a new environment means the introduction of a host-parasite system, while the invasion theory explains the absence of certain parasites upon the introduction of a host to a new environment (temporal release) (Keane and Crawley 2002; Torchin *et al*., 2003). Therefore, the absence of *C. halli* and *S. gravivaginus* in *O. niloticus* in Lake Naivasha could possibly indicate that the *O. niloticus* reintroduced into the lake were not infected by the two parasites species and that the number of lateral transfers of the parasites which are usually observed after the introduction of new hosts in the new environment, are minimal. The *O.*

leucostictus was introduced in 1956 into Lake Naivasha (Gherardi *et al*., 2011) and has had enough time to acquire diverse parasite taxa unlike the *O. niloticus* which was recently reintroduced (2011) into the lake. On the other hand this result could also suggest the difficulty for these parasites species to survive in Lake Naivasha.

This study's findings that *C. sclerosus* is most dominant in *O. leucostictus* while *C. tilapiae* is the most dominant in *O. niloticus* (Berger–Parker index of dominance 0.7296 and 0.5714, respectively) differs from those of Maneepitaksanti and Nagasawa (2012) who found that *C. sclerosus* was the most dominant species on *O. niloticus* and *C. tilapiae* the least dominant in Okinawa Prefecture in Southern Japan. The high prevalence (100 %) of *C. sclerosus* in *O. leucostictus* signals that Lake Naivasha provides good conditions for the spreading/infection by the parasite. Physical factors such as high water temperatures (23.4 °C) and the biomass of the *O. leucostictus* in Lake Naivasha may induce increased fecundity of this parasite (Woo, 1995). The variability of parasite richness in *O. leucostictus* and *O niloticus* (Margalef richness 0.5504 and 0.5139 and diversity: Shannon–Weiner index 0.7901 and 0.2966 respectively) can be associated to factors related to: water quality–eutrophication (Galli *et al*., 2001), host (Morand *et al*., 1999), ecology (Zharikova, 2000), and the phylogeny of the host and parasites (Bush *et al*., 1997; Sasal *et al*., 1997). This study is a continuation of the discovery of ectoparasites from Lake Naivasha (Otachi *et al*., 2014), which have not previously been recorded in the tropical lake (Aloo, 2002) and which they attributed to sensitivity of ectoparasites to poor water quality (Dubinin, 1958; Aloo, 2002).

3.6 Conclusion

This is the first time that these monogeneans have been identified from Lake Naivasha, Kenya, presenting new geographical records. It was concluded that Ancyrocephalids (*Cichilidogyrus* spp.) dominated the two cichlid fish species in Lake Naivasha, Kenya.

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CHAPTER FOUR

4.0 A MORPHOMETRIC ANALYSIS OF GILL MONOGENEANS INFECTING THE REDBELLY TILAPIA, *Tilapia zillii* (Gervais, 1848) FROM LAKE NAIVASHA, KENYA: NEW BIOGEOGRAPHICAL RECORDS

4.1 Abstract

A total of fifty specimens of the introduced Redbelly tilapia, *Tilapia zillii* (Gervais, 1848) were collected from Lake Naivasha, Kenya from January to May 2015, and studied with the aim of collecting and identifying the first data on the monogenean parasites. Standard methods for parasitological examination were used in the identification of gill monogenean species. Some of the collected monogeneans were preserved in absolute ethanol (96%) for later molecular analysis and others in 4% formalin prepared from folmaldehyde solution for morphometric analysis. Seven *Cichlidogyrus* species were identified from the gills based on morphometric features of the opisthaptor and copulatory organs using identification keys. These include: *C. sclerosus* Paperna & Thurston, 1969; *C. tilapiae* Paperna, 1960; *C. digitatus* Dossou, 1982; *C. aegypticus* Ergens, 1981; *C. vexus* Pariselle & Euzet, 1995, *C. arthracanthus* Paperna, 1960 and *C. yanni* Pariselle & Euzet, 1996. These monogeneans form the first biogeographical record on the host (*T. zillii*) in Lake Naivasha, Kenya. *Cichlidogyrus digitatus* was the most dominant *Cichlidogyrus* species in *T. zillii* in Lake Naivasha.

4.2 Introduction

The Redbelly Tilapia, *Tilapia zillii* (Gervais, 1848) was introduced into Lake Naivasha in 1956 to establish a population for commercial use (Hickley *et al*., 2015). Since then, it has established itself and is among the common fishes that are frequently collected in the lake. The fish is economically and ecologically important as food fish in aquaculture, commercial aquarium trade, weed control and recreational fishery (Elias *et al*., 2014). *Tilapia zillii* is not immune to monogenean trematodes belonging to the genus *Cichlidogyrus* Paperna, 1960 which usually infect cichlids and other freshwater fishes (Pariselle and Euzet, 2009). Monogeneans are ectoparasites which are commonly found on host skin and gills (Blahoua *et al*., 2016), but they can also invade the rectal cavity, ureter, body cavity, nostrils, intestine, stomach and even the vascular system (Rohde *et al*., 1992; Pariselle and Euzet, 1998; Whittington *et al*., 2000). They have a direct life cycle and they are mostly spread by way of releasing eggs which hatch into free-swimming

infective larvae known as oncomiracidia (Öztürk and Özer, 2014). Their pathogenicity is important in aquaculture (El Madhi and Belghyti, 2006). When they attack the gills in large numbers they hinder the respiration or perhaps rather the mucous secreted by the host leading to fish mortalities, they form a major component of aquatic biodiversity, and their monitoring is considered an essential element of the management strategies of fish health (Ibrahim, 2012; Blahoua *et al*., 2016). This requires a good taxonomic and biological knowledge. The Food and Agricultural Organization of the United Nations (FAO, 2009) reported that, to satisfy an increasing demand in freshwater fish, extensive research must include studies of their parasites for optimal production levels. In Kenya, there is very little information about monogenean parasites. To the best of our knowledge, there has only been two ichthyoparasitological studies on *T. zillii* in L. Naivasha; Aloo (2002) did not find any ectoparasites, and therefore the only existing data is from Otachi *et al*., (2014) who recorded that monogeneans form the bulk of the parasites in fish in the lake. Recently, Rindoria *et al*., (2015) identified monogeneans on *Oreochromis niloticus* and *O. leucostictus* from the lake. The aim of this study was to identify gill monogeneans infesting *T. zillii* in Lake Naivasha, Kenya using morphometric analysis and also to determine their prevalence and mean intensities.

4.3 Materials and Methods

4.3.1 Study site

The study was carried out in Lake Naivasha in which the fish community comprises of only introduced species (Hickley *et al*., 2015). The lake lies at $00^{0}45'$ S and $36^{0}20'$ E in a closed basin at an altitude of 1890 m above sea level, 190 km south of the equator, within the Eastern Rift Valley of Kenya. It is approximately 160 km^2, with a volume of 4.6 km^3 (Campbell *et al*., 2003), is and has a mean depth of 3.35 m and a maximum depth of 7 m (Hickley *et al*., 2002). It is a freshwater lake without a surface outlet but with substantial exchange with groundwater (Gaudet and Melack, 1981). Riparian ownership of Lake Naivasha is private, and the pressures on the lake's eco-system and fishery are considerable. The most significant riparian activity on Lake Naivasha is the large-scale production of flowers for the European market, and at least 50% of the perimeter of the lake is under irrigated agricultural use (Hickley *et al*., 2015). As the labour intensive flower industry developed, so did the need for housing, water and latrines (Enniskillen, 2002). Furthermore, the lake resources are also of critical importance to geothermal electricity

generation, tourism, wildlife and conservation (Harper *et al*., 1990). A map of the lake and its environs is available in Rindoria *et al*., (2015).

4.3.2 Fish collection

Fifty specimens of the Redbelly Tilapia, *T. zillii* were collected using a fleet of gill nets with mesh sizes 2, 2.5, 3, 3.5, 4, and 4.5 inch from January to May 2015. The fish were transported alive in a fish tank with lake water to the laboratory of the Department of Biological Sciences, Egerton University Njoro, Kenya.

4.3.3 Parasite recovery

In the laboratory the fish were separated according to species to avoid cross contamination, killed by cervical dislocation (Schäperclaus, 1990) and dissected. The gills were removed and examined with a dissecting microscope and a motic BA210 compound microscope. The monogeneans were detached from the gills using a pair of fine forceps. Some of the monogeneans were individually transferred to a drop of ammonium picrate-glycerine (Malmberg, 1957) on a glass slide for observations of the sclerotized structures as per the methods of Ergens (1969). The preparation was covered with a cover slip and sealed with a transparent nail hardener for examination of the anatomy, additional specimens were flattened and fixed in 4% formalin or were preserved in absolute ethanol (96%).

4.3.4 Morphometric analysis

The sclerotized structures such as the opisthaptor and the copulatory complex were drawn using corelDRAW Graphics Suite X6 software (Corel Corporation, 2003). Measurements were made with Motic software in which a motic camera (moticam 2300, 3.0 pixel USB 2.0) was attached to a Motic BA210 compound microscope. All measurements are given in micrometers as the mean ± the standard deviation followed by the range in parentheses, as proposed by Gussev (1962). Identification was done using the identification keys by Pariselle and Euzet (1995; 2009). The method of numbering of the sclerotized parts was that adopted from ICOPA IV (Euzet and Prost, 1981). The terminology used was that proposed by Pariselle and Euzet (1995): uncinulus for the marginal hooklets; gripus for the large median hooks. The measurements made in this study were as follows: gripus (G): a = total length, b = blade length, c = root length, d = shaft length, and e = point length; male apparatus (MA): penis total length (Pe), heel (He), and accessory piece length (AP); auxiliary plate(Pl); dorsal transverse bar (DB): h = length of auricle, w = maximum width,

x = total length and y = distance between auricle; uncinuli length (U); ventral transverse bar (VB): w = maximum width and x = length of one branch; vagina (Vg): L = total length and l = maximum width according to Pariselle and Euzet (1997). The prevalence (P) and mean intensities (MI) were determined according to Bush *et al*., (1997). The measures of monogeneans community structure such as the Shannon–Wiener index, Margalef richness index, Dominance index and Berger–Parker dominance index as proposed by Magurran (1988) were determined using the online Biodiversity calculator (Danoff-Burg and Xu, 2005).

