SPREAD OF *TYLODELPHYS MASHONENSE* (DIGENEA: DIPLOSTOMIDAE) BY GREY HERON *ARDEA CINEREA* AND GREAT WHITE EGRET *A. ALBA* IN LAKE VICTORIA, TANZANIA

Fred D. Chibwana*, Gamba Nkwengulila

Department of Zoology and Wildlife Conservation, University of Dares Salaam,
P.O. Box 35064, Dar es Salaam, Tanzania,
* Corresponding author: fredchibwana@udsm.ac.tz, fredchibwana@yahoo.com

ABSTRACT

Despite the fact that Tylodelphys mashonense, parasites of the cranial cavity of the catfish Clarias gariepinus, are ubiquitous in freshwater systems, little is known on their spread. As such, we examined a total of 152 piscivorous birds, belonging to 6 species; 43 great cormorant Phalacrocorax carbo, 33 grey heron Ardea cinerea, 26 hamerkop Scopus umbretta, 22 great white egret Ardea alba, 15 marabou stork Leptoptilos crumeniferus and 13 pied kingfisher Ceryle rudis foraging in the Lake Victoria. Out of the six bird species only A. cinerea and A. alba were found infected by T. mashonense with prevalence of 42.4% and 9.1%, respectively. These findings report T. mashonense in A. alba for the first time and its occurrence in A. cinerea in Tanzania is the first record outside Zimbabwe.

Keywords: Ardeidae, *Ardea cinerea*, *Ardea alba*, intestinal digeneans

INTRODUCTION

Fish from freshwater bodies such as rivers, lakes, dams and ponds are a significant source of dietary protein to human populations worldwide. As a result, freshwater fisheries are an important economic activity for many rural and urban populations in Africa and throughout the globe. The widening gap between supply and demand for fish products, resulting both from the stagnation or decline of marine and freshwater capture fisheries has resulted in increased search for supplementary proteins as the human populations increase. Also the recent outbreaks of bird flu, bovine and pig diseases further contribute to limiting the supply of protein sources (Webster et al. 2006) which further promotes the importance of fish products. As such, most communities worldwide have responded by venturing into aquaculture to supplement capture fisheries. However, both the natural waters and aquaculture systems face a common problem of fish parasitic diseases (Paperna 1991). Diplostomiasis is one of such parasitic diseases caused by digenetic trematodes of the family Diplostomidae (Niewiadomska 1996).

Diplostomid species have a three host life cycle, which is well documented in Europe and Northern America, but not fully understood in Africa. While snails and fish serve as secondary hosts, piscivorous birds are the final hosts (Sweeting 1974; Chappell 1995; Niewiadomska 1996). Lymnaeid snails (*Lymnaea* and *Radix* spp) and planorbid snails (*Planorbarius corneus*) are the main intermediate hosts (Niewiadomska 1996, Faltýnková 2005) and fishes of different families could be infected. In Tanzania, and large part of Sub-Saharan Africa, diplostomiasis in catfish *Clarias gariepinus* is caused by the infection of *Tylodelphys mashonense* (Barson et al. 2008, Madanire-Moyo and Barson 2010, Chibwana and Nkwengulila

2010, Musiba and Nkwengulila 2006, Mwita and Nkwengulila 2008). The metacercariae of *T. mashonense* were first described from the cranial cavity of catfishes *Clarias gariepinus*, adults from the grey heron *Ardea cinerea* L in Zimbabwe by Beverley–Burton (1963) and intramolluscan stages develop in *Bulinus* species (Chibwana et al. 2015).

