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Short Communication

First record of plerocercoid larvae belong to the order Trypanorhyncha (Diesing 1863) isolated from swordfish (*Xiphias gladius*, Linnaeus 1758) captured off Sri Lanka

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Abstract Parasitism leads to severe economic losses to marine fish exports in Sri Lanka. Swordfish is a seasonal species with high demand both locally and internationally. Presence of a parasite in their flesh confuses the suppliers with zoonotic *Anisarkis* spp. This study is aimed to identify the plerecercoid parasitic larvae found in swordfish and establish a feasible method of diagnosis at fish processing factories. Parasites buried in frozen swordfish (*Xiphias gladius*) muscles were isolated and stored in cold sea water. Samples were washed with normal saline water and observed under bright field light microscope at 40x and 100x magnifications, and the parasites were identified based on their morphological features. For further confirmation, confocalmicroscope was used to record scolex parameters. Total length of the parasites was 1.7 cm– 3.5 cm (2.27 cm). Presence of the elongated, acraspidote scolex (length 6.23 mm, width at *pars bothridialis* 2.4 mm) with four tentacular armature and curved, apically inverted, thick edged bothridia confirmed the parasitic larvae belonged to the Order Trypanorhyncha. Corona of falciform hooks in the basal armature and the heteromorphous hooks in a half spiral arrangement at metabasal armature are characteristics of the genus *Molicola*. This is the first record of the Trypanorhynch plerocercoid larvae (genus:*Molicola*) isolated in swordfishes from Sri Lanka. This study emphasizes that morphological examination of the parasite scolex using bright field microscopy will be helpful to distinguish the parasitic genera and feasible to use in the fish processing factories.

Keywords: Trypanorhyncha, Molicola, Swordfish, scolex, cestode, parasite

INTRODUCTION

Capture fisheries is a major source of protein for the majority of the people in the world. As an island situated in the Indian Ocean, Sri Lanka has a long history of capture fisheries. Fisheries sector in Sri Lanka has a great impact on the economic and social life contributing about 1.8% to the gross domestic production (GDP) and earns over US$ 94.3 million with a production over 0.4 million tonnes of fish annually (Anon. 2015). The quality of the captured fish is crucial to maintain a sustainable market. Although improving post-harvest practices can upgrade the quality of fish, infestation with parasites is an inevitable cause for their rejection by the consumers.

In marine helminth parasites, the intermediate and definitive host specificity are generally lacking. Most of these parasites have a life cycle with very long larval stages within intermediate hosts. The formation of parasitic colonies is based on the food web and the distributional pattern of the marine fish can be predicted by the abundance of parasites (Marcogliese 2002). Many consumers concern about the parasites in fish because of the potential zoonotic species. The most abundant zoonotic parasite is a nematode belonging to the family

Anisarkidae. Other types of parasites infecting humans are *Diphyllobothrium* spp. and digenic trematodes *Heterophyidae, Opisthorchiidae* and *Nanophyetidae* (Adams et al. 1997). In addition to zoonotic parasites, most of the other helminths such as cestodes infect muscles of the fish causing huge economic losses. Order Trypanorhyncha is one of the well-studied cestode species which has intermediate larval stages affecting economically important marine fish. During the course of the life cycle they pass through at least three host species as procercoid, plerocercoid (plerocercus or merocercoid in some species) and finally the adult stage in sharks (Palm and Caira 2008).

Swordfish (*Xiphias gladius* L. 1758) is one of the high-valued fish species exported from Sri Lanka. Presence of an unidentified parasite raises concerns of the fish exporters and the diversity of marine parasites lead to study the organism scientifically. It is time consuming and expensive to send samples to diagnostic laboratories to identify the species by molecular methods. Therefore, the current study was carried out with an intention to identify the parasites present in swordfish by morphological examination and to establish a feasible diagnostic method to perform at fish processing factories.

MATERIALS AND METHODS

Sample collection

Parasitic larvae were extracted from swordfishes (*Xiphias gladius*) that were captured from the FAO zone 57 of the Indian Ocean by multi-day boats and received at frozen state from a fish processing company. The extraction of larval parasites was performed manually by carefully pulling them off from the frozen fish muscles. Nearly 100 parasites were isolated, stored in cold sea water and transported to the laboratory immediately at a storage temperature of 4ºC.

Morphological examination

Parasitic larvae were washed in normal saline water and examined under the light microscope at x40, x100 and x400 magnifications. Out of all collected specimens, 20 larval parasites with scolex were selected for further examination. Samples without scolex or partially damaged scolex were excluded from further investigation. Parasites fixed in 10% formalin were observed for scolex morphology under the bright field microscope (Olympus, CX22LED, USA) and one parasite with complete morphology was stained in bromophenol blue and observed under confocalmicroscope (Fluoview FV1200, Olympus, USA) to measure the structures in the scolex.

Parasite identification key

The parasites were identified following Palm (1997) and the confirmation of the genus was performed by comparison of the morphological features stated by Robinson (1959) for *Molicola thyrsitae* (Robinson 1959) and Knoff et al. (2004) for *Molicola horridus* (Goodsir 1841).