4.4 Results

Seven species of *Cichlidogyrus* (Paperna, 1960, Monogenea, Ancyrocephalidae) were found on the gills of *T. zillii*. Two of them namely; *C. sclerosus* Paperna and Thurston, 1969 and *C. tilapiae* Paperna, 1960 have previously been reported infecting the *O. niloticus* and *O. leucostictus* in the same lake (Rindoria *et al*., 2015). The other five species found included: *C. aegypticus* Ergens, 1981; *C. arthracanthus* Paperna, 1960; *C. digitatus* Dossou, 1982; *C. yanni* Pariselle and Euzet, 1995 and *C. vexus* Pariselle and Euzet, 1995 and forms the first biogeographical records from the lake, the host (*T. zillii*) and from the Republic of Kenya. A summary of their morphometric data is given below.

4.4.1 *Cichlidogyrus digitatus* Dossou, 1982

Description and measurement (µm) **(**Fig. 6). Twenty one specimens were recovered but only 10 measured: Adult individuals 474 ± 48.0 (396-533) long, 103 ± 28.7 (70-144) wide at the level of penis (no visible vagina); Dorsal gripus with guard much longer than shaft; thin blade curved in distal third: a = 41 ± 4.0 (35-47), b = 31 ± 2.9 (26-35), c = 5 ± 1.9 (3-8), d = 14 ± 2.2 (11-18), e = 13 ± 2.2 (9-15). Thick dorsal transverse bar with large auricles: x = 32 ± 4.7 (29-37), w = 8 ± 1.5 (6-10), h = 15 ± 2.1(12-18), y = 10 ± 1.5 (8-12). Large ventral gripus with short guard and shaft. Ventral transverse bar V-shaped: x = 38 ± 6.0 (31-45), w = 4 ± 2.0 (2-8). Large uncinulus and short uncinuli U = 21 ± 3.8 (16-28). Penis as described by Dossou (1982): short and slightly sinuous, with fine, straight heel: Pe = 34 ± 2.5 (31-38). Accessory piece, linked to base of penis, ending in two fine, opposed digitations: AP = 29 ± 5.8 (23-38). No auxiliary plate and no vagina observed.

Type-host: *T. zillii* Gervais, 1848 (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45′ S, 36°20′ E

Site of infection: Gills.

Material studied: 50 individuals.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro Kenya.

Remarks

The finding of *C. digitatus* in this study represents the first biogeographical record from Kenya. This parasite was originally described by Dossou (1982) in Ouémé Benin based on the specimens from the gills of *type-host* and later by Pariselle and Euzet (1996); Pouyaud *et al*., (2006) from Benin, Ivory Coast and Guinea on the same type-host. The species has also been reported from various cichlid fishes (*Tilapia dageti*, *T. guineensis*, *T. louka*, *T. brevimanus* and *T. walteri*) from Senegal, Mali, Ivory Coast, Gambia and Guinea in Africa by Pariselle and Euzet, (1996); Pouyaud *et al*., (2006). The latest record from Africa is by Blahoua *et al*., (2015) in a man-made Lake Ayame I, Ivory Coast from the type-host.

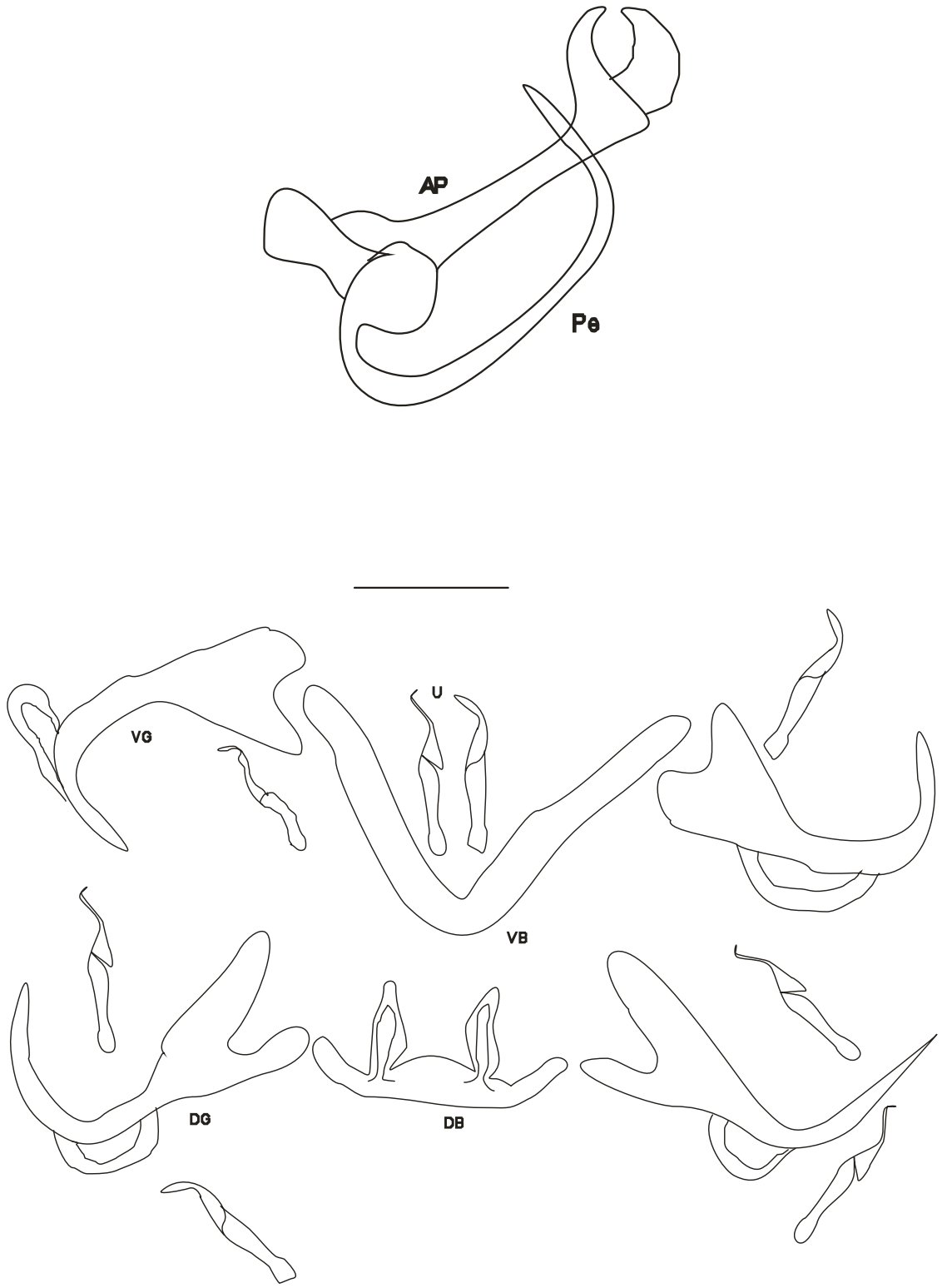

Figure 6: *Cichlidogyrus digitatus* Dossou, 1982: Sclerotized parts of copulatory complex and opisthaptoral structures. *Abbreviations*: AP accessory piece, Pe penis, DB dorsal bar, DG dorsal gripus, VG ventral gripus, VB ventral bar, U uncinuli (marginal hooks) (*Scale-bar*: 50µm)

4.4.2 *Cichlidogyrus yanni* Pariselle & Euzet, 1996

Description and measurement (µm) **(**Fig. 7). Only 1 specimen recovered and measured: Adult individuals 520 long, 82 wide at level of penis (no visible vagina). Dorsal gripus with guard much longer than shaft; thin blade curved in distal third, Large ventral gripus with short guard and shaft and thick dorsal transverse bar with large auricles: x = 28, w = 7, h =12, y = 9. Gripus: a = 37, b = 24, c = 4, d = 13, e = 9. Large uncinulus U = 21. Accessory piece ending in single short, rounded hook AP = 28**.** The auxiliary plate, the vagina and the penis were not observed.

Type-host: *T. Zillii* Gervais, 1848 (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45′ S, 36°20′ E

Site of infection: Gills.

Material studied: 50 individuals of the host.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro Kenya.

Remarks

The finding of *C. yanni* in this study represents the first biogeographical record from Kenya. This parasite was originally described by Pariselle and Euzet (1996) on the type-host in Guinea, Kogon River and Pouyaud *et al*., (2006) in Burkina Fasso Volta Noire River. The parasite has since then been reported on various tilapia hosts: *Tilapia dageti*, (Senegal, Mali); *T. guineensis*, (Ivory Coast, Senegal); *T. louka* (Guinea) *T. walteri and T. mariae* (Ivory Coast) in Africa (Pariselle and Euzet, 1996; Pouyaud *et al*., 2006). The latest record from Africa is by Blahoua *et al*., (2015) in man-made Lake Ayame I, Ivory Coast from the type-host.

Figure 7: *Cichlidogyrus yanni* Pariselle & Euzet, 1995: Sclerotized parts of copulatory complex and opisthaptoral structures. *Abbreviations*: AP accessory piece, DB dorsal bar, DG dorsal gripus, VG ventral gripus, VB ventral bar, U uncinuli (marginal hooks) (*Scale-bar*: 50µm)

4.4.3 *Cichlidogyrus aegypticus* Ergens, 1981

Description and easurement (µm) **(**Fig. 8). Only 2 specimens recovered and measured: Adult individuals: 491 ± 69.3 (442-540) long, 105 ± 26.9 (86-124) wide at level of vagina; Gripus: a = 25 ± 2.1 (23-26), b = 20 ± 1.4 (l9-21), c = 5 ± 1.4 (4-6), d = 12 ± 3.5 (9-14), e = 10 ± 2.8 (8-12). Dorsal transverse bar: x = 33 ± 7.8 (27-38), w = 4 ± 1.4 (3-5), h = 16 ± 2.8 (14-18), y = 15 ± 3.5 (12-17). V-shaped ventral transverse bar: x = 34 ± 2.8 (32-36), w = 6 ± 0.7 (5-6). Uncinulus U = 29 ± 3.5 (26-31) long. Male copulatory organ with arched tubular penis and large basal bulb: Pe = 65 ± 5.7 (61-69) long. Accessory piece (linked to heel), bent at right angle (with digitation at its vertex), ended in large hook: AP = 36 ± 3.2 (32-42). Auxiliary plate and vagina not observed.

Type-host: *T. zillii* Gervais, 1848 (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45′ S, 36°20′ E

Site of infection: Gills.

Material studied: 50 individuals.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro Kenya.

