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Molecular Identification of the Genus *Molicola* Larvae from Swordfish (*Xiphias gladius*) Captured in Sri Lanka by Ribosomal Subunit Gene Sequencing

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Abstract

Swordfish (*Xiphias gladius*) is a migratory fish commercially exploited due to its high export value. The presence of parasites in fish leads to economic losses in the export market and public health issues. This study was conducted to identify the parasite larvae inhabiting swordfish and to determine its phylogenetic origin using ribosomal subunit gene sequence. Parasite samples were extracted from swordfish muscles and five larvae belong to *Molicola* genus, confirmed by scolex morphology, were used for genomic DNA extraction. Polymerase chain reaction (PCR) was performed to amplify 18S and 28S ribosomal RNA (rRNA) subunit genes followed by Sanger sequencing. DNA sequences were edited by BioEdit software and assembled by CLC genomics version 8.0. Consensus sequences were aligned with NCBI blast to determine the species status. Isolated larval sequences were best aligned with genus *Molicola* followed by genus *Gymnorhyncha*. Out of the two published *Molicola* rRNA gene sequences, 99% identity was observed with *Molicola* sp. HP5 isolate from Indonesia. Due to the lack of sequence data on other *Molicola* species (except *M. thyristes*) for comparison, our sequences were published as *Molicola* sp. Sri Lankan isolates. This is the first record of *Molicola* sp. in swordfish from Sri Lanka and the results will enhance the knowledge on the distribution of *Molicola* species while contributing to expanding the genetic information on rRNA coding sequences.

INTRODUCTION

Capture fisheries provide 65% of the animal protein requirement of the people in Sri Lanka. According to the fisheries statistics in 2016, its contribution to gross domestic production (GDP) was around 1.8 % (marine 1.6 %, inland 0.2 %). Among food fish Tuna species is the highest catch while swordfish (*Xiphias gladius*) is one of the seasonal fish which has higher demand from importing countries (Ministry of Fisheries & Aquatic Resources Development, 2019). Swordfish

is also called Broadbill, belongs to the family Xiphiidae of order Perciformes. They can be found in the Indian, Atlantic and Pacific oceans as well as in the Mediterranean, Marmara, Black, and Azov seas (FAO, 2019). Due to over-fishing, this species reduced by 28% over the past two decades (IUCN, 2019). Swordfish migrate to cold waters for feeding and returns to warm waters during summer for spawning. They eat small to relatively large fishes and occasionally consume crustaceans and squids (Williams and Bunkley-Williams, 1996). These feeding and breeding patterns increase their risk of getting exposed to parasites. Most of the marine helminths, especially *Trypanorhyncha* species have multiple hosts during their life cycle including swordfish and crustaceans as intermediate hosts (Palm and Caira, 2008).

The parasitism in fish is an ecological coexistence between two organisms in close contact. Some parasites establish an equilibrium of host-parasitic relationship being encysted or dormant until it gets a favorable condition to multiply (Schaperclaus, 1992). It is found that there are 49 parasitic species infecting swordfish. About 13 of them are host-specific while six are super family-specific. Swordfish harbors about 20 larval tapeworm species particularly due to the consumption of intermediate hosts infested with parasitic larvae. There are 14 cestodes (tapeworms) recorded in swordfish including Trypanorhynch (Williams and Bunkley-Williams, 1996).

Parasites infesting swordfish muscles include *Pennella* sp. (Copepod) (Hogans *et al*., 1985; Castro-Pampillón *et al*., 2002), cestode Trypanorhyncha plerocercoid larvae of *Molicola* (*Gymnorhyncus*) *horridus*, and *Gymnorhynchus gigas*, and flukes, *Maccalumtrema xiphiados*. A zoonotic parasite *Anisakis* nematode larvae were also reported in swordfish muscle (Hogans *et al*., 1983; Castro-Pampillón *et al*., 2002; Garcia *et al*., 2011).

In Sri Lanka, swordfish catch is seasonal, and a large amount of flesh was discarded due to parasitic infestation in muscles. A whitish, thread-like parasite found in muscles often mistook as the zoonotic *Anisakis* species until a preliminary study using morphological evaluation of this parasite revealed as plerocercoid larvae of the genus *Molicola* belong to order Trypanorhyncha (De Silva *et al*., 2017). But due to higher morphological similarity within the genus *Molicola*, this study was aimed at the molecular identification methods to identify up to the species level and to construct its phylogenetic tree.

