

Case Report – *Heteronybelinia* spp. in *Pomatomus saltatrix* (Linnaeus, 1766)

Thatiana Ferrari Ceron¹, Yete Gambarini Ferri Lazarin¹, Silvia Helena Pereira Vergili Sgarbosa¹, Felipe Vaz Rodrigues³, Marianna Vaz Rodrigues^{1,2}

¹ Veterinary School, UNIP, Bauru, São Paulo, Brazil

² Department of Microbiology and Immunology, Biosciences Institute, Univ. Estadual Paulista, Botucatu, São Paulo, Brazil

³ Biomedical Scientist, São Paulo, Brazil

Abstract: *Heteronybelinia* is a genus of cestodes's order frequently find in fishes with fifteen species described in literature. This study aimed report the presence of cestodes of the genus *Heteronybelinia* spp. in *Pomatomus saltatrix*. The fish was purchased from a supermarket in the city of Jaú, São Paulo, Brazil. The cestode larvae found in the internal organs were collected during necropsy with the aid of a needle, fixed in AFA and identified. These parasites have great hygienic sanitary importance, once some species parasitize fishes' musculature generating negative aspects in their appearance and economic losses in aquaculture. In addition, another well-known aggravating factor is the immunogenic reactions that are currently being studied in fish parasitized with *Trypanorhyncha* cestodes.

Keywords: *Heteronybelinia* spp., *Pomatomus saltatrix*, parasitosis in fish, *Trypanorhyncha*.

1. INTRODUCTION

The fish species *Pomatomus saltatrix* [1], popular know as bluefish, belongs to Actinopterygii class, Pomatomidae family, and is find in subtropical, tropical, coastal, and oceanic waters [2]. Helminths and plathelminths -i.e. *Trypanorhyncha* cestodes- are often find into marine environment and has been widely reported in fish species with commercial importance.

The order *Trypanorhyncha* belongs to Plathelminths phylum, which has 290 species described. [3]. It has worldwide distribution [4] and parasitizes both marine fish and invertebrates [5-6], specially from tropical and subtropical regions [4]. Nowadays, there are reports of this cestode in 60 fish species from Brazil [3]. According to São Clemente [7], parasites from *Trypanorhyncha* order found in *P. saltatrix* [1] were *Callitetrarhynchus gracilis* [8], *Callitetrarhynchus speciosum* [9], and *Pterobothrium crassicolle* [10].

Trypanorhyncha has a sanitary and economic importance [11] due to disgusting aspect in parasitized seafood for consumers [12]. Another point is the economic loss to aquaculture, once parasitized animals generates less feed consumption, which is also and important fact for zootechnical losses [11].

According to Decree number 9.013 of March 20th of 2017, which treats about sanitary inspection of seafood, it is considered unfit seafood those with disgusting aspect, such as mutilation, deformation, or with high intensity of parasites that can cause zoonosis or only bad aspect for consumers [12].

This group of parasites has low zoonotic potential [11], however, there are a few cases that reported that some species of this cestode can stimulate immune system of the host. Reactions due to systemic hypersensitivity are not the same for all hosts and in some cases can causes anaphylaxis [3, 13-14]. Cases in humans were reported, which shows the risk of raw seafood intake that could be parasitized [15]. This way, studies about seafood quality has good advances with the objective of guarantee healthy food for consumers [16-17]. Thus, this study aimed report *Heteronybelinia* [18] in *Pomatomus saltatrix* [1].

2. MATERIALS AND METHODS

Necropsy of one *Pomatomus saltatrix* [1] was performed according to Noga [19]. The fish was

acquired from a supermarket in Jaú city, São Paulo, Brazil. For this purpose, fish was put in a bag and transported refrigerated to the laboratory for necropsy and identification of the parasites.

Cestode larvae found in internal organs were sampled during necropsy with a needle, fixed in AFA, and identified as described by [20], followed by photo documentation.

