Kudoa Meglitsch, 1947 (Cnidaria: Myxozoa) in Tunas from Madeira Archipelago and in *Micromesistius poutassou* from Northeast Atlantic

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

MSc

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Abstract

Several *Kudoa* species have economic impact because might cause post-mortem myoliquefaction in fish and some are of public health concern as they can cause food poisoning. *Kudoa* spores can be detected by microscopic observation and identify by morphometric, molecular and phylogenetic analysis.

Tunas have high commercial value and they are an important resource to fisheries and aquaculture industries. Albacore (*Thunnus alalunga*), bigeye tuna (*Thunnus obesus*) and skipjack tuna (*Katsuwonus pelamis*), are important species to fisheries in Madeira archipelago as they are the most abundant tuna species in the area.

Blue whiting (*Micromesistius poutassou*) is a small fish with commercial value, abundant along Portuguese coast.

A survey of *Kudoa* spores was conducted in samples of the dorsal muscle of twenty-two albacore, thirty bigeye and thirty skipjack from Madeira archipelago and also in three regions of the musculature (anterior, median and posterior) of thirty blue whiting from continental Portuguese EEZ. From all examined tunas, only one *Kudoa* spore was detected in one bigeye tuna. There were already records of the occurrence of *Kudoa* spores in this species from Pacific Ocean, but this is the first report of this parasite in bigeye tuna from Madeira archipelago. In blue whiting, *Kudoa* spores were detected with high prevalence, 60%, 70% and 73% in anterior, median and posterior regions respectively. No significant differences were detected in the occurrence of parasites in these regions ($p = 0.236$). Significant differences were detected in abundance between the anterior and posterior regions ($p = 0.040$). This is the first report of *Kudoa* sp. in blue whiting from Northeast Atlantic Ocean.

Kudoid parasites can be used as a tool to improve our knowledge not just about stock discrimination and migrations of tunas, but also to determine flesh quality/levels of infection in commercial important fish stocks.

Resumo

Várias espécies de *Kudoa* têm impacto económico, porque podem provocar mioliquefação post-mortem em peixes e algumas destas espécies são de interesse para a saúde pública, pois podem causar intoxicação alimentar. Os esporos de *Kudoa* podem ser detetados por observação microscópica e identificados por análise morfométrica, molecular e filogenética.

Os tunídeos têm um elevado valor comercial e são um recurso importante para as indústrias das pescas e aquacultura. O voador (*Thunnus alalunga*), o patudo (*Thunnus obesus*) e o bonito (*Katsuwonus pelamis*) são espécies importantes para a indústria pesqueira no arquipélago da Madeira, uma vez que são as espécies de atum mais abundantes na região.

O verdinho (*Micromesistius poutassou*) é um pequeno peixe com valor comercial e abundante ao longo de toda a costa Portuguesa.

Um rastreio de esporos de *Kudoa* foi realizado em amostras do músculo dorsal de vinte e dois exemplares de voador, trinta de patudo e trinta exemplares de bonito provenientes do arquipélago da Madeira. Adicionalmente este parasita foi pesquisado em três regiões da musculatura (anterior, mediana e posterior) de trinta exemplares de verdinho provenientes da EEZ continental Portuguesa. De todos os tunídeos examinados, apenas um esporo de *Kudoa* foi detetado no músculo de um patudo. Existem registos da ocorrência de esporos de *Kudoa* em hospedeiros desta espécie proveniente do Oceano Pacífico, mas esta é a primeira deteção destes parasitas em patudo do arquipélago da Madeira. No verdinho, os esporos de *Kudoa* foram detetados com uma prevalência elevada, 60%, 70% e 73% nas regiões anterior, mediana e posterior, respetivamente. Não foram detetadas diferenças significativas na ocorrência destes parasitas nas diferentes regiões ($p = 0.236$). Diferenças significativas foram detetadas na abundância entre as regiões anterior e posterior ($p = 0.040$). Esta é a primeira deteção de exemplares de *Kudoa* em verdinho do Nordeste Atlântico.

