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Dolops ranarum from *Oreochromis esculentus* and *Protopterus aethiopicus* in Lake Kanyaboli, Kenya: Additional Information Using Scanning Electron Microscopy

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ABSTRACT

Fish parasites are often overlooked, yet they can have detrimental effects on both capture fisheries and aquaculture, leading to significant economic loss. Heavy infestation of external parasites on the fins, skin and gills of fish may lead to pathogenic effects or may create a suitable environment conducive to secondary infections. This study, conducted in Lake Kanyaboli from January to March 2024, aimed to identify the ectoparasites of the endemic species *Oreochromis esculentus* and *Protopterus aethiopicus*, and asses their infection levels and diversity. A total of 60 specimens of each host species were examined for the presence of ectoparasitic crustaceans. The parasites collected were subjected to morphological examination using scanning electron microscopy (SEM). The infection (prevalence and mean intensity) and diversity (Margalef richness, Shannon-Wiener and Simpson's) levels were calculated. Morphological analysis identified the branchiuran (*Dolops ranarum*). A prevalence rate of 76.7% was recorded for *D. ranarum* from *O. esculentus* and 90% from *P. aethiopicus* with a mean intensity of 1.39 and 1.59, respectively. The parasite diversity indices of *D. ranarum* were relatively diverse in *P. aethiopicus*, but dominant in *O. esculentus.* SEM observations revealed additional taxonomic features which have not been previously described. In addition, the study provided an updated taxonomic key to *Dolops* species and extended the geographical locality of *D. ranarum* to Kenya.

INTRODUCTION

Fish parasites are often overlooked, yet they can have significant pathological effects that increase fish susceptibility to secondary infections, leading to substantial economic loss in both capture fisheries and aquaculture **(Tavares-Dias & Martins, 2017)**. Parasitic crustaceans are macroscopic ectoparasites that form a large and diverse group, and they can be found on both fish and amphibians. They generally belong to

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Branchiura, Copepoda or Isopoda. The Branchiura comprises four genera including *Dolops* Audouin, 1837 which is well known for its tolerance to adverse environmental conditions **(Møller, 2009)**. The genus *Dolops* was first mentioned by Audouin in 1837 with *D. lacordairei*, though it is considered a nomen nudum due to the lack of description or illustrations. This was followed by the descriptions of *D. kollari* and *D. longicauda* by Heller in 1857, and *D. doradis* by Cornalia in 1860, which was later synonymized with *D. longicauda*. Bouvier described *D. geayi* in 1897, and in 1899, he added *D. bidentata*, *D. discoidalis*, *D. reperta*, and *D. striata*. Stuhlmann described *D. ranarum* in 1892. Later, Lemos de Castro described *D. carvalhoi* in 1949 and *D. nana* in 1950. Fryer described *D. tasmanianus* in 1969, and da Silva added *D. intermedia* in 1978. Finally, **Walter and Boxshall (2021)** confirmed *D. lacordairei* as a nomen nudum and provided updated taxonomic information. It is worth noting that *D. ranarum* is a unique species which has been widely reported from the African continent **(Avenant & Van As, 1985; Avenant *et al.*, 1989)**.

Members of the genus *Dolops* are considered as major ectoparasites of epidemiological interest in fisheries and fish farms worldwide **(Morey & Arellano, 2019)**. Some potential negative impacts of fish parasites include blood loss, reduced fecundity, retarded growth, and consequently poor productivity **(Hetch & Endemann, 2007)**. Their occurrence has been linked to some factors such as poor environmental conditions and pollution in natural water bodies or fish ponds **(Sures & Milen, 2022)**. Studying freshwater fish parasites is crucial for both ecosystem health and human well-being **(Murrell & Fried, 2007)**. Understanding the types and morphology of these parasites is essential for accurate diagnosis, identification, and the development of effective eradication strategies. While several reports on fish parasitology have been published from various water bodies in Kenya **(Aloo, 2002; Otachi *et al*. 2014; Rindoria *et al*. 2016, 2020, 2023; Kibet *et al*. 2019; Maraganga *et al*. 2024a, b; Rindoria, 2024)**, studies focusing on fish parasites in small lakes and reservoirs, such as Lake Kanyaboli, remain limited. Therefore, this study aimed to identify and determine the infection levels and diversity indices of ectoparasitic crustaceans affecting the endemic *O. esculentus* and *P. aethiopicus* in Lake Kanyaboli, Kenya.