3. RESULTS AND DISCUSSION

During necropsy, it was found cestode larvae of the genus *Heteronybelinia* [18] (Figs.1 and 2). *Heteronybelinia* [18] belongs to Tentaculariidae family and Trypanorhyncha order. In the Trypanorhyncha order exists about 290 species, but few information is available of pathogenesis in the host [3]. Tentaculariidae Family has sixteen genera, which are *Acoeleorhynchus* [21], *Aspidorhynchus* [22], *Congerina* [23], *Heteronybelinia* [18], *Kotorella* [24], *Kotorelliella* [25], *Mixonybelinia* [18], *Nybelinia* [21], *Pleronybelinia* [26], *Rhynchobothrius*, *Ruffieria* [27], *Syngenes* [27], *Tentacularia* [28], *Abothros* [29], *Pierretia* [30], and *Stenobothrium* [10], being the last three genera considered as *Tentacularia* [28]. There are fifteen species of *Heteronybelinia* genus [18] reported, which are *Heteronybelinia annakohnae* [31], *Heteronybelinia australis* [25], *Heteronybelinia elongata* [32], *Heteronybelinia estigmema* [33], *Heteronybelinia eureia* [33], *Heteronybelinia heteromorphi* [18], *Heteronybelinia mattisi* [34], *Heteronybelinia minima* [18], *Heteronybelinia nipponica* [35], *Heteronybelinia overstreeti* [4], *Heteronybelinia palliata* [36], *Heteronybelinia perideraeus* [37], *Heteronybelinia pseudorobusta* [25], *Heteronybelinia robusta* [38], and *Heteronybelinia yamagutii* [33].

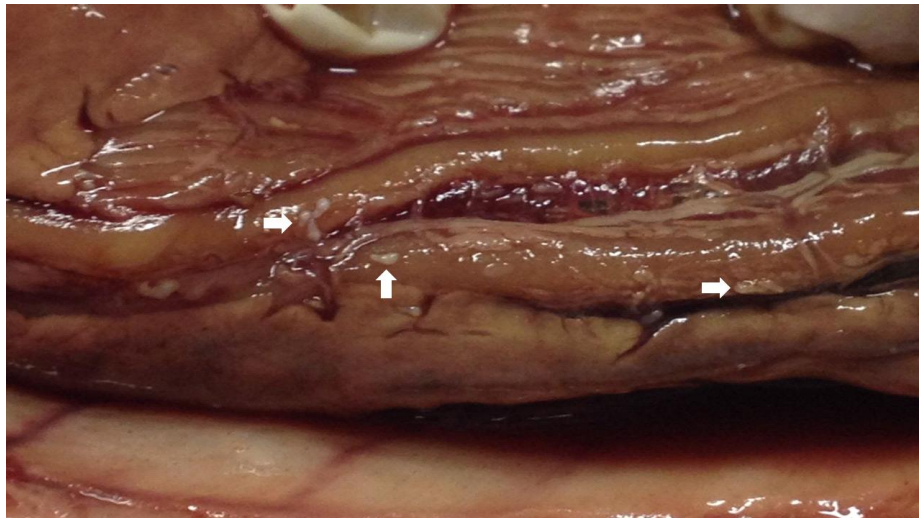


Fig 1. *Heteronybelinia* spp. metacestodes in the serous of the internal organs (arrow) of *Pomatomus saltatrix*