Remarks

The finding of *C. aegypticus* in this study represents the first biogeographical record from Kenya. This parasite was originally described by Ergens (1981) based on the specimens from the gills of type-host in River Nile Egypt, later from by Dossou (1982) in Benin; Burkina Faso and in Ivory Coast by Pariselle and Euzet (1996); Pouyaud *et al*., (2006). The species has so far been reported from other fish hosts from Egypt and Ivory Coast in Africa (*Tilapia dageti*, *Sarotherodon galilaeus galilaeus* (*Tilapia galilaea*); *Oreochromis niloticus niloticus* (*Tilapia nilotica*); *Tilapia busumana*; *T. dageti*; *T guineensis*; *T. walteri* (El-Naggar and Khidr, 1986; Pariselle and Euzet, 1996; Pouyaud *et al*., 2006). The latest record from Africa is by Ibrahim (2012) from Lake Manzalah, Egypt and Blahoua *et al*., (2015) in man-made Lake Ayame I, Ivory Coast from the type-host.

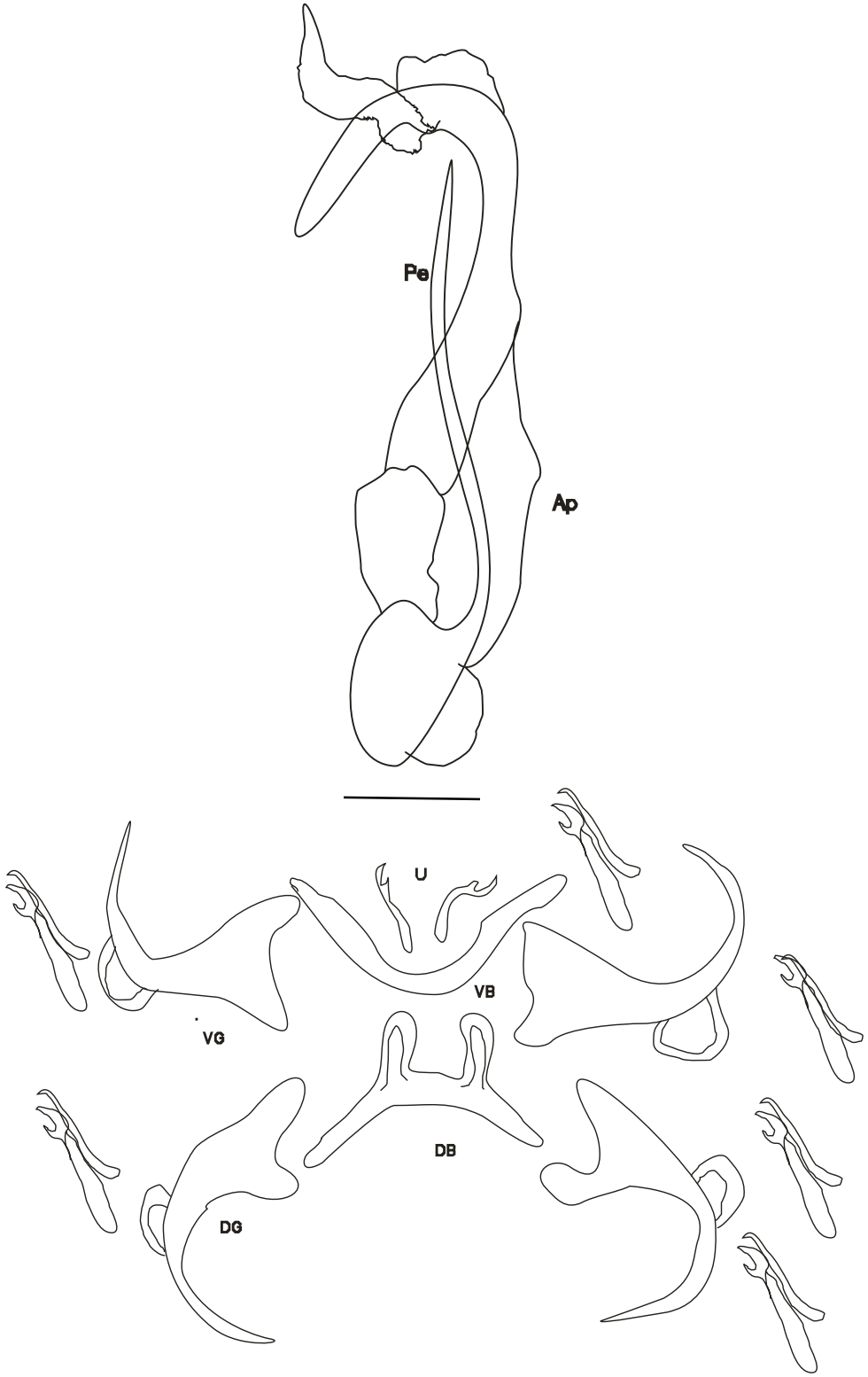

Figure 8: *Cichlidogyrus aegypticus* Ergens, 1981: Sclerotized parts of copulatory complex and opisthaptoral structures. *Abbreviations*: AP accessory piece, Pe penis, DB dorsal bar, DG dorsal gripus, VG ventral gripus, VB ventral bar, U uncinuli (marginal hooks) (*Scale-bar*: 50µm)

4.4.4 *Cichlidogyrus arthracanthus* Paperna, 1960

Description (Fig. 9). Only 1 specimen recovered

Type-host: *T. zillii* Gervais, 1848 (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45′ S, 36°20′ E

Site of infection: Gills.

Material studied: 50 individuals of the host.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro Kenya.

Remarks

The finding of *C. arthracanthus* in this study represents the first biogeographical record from Kenya. This parasite was originally described by Paperna (1960) based on the specimens from the gills of type-host in Dor and near Sea of Galilee Israel and later in South Ghana and North Ghana (Paperna 1965; 1969; Khidr and Hassan 1990), Uganda (Paperna and Thurston, 1969; Pariselle and Euzet, 1996) and Egypt (Ergens, 1981; Pouyaud *et al*., 2006). The species has also been reported from other host fishes from Israel in the Middle East: (*Tristramella simonis simonis* (*Tristramella simonis*); *Tristramella sacra* and Egypt: *Sarotherodon galilaeus galilaeus (Tilapia galilaea) Oreochromis niloticus niloticus* (*Tilapia nilotica*); *Tilapia busumana*; *T. dageti*; *T. guineensis*; *T. walteri* (El-Naggar and Khidr, 1986; Pariselle and Euzet, 1996; Pouyaud *et al*., 2006) in Africa. The latest record from type-host is by Ibrahim (2012) from Lake Manzalah, Egypt, Africa.

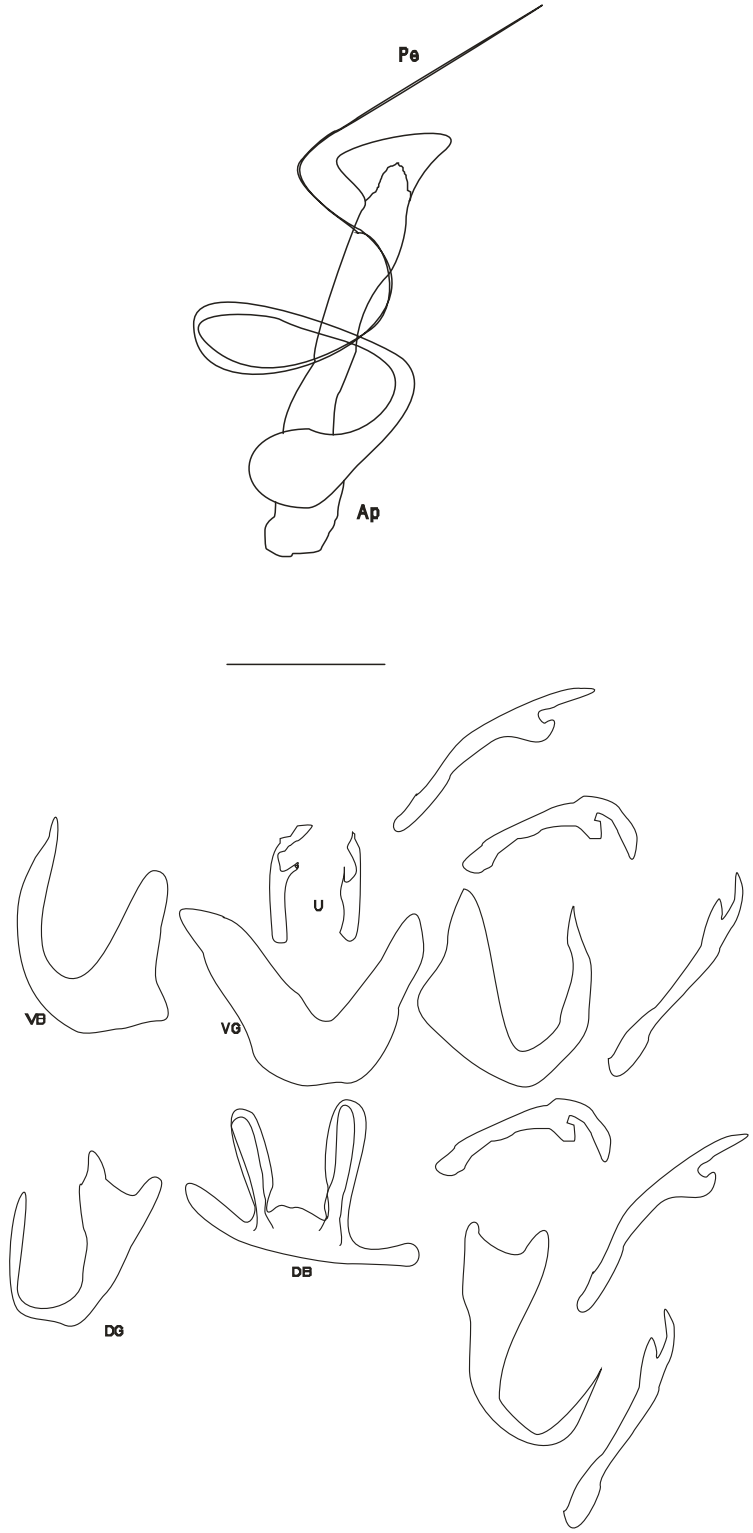

Figure 9: *Cichlidogyrus arthracanthus* Paperna, 1960: Sclerotized parts of copulatory complex and opisthaptoral structures. *Abbreviations*: AP accessory piece, Pe penis, DB dorsal bar, DG dorsal gripus, VG ventral gripus, VB ventral bar, U uncinuli (marginal hooks) (*Scale-bar*: 50µm)

4.4.5 *Cichlidogyrus vexus* Pariselle & Euzet, 1995

Description (Fig. 10) (Only 2 specimens recovered and measured): Adult: 713 ± 32.5 (690–736) long, 237 ± 71.4 (186–287) wide. Two pairs of eyes with lens on the first pair. Haptor is rounded with two pairs of hamuli and seven hamuli (uncinuli). Gripus: a = 26 ± 1.4 (25–27), b = 21 ± 2.1 (19–22), c = 7 ± 1.4 (6–8), d = 11 ± 1.4 (10–12), e = 15 ± 1.4 (14–16). Dorsal transverse bar: x = 37 ± 1.4 (36–38), y = 13 ± 2.1 (11–14), h = 16 ± 1.4 (15–17), w = 12 ± 0.7 (11–12). Ventral transverse bar V-shaped: x = 33 ± 2.8 (31–35), w = 14 ± 2.8 (12–16). Uncinuli: u = 19 ± 2.8 (17–21). Pe = 45 ± 4.2 (58–60). AP = 59 ± 1.4 (36-38). Accessory piece was not observed.