RESULTS

The total length of the parasites examined were measured and it varied from 1.7 cm to 3.5 cm (average 2.27 cm). Parasites with the broken tentacular apparatus, scolex or lack of oral parts such as bothria were excluded from the study to avoid misinterpretations. Under light microscopic examination, the bothridial shape and the presence of four tentacles were considered as the characteristics of Trypanorhyncha pleorocercoid larvae (Figure 1).

Confocal microscopic examination provided the length and width parameters of the Trypanorhyncha scolex. Presence of the elongated, acraspidote scolex (length 6.23 mm, width at *pars bothridialis* 2.4 mm) with four curved, apically inverted, thick edged bothridia and four tentacular armature confirmed the parasitic larvae belong to the Order Trypanorhyncha. Tentacles with unarmed proximal region followed by basal and metabasal armatures were characteristics of the family *Gymnorhynchidae*. The basal armature with corona of hollow, falciform hooks and the metabasal armature with heteromorphous hooks in a half spiral arrangement are significant features of the genus *Malicola*. Length and width of the *pars bulbosa* were 1.12 mm, 1.17 mm respectively. Prebulbar organ was absent in the examined samples and had hyperapolytic strobila with the proglottid length of 1.97 mm.

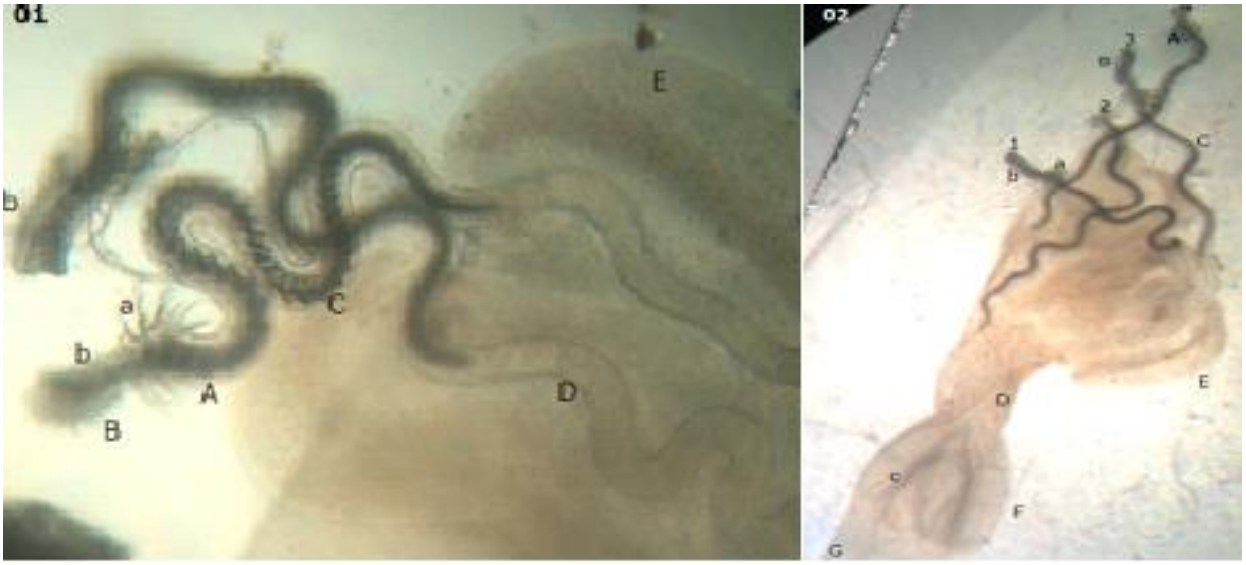

Fig. 1 1. Scolex of the parasitic cestode, Order Trypanorhyncha (genus *Molicola*) larval stage (plerocerci) (LM x40). 2. Scolex and the bulb with four tentacles (LM x10). (A - Corona of long hooks, B - Metabasal armature, C - Unarmed proximal region of the tentacle, D - Tentacle sheath, E - Bothridium (*pars bothridialis*), F - Bulb (*pars bulbosa*), G - Strobila (proglottids), a - long hooks, b - metabasal hooks, c - tentacle bulb, LM - light microscope)

DISCUSSION

Swordfish (*Xiphias gladius*) is the only species of the Family Xiphiidae having a pelagic and migratory behaviour. Many fish parasites such as cestodes and trematodes are transmitted to this fish along with the food they consume. There are about 14 cestodes species (tapeworms) recorded in swordfish including Trypanorhynch parasitic larvae *Molicola* (*Gymnorhyncus*) *horridus* and *Gymnorhynchus gigas* (Panebianco and Gianetto 1994; Williams and Williams 1996; Muscolino et al. 2012). There are a number of *Molicola* species isolated so far from various host species and the accepted *Molicola* (Dollfus 1942) species in World Register of Marine Species (WoRMS) are *Molicola horridus* (Goodsir 1841), *Molicola uncinatus* (Linton 1924 as sited in Palm 2004), *Molicola thyrsitae* (Robinson 1959) and *M. walteri* (Palm 2004) (www.marinespecies.org). The morphology of these species is quite similar to each other and species specific characteristics are generally lacking (Khalil et al. 1994). In this study, parasitic larvae with the broken tentacular apparatus, scolex or oral parts such as bothria were excluded for identification. It is possible that the damages to the parasite can happen during the extraction of the parasites that were buried deep in the swordfish muscles. Therefore by morphological analysis we were able to identify the parasite only up to the genus level. For species identification, it is necessary to perform molecular genetic analysis due to higher morphological similarity among species.