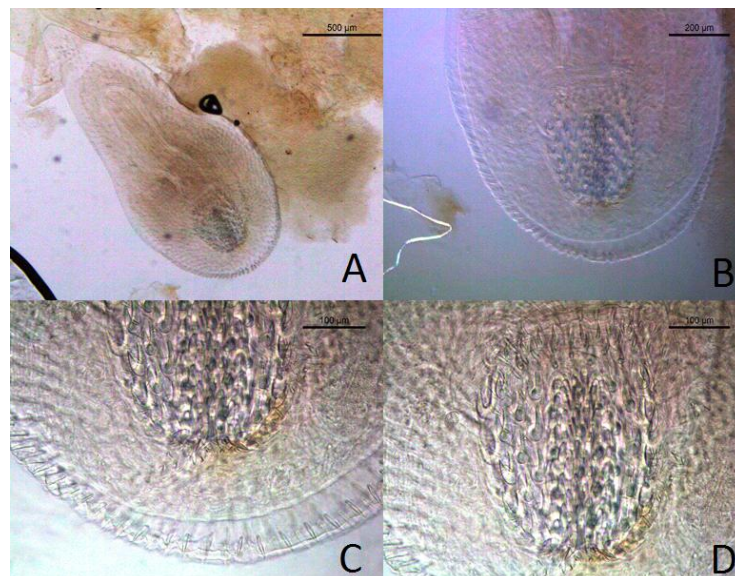


Fig 2. Microscopic observation of *Heteronybelinia*. **A.** *Heteronybelinia* spp. **B.** Basal region. **C.** Aculeus in the basal region. **D.** Aculeus

The oriental cuisine -i.e. Japanese food- have been more popular nowadays, increasing the risk of raw seafood intake, which can cause parasite infections in consumers [39]. Studies about seafood quality have been performed to guarantee safety of the products [16]. Among parasites species, those that belong to Trypanorhyncha order has a sanitary and hygienic importance [11,40].

Parasitized seafood does not present high zoonotic potential, but the presence of the parasites cause fishes' disgusting aspect to consumers and consequently decrease sale of this products generating some economic losses to aquaculture [11].

Adults larvae habits gastrointestinal tract of elasmobranch fishes, such as sharks and rays, which are definitive hosts. Although, metacestodes are found in teleosts, elasmobranchs, and marine invertebrates, acting as intermediate hosts. In this stage, the parasites could be found in the cavity, organs serous, mesentery, and muscle [5, 41-42].

Some researchers, such as Rodero and Cuellar [43] reported that this cestodes can cause allergy and anaphylactic reactions. This process can occur due to seafood ingestion or contaminants substances, such as histamine, toxins, and parasites [44]. In sensitized hosts, this reaction could even be caused by cooked parasitized seafood, once allergens are resistant to the heat [45].

Tropomyosin and parvalbumin isoforms are the main molecules involved in allergy process of crustacea and fish intake, respectively [46]. In a study, people in Spain presented seroprevalence of antibodies against *Gymnorhynchus gigas* cestode [47], which causes modulation of cholinergic activity and alterations in the gastrointestinal tract [14].

Studies performed with Trypanorhyncha order occur since 1990, describing allergical reactions and hypersensitivity in murine model [13, 43, 48-49]. Infections by these parasites in humans are considered rare, once there are only three cases reported in the literature [3, 13, 43, 48-50]. Two cases were caused by *Hepatoxylon trichuri* [51], being one in the South Africa and other in Mozambique [52-53]. The other report was caused by *Nybelinia surmenicola* [33], that described the presence of the parasite in the palatine tonsil in a Japanese man [55].

Due to the allergic response and sensitivity in the host is caused by these cestodes by oral via, lead to parasite resistance to digestive process, which causes IgE response. In all cases reported in humans, larvae were found in the feces, showing resistance to gastrointestinal tract resistance [52-53].

The prevalence and quantity of infections caused by Trypanorhyncha vary due to fish age, food habits, and immune response [55].

There are no reports of *Heteronybelinia* [18] parasiting *P. saltatrix* [1] until the moment of this study.

According to Alves *et al.* [56], *Nybelinia* [21] was found in the mesentery, although, São Clemente *et al.* [57] reported this same genus in mesentery, stomach serous, and celomatic cavity. *Heteronybelinia rougetcampanae* [33] and *Myxonybelinia beveridgei* [58], which also belongs to Tentaculariidae family, were found in celomatic cavity and mesentery, stomach serous and liver, respectively [57]. As described by Amato *et al.* [59] and São Clemente *et al.* [7, 40], infection prevalence and intensity, infection intensity variation, number of species by infection site, and number of parasites in the muscle or others places are related to disgusting aspect in parasitized seafood.