MATERIAL AND METHODS

Study area

This study was carried out in Lake Kanyaboli of the Lake Victoria Basin of Western Kenya (Fig. 1). It is the largest freshwater oxbow lake in Kenya and second largest in Africa. It covers an area of 10.5km^2 with a mean depth of about 3 meters. The lake plays an important economic role to the local community in terms of provision of water for agriculture and domestic uses. This lake harbors endemic fish species including

Oreochromis niloticus, *Haplochromis* spp., *Clarias gariepinus*, *O. esculentus*, and *P. aethiopicus*, which support artisanal fishing activities.

Ethical standards

A research permit was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) with licence number NACOSTI/P/24/32294 and ethical clearance from the Kisii Teaching and Referral Hospital Institutional Science and Ethical Review Committee (KTRH ISERC) approval number ISERC/KTRH/025/23.

Sample collection and examination

A total of 120 fish specimens,comprising sixty individuals of each species (*O. esculentus* and *P.aethiopicus*) were collected from Lake Kanyaboli from January to March 2024. They were caught using gill nets of mesh sizes 2, 3, 4 and 5 inches. Identification of sampled fish was carried out following the guidelines of **Skelton (2001)** and **Okeyo and Ojwang (2015)**. Fish were temporarily held in aerated tanks filled with water from the lake. They were ethically killed through cervical dislocation following the methods of **Gupta and Mullins (2010)**. The skin, fins and gills were examined for ectoparasites using a LEICA EZ4 stereo microscope (Leica Microsystems, Heerbrugg, Switzerland). All the parasites recovered were fixed in 70% ethanol for scanning electron microscopy (SEM).

Scanning electron microscopy

The ethanol-fixed specimens were prepared following the method of **Dos Santos and Avenant-Oldewage (2015)** and were dehydrated in ascending series of ethanol following the timings recorded in **Rindoria *et al*. (2023)**. The specimens were then mounted on a strip of carbon conductive tape fixed to a microscope slide, dried in a portable glass desiccator, coated with gold using an Denton Vacuum Desk V sputter coater (Quorum Technologies, Newhaven, U.K.), and examined using a Zeiss Sigma 560VP scanning electron microscope (Zeiss, Jena, Germany) at 6kV.