Estes parasitas podem ser utilizados como uma ferramenta para melhorar o nosso conhecimento não só sobre descriminação de stocks e migrações de tunídeos, mas também na determinação da qualidade do pescado/níveis de infeção de stocks de peixes comercialmente importantes.

Keywords

Kudoa, economic impact, public health, albacore, *Thunnus alalunga*, bigeye, *Thunnus obesus*, skipjack, *Katsuwonus pelamis*, Madeira archipelago, blue whiting, *Micromesistius poutassou*, Northeast Atlantic.

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1. Introduction

Importance of fish and fisheries

The world population is about 6.8 billion people and it is expected to grow to about 9 billion by 2050 (UN-DESA, 2009). With a growing world population, the demand for food supply is urgent, mostly in developing countries with hunger and malnutrition issues. Fisheries contribute to society by providing sources of protein, vitamins and micronutrients, while promoting employment and economic growth. So, their sustainable use for future global food security it is a matter of concern for public policy (Garcia & Rosenberg, 2010; Dueri et al. 2016).

According to Food and Agriculture Organization of the United Nations (FAO, 2016), global world capture fisheries production in 2014 was 93.4 million tonnes, being 81.5 and 11.9 million tonnes from marine and inland waters respectively.

In marine fisheries, China remain the leading producer between the 25 major fishing countries, followed by Indonesia and Myanmar (Asia), Norway (Europe) and Chile and Peru (South America). Northwest Pacific remained the most productive area for fisheries, followed by the Western Central Pacific, the Northeast Atlantic and the Eastern Indian Ocean. Four highly value groups (tunas, lobsters, shrimps and cephalopods) registered new catches records in 2014, being the total captures of tunas and tuna-like species about 7.7 million tonnes (FAO, 2016).

In inland waters, world catches increased 37% since the last decade. Sixteen major countries have annual catches exceeding 200 000 tonnes, which represent 80% of the world total (FAO, 2016).

Increase in the global supply of fish for human consumption has exceeded population growth over the last five decades, resulting in increasing average fish consumption of about 21 Kg per capita in 2014 (Fig. 1). Some factors such as reductions in wastage, improved distribution channels and international trade contributing to rising consumption of fish per capita worldwide (FAO, 2016).