During necropsy, it was found some alterations, such as steatosis in the liver, liquefaction necrosis in the kidney, hepatic congestion, lamellae fusion in the gills, necrosis in the stomach, and opacity of the eyes.

São Clemente [7], reported the species of Trypanorhyncha order found in *P. saltatrix* [1], which were *Callitetrarhynchus gracilis* [8], *Callitetrarhynchus speciosum* [9], and *Pterobothrium crassicolle* [10]. This information supports that this is the first report of *Heteronybelinia* in *P. saltatrix* [1] until this study.

4. CONCLUSION

We conclude that the parasites of Trypanorhyncha order, genus *Heteronybelinia* can parasite *Pomatomus saltatrix* [1], being this the first report of this cestode in this host.

REFERENCES

- [1] Linnaeus, C.1766.Systema naturae sive regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Holmiae, Laurentii Salvii, (12th), pp. 1-532.
- [2] Juanes, F., Hare, J. and Miskiewics, A.1996.Comparing early life history strategies of *Pomatomus saltatrix* : a global approach. Marine and Freshwater Research, 47:365–379.
- [3] Mattos, D.P.B.G. Aspecto sanitário e potencial alergênico de helmintos parasitos de peixes teleósteos marinhos do estado do rio de janeiro, Brasil. 2012. 98f. Tese de doutorado em medicina veterinária. Programa de pós-graduação em higiene veterinária e processamento tecnológico de produtos de origem animal. Universidade federal fluminense, Niterói, RJ. <http://www.marinespecies.org/aphia.php?p=taxdetails&id=104922>.
- [4] Palm, H.W.2004.The Trypanorhyncha Diesing, 1863. PKSPL-IPB Press, Bogor, Indonesia, pp. 710.
- [5] Campbell R. A.1994.Beveridge I. Order Trypanorhyncha Diesing, 1863. In Khalil LF, Jones A, Bray RA (eds) Chapter 7 keys to the cestodes parasites of vertebrates. Commonwealth Agriculture Bureaux International, Wallingford, pp 51–148.
- [6] Knoff, M., São Clemente, S. C., Pinto, R. M. and Gomes, D. C.2002.Prevalência e intensidade de infecção de cestóides Trypanorhyncha de elasmobrânquios nos estados do Paraná e Santa Catarina, Brasil. Parasitologia latino-americana, Santiago, 57(3-4):149-157.
- [7] São Clemente, S. C., Silva, C. M. and Gottschelk, S.1997.Prevalência e intensidade de infecção de cestóides trypanorhyncha em anchovas, *Pomatomus saltatrix* (L.) do litoral do Rio de Janeiro, Brasil. Parasitologia al día, 21:54 - 57.
- [8] Rudolphi, C. A.1819.Sumptibus Augusti Ru'cker. In Entozoorum synopsis cui accedunt mantissa duplex et indices loupletissimi, Berlin, Germany, pp. 811.
- [9] Linton, E.1897.Notes on larval cestodes parasites of fishes. Proceedings of the United States National Museum, 19:787-824.
- [10] Diesing, C. M.1850.*Systema helminthous*, 1.
- [11] São Clemente, S. C., Knoff, M., Lima, F. C., Andrada, C. G., Felizardo, N. N., Padovani, R. E. S. and Gomes, D. C.2007.Cestóides trypanorhyncha parasitos de peixe sapo-pescador, *Lophius gastrophysus* Miranda-Ribeiro, 1915 comercializados no estado do Rio de Janeiro, Brasil. Revista Brasileira de Parasitologia Veterinária, 16(1): 37–42.
- [12] BRASIL. Ministério da Agricultura. Secretaria Nacional de Defesa Agropecuária. Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal. Aprovado pelo Decreto nº 9.013, de 29/03/17. Brasília, DF, 2017.
- [13] Gómez-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):314–320.
- [14] Pelayo, V., García-Hernández, P., Puente, P., Rodero, M. and Cuéllar, C.2009.Seroprevalence of anti-*Gymnorhynchus gigas* (trypanorhyncha, gymnorhynchidae) antibodies in a Spanish population. The Journal of Parasitology, 95(3):778–780.
- [15] Bates, R. M.1990.A checklist of the Trypanorhyncha (Platyhelminthes: Cestoda) of the world (1935-1985). National Museum of Wales, Zoological series, 1:218.
- [16] São Clemente, S. C.1993.Estudo de nematóides da família Anisakidae em peixe exportado pelo Brasil *Pagrus pagrus* com importância na inspeção sanitária e seu controle através da utilização de baixas temperaturas. 31 f, tese (doutorado) – faculdade de veterinária – universidade federal fluminense, Niterói, RJ.
- [17] Oliveira, V. M., Silva, C. M. and São Clemente, S. C.2003.Ocorrência de anisquídeos em *Gadus morhua* (bacalhau) no mercado varejista do município do Rio de Janeiro e sua importância para a saúde pública. Revista Universidade Rural Série Ciências da Vida, 23:334.
- [18] Palm, H.W.1999.*Nybelinia* Poche, 1926, *heteronybelinia* gen. Nov. And *myxonybelinia* gen. Nov. (Cestoda: Trypanorhyncha) in the collections of the natural history museum, London. Bulletin of the Natural History Museum, Zoology Series, 65(2):133-153.