Type-host: *T. zillii* Gervais, 1848 (Perciformes: Cichlidae)

Type-locality: Lake Naivasha, Kenya, 00°45′ S, 36°20′ E

Site of infection: Gills.

Material studied: 50 individuals of the host.

Deposition of types: Preserved at Department of Biological Sciences in Egerton University, Njoro, Kenya.

Remarks

The finding of *C. vexus* in this study represents the first biogeographical record from Kenya. This parasite was originally described by Pariselle and Euzet (1995c, 1996; Pouyaud *et al*., 2006) based on the specimens from the gills of *Tilapia guineensis* in Bandama River, Ebrié Lagoon, Ivory Coast in Africa. The latest record from Africa is by Blahoua *et al*., (2015) in man-made Lake Ayame I, Ivory Coast from the type-host.

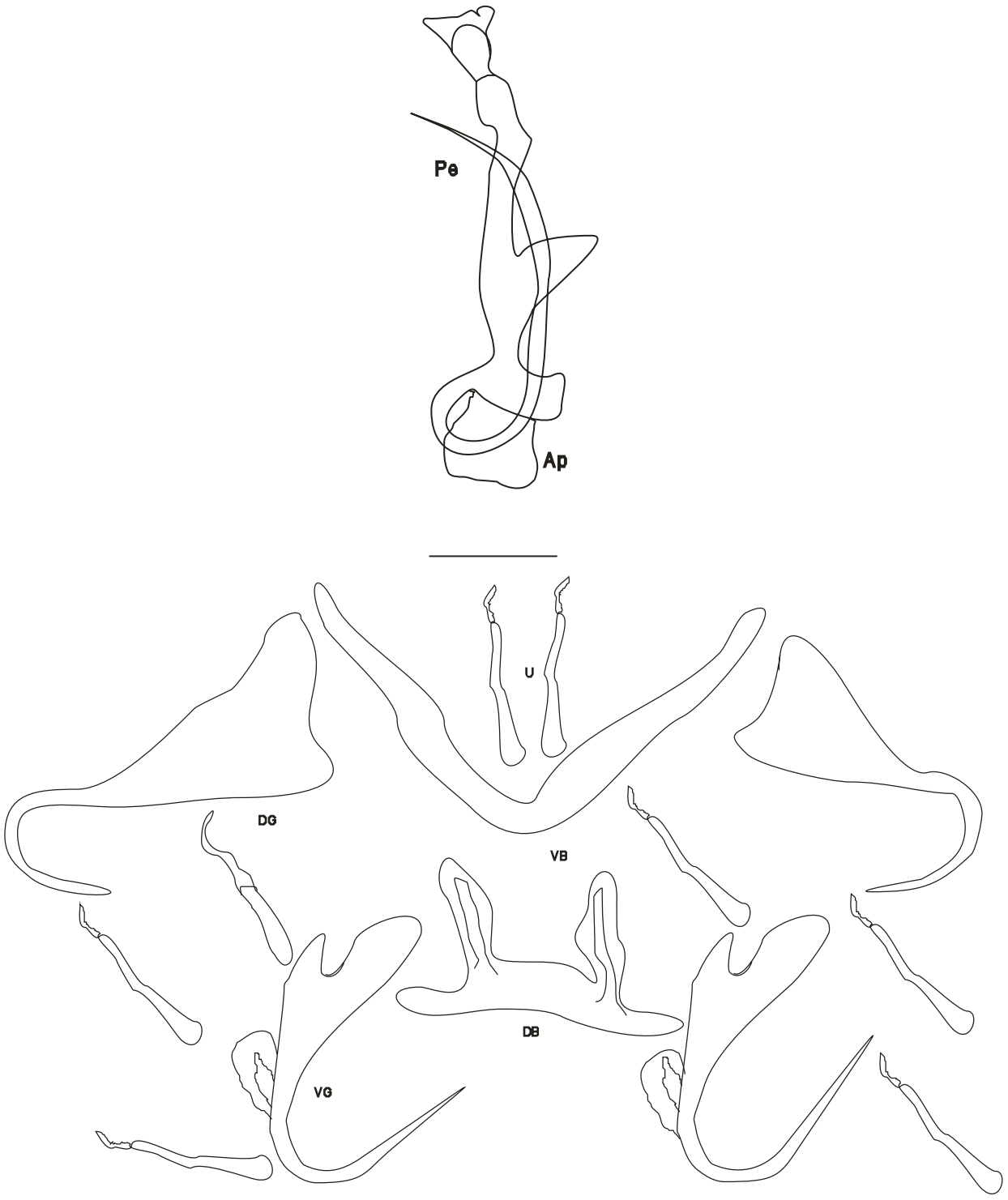

Figure 10: *Cichlidogyrus vexus* Pariselle & Euzet, 1995: Sclerotized parts of copulatory complex and opisthaptoral structures. *Abbreviations*: AP accessory piece, Pe penis, DB dorsal bar, DG dorsal gripus, VG ventral gripus, VB ventral bar, U uncinuli (marginal hooks) (*Scale-bar*: 50µm)

Parasitological parameters

The prevalence (P %) and mean intensity (MI) were calculated and *C. digitatus* recorded the highest P = 42%; and MI = 1.91 whereas *C. arthracanthus*, *C. sclerosus*, *C. tilapiae*, *C. vexus* and *C. yanni* had equal P = 2% and MI = 1.00. The *C. vexus* had a P =4% and MI=2.00 (Fig 11 and 12)

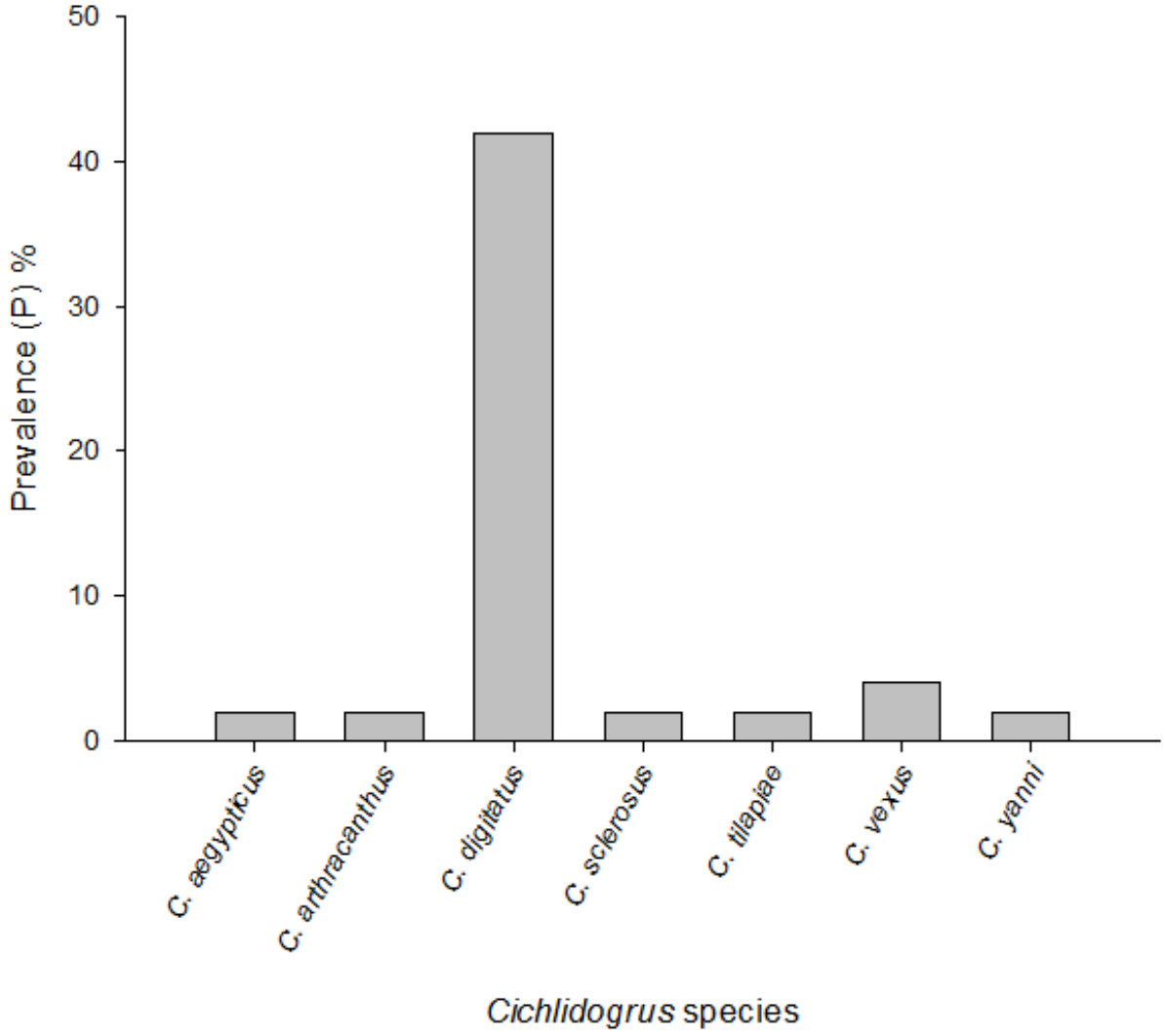

Figure 11: Prevalence (P) % of the *Cichlidogyrus* species in *T. zillii* from L. Naivasha, Kenya