Since 1963, the metacercariae of *T. mashonense* have been frequently reported from *C. gariepinus* in most countries of Sub-Saharan Africa (Mashego and Saayman 1989, Moema et al. 2013), but their adults were not recorded again, even when intestinal examinations of piscivorous birds including the grey heron in Zimbabwe or elsewhere was carried out. In addition, the genus *Clarias* was reviewed by Teugels (1986), which resulted in several widespread species being synonymized i.e *C. ngamensis*, *C. melandi* and *C. capensis* of southern Africa, *C. mossambicus* of central Africa and *C. lazera* of west and north Africa under the name *C. gariepinus*. In all these fish host species, parasites similar to *T. mashonense* have been reported. However, the means of spread of *T. mashonense* and their conspecifics in Africa is not clearly understood.

In Tanzania the metacercariae of *T. mashonense* are ubiquitous in almost all freshwater bodies where *C. gariepinus* are prominent (Musiba and Nkwengulila 2006, Mwita and Nkwengulila 2008, Chibwana and Nkwengulila 2010), but their means of spread was unknown. Therefore, the abundance of piscivorous birds in almost all areas where previous diplostomid researches where conducted, coupled with lack of specificity of diplostomid species to their definitive hosts (Niewiadomska 1996) formed the basis of the present study. Thus the present study aimed to report ardeids, i.e. *Ardea alba* and *A. cinerea*, as the definitive hosts responsible for the spread of *T. mashonense* infecting *C. gariepinus* in Lake Victoria in Tanzania.

MATERIAL AND METHODS

Study area

Lake Victoria is the world's largest tropical freshwater lake, set in the interior of equatorial Africa covering an area of 68,800 sq km. It is situated at 1º N, 4º S and between longitudes 31º and 35º E at an elevation of 1134 km above sea level. The depth however is relatively shallow, approximately 40 m on average and a maximum of 79 m. Three nations share the waters of the lake - Kenya, Tanzania and Uganda, but Tanzania has the largest share (>50%). The main inflowing rivers are Nzoia, Simiyu, Kagera and Mara with several streams associated with swamps near the lakeshore within Tanzania. The shoreline length is 3,440 km with greater part of the coastline being very irregular and largely characterized by shallow bays and gulfs, especially at the northern and southern part of the lake.

Birds were collected along the shores of the Mwanza gulf, which is the largest gulf located at the southernmost end of the lake and is one of the main fishing grounds in Lake Victoria. The shape of the gulf elongates from north to south (Figure 1). The papyrus swamps occupy some parts of the gulf and the rest consists of hills sporadically covered with big granite rocks. The surface area is about 500 km² and the maximum depth is about 18 m.