- [19] Noga, E. J. 2010. Fish disease: diagnosis and treatment. Missouri, Mosby.
- [20] Amato, J. F. R.; Boeger, W. A.; Amato, S. B. 1991. Protocolos para laboratório – coleta e processamento de parasitos de pescado, (1st), 14-32.
- [21] Poche, F. 1926. Das system der Platyhelminthes. Archiv Fur Naturgeschichte, (91th), pp. 1-458.
- [22] Molin, R. 1858. Prospectus helminthum, quae in prodromo fauna helminthologicae Venetiae continentur. Sitzungsb. D. K. Akad. D. Wissensch. Wien, math.-naturg., 30:127-138.
- [23] Dollfus, R. P. 1942. Études critiques sur les tétrarhynques du Muséum de Paris. Archives du Musée National d'Histoire Naturelle, v. 19, p. 1-466, 1942.
- [24] Euzet, L. and Radujkovic, B. M. 1989. *Kotorella pronosoma* (Stossich, 1901) n. gen., n. comb. type des Kotorellidae, nouvelle famille de Trypanorhyncha (Cestoda), parasite intestinal de *Dasyatis pastinaca* (L., 1758). Annales De Parasitologie Humaine Comparee, 64: 420-425.
- [25] Palm, H. W. and Beveridge, I. 2002. Tentaculariid cestodes of the order Trypanorhyncha (Platyhelminthes) from the Australian region. Records of the South Australian Museum, 35(1):49-78.
- [26] Sezen, Y. and Price, C. E. 1969. The parasites of Turkish fishes. Part II. Proposal of a new genus to contain the plerocercoids of *Nybelinia* Poche, 1926 (Cestoda: Trypanorhyncha). Rivista Di Parassitologia, 30(1):35-38.
- [27] Guiart, J. 1927. Classification des Tétrarhynques. Comptes rendus de la 50. Session de l'Association Française pour l'Avancement des Sciences, Lyon, 397-401.
- [28] Bosc, L. A. G. 1797. Description des objets nouveaux d'Histoire naturelle trouvés dans une traversée de Bordeaux à Charles-Town. Bulletin des Sciences par la Société philomatique, Paris 2, 2:9-10.
- [29] Welch, F. H. 1876. The Anatomy of two Parasitic Forms of the Family Tetrarhynchidae. Journal of the Linnean Society of London, Zoology, 12:329-342.
- [30] Pérez-Ponce De León, G., Mendoza, L.G.B., León-Règagnon, V., Pulido, G., Aranda, C., García, F. 1999. Listados Faunísticos de México IX. Biodiversidad de Helminthos Parásitos de Peces Marinos y Estuarinos de la Bahía de Chamela, Jalisco, México. México, DF: Serie Listados Faunísticos del Instituto de Biología, Universidad Autónoma de México, 48 pp.
- [31] Pereira, J. and Boeger, W. A. 2005. Larval tapeworms (Platyhelminthes, Cestoda) from sciaenid fishes of the southern coast of Brazil. Zoosystema, 27(1):5-25.
- [32] Shah, M. and Bilquees, F. M. 1979. *Nybelinia elongata*, new species from the fish *Pellona elongata* of Karachi coast. Pakistan Journal of Zoology, 11:231-233.
- [33] Dollfus, R.P. 1960. Sur une collection de tétrarhynques homeacanthes de la famille de Tentaculariidae, récoltés principalement dans la région de Dakar. Bulletin de l'Institut Français d'Afrique Noire, 22(A3):788-852.
- [34] Menoret, A. and Ivanov, V. 2013. A new species of *Heteronybelinia* (Cestoda: Trypanorhyncha) from *Sympterygia bonapartii* (Rajidae), *Nemadactylus bergi* (Cheilodactylidae) and *Raneya brasiliensis* (Ophidiidae) in the south-western Atlantic, with comments on host specificity of the genus. Journal of Helminthology, 87(4):467-482.
- [35] Yamaguti, S. 1952. Parasitic worms mainly from Celebes. Part 1. New digenetic trematodes of fishes. Acta Medicinæ Okayama, 8(2):146-198.
- [36] Linton, E. 1924. Notes on cestode parasites of sharks and skates. Proceedings of the United States National Museum, 64(2511):1-114.
- [37] Shipley, A. E. and Hornell, F. L. S. 1906. Report on the cestode and nematode parasites from the marine fishes of Ceylon. Ceylon Pearl Oyster Fisheries Marine Biological Reports, 5:43-96.
- [38] Linton, E. 1890. Notes of Entozoa of marine fishes of New England, with description of several new species - Part II. Annual Report of the U.S. Commissioner of Fish and Fisheries for 1887, 719-900.
- [39] Broglia, A. and Kapel, C. 2011. Changing dietary habits in a changing world: Emerging drivers for the transmission of foodborne parasitic zoonoses. Veterinary Parasitology, 182(1):2-13.
- [40] São Clemente, S. C., Lima, F. C. and Uchoa, C. M. A. 1995. Parasitos de *Balistes vetula* (L.) e sua importância na inspeção do pescado. Revista Brasileira de Ciência Veterinária, 2(2):39-41.

