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M. Videira
https://orcid.org/0000-0002-9559-8521
E. Matos
https://orcid.org/0000-0002-9918-1408
M. Velasco
https://orcid.org/0000-0002-2525-423X
O. Sanches
https://orcid.org/0000-0002-4939-8123
P. Matos
https://orcid.org/0000-0002-7175-4956
P.S. Santos
https://orcid.org/0000-0003-4858-1601

# First report of *Kudoa* sp. in the palate and pharyngeal musculature of *Gobioides grahamae* Palmer and Wheeler, 1955 (Perciformes, Gobiidae) from Marajó Island, Brazil

[*Primeiro registro de Kudoa sp. na musculatura do palato e faringeal de Gobioides grahamae Palmer e Wheeler, 1955 (Perciformes, Gobiidae) da Ilha do Marajó, Brasil*]

M. *Videira*[1], M. *Velasco*[2], O. *Sanches*[3], P. *Matos*[4], P.S. *Santos*[5], E. *Matos*[5]

[1]Laboratório de Morfofisiologia e Sanidade Animal - Universidade do Estado do Amapá - Macapá, AP
[2]Universidade Federal Rural da Amazônia - Tomé-Açu, PA
[3]Laboratório de Patologia Animal - Universidade do Oeste Paulista - Presidente Prudente, SP
[4]Laboratório de Pesquisa Edilson Matos - Universidade Federal do Pará - Belém, PA
[5]Laboratório de Pesquisa Carlos Azevedo - Universidade Federal Rural da Amazônia - Belém, PA

## ABSTRACT

The aim of this study was to describe the first occurrence of *Kudoa* sp. in *Gobioides grahamae*, contributing to the understanding of this group of parasites in the Amazonian ichthyofauna. Forty specimens of *G. grahamae* collected from the natural environment were analyzed. Cysts of *Kudoa* sp. were diffusely distributed through the striated skeletal muscle fibers with severe edema and inflammatory infiltrate composed of lymphocytes were observed in 30% of the specimens. Edema and marked coagulation necrosis of the muscle fibers was associated with infection by *Kudoa* sp. spores, which had accumulated inside the skeletal muscle fibers. Although there are no records of foodborne outbreaks caused by *Kudoa* spp. in Brazil, it is of paramount importance that we evaluate its occurrence, since the consumption of fish, especially raw fish, has increased because of the adoption of Japanese cuisine. To minimize the economic impacts on the fisheries market and the risk of this parasite to public health, it is necessary to initiate a program to monitor the presence of this likely underdiagnosed, emerging parasite.

Keywords: muscle fibers, parasite, *Myxozoa*, *Multivalvulida*

## RESUMO

*O objetivo deste estudo foi descrever a primeira ocorrência de* Kudoa *sp. em* Gobioides grahamae, *contribuindo, assim, para a compreensão desse grupo de parasitas na ictiofauna amazônica. Foram analisados 40 espécimes de* G. grahamae *coletados de ambiente natural. Cistos de* Kudoa *sp. foram distribuídos difusamente através das fibras musculares esqueléticas estriadas com presença de edema grave e infiltrado inflamatório composto de linfócitos, que foram observados em 30% dos espécimes. Edema e necrose de coagulação acentuada das fibras musculares foram associados com a infecção por esporos de* Kudoa *sp., acumulados no interior das fibras musculares da faringe. Apesar de não haver registros de surtos de origem alimentar causada por* Kudoa *spp. no Brasil, é de suma importância a avaliação de sua ocorrência, uma vez que o consumo de peixe, especialmente peixe cru, aumentou por causa da adoção da culinária japonesa. Para minimizar os impactos econômicos no mercado da pesca e o risco desse parasita para a saúde pública, é necessário iniciar um programa para monitorar a presença desse parasita emergente, possivelmente subdiagnosticada.*

*Palavras-chave: fibra muscular, parasita*, Myxozoa, Multivalvulida

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E-mail: marcela.videira@ueap.edu.br

## INTRODUCTION

The host range of parasites belonging to the phylum Myxozoa comprises mainly fish. The taxonomy of this phylum is based primarily on spore morphology and structural characteristics, such as the number of valves, number of polar capsules, and size of spores, which provide data for the classification of these organisms (Whipps *et al*., 2004).