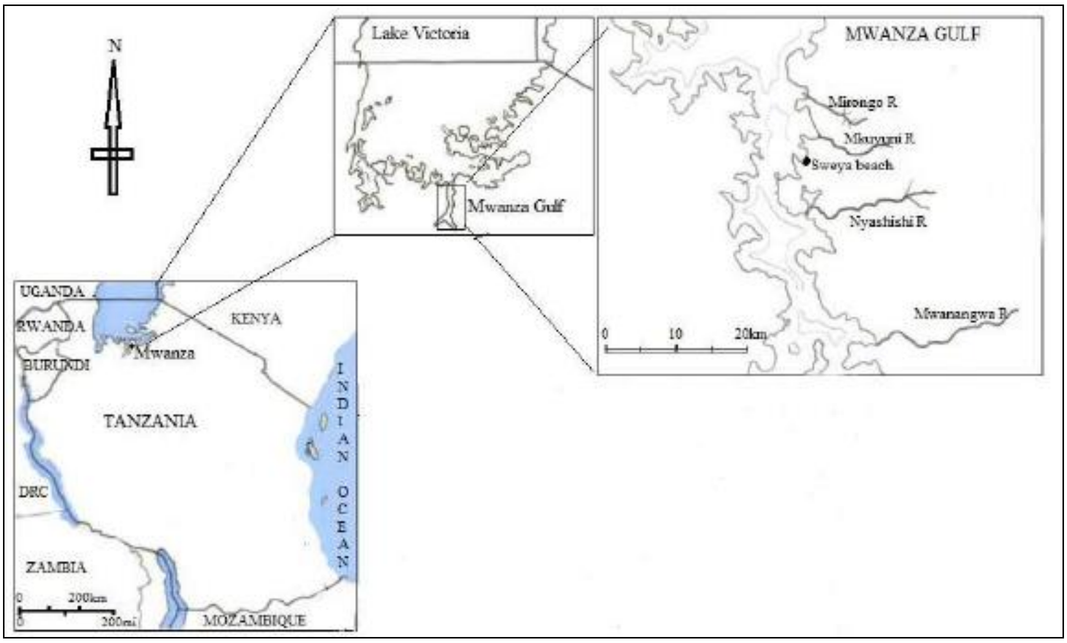

Figure 1: Map of Tanzania showing the Mwanza gulf of Lake Victoria

Collection of birds, parasite recovery and processing

Piscivorous birds, belonging to six species: great cormorant *Phalacrocorax carbo*, grey heron *Ardea cinerea*, hamerkop *Scopus umbretta*, great white egrets *Egretta alba*, marabou storks *Leptoptilos crumeniferus* and pied kingfishers *Ceryle rudis*, were collected along the shores of the Mwanza gulf by using local traps (snares and hooks) and examined for *T. mashonense* in the laboratory, at the Tanzania Fisheries Research Institute (TAFIRI). The permit to capture birds was acquired from the Ministry of Natural Resources and Tourism of United Republic of Tanzania. The necropsy examination was based on the entire digestive tract of birds, including oesophagus, stomach, gizzard and intestines covering the duodenum, jejuno-ileum caeca and cloaca. The techniques detailed by Krone (2007) were used in the collection and counting of worms. Birds handling and anesthetization followed Cooper (2004). Birds' carcasses were buried after examination. The worms intended for measurements were fixed in 70% alcohol. Preceding staining, the worms were hydrated in a decreasing concentration of alcohol 70%, 50%, 30% and finally distilled water. Whole-mount specimens were then stained in acetocarmine for 12-24 hours, dehydrated in an increasing concentration of alcohol; using 30%, 50%, 70%, 95% and followed by two changes of absolute alcohol, cleared in xylene and mounted in permount (Lunaschi and Drago, 2006). Measurements were taken with the aid of an inbuilt motic microscope camera with Motic Image Plus 2 software. Drawings for the specimens were made with the aid of a camera lucida. The specimens have been stored in the Department of Zoology and Wildlife Conservation at the University of Dar es Salaam.

The taxonomy of digenean worms is given in accordance to Dubois (1970); Yamaguti

(1971), Niewiadomska (2002); identification of birds follows a field guide by Stevenson & Fanshawe (2004) and the parasitic indices used were based on Bush et al. (1997).

RESULTS

Out of the six species examined only two species, *A. cinerea* and *A. alba*, were infected with *Tylodelphys mashonense*. From the 33 grey heron examined, 22 were infected (i.e. prevalence of 42.42%), while from 22 great white egret examined only three (3) were carrying *T. mashonense* with a prevalence of 9.1%. Intensity ranges were 2 - 246 and 12 – 18 for *A. cinerea* and *A. alba*, respectively. The other four species, namely, *P. carbo*, *S. umbretta*, *L. crumeniferus* and *C. rudis* were not infected with *T. mashonense*.

Morphological description of the *T. mashonense* recovered from grey heron

The parasites are small in size and white in colour. The worms measure 1066.1 µm long by 363 µm wide. The body is divided into fore and hind body but separation is not clearly distinct. The oral sucker is subterminal, measuring 54.3 µm x 50.6 µm, while the ventral sucker measures 47.7 µm x 53.3 µm. Pseudosuckers are present on both sides of the oral sucker measuring 98.7 µm x 24.2 µm. The pharynx is small about 45.3 µm x 32.8 µm. The oesophagus is long leading to two intestinal caeca, which bifurcate anterior to the ventral sucker running posteriorly on each side of the Brandes organ to as far as touching the genital cone. The Brandes organ is oval with a longitudinal median slit measuring 201.2 µm x 198.3 µm. The hind body is cylindrical and cone shaped containing the sex organs. The ovary is ovoid, measuring 84.4 µm x 106.5 µm, located at the integumentary boundary between the fore and hind bodies. The testes are tandem in position and lie caudally in the last third of the body. The claviform anterior testis measures 128.7 µm x 225.9 µm; while the bilobed posterior testis measures 132.8 µm x 232.4 µm, the seminal vesicle and genital cone lie behind the posterior testis. The vitellaria occur in both the fore- and hind body, and extend briefly anteriorly to the ventral sucker and posteriorly to the level of the genital cone. A few oval and operculated eggs were present.