- [41] Knoff, M., São Clemente, S. C., Pinto, R. M. and Gomes, D. C. 2002. Prevalência e intensidade de infecção de cestóides Trypanorhyncha elasmobrânquios nos Estados do Paraná e Santa Catarina, Brasil. *Parasitologia Latinoamericana*, 57:149m-157.
- [42] Lima, F. C. 2004. Cestóides da ordem Trypanorhyncha em peixes teleósteos comercializados no estado do Rio de Janeiro, Niterói. 86 f. Tese (Doutorado em Medicina Veterinária) – Faculdade de Veterinária, Universidade Federal Fluminense, Niterói.
- [43] Rodero, M. and Cuéllar, C. 1999. Humoral responses induced by *Gymnornhynchus gigas* extracts in BALB/ c mice. *Journal of Helminthology*, 73:239-273.
- [44] Nieuwenhuizen, N., Lopata, A. L., Jeebhay, M. F., Herbert, D. B. R., Robins, T. G. and Brombacher, F. 2006. Exposure to the fish parasite *Anisakis* causes allergic airway hyperreactivity and dermatitis. *Journal of Allergy and Clinical Immunology*, 117(5):1098–1105.
- [45] Audicana, M. T. and Kennedy, M. W. 2008. *Anisakis simplex*: From obscure infectious worm to inducer of immune hypersensitivity. *Clinical Microbiology Reviews*, 21(2):360–379.
- [46] Hajeb, P. and Selamat, J. 2012. A contemporary review of seafood allergy. *Clinical Reviews in Allergy and Immunology*, 42(3):365–385.
- [47] Cuvier, G. 1817. Les crustacés, les arachnides et les insectes. *Museum of Comparative Zoology*, Chez Deterville, 3(rd).
- [48] Vazquez-Lopez, C., Armas-Serra, C., Bernardina, W. and Rodriguez-Caabeiro, F. 2001. Oral inoculation with *Gymnornhynchus gigas* induces anti-parasite anaphylactic antibody production in both mice and rats and adverse reactions in challenge mice. *International Journal of Food Microbiology*, 64(3):307–315.
- [49] Vazquez-Lopez, C., Armas-Serra, C., Bernardina, W. and Rodriguez-Caabeiro, F. 2002. A 24-kDa collagenase from *Gymnornhynchus gigas* elicits rat ileum hyperreactivity and is a target of humoral responses in mice previously given a single oral dose of parasite extract. *Digestive Diseases and Sciences*, 47(4):935–942.
- [50] Mattos, D. P. B. G., Verícimo, M. A., Lopes, L. M. S. and São Clemente, S. C. 2013. Immunogenic activity of the fish tapeworm *Pterobothrium heteracanthum* (Trypanorhyncha: Pterobothriidae) in BALB/c mice. *Journal of Helminthology*, 88:1-5.