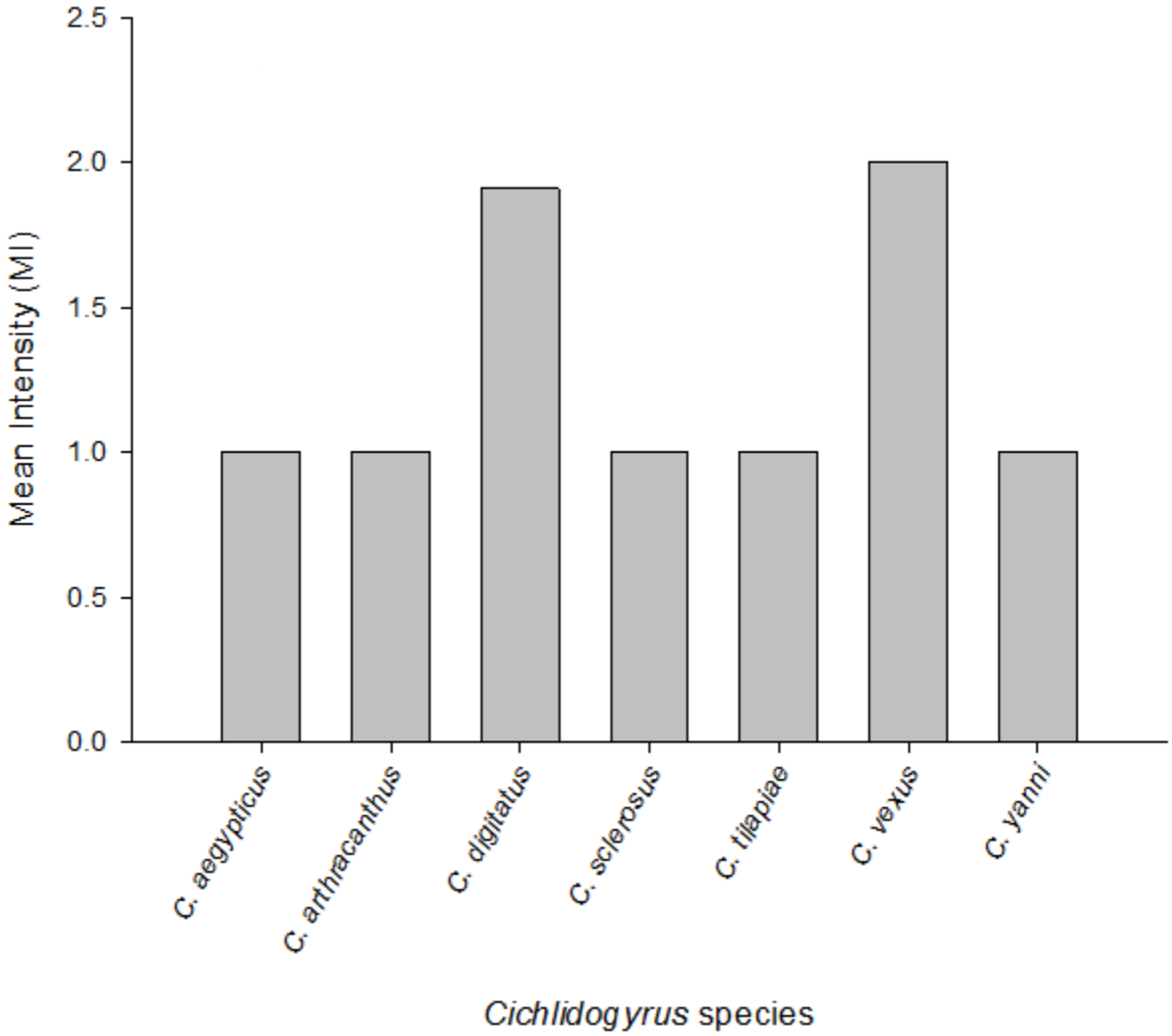

Figure 12: Mean intensities (MI) of the *Cichlidogyrus* species in *T. zillii* from L. Naivasha, Kenya

Species richness

The species richness of the parasite infracommunities harboured by the host was 1.80 which almost agrees with Ibrahim, (2012) who recorded a richness of 1.87 on the same host. The diversity of parasite communities was 0.442 which is comparable with Ibrahim, (2012) who reported a Brillouin index of 2.050. The Berger-Parker index value was 0.750 which is also comparable with what Ibrahim (2012) who reported 0.410 (Table 4). The slight differences in these indices in the two studies may be attributed to different sample sizes and geographical locations.

Table 4: Diversity indices of the *Cichlidogyrus* species on *T. zillii* from L. Naivasha, Kenya

Total component communities	***T. zillii* (n = 50)**
Total number of species	7
Total number of organisms	28
Shannon–Wiener index	0.999
Berger–Parker index	0.750
Margalef richness	1.801
Dominant species	*C. digitatus*

Table 5: Comparison between the current study and other studies of the *Cichlidogyrus* species on *T. zillii* in Africa

Cichlidogyrus species	Authors, Year and Country							
	Ergens (1981) R. Nile, Egypt		Paperna (1996) West Africa		Ibrahim (2012) Lake Manzalah, Egypt		Present study Lake Naivasha, Kenya	
	P (%)	MI	P (%)	MI	P (%)	MI	P (%)	MI
C. aegypticus	NR	NR	NR	NR	33.82	12.29	2	1.00
C. arthracanthus	NR	NR	-	-	47.94	15.50	2	1.00
C. digitatus	-	-	NR	NR	-	-	42	1.91
C. yanni	-	-	NR	NR	-	-	2	1.00
C. sclerosus	-	-	-	-	9.12	5.51	2	1.00
C. tilapiae	NR	NR	-	-	-	4.97	2	1.00
C. vexus	-	-	NR	-		-	4	2.00
Prevalent taxon	NR		NR		*C. arthracanthus*		*C. digitatus*	

NR Studied but not recorded, - Not Studied

4.5 Discussion

The present study recorded seven species of the monogenean trematode of the genus *Cichlidogyrus* on *Tilapia zillii*: *Cichlidogyrus digitatus, C. yanni, C. vexus, C. arthracanthus, C. aegypticus, C. sclerosus* and *C. tilapiae*. This species richness is close to that reported in previous studies (Table 4). Ergens (1981) found three monogenean species in 15 specimens on the same host in River Nile Cairo Egypt, Paperna (1996) found five monogeneans on the same host in West Africa, Ibrahim (2012) recovered four similar monogenean species to our study from the same host in Lake Manzalah, Egypt, while Blahoua *et al*., (2015) found four monogenean species in the same host in man-made Lake Ayame I, Ivory Coast (Table 5). The larger number/variety of *Cichlidogyrus* species observed in a single host from Lake Naivasha shows the exploitation of a host fish by several species of monogenean (polyparasitism) which has been reported by various authors in mouth-brooding tilapias. For instance, Blahoua *et al*., (2009) reported the presence of *Scutogyrus* and three species of *Cichlidogyrus* in *Sarotherodon melanotheron*. Ibrahim (2012) also showed that eight monogenean species colonized the gills of *T. zillii* in Lake Manzalah Egypt (Table 5). Moreover, the colonization of hosts by several congeneric species was also reported by Boungou *et al*., (2008), Bittencourt *et al*., (2014) and Tombi *et al*., (2014). This study confirms that a great diversity of monogenean parasites occur in African cichlids as reported by Pariselle (1996). This polyparasitism could be explained by the fact that in the natural environments, the parasitic densities are generally low and therefore, niches are always available on the gill biotope thereby facilitating simultaneous colonization of the same host by several species of Monogenea (Buchmann and Lindenstrom, 2002; Simkova *et al*., 2006; Nack *et al*., 2010; Ibrahim, 2012). In most cases, regarding parasite dispersion, they are almost evenly aggregated between their hosts (Krasnov and Poulin, 2010). Indeed, most of the hosts have few if any parasites, while a small number of hosts are infected with many parasites (Poulin, 1993). This pattern is expected in most animals in nature, as observed within monogeneans (*C. aegypticus*, *C. arthracanthus*, *C. digitatus*, *C. yanni*, *C. vexus*, *C. sclerosus* and *C. tilapiae*) parasitizing the gills of *T. zillii* in the present study. This trend has also been reported by Ibrahim (2012). For example *C. arthracanthus, C. aegypticus, C. ergensi, C. sclerosus, C. tilapiae, C. halli typicus, C. tiberianus* and *Gyrodactylus cichlidarum* gill parasites of the same type-host have been reported from Lake Manzalah, Egypt. According to Combes (1995), an aggregative distribution may indicate heterogeneity in the relationship between the host and the parasite populations. The probability for a parasite to meet

its host and its chances of surviving in the latter may vary from one host to another. In addition, Kennedy (1977) stated that aggregative distribution increases the opportunities for parasites to meet a partner in order to reproduce. Our results suggest that the water of Lake Naivasha may provide better eco-climatic conditions such as temperature (23.4^0C) for the development of these parasites and facilitate contact between the infecting stages (oncomiracidium) of these monogeneans and the host (Rindoria *et al*., 2015). The occurrence of many monogenean parasites on the same host can also be as a result of changes in abiotic factors such as changes in concentration of suspended solids, conductivity and water transparency (Blahoua, 2013).

4.6 Conclusion

This study demonstrates that gill parasitizing monogeneans on *T. zillii* exhibit polyparasitism in natural water systems. The five monogenean species (*C. digitatus*, *C. yanni*, *C. vexus*, *C. arthracanthus* and *C. aegypticus*) recorded in this study form the first biogeographical record from Kenya; however the *C. sclerosus* and *C. tilapiae* have been previously recorded from the *O. leucostictus* and *O. niloticus* from the same lake. The *C. digitatus* had the highest prevalence while the *C. vexus* had the highest mean intensity.