Remarks: The material of the present study was recovered in both *A. cinerea* and *A. alba* which closely resembles the original material described by Beverley-Burton (1963) from *A. cinerea* as *Diplostomum* (*Tylodelphys*) *mashonense* (see Table 1; Figure 2). However, a comparison with material described as *D. tregenna* (Nazmi 1932), and *D. marahoueense* (Baer 1957) shows a very strong similarity in shape, size of body and shape of posterior testis. Unfortunately there is no remarkable morphological difference among these materials with the exception of size and the utilisation of different hosts (Table 1). The reproductive structures, the presence of a genital cone and an asymmetrical anterior testis, of the present specimen resemble those of *Dolichorchis lacombeensis* prompting Lunaschi and Drago (2006) to consider it as one of the species of the genus *Dolichorchis*. However the present study considers the present material as *Tylodelphys* due to molecular evidence reported by Chibwana et al. (2013).

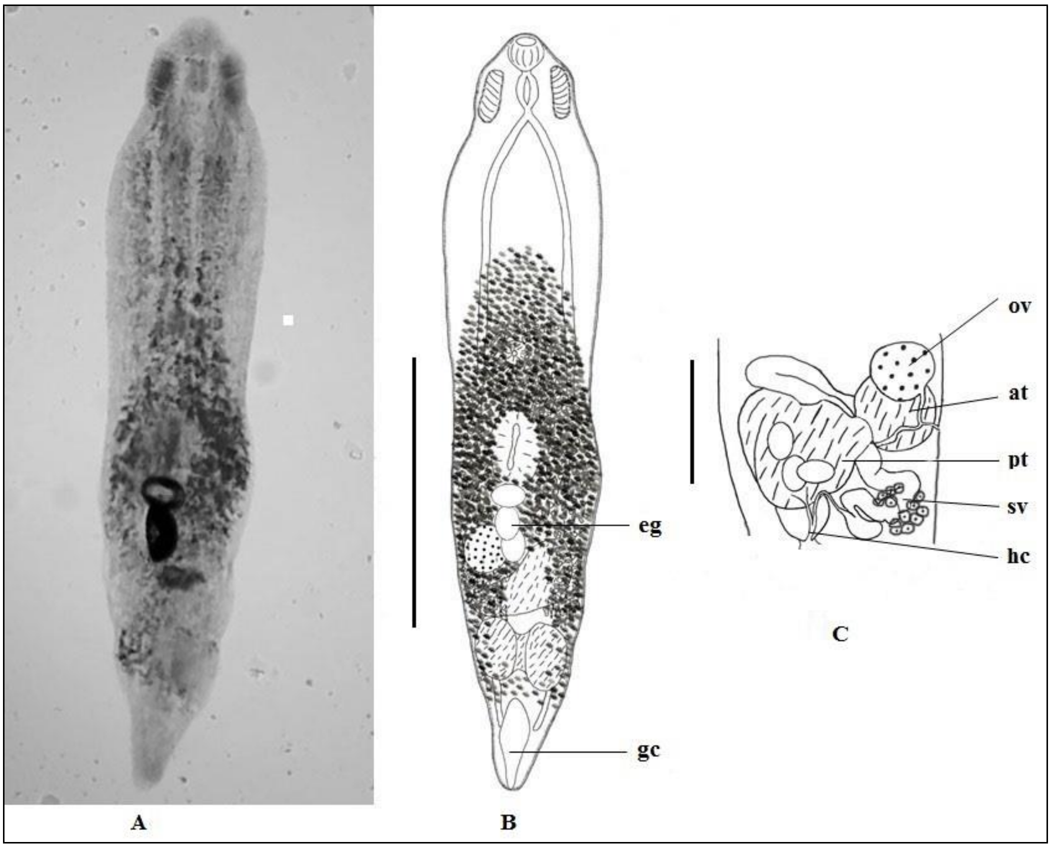

Figure 2: *Tylodelphys mashonense* from *Ardea alba*. A**.** Photo, entire worm ventral view. Scale bar = 350 μm. B**.** Drawing, entire worm ventral view. Scale bar = 350 μm. C**.** Enlarged side view of hind body showing genital organs and some genital ducts. Scale bar = 50 μm. Abbreviations: at – anterior testis, eg – egg, gc – genital cone, hc – hermaphrodite canal, ov – ovary, pt – posterior testis, sv – seminal vesicle.

Table 1: Comparison of measurements of adult *Tylodelphys mashonense* from the grey heron and great white egret and other diplostomid species described from other piscivourous birds in Africa