- [51] DOLLFUS, R. P. 1942. Études critiques sur les tetrarhynques du Muséum de Paris. *Archives du Muséum national d'Histoire naturelle (Paris)*, 19:1-466.
- [52] Heinz, H. J. 1954. A case of tetrarhynchid (cestode) infection in man. *Revista Ecuatoriana de Entomología y Parasitología*, 2:227-230.
- [53] Fripp, P. J. and Mason, P. R. 1983. Spurious human infection with a Trypanorhynchiid tapeworm. *South African Journal of Science*, 79:473.
- [54] Kikuchi, Y., Takenouchi, T., Kamiya, M. and Ozaki, H. 1981. Trypanorhynchid cestode larva found on the human palatine tonsil. *Japanese Journal of Parasitology*, 30(5): 497-499.
- [55] Ferreira, M. F., São Clemente, S. C., Tortelly, R., Lima, F. C., Nascimento, E. R., Oliveira, G. A. and Lima, A. R. 2006. Parasitas da ordem Trypanorhyncha: sua importância na inspeção sanitária do pescado. *Revista Brasileira de Ciência Veterinária*, 13(3):190–193.
- [56] Alves, D. R., Luque, J. L. and Paraguassú, A. R. 2002. Community ecology of the metazoan parasites of pink cusk-eel, *Genypterus brasiliensis* (Osteichthyes: Ophidiidae), from the coastal zone of the State of Rio de Janeiro, Brazil. *Memorias do Instituto Oswaldo Cruz*, 97(5):683–689.
- [57] São Clemente, S. C., Knoff, M., Padovani, R. E. S., Lima, F. C., and Gomes, D. C. 2004. Cestóides trypanorhyncha parasitos de congro-rosa, *Genypterus brasiliensis* Regan, 1903 comercializados nos municípios de Niterói e Rio de Janeiro, Brasil. *Revista Brasileira de Parasitologia Veterinária*, 13(3):97–102.
- [58] Palm, H. W., Walter, T., Schwerdtfeger, G. and Reimer, L. W. 1997. *Nybelinia* Poche, 1926 (Cestoda: Trypanorhyncha) from the Moçambique coast, with description of *N. beveridgei* sp. nov. and systematic consideration on the genus. *South African Journal of Marine Science*, 18:273-285.
- [59] Amato, J. F., São Clemente, S. C. and Oliveira, G. A. 1990. *Tentacularia coryphaenae* Bosc, 1801 (Eucestoda: Trypanorhyncha) in the inspection and technology of the skipjack tuna, *Katsuwonus pelamis* (L.) (Pisces: Scombridae). *Atlântica*, 12(1):73-77.