The family Kudoidae (Myxozoa: Multivalvulida) includes the genus *Kudoa* (Meglitsch, 1947) whose principle characteristic is the presence of four valves (in most cases), each one containing one polar capsule (Moran *et al*., 1999; Casal *et al*., 2008). This genus has a worldwide distribution and is composed of approximately 95 reported species that parastitize marine and estuarine fishes (Eiras *et al*., 2014a). Most of these species are histozoic parasites that infect skeletal muscle and form macroscopic cysts, although they are reported to be present in other organs such as the brain, pericardium, digestive tract, kidneys, and ovaries (Matsukane *et al*., 2010; Casal *et al*., 2008; Mansour *et al*., 2013).

The identification and study of these parasites is highly important in fisheries and aquaculture because infection with this pathogen reduces the market value of products and generates economic losses for the industry (Shirakashier *et al*., 2014). In addition, the effects on public health should be taken into account, because *Kudoa* parasitism can cause food poisoning and allergic reactions, as previously described for *Kudoa septempunctata* (Kawai *et al*., 2012; Iwashita *et al*., 2013). The aim of this study was to describe the first occurrence of *Kudoa* sp. in *Gobioides grahamae*, contributing to the understanding of this group of parasites in the Amazonian ichthyofauna.

## MATERIALS AND METHODS

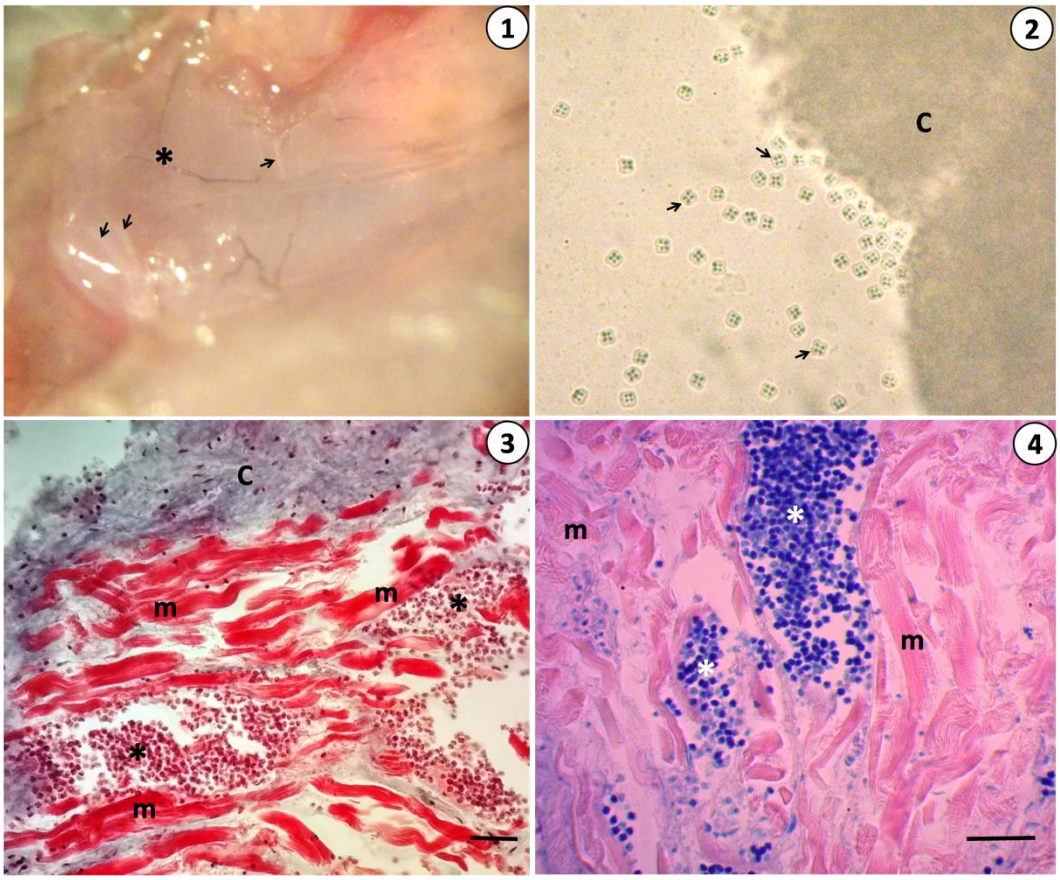
Forty specimens of *G. grahamae* were collected from the natural environment of an area adjacent to the town of Salvaterra (00 of 45'21"S 48°30' 54" W) on the Island of Marajó, in the state of Pará, Brazil. Live specimens were brought to the Laboratório de Pesquisa Carlos Azevedo (LPCA) at Universidade Federal Rural da Amazônia (UFRA) (Belém/PA) and kept in aquariums at a temperature of 28–30ºC. The animals were anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 50mg/L and dissected using a stereoscopic microscope. This study was approved by the Ethics Committee for Experiments with Animals/UFRA (report number 013/2014).