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CHAPTER FIVE

5.0 DESCRIPTION OF *Dactylogyrus* sp. FROM STRAIGHTFIN BARB *Barbus paludinosus* (Peters, 1852) (PISCES: CYPRINIDAE) IN LAKE NAIVASHA, KENYA

5.1 Abstract

The straightfin barb *Barbus paludinosus* (Peters, 1852) is a species of ray-finned fish in the cyprinid family which invaded Lake Naivasha from inflowing rivers since 1982. The fish is a host to gill monogeneans of the genus *Dactylogyrus* which are predominantly parasitic gillworms on cyprinid fishes. A highly diverse group, with a distribution and zoogeography linked to the evolutionary history of their hosts. In Africa, more than 92 species have been described as compared to the more than 900 nominal species described worldwide, consequently causing confusion within this largest helminth genus. In Kenya only one study records *Dactylogyrus* sp. from the same host in the same lake. In the present study undertaken in Lake Naivasha between October 2015 and January 2016, fifty specimens of *B. paludinosus* were collected using gill nets and killed by severing the spinal cord. The gills were removed and examined with dissecting and compound motic-microscopes. The collected specimens were stored in either 4% buffered formalin for morphometric analysis or in 70 % ethanol for molecular analysis; the formalin fixed specimens were later mounted using glycerine jelly. Identifications were based on morphometric analyses with the help of drawings, micrographs and dimensions. One *Dactylogyrus* sp. was found with a P = 22 and MI = 1.375. The *Dactylogyrus* sp. differed with all *Dactylogyrus* species described from the same host, and was suspected to be a new species. Therefore, this study describes the new species which was named *Dactylogyrus hansi*.

5.3 Materials and Methods

5.2 Introduction

The Cyprinidae is one of the largest families of teleosts in the world comprising at least 1700 species and over 200 genera (Abdullah, 2009). Natural populations of cyprinids are widely distributed in most freshwater rivers, lakes and ponds (Hoole *et al.*, 2001). The cyprinids parasites including monogeneans have been intensively studied and documented in books (Bykhovskaya-Pavlovskaya *et al.*, 1962). The monogenean infections on the cyprinids generally have negligible

impacts on natural host populations in the wild, where host densities are low (Cusack, 1986), but their study is important because the knowledge on monogenean taxonomy and biology is essential in aquaculture and fisheries management for potential aquaculture species (Swanepoel, 2015). Monogenea parasite infections may cause mortalities (Paperna, 1968) and retard growth in aquaculture and aquarium conditions (Crafford *et al.*, 2014). In terms of the genus *Barbus* Cuvier and Cloquet, 1816, knowledge is widely recognised for the ornamental fish trade and for their effects on biological diversity (Crafford *et al.*, 2012). The current study was an investigation into the monogenean species of the cyprinid *B. paludinosus* in Lake Naivasha, Kenya. The Monogenea fauna of Kenya on genus *Barbus* has been studied since 1968 through the work of Paperna and Thurston, 1968 (from *B. altaianalis* and recovered *D. spinicirrus*); Paperna, 1979 (from *B. nyanzae* and also recovered *D. spinicirrus*) but this is still far from complete. A recent increase in interest in monogeneans of Kenya is evident from the number of recent publications (Aloo, 2002, Otachi *et al*., 2014, Rindoria *et al*., 2015), though none of these publications focusses on monogeneans of the genus *Barbus*. Most publications of the Monogenea of the genus *Barbus* in Africa are from South Africa (Mbokane, 2011; Matla, 2012; Crafford *et al*., 2012 Mbokane *et al*., 2015; Swanepoel, 2015). The majority of monogeneans described from *Barbus* belong to the genus *Dactylogyrus* Diesing, 1850, which is the largest genus with more than 900 species described. Ninety five percent of all *Dactylogyrus* species were collected from cyprinid fish, but only 11.5 % are known from African fish (Gibson *et al.*, 1996). More than 60 species have been described from the genus *Barbus* in Africa with most deriving from western Africa through the work of Guégan and Lambert (1990) and East Africa (Uganda and Kenya) by Paperna (1979). Apart from the barb monogeneans of the genus *Dactylogyrus*, other monogeneans also parasitize this fish, for example, *Afrodiplozoon polycotyleus* (Paperna, 1973) and *Gyrodactylus* von (Nordmann, 1832) sp.; *Dogielius* sp. (Bychowski, 1957); *Dactylogyrus myersi (*Price, McClellan, Druckenmiller and Jacobs, 1969), *Dactylogyrus teresae*, *Dactylogyrus enidae* and *Dactylogyrus dominici* all by Mashego, (1983); Christison (2002) recorded *Dactylogyrus barrilus* and *Dactylogyrus viviersii*, from the Okavango River System. Matla (2012) described two species of the genus *Dactylogyrus.* Most of the descriptions of these monogenean species are associated with South African freshwaters barbs (Swanepoel, 2015). However, in Kenya, it is only in the studies of Otachi *et al*., (2014) and Rindoria *et al*., (2015) that cichlid monogeneans were first identified

and reported in the lake. Therefore, this study aimed at identifying the monogeneans infecting *B. paludinosus* from Lake Naivasha and provide their infection parameters.

5.3.1 Study area

The study was conducted in Lake Naivasha, Kenya from October 2015 to January 2016. The lake is situated at 00º45'S and 36º20'E (Kamau *et al.,* 2008) in a closed basin at an altitude of 1890m above sea level and covers approximately 160km^2 in the eastern Rift Valley of Kenya (The detailed map of the study site is in Fig. 1). It is the only freshwater lake in the Rift Valley without a surface outlet but with a substantial exchange with groundwater (Gaudet and Melack 1981; Clarke *et al*., 1990). It is shallow (approximately 6 m mean depth) with a volume of 4.6km^3 (Campbell *et al*., 2003). It is bordered by papyrus *Cyperus papyrus* in some sections and the overall composition of aquatic macrophytes are in a state of change (Tarras-Wahlberg *et al*., 2002) probably due to anthropogenic influence such as clearance of littoral vegetation, eutrophication and plant and animal introductions (Kitaka *et al.,* 2002; Gherardi *et al*., 2011). Most of its fresh water inflow (approximately 80-90%) comes from River Malewa (Hickley *et al*., 2002; Kamau *et al*., 2008) with an estimated mean annual flow of 153 million m^3 and a catchment area of 1730 km^2, followed by River Gilgil with an estimated average annual flow of 24 million m^3 and a catchment area of 420 km^2 while the River Karati flows only intermittently (Ase *et al*., 1986; Ase 1987; Abila *et al*., 2008; Harper *et al*., 2011). The Lake Naivasha basin has three distinct components. The first component is the main lake with an area of 145 km^2 and is the most important for fisheries (Harper *et al.,* 1990). The second component is the Oloidien Lake with an area of 5.5 km^2 (Harper *et al*., 1990). This lake had been separated from the main lake due to receding water levels and now has a considerably higher pH than the main lake (Harper *et al*., 1990). The third component is the Crescent Island Lake with an area of 2.1 km^2 (Harper *et al*., 1990). It is the deepest lake (approximately 12-15 m deep) but is constantly connected to the main lake (Abiya, 1996; Harper *et al*., 2011). The basin area is generally semi-arid; receiving a mean annual rainfall of 620 mm while the mean annual evaporation is estimated at 1735 mm. Evaporation generally exceeds precipitation throughout the year except at peak rainfall with the rainfall trend being bimodal with a major peak in April-May and a minor peak in October-November (Abiya, 1996). The water from Lake Naivasha is used extensively for agriculture (horticultural farms: approx. 77 million m^3/year), geothermal power generation (approx. 1 million m^3/year) (WWF 2011), domestic water supplies, commercial fishing, tourism and recreation as

well as ranching and game farming (Abiya, 1996). The key environmental problems facing the lake are water abstraction leading to changes in water level, eutrophication, pollution, invasive species, decline in fish stocks and biodiversity (Otiangà-Owiti and Oswe 2007; Abila *et al*., 2008).

5.3.2 Fish collection and parasite recovery

Fish were collected from Lake Naivasha using a seine net during the period October 2015 and January 2016. The fish were transported alive in a fish tank with lake water to the laboratory of the department of Biological sciences, Egerton University, Njoro. They were killed by cervical dislocation followed by a dissection as described by Schäperclaus (1990). Gill smears were prepared for examination under high magnification (×40-×100). The monogeneans observed were carefully removed from gills using fine forceps, fixed and preserved in either 4% buffered formalin or (70%) absolute ethanol. The monogeneans were mounted in either glycerine ammonium picrate (GAP) or glycerine jelly on slides and cover slips gently placed on top to flatten the worms and sealed with clear nail polish. Some monogeneans were identified directly using identification keys and key literature (Bykhovskaya-Pavlovskaya *et al*., 1962, Paperna 1979; 1980; 1996, Guegan and Lambert 1990; 1991; Pariselle and Euzet, 1995; 1998; 2009; Musilová *et al*., 2009; Le Roux and Avenant-Oldewage, 2010; Crafford *et al*., 2012; Gillardin *et al*., 2012; Bukinga *et al*., 2012; Vanhove *et al*., 2011a, b; Barson *et al*., 2010; Řehulková *et al*., 2013).

5.3.3 Measurements of sclerotized structures

For new species; detailed morphometric data was obtained using an Olympus BX40 compound transmitted light microscope with phase contrast fitted with 35 mm camera system connected to a computer with digital software. Although, recommendations made by Gussev (1979) were taken into consideration during measurement and examination of relevant structures reported in this paper, the terminology used followed Pariselle and Euzet (1998) in order to use the identification key dedicated to dacylogyridean parasites from cichlid fishes in Africa (Pariselle and Euzet, 2009). However, for the description of the new species, more acceptable terms such as anchor and hooks instead of ``gripus`` and ``uncinuli``, were used following Pariselle and Euzet (2009) and Řehulková *et al*., (2013). The numbering of hooks follows the convention adopted at ICOPA IV (Euzet and Prost, 1981).

5.3.4 Deposition of type material

Type specimens (holotypes) shall be deposited in the Iziko Museum Cape Town, South Africa. Additional specimen (paratypes) shall be maintained in the corresponding author`s collection at Department of Biological Sciences Egerton University, Kenya.

5.4 Results

All the monogeneans recovered belonged to a single species of *Dactylogyrus.* The species differed from all known *Dactylogyrus* species from the same host in several studies (Table 6). Therefore, it was suspected to be a new species and named *Dactylogyrus hansi*. The new species is recognized on the basis of its differences in opisthaptor the copulatory organ and anchors with known species: Its morphometric data: a (gripus total length), b (gripus blade length), c (gripus shaft length), d (gripus guard length), e (gripus point length), L (overall length), W (width at level of penis) is provided.

Type host : *Barbus paludinosus* (Peters, 1852) (Pisces: Cyprinidae)

Type locality : Lake Naivasha, Kenya, 00º45'S and 36º20'E

Site of infection : Gills.