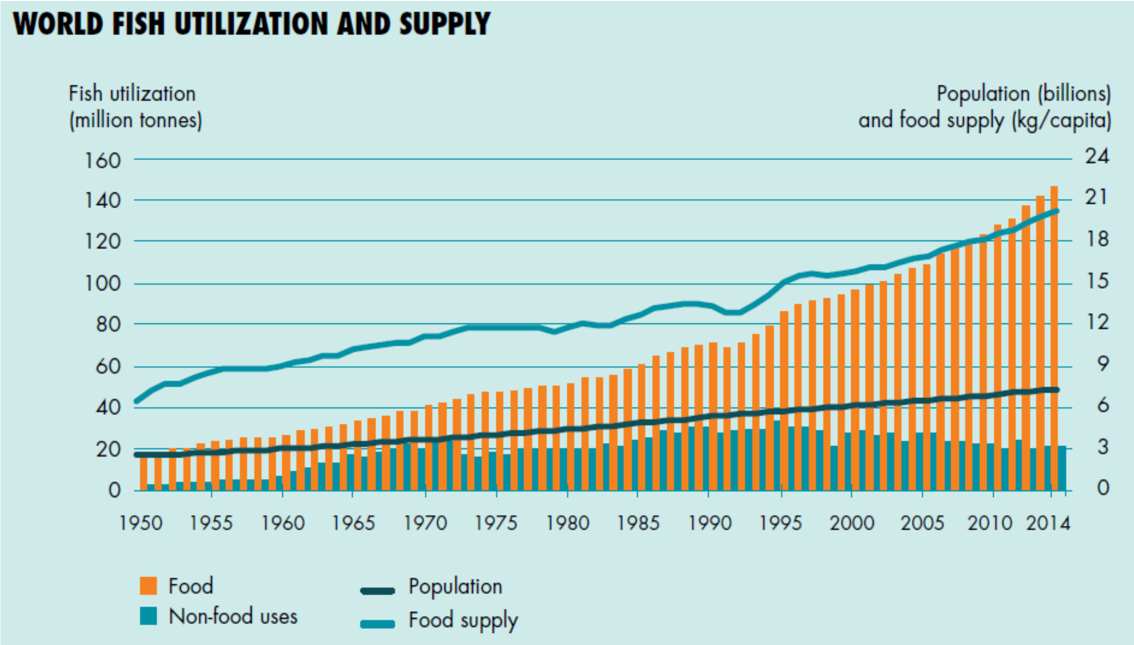

Fig. 1 – World fish utilization and supply since 1950 to 2014 (FAO, 2016).

Portugal is the third highest consumer of fish per capita in the world with an average of 59 kg in 2015 (Failler, 2007; Gamito et al. 2016). In the same year, 194 164 tonnes of fish were caught by Portuguese fleet. The main species caught were european hake (*Merluccius merluccius*), atlantic horse mackerel (*Trachurus trachurus*) and cephalopods, mostly caught by trawl fisheries and atlantic chub mackerel (*Scomber colias*) and european pilchard (*Sardina pilchardus*) caught by purse-seine fisheries (Instituto Nacional de Estatística, 2016; Gamito et al. 2016).

The Portuguese Autonomous Region of Madeira is a small oceanic archipelago composed of two inhabited islands (Madeira and Porto Santo) and several small islands and islets, which are, in some cases nature reserves (Desertas and Selvagens). This archipelago is located to the northwest of Africa and surrounded by Atlantic Ocean deep waters. Its Exclusive Economic Zone (EEZ) is nearly 454.500 km² (Fig. 2). Waters around Madeira are very deep, with low productivity and the islands are characterized for their narrow platforms and steep incline of the slopes, which set limits for potential catches (Shon et al. 2015; Hermida & Delgado, 2016). Total accumulated landings (fish, molluscs and crustaceans) in Madeira archipelago for the period 1938-2014 reached 441.675 tonnes. Before 1950 at around 2000, total landings were stable in the region (Hermida & Delgado, 2016).