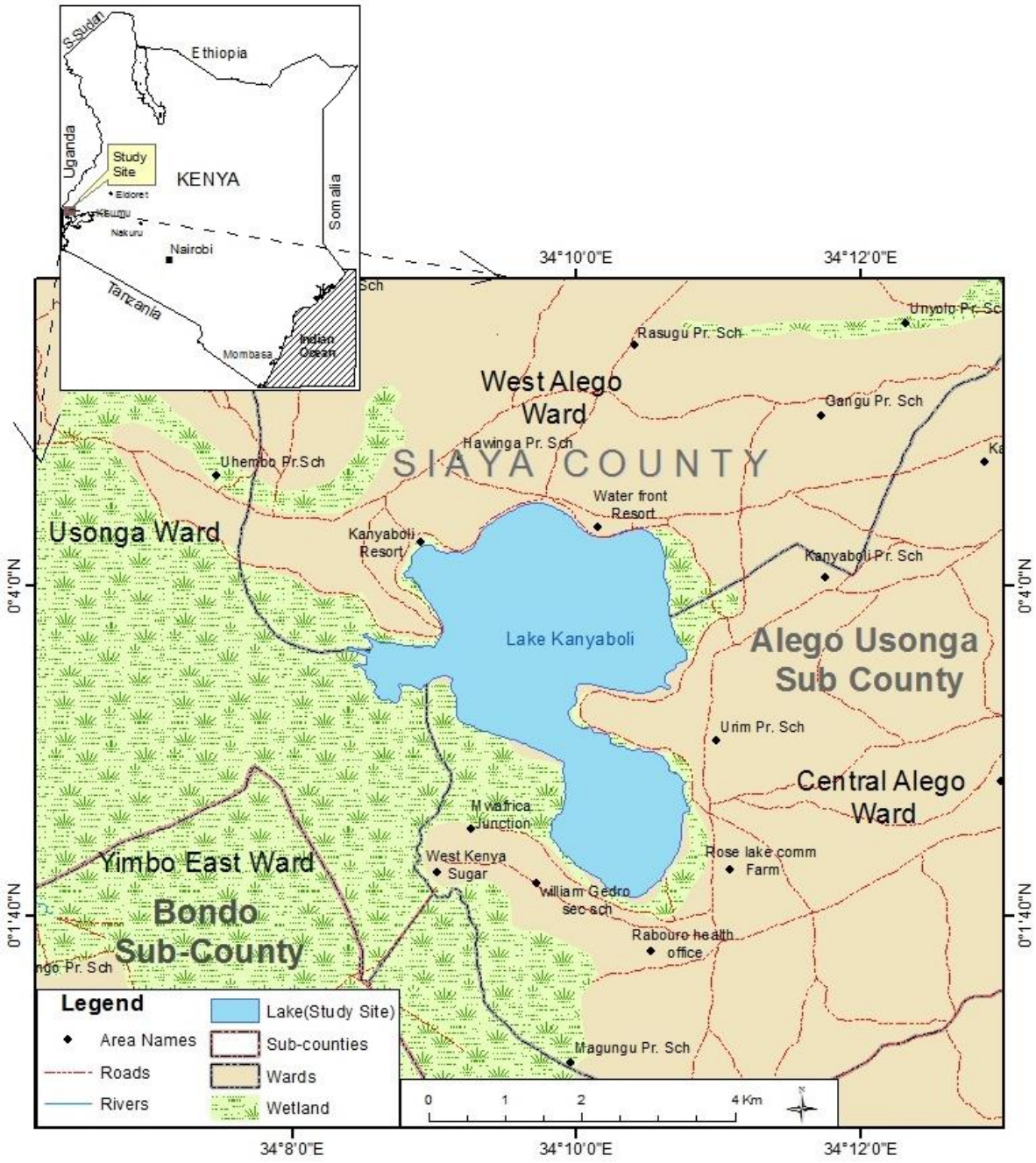

Fig. 1. A map showing the study area, Lake Kanyaboli in Siaya county, Kenya

Data analyses

Infection levels (prevalence, mean intensity) of parasites on fish hosts were determined according to **Bush *et al.* (1997)**. The analysis of the parasite community structure of *O. esculentus* and *P. aethiopicus* were calculated using various formulae. Shannon-Wiener index was calculated according to the formula of **Shannon (1948)** as follows:

$$Shannon - Wiener\ index\ (H') = -\Sigma\ pi \times ln(pi) \quad \text{.....i}$$

Where, Pi is the proportion of each parasite species in the samples collected, and ln is the natural logarithm.

Simpson's index was calculated according to the formula of **Simpson (1949)** as follows:

$$Simpson's\ index\ (D) = \Sigma ni(ni-1)/N(N-1) \quad \text{.....ii}$$

Where, N is the total number of all parasites collected and ni is the number of parasites per taxa.

The Margalef richness index was calculated using the formula of **Margalef (1958)** as follows:

$$Margalef\ richness\ index = (S-1)\ /\ Log\ (n) \quad \text{.....iii}$$

Where, S is the total number of taxa recorded, and n is the total number of parasites recovered.

RESULTS

A total of 150 *D. ranarum* specimens were recovered; 64 from *O. esculentus* and 86 from *P. aethiopicus*.

Taxonomic summary

Dolops ranarum Stuhlmann, 1892.

Type host: Tadpole.

Type locality: Bukoba Tanzania.

Other hosts: *O. esculentus* and *P. aethiopicus* (present study).

Locality: Lake Kanyaboli, Kenya (present study).

Sites of infection: Buccal cavity, gill chambers, fins, and skin.

Material studied: 150 specimens for morphometric of which 6 were for SEM.