Material studied : 50 individual of the host.

Description and measurement (µm): Body length 311 ± 54.5 (212-373), width 76 ± 24.4 (42-108). Gripus total length 49± 7.3 (45-69), gripus blade length 35 ± 8.7 (27-58), gripus shaft length 9 ± 6.1 (4-24), gripus guard length 22 ± 4.3 (18-33) and gripus point length 12 ± 3.0 (8-19). Marginal hooklets I 12 ± 0.73 (11-13), II 15 ± 2.1(12-17), III 18 ± 3.4(12-21), IV 16 ± 3.3(11-20), V 16 ± 3.4(12-20), VI 14 ± 2.2(12-17), VII 15 ± 2.9(12-17). Copulatory organ and vagina are visible.

Differential diagnosis: The major differences of this specimen with other *Dactylogyrus* species are highlighted in Table 6.

Table 6: Highlights of the specific differences between *D. hansi* n. sp and species of *Dactylogyrus* Diesing, 1850 previously described from *B. paludinosus** mainly from Africa and the rest of the world as well as other *Dactylogyrus* species known from other fish species from Kenya

Species of *Dactylogyrus* Diesing, 1850	Specific differences
D. afrochelatus Paperna, 1973*	Differ in shape of bar (robust, subdivided by two submedian folds into one central and two lateral plates), small copulatory organ, shape of vaginal prop elongated and dentated distally, shape of penis and accessory piece, has very short roots on anchor.
D. afrofluviatilis Paperna, 1973	Differ in shape of bar plate-straight in this sp. While it is arched in *D. hansi* sp. nov. also in shape of vaginal prop which is digitiform with small tuberculi.
D. afropsilovaginus Paperna, 1973*	Differ in lack of vaginal prop in this sp., has large distal hook on accessory piece large and movable, has short roots of anchor. There is a striking resemblance of the opisthaptor with the current species.
D. clavatovaginus Paperna, 1973*	Differ in shape of copulatory organ which appears to have bifurcated distal hooks 2, shorter anchors and roots, differ in shape of vaginal prop. There is similarity in shape of bar.
*D. cf. clavatovaginus** Paperna, 1973	Differ in shape of vaginal prop, shape of copulatory organ shorter roots of anchor. Overall, there is similarity in shape of bar.
D. cyclocirrus Paperna, 1973	Differ in shape of opisthaptor and copulatory organ.
D. longiphallus Paperna, 1973	Differ in shape of opisthaptor and copulatory organ.
D. brachydiscus Paperna, 1973	Differ in shape of opisthaptor and copulatory organ.
D. brevicirrus Paperna, 1973	Differ in shape of opisthaptor and copulatory organ.

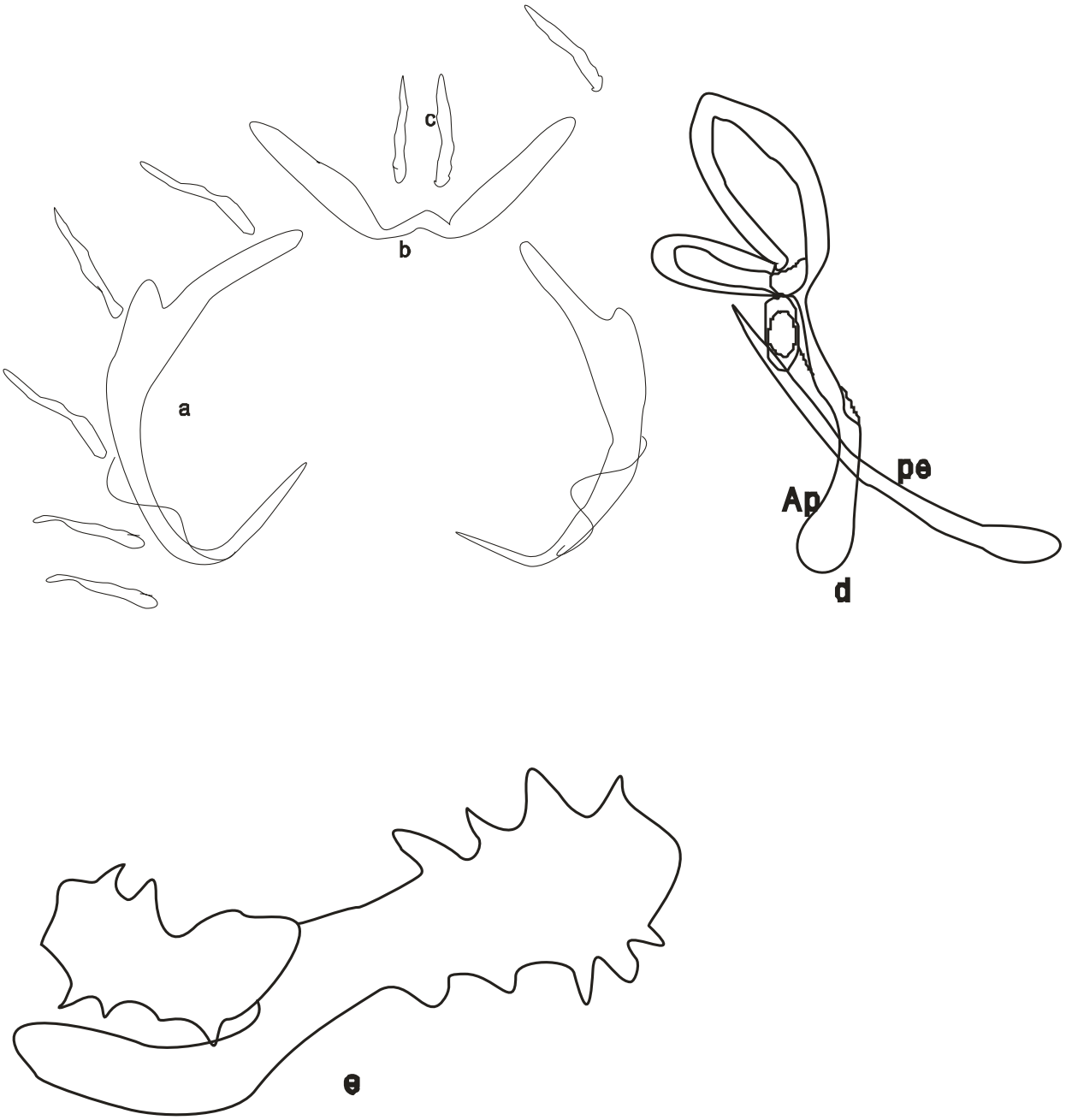

Figure 13: *Dactylogyrus hansi sp. nov*: Sclerotized parts of copulatory complex and opisthaptoral structures. *Abbreviations*: a anchor, b bar, c marginal hook, d copulatory organ, e vagina (*Scale-bar*: 50µm)

The *D. hansi* sp. nov. had a P = 22% and MI = 1.375. This was the only monogenean species identified on the gills of the host fish during the entire study period.

Remarks: The parasite species represents the first record of monogenean on *B. paludinosus* in Lake Naivasha, Kenya.

Etymology: the species name *hansi*, is from the latinized name Hans, who is the son of Dr. Elick Otachi, who conceptualized and guided the whole study.

5.5 Discussion

In the present study one species of *D. hansi* sp. nov was recorded as a new species. This was the only species recorded from the 21 fish out of the 50 which were examined. This species resembles *D. dominici* Mashego, 1983 in the shape of anchor, bar and marginal hooks (I-VII) but differ significantly in the shapes of the copulatory tube and the vagina. In Africa six species already have been described from *B. paludinosus*: *D. afrochelatus, D. afropsilovaginus, D. afrosclerovaginus, D. clavatovaginus, D. dominici* and *D. teresae* which seem to be restricted to their systems and not to the distribution of the host, except *D. dominici* that has a wider distribution. Surprisingly none of those species have been reported in Kenya.

5.6 Conclusion

This study erects a new species named *Dactylogyrus hansi.*

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CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study, ten monogenean parasites were identified: Nine from Cichlids (*S. gravivaginus*, *C. halli*, *C. tilapiae*, *C. sclerosus*, *C. digitatus*, *C. aegypticus*, *C. vexus*, *C. arthracanthus* & *C. yanni*) and one from Cyprinid *D. hansi* sp.nov. The *Cichlidogyrus* species recorded high prevalences and mean intensities as compared to *Dactylogyrus* species. All the monogeneans recorded under this study form the first biogeographical records from Lake Naivasha, Kenya.

6.2 Recommendations

Further studies on the identified monogenean ecology, seasonality and water quality should be carried out to determine whether they facilitate the spread of these parasites in Lake Naivasha. Additionally, more studies should be done to identify monogeneans infecting the other fish species which were not part of the present study.

APPENDICES

Appendix I Publications

Rindoria, N. M., Mungai, L. K., Yasindi, A. W. and Otachi, E. O. (2015). Gill monogeneans of *Oreochromis niloticus* (Linnaeus, 1758) and *Oreochromis leucostictus* (Trewavas, 1933) in Lake Naivasha, Kenya. *Parasitology Research* **115** (4): 1501-1508. DOI 10.1007/s00436-015-4883-3.