The main morphological characteristics of order Trypanorhyncha are, scolex (with two or four bothria) and tentacular apparatus (consists of four tentacles covered with hooks and attached to four bulbs by tentacle sheaths) (Palm *et al*., 2009). There are about 277 valid Trypanorhyncha species identified so far and new species are being added continuously. Based on a more detailed cladistic analysis, five Trypanorhyncha superfamilies and 15 families were found. The five super families are, Tentacularioidea, Eutetrarhynchoidea, Gymnorhynchoidea, Lacistorhynchoidea, and Otobothrioidea (Palm, 1997).

Taxonomic reports of the genus *Molicola* indicated the presence of parasitic larvae in teleost muscles and liver. Sunfish (*Mola mola*) were reportedly infected by *Molicola* in France, the Mediterranean region, Japan, New Zealand, India, and Canada. *Molicola* was also reported on muscles of *Thyrsites* sp. from Holland (Knoff *et al*., 2004). *Molicola horridus* possess elongated scolex, four auriculate and elongated bothridia which is curved and apically inclined with round-edged, thick rims that are highly similar to the morphological examination of the Sri Lankan isolate of *Molicola* sp. reported previously (Knoff *et al*., 2004; De Silva *et al*., 2017). But there are other *Molicola* species (*Molicola uncinatus* (also called *Molicola thyrsitae*) (Johns *et al*., 2009) and *Molicola walteri*) which share similar morphological characteristics (Palm, 2004), indicating the necessity of

molecular identification for the determination of species.

In Sri Lanka, very few studies have been conducted on marine capture fish diseases. The presence of cestode and nematode parasites from Sri Lanka was reported as early as 1906 (Shipley and Hornell, 1906). The earliest report of Trypanorhyncha species from Sri Lanka, named *Halysiorhynchus microcephalus* was isolated from *Himantura imbricate* (Scaly Whipray) from the Sri Lankan coast (Southwell, 1929). A specimen at the British Museum of Natural History, which was received from Sri Lanka had been identified as another Trypanorhynch larva, *Pseudo-gilquinia pillersi* (Beveridge *et al*., 2007). Since there are a number of Trypanorhynch parasites inhabiting various host species, it was a timely necessity to identify the parasitic species present in swordfish muscles.

This study focused on the identification of *Molicola* plerocercoid larvae up to the species level and to develop a phylogenetic tree to determine its origins. Ribosomal RNA genes of 18S and 28S subunits were sequenced for comparative analysis due to their conserved nature during evolution. Based on this study *Molicola* sp. Sri Lankan isolates found in swordfish enhances the rRNA gene database of Trypanorhyncha parasites.

METHODOLOGY

Place and Time

Parasitic samples from the muscles of swordfish (captured from the FAO statistical zone 57 of the Indian Ocean), were obtained from a fish processing factory in Sri Lanka. Samples were collected twice during 2015 and 2016, directly from the fish processing factory and transferred on ice to the Center for Biotechnology, Department of Zoology, University of Sri Jayewardenapura, Sri Lanka, where all morphological analysis, DNA extraction and polymerase chain reaction (PCR) were performed. PCR products were sent to Microgen, Korea for the Sanger sequencing.

Research Material

The whitish, thread-like parasitic samples obtained from swordfish muscle were confirmed as *Molicola* plerocercoid larvae by morphological analysis. To examine the morphology of the parasite using wet mounts, compound light microscope (Olympus, CX22LED, U.S.A.) and microscope slides were utilized and the parasitic samples were fixed in 10% formalin 1.5 ml. Five larvae were confirmed as genus *Molicola* based on their scolex characteristics and used for genomic DNA extraction. For DNA extraction, reagents mentioned in the methods of Ballinger-Crabtree *et al.* (1992) were used.

In order to amplify ribosomal subunit genes, primers were designed for 18S rRNA (ssrRNA) and 28S rRNA (lsrRNA) gene sequences as shown in Table 1. Primer sequences and PCR protocols for 18S rRNA and 28S rRNA gene amplification were based on Palm *et al*. (2009) with slight modifications.