The parasitized tissues were cut into 0.5cm-thick sections, fixed in Davidson solution (formaldehyde PA, acetic acid PA, alcohol 95%, distilled water) for 24h, dehydrated in an increasing series of absolute ethanol, diaphanized in xylol, embedded in paraffin, and then stained with hematoxylin-eosin, Ziehl-Neelsen, and Masson's trichrome. These sections were used for histopathological analyses and results were documented. The measurements of spores were made according to Lom and Dyková (1992) under 1000X magnification in a light microscope with a Zeiss Primo Star photographic camera AxioCam ERC 5s using the AxioVision 5.1 software.

## RESULTS AND DISCUSSION

The analysis of the specimens revealed the presence of filamentous cysts in the pharyngeal muscles. Cysts were diffusely distributed through the striated skeletal muscle fibers of 30% (12/40) of the specimens (Figure 1). Similar to that described by Eiras *et al*. (2016) and Oliveira *et al*. (2015), the parasites were located inside and between the muscle fibers. The spores were identified by light microscopies belonging to the genus *Kudoa*, based on characteristics including the quadrangular or pseudoquadrangular format, with the presence of four equal and pyriform polar capsules located side-by-side and the apex converging on the apical pole of spores, as described by Casal *et al*. (2008) and Azevedo *et al*. (2015) (Figure 2).

Microscopic analysis showed a degenerated epithelium of the palate and multifocal areas of necrosis. Cysts of *Kudoa* sp. with severe edema and inflammatory infiltrate composed of lymphocytes were observed in the submucosa. Edema and marked coagulation necrosis of the muscle fibers was associated with infection by *Kudoa* sp. spores (Figure 3 and 4).




Figures 1-4**.** *Kudoa* sp. in musculature of *Gobioides grahamae*. 1. Region palate pharyngeal (*) with filamentous cysts (arrows). *Kudoa* sp. fresh spores observed (Objective 2X). 2. Cyst (C) and spores (arrows) of *Kudoa* sp. in fresh muscle (Objective 40X). 3. *Kudoa* sp. spores (*) located between muscle fibers (m=skeletal muscles, C=conjuntive tissue) (Masson's Trichrome Stain), scale bar: 100µm. 4. *Kudoa* sp. spores (*) located inside the skeletal muscles fibers of the palate. spores (*) fibers muscles (m) (Staining in Ziehl-Neelsen). Scale bar: 100µm.

Similar histopathological aspects as described here were observed in *Morone americana* (Bunton and Poynton, 1991), in *Trichiurus lepturus* (Andrada *et al*., 2005), and in *Sebastes paucispinis* (Heckmann and Jensen, 1978). Casal *et al*. (2008) described the disintegration of the myofibrils in *Aequidens plagiozonatus* associated with parasitism by *Kudoa aequidens*, without observing an inflammatory reaction. In addition, muscular necrosis has been described as a result of *Kudoa* parasitism in two other species of the Gobiidae family (*Gobius paganellus* and *Pomatoschistus micros*), in a study carried out by Davies *et al*. (1998).

Barja and Toranzo (1993) and Henning *et al*. (2013) suggested that the myoliquefaction of skeletal muscle is related to the fact that these parasites produce proteolytic enzymes that, after the death of the host, act to break down muscle. On the other hand, Eiras (2016b) describes the absence of myoliquefaction in the species *Lutjanus analis, Bagre marinus, Aspistor luniscutis* and *Lutjanus jocu*, parasitized by *Kudoa* sp.