Since the gross appearance of Molicola parasite is similar to zoonotic *Anisarkis* species, fish processing factories discard large quantities of fish and experience a huge economic loss (personal communication). Nevertheless, this study revealed the presence of *Molicola* which is not zoonotic and does not harm the consumers. A study done on mouse models has indicated that the allergenic activity of *Molicola horridus* crude extract by emphasizing the increase of immunoglobulins (Gomez-Moralez et al. 2008). Furthermore, a broad survey carried out by Muscolino et al. (2012) on FAO Fishery Zones including Indian Ocean identified swordfish muscles infested with *Molicola* and *Gymnorhynchus* larvae, which do not cause public health risk. Therefore, the results of this study will help capture fishery industry at ease but due to the consumer preference, removal of parasites from the flesh is still needed to be practiced.

This is a preliminary study and a good initiative to investigate marine parasites in capture fishery in future. Our findings confirmed the presence of plerocercoid larvae of genus *Molicola,* affecting swordfish captured from Sri Lankan deep sea areas. It is more similar morphologically to *Molicola horridus* isolated from Brazil (Knoff et al. 2004).

CONCLUSION

The current study revealed the parasites in swordfish muscles as pleorocercoid larvae of the genus *Molicola* belonging to family Gymnorhynchidae of order Trypanorhyncha. To our knowledge, this is the first record of *Molicola* spp. larvae isolated from *Xiphias gladius* captured off Sri Lanka.

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REFERENCES

Adams A.M., K.D. Murrell and J.H. Cross 1997. Parasites of fish and risks to public health. Revue scientifique et technique (International Office of Epizootics) 16(2): 652-660.

Anon. 2015. Export and Import, Fisheries Statistics, Ministry of Fisheries and Aquatic Resources Development. http://www.fisheries.gov.lk/content.php?cnid=ststc Accessed on 21/07/2015

Gomez-Moralez M.A., A. Ludovisi, A. Giuffra, M.T. Manfredi, G. Piccolo and E. Pzio 2008. Allergenic activity of *Mollicola horridus* (Cestoda, Trypanorhyncha), a cosmopolitan fish parasite, in a mouse model. Veterinary Parasitology 157 (3-4): 314-320. doi:10.1016/j.vetpar.2008.07.010

Khalil L.F., A. Jones and R.A. Bray 1994. Keys to the cestode parasites of vertebrates. CAB international, Willingford.

Knoff M., S.C. de S. Clemente, R.M. Pinto, R.M. Lanfredi and D.C. Gomes 2004. Taxonomic reports of Otobothrioidea (Eucestoda, Trypanorhyncha) from elasmobranch fishes of the southern coast of Brazil. Memórias do Instituto Oswaldo Cruz, Rio de Janeiro 99(1): 31-36. doi:10.1590/S0074-02762004000100006

Marcogliese D.J. 2002. Food webs and the transmission of parasites to marine fish. Parasitology 124 (Supplement): S83-S99.

Musculino D., F. Giarratana, A. Giuffrida and A. Panebianco 2012. Inspective investigation on Swordfish (*Xiphias gladius*) frozen slices of commerce: anatomical – histopathological findings. Czech Journal of Food Science 30: 206-210.

Palm H.W. 1997. An alternative classification of trypanorhynch cestodes considering the tentacular armature as being of limited importance. Systematic Parasitology 37: 81-92. doi: 10.1023/A:1005765126294

Palm H.W. and J.N. Caira 2008. Host specificity of adult versus larval cestodes of the elasmobranch tapeworm order Trypanorhyncha. International Journal for Parasitology 38: 381-388. doi: 10.1016/j.ijpara.2007.08.011

Panebianco A. and S. Giannetto 1994. Osservazioni sull'infestione da *Macallumtrema xiphiados* (Yamaguti 1970) in *Xiphias gladius*. Argomenti di Patologia Veterinaria ed Fondazione Iniziative Zooprofilattiche e Zootecniche 36: 243–251.

Williams Jr. E.H. and L.B. Williams 1996. Parasites of offshore big game fishes of Puerto Rico and the Western Atlantic. Sportfish Disease Project, University of Puerto Rico, Mayaguez, Puerto Rico. 382 p.

http://www.marinespecies.org/ (accessed on 21/09/2015)