General description (Based on 6 SEM specimens): Body divided into three parts: cephalon, thorax, abdomen (Fig. 2A). Cephalon and thorax fused but easily distinguishable. Cephalon bears fully fused segments, almost round, with a deep incision posteriorly appearing ridged. Dorsally covered by a tough protective carapace bears a wide incision that exhibits the the median dorsal surface of thorasic segments (Fig. 2A). The cephalon bears antennulae, antennae, maxillulae, maxillae and mandibles (Fig. 2B). Some specimens exhibited epibiont protozoan peritrichs (Fig. 2C). Two sharp spines at basal part of antennulae and antennae with sharper distal spine. Small unsegmented structure with setae on terminal end between antennulae and antennae (Fig. 2D). Antennae lie immediately after antennules and made up of five cylindrical segments. First and second segments short; third and fourth segments longer and slender. Setae on the fourth segment longer than those on the fifth; the latter is the smallest. Proximal end of antenna bears setae and single solonidium (Fig. 2E). Mouth bears a pair of laterally located maxillulae, each of four segments, end with two segmented heavily sclerotized hooks and a small protuberance at the base. Proximal segments cylindrical, non-sclerotized, and tapers distally. Maxillulae are sharp and curved (Fig. 2F). Maxillae locate

posterior to maxillulae with 6 leg segments of different shapes and sizes. Basal plate with 3 spines, two at the proximal end (sharper and more slender) and one at the distal end (shorter and blunt). Setae packed at the basal podomere above the middle spine, some on third and fourth podomeres (Fig. 2G). A single solonidium terminal end (Fig. 2G). Each segment bears 2 smaller distal and 2 larger proximal seta (Fig. 2G). Fifth podomere with eleven setae extending beyond the sixth podomere(Fig. 2G, H).

Thoracic region has four distinguishable segments, each bears a pair of swimming legs (Fig. 2J). The leg is formed of a coxopodite, basipodite, endo- and exopodite. The fully developed flagellum on the upper surface of the basipodite of the first 2 pairs of legs, with the flagellum on the second pair being longer than those of the first pair of legs. Serrated setae scatter on opposite sides of the flagellae, endopodites, and exopodites of all the legs. Setae also are present on the postero-ventral side of the coxo- and basipodites of all the four pairs of legs (Fig. 2J). Legs bear ventrally located small protrusions. Genital pores covered by natatory lobes ventrally located on the coxopodites of the fourth legs (Fig. 2J).

The abdominal segment with 2 bean-shaped lobes anteriorly coalesced with the fourth thoracic segment; fusion visible when carapace lobes displaced. Abdomen engraved for two-thirds of its length with a conspicuous pocket-shaped sinus (Fig. 2K). Dorsally fusion of the abdominal lobes extends further posteriorly to form a visible triangular anal sinus ventrally. Anus located in minute corner of isosceles sinus. Furcal rami located between edges of the abdominal sinus (Fig. 2K).