According to Lom and Dyková (1992), myxosporidia infection in a particular host is a result of the interaction of physiological (host resistance) and ecological (environment, nutrition) factors. They report that prevalence of myxospordia can be very high, reaching up to 100%, despite the fact that only a few fish within a stock may be infected initially. They report prevalence of two species, *Kudoa*

*paniformis* and *Kudoa thyrsites*, up to 90% in *Merluccius productus.* Bunton and Poynton (1991) also observed a high prevalence (85%) of *Kudoa* spp. in *Morone americana*, Vaz (2000) reported prevalence of 84.7% in *Trachurus trachurus* and Oliveira *et al*. (2015) reported prevalence of 100% by *Kudoa* sp in *Plagioscion squamosissimus.*

This reported prevalence is higher than that found in this study. Values closer to ours were reported by Casal *et al*. (2008) for *K. aequidens* (35.7%) and by Menezes *et al*. (1989) for *Sardina pilchardus* (30%). Andrada *et al*. (2005) reported a lower prevalence (19%) in *Trichiurus lepturus*. The majority of *Kudoa* species have specific hosts. However, *K. thyrsites* seems to have less host specificity, with reports of this parasite in at least 23 species in several families of fish (Moran *et al*., 1999).

In Brazil, this genus is recorded in 14 species of fish, in the North, Northeast, South and Southeast (Table 1). There is no record of parasitism of the *Kudoa* genus in Gobiidae family fish in Brazil. Velasco *et al*. (2012) described necrotizing myositis in the muscles of the palate of *Gobioides broussonetii*, due to parasitism by *Myxobolus* sp. captured in the same location as the hosts of this study. Many authors describe infections by *Kudoa* as subclinical, and cysts may not be detected by the naked eye. Therefore, a fish infected with thousands of spores in its muscles can go unnoticed and be consumed (Cruz *et al*. 2003).

Table 1. Comparative data of occurrence of *Kudoa* genus in brazilian fishes

| Host | Parasite | Locality | Prevalence | Autor |
|---|---|---|---|---|
| *Mugil platanus* | *Kudoa* sp. | Rio de Janeiro-RJ | - | Knoff and Serra-Freire (1993) |
| *Trichiurus lepturus* | *Kudoa* sp. | Niterói-RJ | 19,15% | Andrada *et al.* (2005) |
| *Aequidens plagiozonatus* | *Kudoa aequidens* | Peixe Boi-PA | 35,7% | Casal *et al.* (2008) |
| *Scomberomorus brasiliensis* | *Kudoa* sp. | Mossoró-RN | 80% | Eiras *et al.* (2014b) |
| *Plagioscion squamosissimus* | *Kudoa* sp. | Outeiro-PA | 100% | Oliveira *et al.* (2015) |
| *Chaetobranchopsis orbicularis* | *Kudoa orbicularis* | Cachoeira do Arari (Marajó Island)-PA | 47,6% | Azevedo *et al.*(2016) |
| *Mugil liza*, *Odontesthes bonariensis* and *Micropogonias furnieri* | *Kudoa* sp. | Rio Grande-RS | - | Eiras *et al.* (2016a) |
| *Lutjanus analis*, *Bagre marinus*, *Aspistor luniscutis* and *Lutjanus jocu* | *Kudoa* spp. | Aracaju-SE | - | Eiras *et al.* (2016b) |
| *Cathorops spixii* and *Cathorops agassizii* | *Kudoa* sp. | Vigia de Nazaré-PA | | Santos *et al.* (2019) |
| *Gobioides grahamae* | *Kudoa* sp. | Salvaterra (Marajó Island)-PA | 30% | Present study |

Martinez de Velasco *et al*. (2008) reported that *Kudoa* spp. can induce an allergic reaction in some people, and through epidemiological analyses and animal tests, Kawai *et al.* (2012) reported *Kudoa septempunctata* as a causative agent in outbreaks of food poisoning. Jeon *et al.* (2014) suggested that, owing to the outbreaks that have been described, it is necessary to develop effective, feasible methods to detect *K. septempunctata* to ensure food safety and to facilitate marketing. According to Lee (2017), in 2015, a total of 11 outbreaks caused by *K. septempunctata* were described associated with the consumption of sushi and sashimi. Diarrhea and vomiting were the most common symptoms observed in these outbreaks.

Although there are no records of foodborne outbreaks caused by *Kudoa* spp. in Brazil, it is of paramount importance that we evaluate its occurrence, since the consumption of fish, especially raw fish, has increased because of the adoption of Japanese cuisine. To minimize the economic impacts on the fisheries market and the

risk of this parasite to public health, it is necessary to initiate a program to monitor the presence of this likely underdiagnosed, emerging parasite. The present study was approved by the Ethics Committee for Animal Experimentation of the Universidade Federal Rural da Amazônia (UFRA) (CEUA approval no. 013/2014).