Reference **Parasite** **Host**	Nazmi (1932) *D. tregenna* Egyptian kite	Baer (1957) *D. marahoueense* Pel's fishing owl	Beverley- Burton (1963) *D* (*T*). *mashonense* Grey heron	Nkwengulila (1995) *T. mashonense* Chicken	Musiba (2004) *T. mashonense* Chicken	Present study *T. mashonense* Grey heron	Present study *T. mashonense* Great white egret
BL	1080-1120	1300-1550	700-960	986-1330	876-1171	876-1096	964-1271
FbL	-	850-910	380-420	546-784	531-826	511-723	630-921
FbW	520-600	-	250-350	223-451	286-390	286-390	362-491
HbL	220-230	460-590	230-560	392-558	303-429	360-510	401-522
HbW	480-500	-	120-220	241-297	238-286	146-234	332-482
OsL	90-100	110-119	52-58	39-75	29-71	47-66	59-82
OsW	90-100	100-110	47-56	46-70	29-67	49-63	59-79
VsL	60-80	55-78	43-56	46-61	38-67	52-63	62-97
VsW	60-80	96-128	53-62	46-68	25-57	51-70	64-97
BoL	160-240	-	86-130	121-223	105-152	129-192	125-173
BoW	160-240	69-105	92-173	103-251	95-200	98-201	102-250
PhL	70-90	42-82	36-46	38-57	29-57	45-58	49-68
PhW	60-70	-	25-33	42-51	19-48	43-57	38-72
O	-	-	-	304-475	248-257	302-450	341-452
OeS	30	-	25	23-70	-	30-42	33-49
PtL	230	-	78-128	70-163	95-200	102-162	101-232
PtW	450	-	138-148	121-196	143-219	112-168	144-230
AtL	160-170	-	76-84	79-153	114-152	94-138	116-161
AtW	360-380	-	155-174	70-196	133-209	140-196	134-219
OvL	60-100	114	36-65	51-98	67-152	57-86	69-172
OvW	170-200	80	51-65	51-100	76-143	51-82	78-146
Ratios							
O:BL				0.28-0.41	0.26-0.41	0.23-0.39	0.25-0.42
HbL:FbL				0.58-0.97	0.37-0.78	0.60-0.88	0.36-0.72
Os:Vs				0.55-1.52	0.36-1.68	0.79-1.41	0.35-1.58
Os:Ph				0.99-2.07	0.50-1.60	1.19-1.67	0.51-1.61

DISCUSSION

The present study is the first in Tanzania of its kind on the digenean fauna occurring in piscivorous birds, although there are many similar studies in Africa (Nazmi 1932; Beverley-Burton 1963; Ukoli 1968). The vast majority of these studies have produced a tremendous number of new species, despite the fact that the validity of some of the species is fragile (Nazmi 1932; Beverley-Burton 1963).