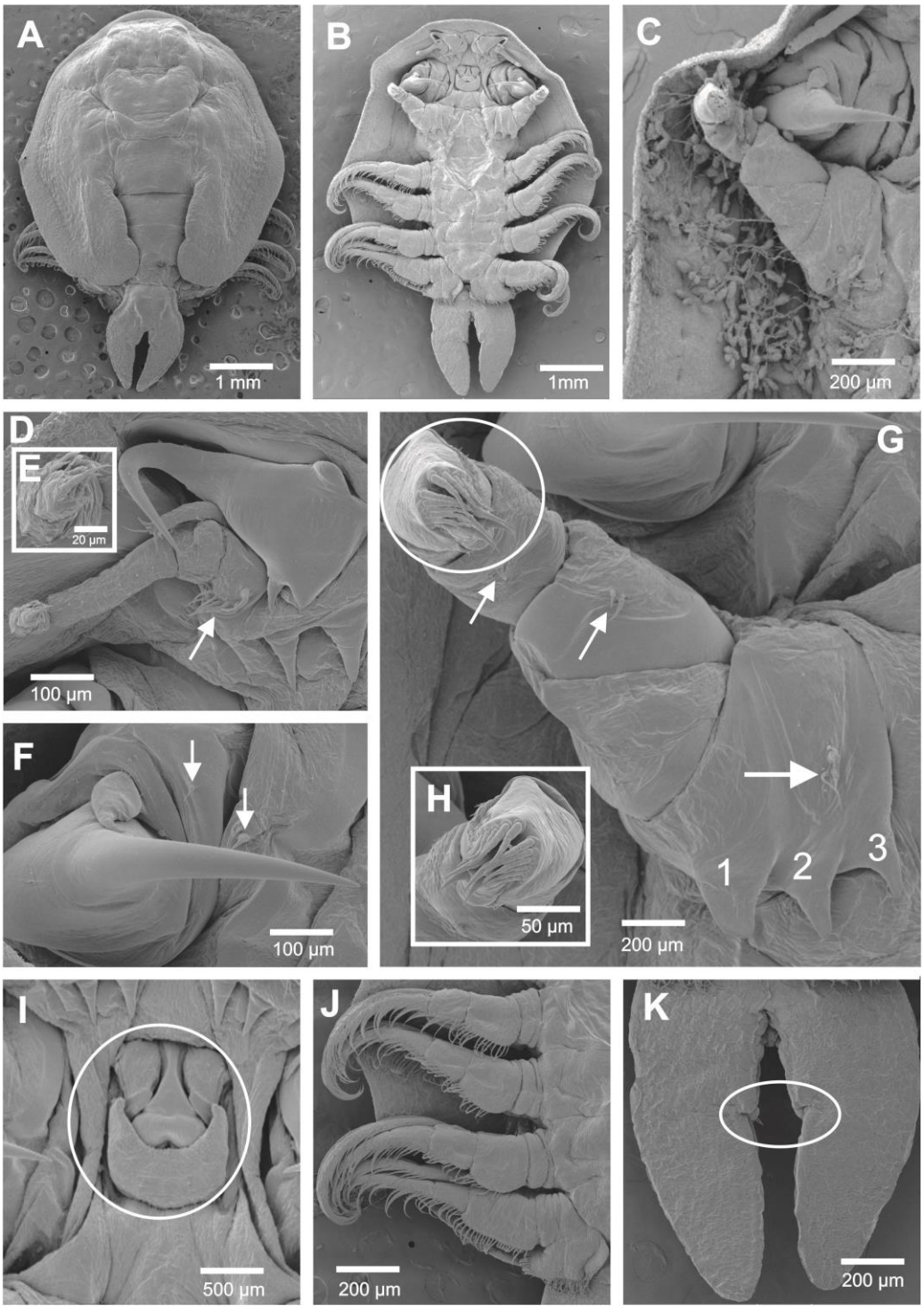

Fig. 2. Scanning electron micrographs of *Dolops ranarum* (Stuhlmann, 1892) from the external surface of *Oreochromis esculentus* Graham, 1928 and *Protopterus aethiopicus* Heckel, 1851 in Lake Kanyaboli, Kenya. **A**: Dorsal view with cephalone and thorax covered by the carapace, **B:** Ventral view, **C**: epibiont protozoan peritrichs below the carapace of an adult female, **D:** Right antennule,and antenna, each with two basal spines and a small cuteneouse projection in between, (white arrow shows group of seta on basal segment of antennae) **E:** Proximal end of right antenna, **F:** Right maxillule and a small protuberance at the

base (shown by the two white arrows), **G:** Right Maxilla(3 spines on basal plate labelled 1,2,3; each segment bears some setae dorsally shown by three white arrows); **H:** Proximal podomere of the maxillae, **I:** Mouth part showing mandibles shown in circle, **J:** Legs frined with flagellae, **K:** Abdominal sinus.

Remarks: The present study presents additional taxonomic features not previously recorded on some structures which includes: (i) Antennule: A small projection bearing a seta medially occurs medio-ventrally on the basal segment. It consists of cutinized material bearing 6 terminal setae with 1 longer than the rest; (ii) Antennae: 11 setae of almost the same size on the lateral-ventral side of the first segment 1 seta laterally on second segment. Segment 3 longest. Fourth and fifth segments terminate with 7 setae. (iii) Maxillulae: 2 setae and 1 seta laterally on first and second segment, respectively. Setules on the inner margin of the third segment; (iv) Maxillae: A set of 4 setae on the basal plate (first podomere) above the middle spine. 2 lateral setae on the third podomere. Numerous minute setae on the lateral surface of fourth, fifth and sixth segments with openings. A single ventral seta on the fourth and 2 setae on the fifth segment. The sixth segment terminates with 4-spine like setae and 12 similar sized setae, with 2 small setae facing the lateral side; (v) Mandibles: bearing oral apparatus between the bases of the maxillulae, with lateral duadrant-shaped structures; (vi) Third swimming leg: Flagellum longer than the rest; (vii) Furcal rami: Each terminates with a seta situated in the angles on the margin of the abdominal sinus.