PCR reagents such as magnesium chloride ($MgCl_2$), deoxyribose nucleotide triphosphate (dNTP), buffer (5x), *Taq* DNA polymerase (University of Colombo, Sri Lanka) were used to prepare samples for amplification. Thermal cycler (Master cycler personal, Eppendorf, USA) was used to run the PCR. Agarose gel (Promega, USA) was used in gel electrophoresis. ABI sequencer (U.S.A.) was utilized to perform Sanger sequencing at Microgen, Korea.

Table 1. Primers used to amplify and sequencing of 18S rRNA and 28S rRNA gene segments.

Primer type	Primer ID	Primer sequence
ssrDNA (18S rRNA gene)		
Forward primer	ssrDNA F1	5`-GCGAATGGCTCATTAAATCAG-3`
Reverse primer	ssrDNA R1	5`-CTTGTTACGACTTTTACTTCC-3`
Internal primers	300F2	5`-AGGGTTCGATTCCGGAG-3`
	600R	5`-ACCGCGGCKGCTGGCACC-3`
	1270F	5`-ACTTAAAGGAATTGACGG-3`
	930F	5`-GCATGGAATAATGGAATAGG-3`
	1200F	5`-CAGGTCTGTGATGCCC-3`
lsrDNA (28S rRNA gene)		
Forward primer	lsrDNA F1	5`-ACCCGCTGAATTTAAGCATAT-3`
Reverse primer	LsrDNA R1	5`-GCTATCCTGAGGGAAACTTCG-3`
Internal primers	300F	5`-CAAGTACCGTGAGGGAAAGTTG-3`
	ECD2	5`-CTTGGTCCGTGTTTCAAGACGGG-3`
	400R	5`-GCAGCTTGACTACACCCG-3`
	1090F	5`-TGAAACACGGACCAAGG-3`

Research Design

Parasitic samples from swordfish muscles were obtained by adhering to the complete randomization method. A total of 20 parasitic samples was obtained for morphological diagnosis and from them, five confirmed *Molicola* samples (by scolex morphology) were selected for DNA extraction followed by a molecular examination. Each parasitic sample was tested for both 18S rRNA and 28S rRNA genes and sequences were compared for determination of their individual variations and phylogenetic distance.

Work Procedures

Genomic DNA from five parasites was separated into two sets for the detection of two regions of the ribosomal RNA gene, 18S rRNA and 28S rRNA respectively. PCR reaction was performed using the reagents and amounts as below: Total volume of the PCR product was 25 μl. Genomic DNA 2 ng were used from each sample as the template. Double distilled water was added as the negative control. PCR master mix was prepared by adding 1 μl each from forward and reverse primers, 6 μl of magnesium chloride ($MgCl_2$), 1.25 μl deoxyribose nucleotide triphosphate (dNTP), buffer (5x) 5 μl, *Taq* DNA polymerase (University of Colombo, Sri Lanka) 0.2 μl into 8.55 μl of double distilled water to make it 25μl of total volume.

The thermocycler was run according to the following conditions: To amplify 18S rRNA gene, initial denaturation at 94°C for 2 minutes followed by 40 cycles of 94°C, 30 seconds, 54°C, 30 seconds and 72°C, 2 minutes. For 28S rRNA gene amplification, 95°C, 5 minutes initial denaturation followed by 40 cycles of 95°C, 30 seconds, 55°C, 30 seconds and 72°C, 2 minutes. Both PCRs were finally extended at 72°C for 7 minutes before it was kept at 4°C until electrophoresis. Final PCR products were observed by one percent (1%) Agarose gel (Promega, USA) electrophoresis.

All amplicons of 18S rRNA and 28S rRNA genes were sequenced by the Sanger method (ABI sequencer at Microgen, Korea) and sequences were analyzed to confirm the species and constructed the phylogenetic tree.

Data Analysis

Poor quality bases on both ends of the sequences were trimmed by using Bio Edit software version 7.2.5 (Hall, 1999). Multiple alignments was performed using Clustal W and assembled by *de novo* assembly of contigs by CLC Bio genomics work bench version 8.0 (www.clcbio.com). Consensus sequences were compared with the reference sequences

deposited in National Center for Biotechnology Information (NCBI) by basic local alignment tool (BLAST). Phylogenetic analysis and the taxonomic tree were performed by using Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 (Tamura *et al*., 2013) neighbour-joining method (Saitou and Nei, 1987).