In the grey heron, *T. mashonense* was the most abundant species in terms of prevalence (42%) and intensity (up to 246). *T. mashonense* was reported and described for the first time in the same host, the grey heron, in Zimbabwe by Beverley-Burton (1963) as *Diplostomum (Tylodelphys) mashonense*. Since then *T. mashonense* adults have not been reported in *A. cinerea* either in Zimbabwe or elsewhere worldwide, although the grey heron have been frequently examined for trematodes (Nogueserola et al. 2002; Navarro et al. 2005). So, the present study is only the second to report the presence of *T. mashonense* in the grey heron and the first to report its adults in their natural environment outside Zimbabwe. However, *T. mashonense* metacercariae have been ubiquitously reported in the catfish *Clarias gariepinus* in Zimbabwe (Beverley-Burton 1963; Barson et al. 2008), in Tanzania (Musiba and Nkwengulila 2006, Chibwana and Nkwengulila 2010, Mwita and Nkwengulila 2010) and South Africa (Moema et al. 2013). As such, it can be surmised that the grey heron is responsible for the abundance and spread of *T. mashonense* metacercariae in catfish in freshwaters of Africa.

In the present study, *T. mashonense* have also been recorded in the great white egret. Despite the fact that the surveys for trematodes in egrets is commonplace worldwide (Poulin and Latham 2003, Sitko et al. 2006, Abd-Al-Aal et al. 2008, Drago 2011, Sitko 2012), there are no records of *T. mashonense* in egrets. With this regard this study reports *T. mashonense* in *A. alba* for the first time. However, the prevalence (9.1) and intensity (2 to 18) are relatively lower than those reported from the grey heron (42.42% and 2-246, respectively), suggesting that great white egret may not be a common or very suitable host for *T. mashonense*. This findings corroborate the statement that if other host species (larger taxon) are available, parasites would try to expand their chances of transmission by colonizing those new host species, in which case they have to adapt physiologically and morphologically to overcome hosts' defenses (Poulin and Mouillot 2004). It is, therefore, more likely that the great white egret is not a common host to *T. mashonense*, and it could be a trial to widen the host range. As a consequence, they have not been as successful as they are in the grey heron.

The lack of *T. mashonense* in other trapped and examined birds could be explained by both ecology and physiology. For instance cormorants, kingfishers and hamerkops feed in open waters unlike egrets and herons, which feed in wetlands or in edges of water bodies (Willard 1985). Since *T. mashonense* matures as metacercariae in catfish (Chibwana and Nkwengulila 2010), which prefers shallow and swampy areas with a soft muddy substrate (Mbalassa et al. 2015) favours the ardeid feeding behaviour. Marabou stork on the other hand, besides feeding fish in shallow waters is a scavenger. In the present study marabous have been found feeding in human garbage. Thus the results may have been influenced by the toxins developed in the alimentary tract killing not only *T. mashonense* but also other intestinal parasites. The authors observed that other bird species namely hamerkop, cormorant and kingfisher had other parasites instead of *T. mashonense*, but marabou storks were free of parasites.

In conclusion, the present study has shown that piscivorous birds under the family Ardeidae are responsible for the distribution of *T. mashonense* in freshwaters of Africa. It is particularly possible because the present study was only able to catch *A. cinerea* and *A. alba*, suggesting that other ardeids could as well facilitate the wide range of *T. mashonese*, the parasite of the cranial cavity of the catfish *Clarias gariepinus*, in Africa. Despite the fact that Tanzania, being in the tropics, is reach in biodiversity birds included, this study is the only one so far to examine intestines of birds for parasites. As such the present study recommends that more similar studies should be carried out extensively to recover more potential hosts and/or determine the distribution of the already known species responsible for reducing the efficacy of fishes in aquaculture systems.

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