Updated identification key for the *Dolops* species

The present study provided an updated taxonomic key to the 12 nominal species of Dolops. The key of **Suárez-Morales (2020)** was adopted and expanded to include *D. ranarum* and *D. tasmanianus* (Fryer, 1969). The information updated is presented in bold.

1a. Carapace ventral surface with spines; first maxilla (**maxillulae**) terminal hooks with long, conspicuous adjacent appendage 2

1b. Carapace ventral surface without spines; first maxilla (**maxillulae**) terminal hooks without long adjacent appendage, or if present it is small 7

2a(1) Second maxilla with three teeth (**spines/denticles)**..... 3

2b. Second maxilla with two teeth **(spines/denticles)**; major respiratory area with lateral minor area…..... *Dolops bidentata* (Bouvier, 1899) [French Guiana, Brazil]

3a(2) Carapace lateral areas with submarginal spines arranged in a single row, or absent 4

3b. Carapace lateral areas with submarginal spines arranged in more than one row, irregularly distributed; abdominal lobes length subequal to width; first maxilla (**maxillulae**) hook.....*Dolops reperta* (Bouvier, 1899) [French Guiana]

4a(3) Carapace width length, discoid 5

4b Carapace width < length; first maxilla (**maxillulae**) hooks……...*Dolops kollari* (Heller, 1857) [Brazil]

5a(4) Carapace lateral areas with posterior margin lacking submarginal row of ventral spines; respiratory areas not as below; second maxilla (**maxillae**) teeth (**spines/denticles**) unequal, external tooth (**spine/denticle**) of different size than remaining two..... 6
5b. Carapace lateral areas posterior margin with submarginal row of ventral spines; major respiratory area with two notches; carapace discoid; first maxilla (**maxillulae**) acute; second maxilla (**maxillae**) teeth (**spine/denticle**) rounded, subequal, with row of small spinules ..... *Dolops discoidalis* (Bouvier, 1899) [Venezuela, Brazil, Bolivia]
6a(5) First maxilla (**maxillulae**) appendage blunt, smooth; second maxilla (**maxillae**) teeth (**spines/denticles**) with external tooth (**spines/denticles**) size ~ 2x remaining two.... *Dolops striata* (Bouvier, 1899) [Venezuela, French Guyana, Brazil. Bolivia. Paraguay, Argentina]
6b. First maxilla (**maxillulae**) appendage with spinules; second maxilla (**maxillae**) teeth (**spines/denticles**) with external tooth length < remaining two; carapace widely rounded ..... *Dolops intermedia* (da Silva, 1978) [Brazil]
7a(1b) Second maxilla (**maxillae**) with three teeth (**spines/denticles**) 8
7b. Second maxilla (**maxillae**) with two teeth (**spines/denticles**), both acute, ... *Dolops nana* Lemos de Castro, 1950[Brazil]
8a. First and second pair of swimming legs bear fully developed flagellum……..…. 9
8b. First, second and third pairs of swimming legs with fully developed flagellum...10
9a. Furcal rami situated on the angles formed on the margin of the abdominal sinus, terminating with 1 seta……...……*D. ranarum* Stuhlmann, 1892
9b. Furcal rami small but distinct and terminating in 5 setae……. *D. tasmanianus* (Fryer, 1969).
10a(8) Carapace rounded; first three swimming legs with flagellum .....…11
10b. Carapace elliptical, abdomen acuminate, longer in male than in female; first two swimming legs with flagellum; second maxilla (**maxillae**) teeth (**spine/denticle**) robust, flat, one smaller than other two.....*Dolops geayi* (Bouvier, 1897) [Venezuela, French Guyana, Paraguay, Argentina]
11a. (10) Abdomen length > carapace; abdominal lobes, lanceolate, acuminate, first maxilla (**maxillulae**) hook robust, acute, curved..... *Dolops longicauda* (Heller, 1857) [Ecuador, Brazil, Paraguay, Argentina, Uruguay]
11b. Abdomen length ~ ¼x carapace; abdominal lobes not acuminate..... *Dolops carvalhoi* Lemos de Castro, 1949 [Brazil, Bolivia]

Infection and diversity indices

Results of the infection and diversity indices are provided in Table (1).