RESULTS AND DISCUSSION

Morphological identification of the parasitic larvae found on swordfish muscle was reported previously by our research group as *Molicola* plerocercoid larvae belong to the Order Trypanorhyncha (De Silva *et al*., 2017).

The amplicon for 18S rRNA and 28S rRNA gene, obtained by PCR using region-specific universal primers for trematodes showed electrophoresis band size over 1500 bp which formed the consensus sequences of 18S rRNA and 28S rRNA genes of about 2030 bp and 1500 bp respectively. Based on the sequence analysis of 18S rRNA and 28S rRNA gene, all five isolates from Sri Lanka were aligned to *Molicola* sp. HP5 (Indonesia) with 99% and 100% identity, followed by *Molicola thyristes* isolate Moli (Australia) and *Gymnorhynchus isuri*. All three species belonged to the family Gymnorhynchidae (Table 2).

Table 2. Species comparison using NCBI blast of 18S and 28S rRNA gene sequences of Sri Lankan isolates with species belong to superfamily Gymnorhynchoidea.

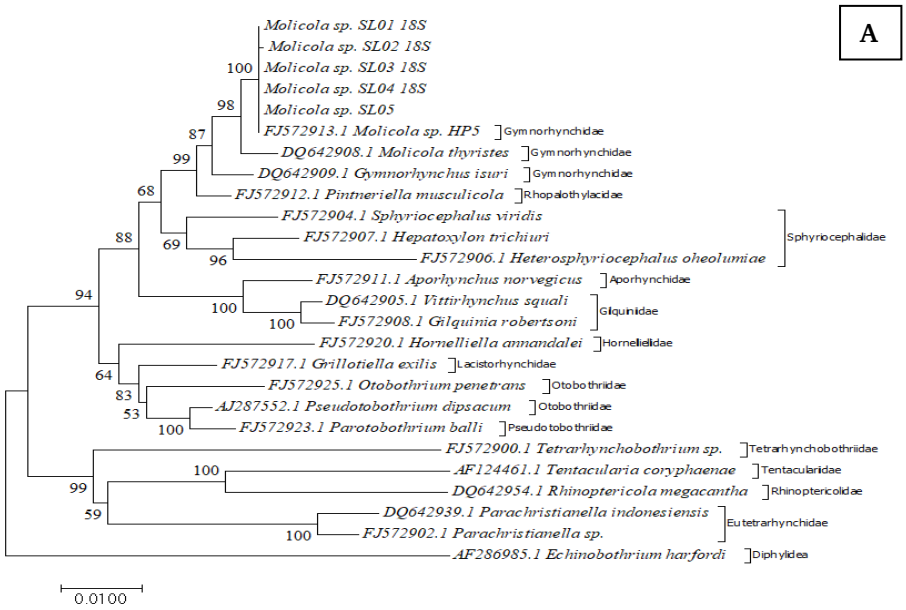
Species name	Family	18S rRNA		28S rRNA	
		Identity	NCBI accession	Identity	NCBI accession
Molicola sp. HP5	Gymnorhynchidae	99%	FJ572913	100%	FJ572949
Molicola thyristes	Gymnorhynchidae	99%	DQ642908	98%	DQ642746
Gymnorhynchus isuri	Gymnorhynchidae	98%	DQ642909	97%	DQ642747
Pintneriella musculicola	Rhopalothylacidae	98%	FJ572912	96%	FJ572948
Chimaerarhynchus rougetae	Gymnorhynchidae	95%	DQ642906	93%	DQ642744
Vittirhynchus squali	Gilquiniidae	95%	DQ642905	93%	DQ642743
Sagittirhynchus aculeatus	Gilquiniidae	95%	DQ642907	93%	DQ642745
Gilquinea robertsoni	Gilquiniidae	95%	FJ572910	93%	FJ572944
Aporhynchus norvegicus	Aporhynchidae	95%	FJ572911	93%	FJ572947

This clearly confirmed that the plerocercoid larvae isolated from swordfish from Sri Lanka (Indian ocean) belonged to the genus *Molicola,* family Gymnorhynchidae. There were point mutations and insertions in 18S and 28S rRNA gene sequences possibly due to the individual variations. The evolutionary distance between the sequences of five isolates was not significantly different ($p > 0.05$). Therefore, all the parasitic larvae isolated from swordfish in this study belong to the same species.