Rindoria, N. M., Mungai, L. K., Yasindi, A. W., Otachi, E. O. and Oldewage, A. A. (2016). A morphometric analysis of gill monogeneans infecting the Redbelly tilapia, *Tilapia zillii* (Gervais, 1848) from Lake Naivasha, Kenya: New biogeographical records (submitted to *Parasitology Research*)

Appendix II Photos of the fish species sampled

Tilapia zillii

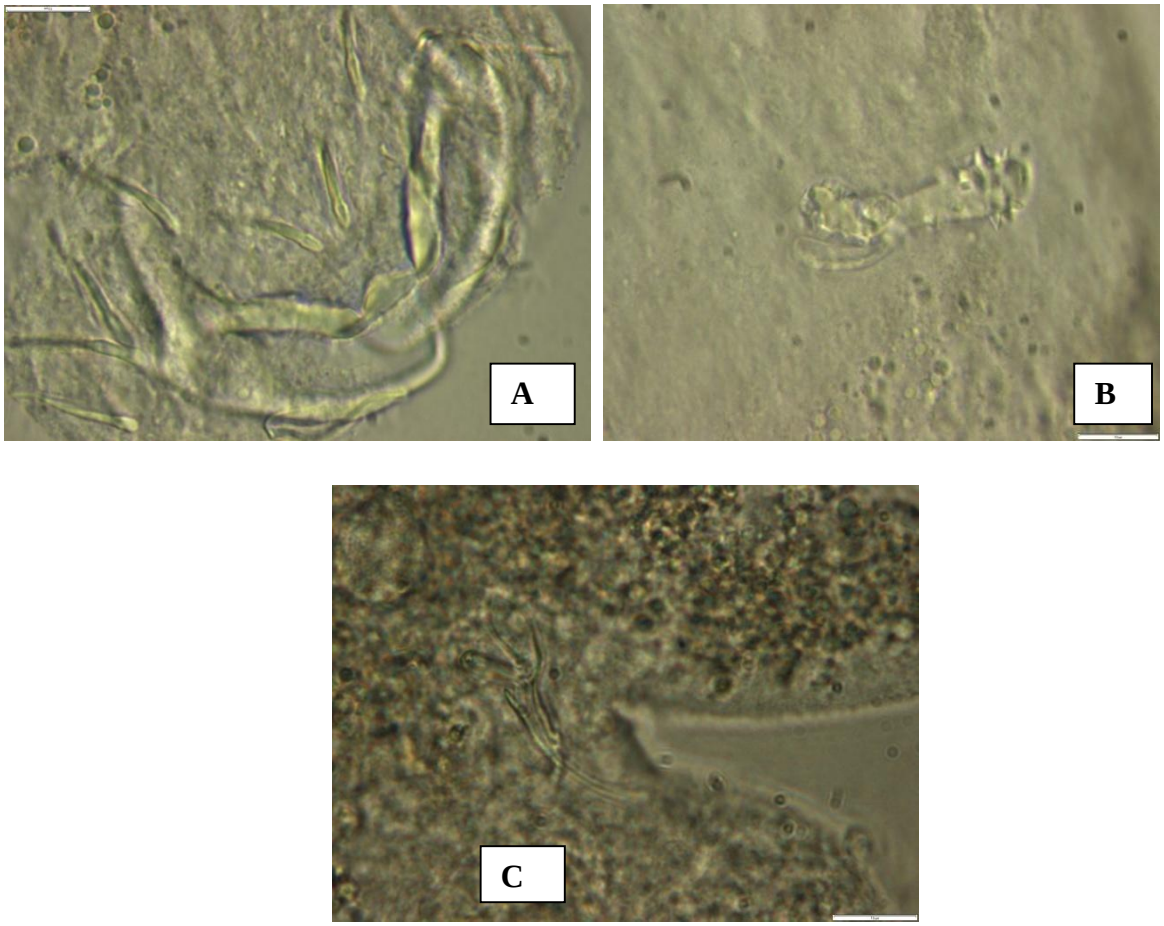
Oreochromis niloticus

Barbus paludinosus

(Source: Author, 2016)

O. leucostictus (Source: www.fishbase.org 01/2016)

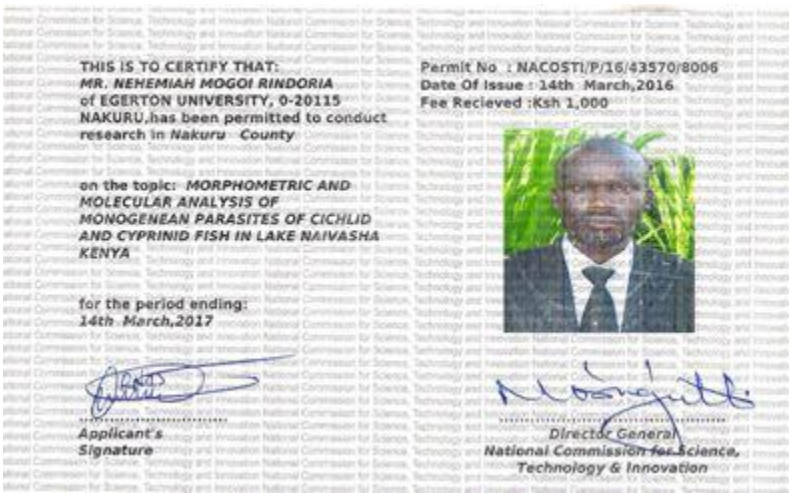
Appendix III Photos of the *Dactylogyrus hansi* sp. nov.



(Source: Author, 2016)

A (anchors, marginal hooks and bars), **B** (vagina), **C** (male copulatory organ)

Appendix IV. Research Permit



THIS IS TO CERTIFY THAT:
MR. NEHEMIAH MOGOI RINDORIA
of EGERTON UNIVERSITY, 0-20115
NAKURU,has been permitted to conduct
research in *Nakuru County*

on the topic: *MORPHOMETRIC AND MOLECULAR ANALYSIS OF MONOGENEAN PARASITES OF CICHLID AND CYPRINID FISH IN LAKE NAIVASHA KENYA*

for the period ending:
14th March,2017

.....
Applicant's Signature

Permit No : NACOSTI/P/16/43570/8006
Date Of Issue : 14th March,2016
Fee Recieved :Ksh 1,000

.....
Director General
National Commission for Science, Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

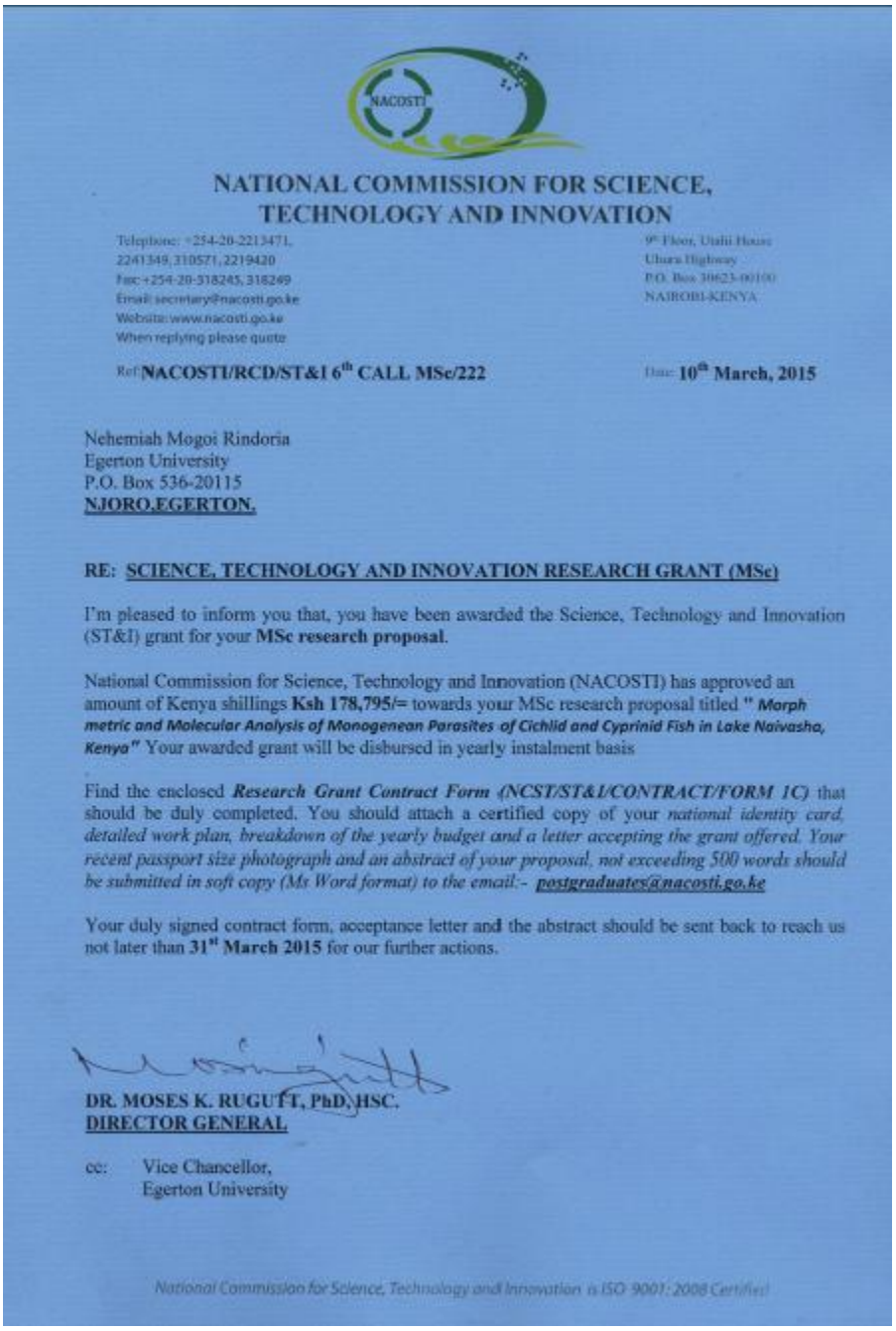
National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A 8134

CONDITIONS: see back page

Appendix V. Research funds



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Date: **10th March, 2015**

Nehemiah Mogoi Rindoria
Egerton University
P.O. Box 536-20115
NJORO,EGERTON.

RE: SCIENCE, TECHNOLOGY AND INNOVATION RESEARCH GRANT (MSc)

I'm pleased to inform you that, you have been awarded the Science, Technology and Innovation (ST&I) grant for your **MSc research proposal**.

National Commission for Science, Technology and Innovation (NACOSTI) has approved an amount of Kenya shillings **Ksh 178,795/=** towards your MSc research proposal titled **" *Morph metric and Molecular Analysis of Monogenean Parasites of Cichlid and Cyprinid Fish in Lake Naivasha, Kenya*"** Your awarded grant will be disbursed in yearly instalment basis

Find the enclosed ***Research Grant Contract Form (NCST/ST&I/CONTRACT/FORM 1C)*** that should be duly completed. You should attach a certified copy of your *national identity card, detailed work plan, breakdown of the yearly budget and a letter accepting the grant offered. Your recent passport size photograph and an abstract of your proposal, not exceeding 500 words should be submitted in soft copy (Ms Word format) to the email:-* ***postgraduates@nacosti.go.ke***

Your duly signed contract form, acceptance letter and the abstract should be sent back to reach us not later than **31st March 2015** for our further actions.

DR. MOSES K. RUGUTT, PhD, HSC.
DIRECTOR GENERAL

cc: Vice Chancellor,
Egerton University

National Commission for Science, Technology and Innovation is ISO 9001: 2008 Certified