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## REFERENCES

ANDRADA C.G.; TORTELLY, R.; NOGUEIRA, P.P. *et al*. Infecção por *Kudoa* Meglitsch, 1947 (Myxozoa: Multivalvulida) em musculatura esquelética de espada *Trichiurus lepturus* L. (Teleostei: Trichiuridae). *Parasitol. Latinoam.,* v.60, p.150-153, 2005.

AZEVEDO, C.; ROCHA S.; MATOS E. *et al*. Ultrastructural and phylogenetic description of *Kudoa orbicularis* n. sp. (Myxosporea: Multivalvulida): a parasite infecting the muscle of the fish *Chaetobranchopsis orbicularis* (Teleostei: Cichlidae) in the Amazon Region. *J. Eukaryotic Microbiol.,* v.63, p.27-36, 2016.

BARJA, J.L.; TORANZO, A.E. Myoliquefaction post-mortem caused by the Myxosporean *Kudoa thyrsites* 'in Atlantic Salmon in Spain. *Bull. Eur. Assoc. Fish Pathol.,* v.13, p.86-88, 1993.

BUNTON, T.E.; POYNTON, S.L. *Kudoa* sp. (Myxosporea, Multivalvulida) infection in juvenile white perch, *Morone americana* (Gmelin): histopathology and spore morphology. *J. Fish Dis.,* v.14, p.589-594, 1991.

CASAL, G.; MATOS, E.; MATOS, P. *et al*. Ultrastructural description of a new myxosporean parasite *Kudoa aequidens* sp. n. (Myxozoa, Myxosporea), found in the Sub-Opercular Musculature of *Aequidens plagiozonatus* (Teleostei) from the Amazon River. *Acta Protozool.,* v.47, p.135-141, 2008.

DAVIES, A.J.; ANDREWS, T.; UPTON, N.P.D. *et al*. *Kudoa* as a contributary cause of necrosis in gobies. *Bull. Eur. Assoc. Fish Pathol.,* v.18, p.62, 1998.

EIRAS, J.C. SARAIVA, A.; CRUZ, C. Synopsis of the species of *Kudoa* Meglitsch, 1947 (Myxozoa: Myxosporea: Multivalvulida). *Syst. Parasitol.,* v.87, p.153-180, 2014a.

EIRAS, J.C. 1947 (Myxozoa: Myxosporea: Multivalvulida). *Syst. Parasitol.,* v.87, p.153-180, 2014a.

EIRAS, J.C.; LIMA, J.T.A.X.; CRUZ, C.F.; SARAIV, A. A note on the infection of. A note on the infection of *Scomberomorus brasiliensis* (Osteichthyes, Scombridae) by *Kudoa* sp. (Myxozoa: Multivalvulida). Braz. *J. Vet. Parasitol.,* v.74, p.164-166, 2014b.

EIRAS, J.C.; PEREIRA JR., J.; SARAIVA, A. CRUZ, C.F. Observations on the Infection by *Kudoa* sp. (Myxozoa, Multivalvulida) in fishes caught off Rio Grande, Rio Grande do Sul State, Brazil. *Acta Sci. Biol. Sci.,* v.38, p.99-103, 2016a.

EIRAS, J.C.; FUJIMOTO, R.Y.; MADI, R.R. *et al*. *Kudoa* spp. (Myxozoa, Multivalvulida) parasitizing fish caught in Aracaju, Sergipe, Brazil. *Braz. J. Vet. Parasitol.,* v.25, p.429-434, 2016b.

HECKMANN, R.A.; JENSEN, L.A. The histopathology and prevalence of *Henneguya sebasta* and *Kudoa clupeidae* in the rockfish, *Sebastes paucispinis* of southern california. *J.* Wildl. *Dis.,* v.14, p.259-262, 1978.

HENNING, S.S.; HOFFMAN, L.C.; MANLEY, M. A review of *Kudoa*-induced myoliquefaction of marine fish species in South Africa and other countries. *S. Afr. J. Sci.,* v.109, p.1-5, 2013.

IWASHITA, Y.; KAMIJO, Y.; NAKAHASHI, S.; SHINDO, A. Food poisoning associated with *Kudoa septempunctata*. *J. Emerg. Med.,* v.44, p.943-945, 2013.