Though the analysis of 18S rRNA and 28S rRNA gene sequences were performed in five individual parasitic samples, there were less than ten nucleotide variations found (more than 95% identity) indicating that they all belong to the same species. The significant identity of Sri Lankan isolates with *Molicola* sp. Hp5 from Indonesia obtained from *Taractes rubescens,* suggests that swordfish captured near the Sri Lankan coast might have migrated via Indonesian coasts. Furthermore, both *T. rubescens* and swordfish are pelagic fish that shows similar feeding patterns (Scott and Tibbo, 1968; Gòmez-Morales *et al*., 2008), justifying the possible entry of *Molicola* larvae through their food chain. The other species of close identity with Sri Lankan

isolates was *Molicola thyristes* isolated from Australia in *Thyrsites atun*. It is also an intermediate host of *Molicola* sp. which feeds on pelagic crustaceans (Nakamura and Parin, 1993).

Phylogenetic analysis of 18S rRNA and 28S rRNA sequences of *Molicola* Sri Lankan isolates were compared with 20 Trypanorhyncha species belonged to different families. Based on the phylogenetic analysis, the highest significance with *Molicola sp.* HP5 isolate from Indonesia was observed and Sri Lankan isolates shared the common ancestor with members from the Gymnorhynchidae family (Figure 1). The comparison of both phylogenetic trees showed similarity in 18S rRNA and 28S rRNA genes. Accordingly, it confirms the morphologically recognized genus of the parasite. Nucleotide sequences of 18S and 28S ribosomal subunit gene of *Molicola* sp. Currently, in the NCBI database only two *Molicola* species 18S and 28S rRNA gene sequences were available for comparison. Therefore, our results were submitted as *Molicola* sp. SL 01 to 05. The country of origin, Sri Lanka was stand by 'SL' and one to five indicates the individual plerocercoid larval isolates. The Sri Lankan isolates identified from this study were deposited at NCBI GenBank with the accession numbers for 18S rRNA partial gene sequences starting from KX712332 to KX 712336 and for 28S rRNA partial gene sequences from KX712337 to KX712341. This is the first study on rRNA genes of *Molicola* sp. infested in the muscles of swordfish captured from the Sri Lankan coast to the best of our knowledge. Further studies on other *Molicola* species are needed to develop a better sequence comparison and speciation of the genus *Molicola*.



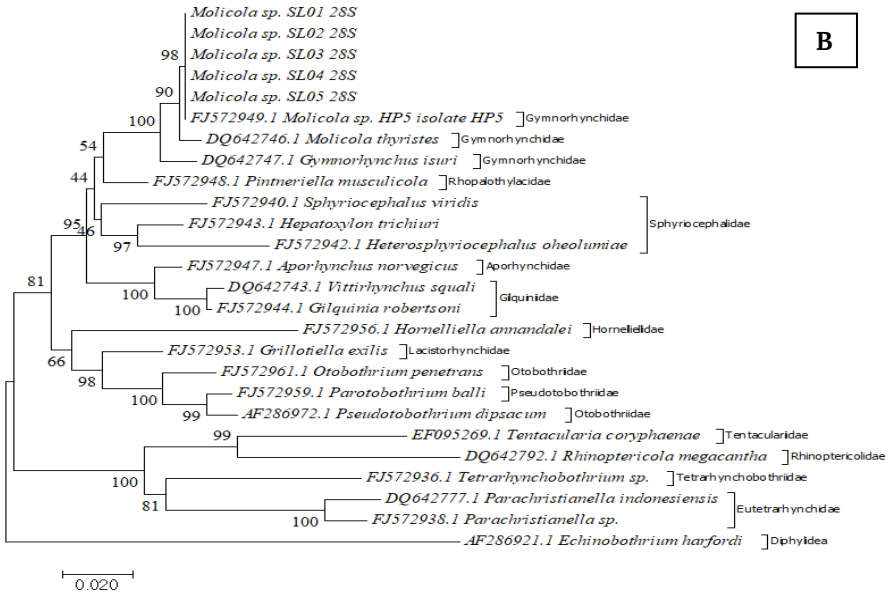


Figure 1. Evolutionary relationships of *Molicola* Sri Lankan isolates with other species belong to different taxonomic families of order Trypanorhyncha, obtained by Neighbor-joining method using (A) 18S rRNA and (B) 28S rRNA gene sequences. Scientific names of the species and their NCBI accession numbers were written at respective nodes and the branch lengths indicate the evolutionary distance drawn to a scale bar of 0.01 for 18S and 0.02 for 28S rRNA gene. *E. harfordi* belong to the order Diphyllidea was used as an outgroup.