Table 1. Infection and diversity indices of *Dolops ranarum* from *Oreochromis esculentus* and *Protopterus aethiopicus* from Lake Kanyaboli

Infection/diversity indices	*O. esculentus*	*P. aethiopicus*
Prevalence (%)	76.7	90
Mean intensity	1.39	1.59
Shannon-Weiner	0.37	0.89
Simpson Dominance	0.78	0.46
Margalef richness	0.23	0.4

DISCUSSION

In this study several specimens of *D. ranarum* were recovered from both *O. esculentus* and *P. aethiopicus* in Lake Kanyaboli, Kenya. These findings confirm the reports that *D. ranarun* is endemic to Africa, and it infests several freshwater fish species across the continent including *Clarias griepinus*, *Oreochromis mossambicus* **(Avenant & Van As, 1985; Avenant *et al*., 1989; Ibraheem, 1998; Møller 2009)**. An initial study had reported the occurrence of *D. ranarum* on tadpoles **(Stuhlmann, 1892)**.

Dolops ranarum, like most of the members in the genus *Dolops* presents diverse morphological features which are unique and useful for species identification. This study agrees in most aspects with the first description of *D. ranarum* given by **Stuhlmann (1892)** and the subsequent redescriptions. However, based on the redescriptions detailed by **Avenant *et al*. (1989)**, keen examination of *Dolops* specimens from Lake Kanyaboli revealed additional information on the morphological features of various body structures. These include: the antennule which had 6 terminal setae with 1 being longer than the rest and the antennae with 11 setae on the lateral-ventral part of the first segment and 7 setae terminating on the fourth and fifth segments. The study was able to account for the number of setae on other structures such as maxillullae and maxillae as provided in the remarks. These details had not been provided in previous studies. Additional observations include oval shaped epibiont protozoan peritrichs beneath the carapace (Fig. 2C). This feature had not been previously reported in literature and form an interesting area for future observations of *D. ranarum* life strategy. Others include the mandibles bearing oral apparatus between bases of maxillulae, with the laterally located duadrant-shaped structures (Fig. 2I); a developed flagellum on the first three swimming legs where the third leg bears the longest (Fig 2J); furcal rami terminate with a seta (Fig. 2K). Despite the additional information, the specimen had the principal identification features which

were documented by **Avenant *et al*. (1989)**. To date, 12 nominal species of *Dolops* are known, but an identification key by **Suárez-Morales (2020)** accounts for only 10 species. Therefore, this study uses the additional morphological information using SEM on *D. ranaram* to distinguish the species from *D. tasmanianus* (Fryer, 1969) which had not been included in the key.

Although not all the fish samples collected form Lake Kanyaboli were infested by *D. ranarum*, the infection levels and diversity indices were consistently different between the two species investigated. Both the prevalence level and mean intensity of the parasites was higher in *P. aethiopicus* than *O. esculantus*. The differential trend could be attributed to the host specificity preferences and vulnerability chareteristics. In early parasitological surveys conducted on 25 fish species in South Africa **(Avenant & Van As, 1985)**, only eight of the sampled species were infected by *D. ranarum* with prevalence ranging from 4 to 50%. Another study had observed the influence of seasonality on the occurrence of *D. ranarum* with higher prevalence occurring in warmer seasons **(Avenant & Van As, 1985)**. Lake Kanyaboli, is a small equatorial water body in the basin of Lake Victoria and usually warm (17^{o}C-29^{o}C) throughout the year, a factor that could help explain the high infestation levels observed in this study. Analysis of diversity indices for *Dolops* revealed that the parasite was relatively diverse in *P. aethiopicus*, but dominant in *O. esculentus*. Epidemiologically, the diversity and richness of parasitic species are associated with their ecological evolution, abundance and habitat ranges of the host, their life cycles characteristics and their reproductive seasons **(Morand, 2015)**. The two host species are endemic to the study area. Therefore, their population dynamics is a key factor that might influence the prevalence and diversity of *D. ranarum* in that ecosystem.

CONCLUSION

Dolops ranaram is a prevalent ectoparasite infesting *O. esculentus* and *P. aethiopicus* in Lake Kanyaboli. Some of the morphological features recorded on *D. ranaram* provide insightful data for its accurate identification. Specifically, the location of flagellum on the swimming legs and the structure of furcal rami, which terminate with a single seta, are unique characteristics that distinguish *D. ranaram* from *D. tasmanianus* and other *Dolops*.

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