JEON, C.H.; WI, S.; SONG, J.Y. *et al*. Development of loop-mediated isothermal amplification method for detection of *Kudoa septempunctata* (Myxozoa:Multivalvulida) in olive flounder (*Paralichthys olivaceus*). *Parasitol. Res.;* v.113, p.1759-1767. 2014.

KAWAI, T.; SEKIZUKA, T.; YAHATA, Y.; KURODA, M. Identification of *Kudoa septempunctata* as the causative agent of novel food poisoning outbreaks in Japan by consumption of *Paralichthys olivaceus* in rawfish. *Clin. Infec. Dis.*, v.54, p.1046-1052, 2012.

KNOFF, M.; SERRA-FREIRE, N.M. Protozoários parasitos de *Mugil platanus* Günther, 1880 do litoral do Estado do Rio de Janeiro, Brasil. *Braz. J. Vet. Parasitol.*, v.2, p.25-28, 1993.

LEE, S.U. Analysis of *Kudoa septempunctata* as a cause of foodborne illness and its associated differential diagnosis. *Epidemiol. Health*, v.39, p.1-5, 2017.

LOM, J.; DYKOVÁ, I. *Protozoan parasites of fishes*. *Developments in aquaculture and fisheries science*. Amsterdam: Elsevier, 1992. v.26, p.159-235.

MANSOUR, L.; THABET, A.; CHOURABI, K. *et al*. *Kudoa azevedoi* n. sp. (Myxozoa, Multivalvulida) from the oocytes of the Atlantic horse mackerel *Trachurus trachurus* (Perciformes, Carangidae) in Tunisian coasts. *Parasitol. Res.*, v.112, p.1737-1747, 2013.

MARTINEZ DE VELASCO, G.; RODERO, M.; CUÉLLAR, C. *et al*. Skin prick test of *Kudoa* sp. antigens in patients with gastrointestinal and/or allergic symptoms related to fish ingestion. *Parasitol. Res.*, v.103, p.713-715, 2008.

MATSUKANE, Y.; SATO, H.; TANAKA, S. *et al*. *Kudoa iwatai* and two novel *Kudoa* spp., *K. trachuri* n. sp. and *K. thunni* n. sp. (Myxosporea: Multivalvulida), from daily consumed marine fish in western Japan. *Parasitol. Res.*, v.108, p.913-926, 2010.

MORAN, J.D.W.; WHITAKER, D.J.; KENT, M.L. A review of the myxosporean genus *Kudoa* Meglitsch, 1947 and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture* v.172, p.163-196, 1999.

OLIVEIRA, J.C.; VALESCO, M.; SANTOS, P. *et al*. *Kudoa* spp. (Myxozoa) infection in musculature of *Plagioscion squamosissimus* (Sciaenidae) in the Amazon region, Brazil. *Braz. J. Vet. Parasitol.*, v.24, p. 235-240, 2015.

SANTOS, J.L.F.; ABRUNHOSA, J.P.; SINDEAUX-NETO, J.L. *et al*. Seasonal patterns of infeccionar by Kudoa *sp.* in the catfishes in the brazilian Amazon region. Bol Inst Pesca, v. 45(2): e449. 2019.

SHIRAKASHI, S.; YAMANE, K.; ISHITANI, H. *et al*. First report of *Kudoa* species in the somatic muscle of the Japanese parrot fish *Calotomus japonicus* (Scaridae) and a description of *Kudoa igami*, n. sp. (Myxozoa: Multivalvulida). *Parasitol. Res.*, v.113, p.2515-2524, 2014.

VAZ, A.F.C. *Parasitoses provocadas por Kudoa (Myxosporea) e larvas de Anisakidae em Trachurus trachurus em Portugal*. 2000. 97f. Dissertação (Mestrado) - Curso de Pós-graduação em Ecologia Aplicada, Universidade do Porto, Portugal.

VELASCO, M.; MATOS, P.; SANCHES, O. *et al*. Necrotizing myositis associated with parasitism by *Myxobolus* sp. (Myxozoa) in the palate of the violet goby, *Gobioides broussonnetii* (Gobiidae), from Marajó Island, Brazil. *Aquaculture*, v.358-359, p.129-131, 2012.

WHIPPS, C.M.; GROSSEL, G.; YOKOYAMA, H. *et al*. Phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based on comparative ribosomal DNA sequence analysis. *J. Parasitol.*, v.90, p.618-622. 2004.