It is mandatory to perform fish quality checks before exporting to other countries and the difficulty arose when both Trypanorhyncha and *Anisakis* species look alike grossly in color and consistency (Muscolino *et al*., 2012). Although *Molicola* is not a zoonotic parasite as *Anisakis* studies on mice have shown anaphylaxis due to *Molicola horridus* allergens (Gomez-Morales *et al*., 2008). However, the chance of allergic reactions to occur in consumers by using frozen swordfish slices is very low, but the parasitized flesh reduces the consumer preference (Muscolino *et al*. 2012). Hence this method can be used for confirmatory and differential diagnosis of Trypanorhyncha parasites from other fish parasites.

CONCLUSION

The whitish parasite isolated from swordfish muscles belonged to the genus *Molicola*, order Trypanorhyncha. Molecular phylogenetic analysis and sequence comparison of 18S and 28S ribosomal subunit genes revealed a higher similarity to *Molocola* sp. HP5 from Indonesia. This is the first record of molecular analysis of ribosomal subunit genes in *Molicola* species isolated from swordfish captured near the Sri Lankan coast.

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REFERENCES

Ballinger-Crabtree, M.E., Black, W.C. and Miller B.R., 1992. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. *The American Journal of Tropical Medicine and Hygiene, 47*(6),

pp.893-901. https://doi.org/10.4269/ajtmh.1992.47.893

Beveridge, I., Chauvet, C. and Justine, J.L., 2007. Redescription of *Pseudogilquinia pillersi* (Southwell, 1929) (Cestoda, Trypanorhyncha) from serranid and lethrinid fishes from New Caledonia and Australia. *Acta Parasitologica, 52*(3), pp.213-218. https://doi.org/10.2478/s11686-007-0029-9

Castro-Pampillón, J.A., Rodríguez-Domínguez, H., Soto-Búa, M., Mejuto-García, J., Arias Fernández, C. and García-Estévez, J.M., 2002. Parasites of swordfish from the Gulf of Guinea. *Journal of Parasitology, 88*(1), pp.188–189. https://doi.org/10.1645/0022-3395(2002)088[0188:POSFTG]2.0.CO;2

CLC Bio Genomics Workbench version 8.0 (http://www.clcbio.com), Accessed on 2nd May 2015.

De Silva, D.P.N., Fernando, H.S.D., Ranatunga, R.R.M.K.P. and De Silva, B.G.D.N.K., 2017. First record of plerocercoid larvae belong to the order Trypanorhyncha (Diesing 1863) isolated from swordfish (*Xiphias gladius*, Linnaeus 1758) captured off Sri Lanka. *Sri Lanka Journal of Aquatic Sciences, 22*(1), p.67–70. https://doi.org/10.4038/sljas.v22i1.7518

Food and Agriculture Organization of the United Nations, *Xiphias gladius*. *Website*. http://www.fao.org/fishery/species/2503/en. Accessed on 12th July 2019.

Garcia, A., Mattiucci, S., Damiano, S., Santos, M.N. and Nascetti, G., 2011. Metazoan parasites of swordfish, *Xiphias gladius* (Pisces: Xiphiidae) from the Atlantic Ocean: implications for host stock identification. *ICES Journal of Marine Science, 68*(1), pp.175-182. https://doi.org/10.1093/icesjms/fsq147

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

Hall, A.T., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, pp.95-98. http://www.academia.edu/download/29520866/1999hall1.pdf

Hogans, W.E., Brattey, J., Uhazy, L.S. and Hurley, P.C.F., 1983. Helminth parasites of swordfish (*Xiphias gladius* L.) from the northwest Atlantic Ocean. *Journal of Parasitology, 69*(6), pp.1178–1179.

Hogans, W.E., Brattey, J. and Hurlbut, T.R., 1985. *Pennella filosa* and *Pennella instructa* (Copepoda, Pennellidae) on swordfish (*Xiphias gladius* L.) from the northwest Atlantic Ocean. *Journal of Parasitology, 71*(1), pp.111–112.

IUCN Red List of Threatened Species, *Xiphias gladius*. Website: http://www.iucnredlist.org/details/23148/0. Accessed on 5th May 2019.

Johns, P., Newman, L.J., Holleman, J.J., Dawson, E.W., Sterrer, W., Allison, F.R., Diggles, B.K., Andrews, J.R.H., Hine, P.M., McKenna, P.B. and Poulin, R., 2009. Phylum Platyhelminthes: flatworms, tapeworms, flukes. In: *New Zealand inventory of biodiversity: 1. Kingdom Animalia: Radiata, Lophotrochozoa, Deuterostomia* (ed. by D.P. Gordon), Canterbury University Press, Christchurch, New Zealand. pp.102-128.

Knoff, M., Clemente, S.C. de S., Pinto, R.M., Lanfredi, R.M. and Gomes, D.C., 2004. Taxonomic reports of Otobothrioidea (Eucestoda, Trypanorhyncha) from elasmobranch fishes of the Southern coast of Brazil. *Memorias do Instituto Oswaldo Cruz, 99*(1), pp.31-36.

https://doi.org/10.1590/S0074-02762004000100006

Muscolino, D., Giarratana, F., Giuffrida, A. and Panebianco, A., 2012. Inspective investigation on swordfish (*Xiphias gladius*) frozen slices of commerce: anatomical-histopatological findings. *Czech Journal of Food Sciences, 30*, pp.206–210. https://doi.org/10.17221/151/2011-CJFS

Nakamura, I. and Parin, N.V., 1993. Snake mackerels and cutlassfishes of the world (families Gempylidae and Trichiuridae): An annotated and illustrated catalogue of the snake mackerels, snoeks, escolars, gemfishes, sackfishes, domine, oilfish, cutlassfishes, scabbardfishes, hairtails and frostfishes known to date. *Food and Agriculture Organization* (FAO), *125*(15), p.136.

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

Palm, H.W., 2004. *The Trypanorhyncha Diesing, 1863*. SPL-IPB Press, Bogor Agricultural University, West Java, Indonesia. p. 710.

Palm, H.W. and Caira, J.N., 2008. Host specificity of adult versus larval cestodes of the elasmobranch tapeworm order Trypanorhyncha. *International Journal for Parasitology, 38*(3-4), pp.381-388. https://doi.org/10.1016/j.ijpara.2007.08.011

Palm, H.W., Waeschenbach, A., Olson, P.D. and Littlewood, D.T.J., 2009. Molecular phylogeny and evolution of the Trypanorhyncha Diesing, 1863 (Platyhelminthes: Cestoda). *Molecular Phylogenetics and Evolution, 52*(2), pp.351-367. https://doi.org/10.1016/j.ympev.2009.01.019

Saitou, N. and Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*(4), pp.406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Schaperclaus, W., 1992. Causes, development and prevention of fish diseases. *Fish diseases, 5th edn. AA Balkema Publisher, Rotterdam*, pp.3-42.

Scott, W.B. and Tibbo, S.N., 1968. Food and feeding habits of Swordfish, *Xiphias gladius*, in the Western North Atlantic. *Journal of the Fisheries Research Board of Canada,* 25(5), pp.903-919. https://doi.org/10.1139/f68-084

Shipley, A.E. and Hornell, J., 1906. Report on the cestode and nematode parasites from Ceylon. Report to the Government of Ceylon on the pearl oyster fisheries of the Gulf of Mannar. Royal Society, London. Part V, pp.43-96.

Southwell, T., 1929. A monograph on cestodes of the order Trypanorhyncha from Ceylon and India. Part I. *Ceylon Journal of Science,* 15, pp.169-312.

Statistics, Ministry of Fisheries and Aquatic Resource Development, Sri Lanka. Website. http://www.fisheries.gov.lk/content.php?cnid=ststc, Accessed on 25th October 2019.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA 6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*(12), pp.2725-2729. https://doi.org/10.1093/molbev/mst197

Williams, Jr. E.H. and Bunkley-Williams, L., 1996. Parasites of offshore big game fishes of Puerto Rico and the Western Atlantic. Sport fish Disease Project, University of Puerto Rico, PR. pp.330-332.