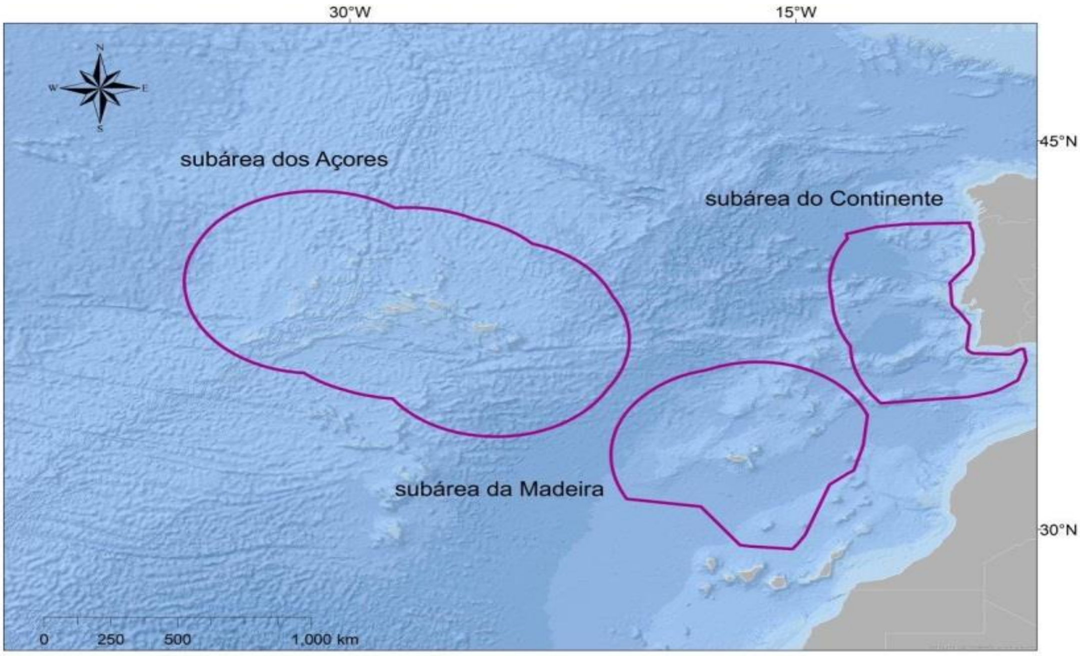

Fig. 2 – Madeira exclusive economic zone (EEZ) and the two other subareas, continent and Azores, of the Portuguese EEZ (DGRN, 2018).

It is well known that fish are highly nutritious, rich in essential micronutrients, minerals, essential fatty acids and proteins, representing the main food supply for some developing countries, mostly for low-income people (Garcia & Rosenberg, 2010). In the wild, fish are often infected by parasites. These parasites can cause diseases or malformations in live fish, or post-mortem lesions as in the case of the infection by myxosporidians (Tejada, 2011). On the other hand, some fish parasites are zoonotic, and humans can be infected by ingesting raw or undercooked fish. (Ribeiro, 2012). For this reason, there are internationally accepted standards sanitary inspection and quality control of fishery products. According to the article 33.º, 9.º, of the decree law nº 9/2011 of June 7, of the Fish Inspection Regulations, fishery products should be visually inspected for the detection of visible parasites before they are available for consumption. Visual control should be done by sampling with a significant number of individuals. When fish or part of it have visible parasites, they should be removed from the market. Regulation (EC) No 853/2004 of 29 April 2004 states that raw or finished fishery products must be frozen at a temperature under -20 °C for a period of at least 24 hours.

The economic and public health importance of the genus *Kudoa* Meglitsch, 1947 (Cnidaria: Myxozoa)

Kudoa are parasites typically histozoic that can be found in several organs like heart, intestines, gills, brain, kidney, ovary, gall bladder, but mostly in the dorsal muscle of wild and cultured fish (Heiniger et al. 2012; Abdel-Baki et al. 2016; Abdel-Ghaffar et al. 2016). This parasite can produce white or black visible cysts containing spores, and some species can cause post-mortem myoliquefaction, frequently referred as post-harvest soft flesh or jellied meat (Yokoyama et al. 2014a; Kasai et al. 2016a). The degree of post-mortem myoliquefaction seems to be affected by several factors like intensity of the infection, fish host inflammatory response, fish storage temperature, etc (Li et al. 2013). These parasites can cause significant economic losses and drastic reduction of the commercial value of the infected fish in aquaculture and fisheries (Moran et al. 1999; Li et al. 2013; Yokoyama et al. 2014a; Griffin et al. 2014; Azevedo et al. 2016; Eiras et al. 2016). Besides the economic impact, some *Kudoa* sp. can be a threat to public health, provoking allergic and/or gastrointestinal symptoms (Martinez de Velasco et al. 2008; Li et al. 2013; Yokoyama et al.2014a). "*Kudoa* food poisoning" is a disease caused by *Kudoa septempunctata* and is manifested by vomiting, diarrhea and abdominal pain 4 to 19 hours after consumption of raw infected fish (Sugita-Konishi et al. 2014; Yahata et al. 2015; Kasai et al. 2017*).* Several cases of food poisoning were reported due to *K. septempunctata* that was obtained through ingestion of infected olive flounder, a fish frequently consumed in Japan. So, since the consumption of raw fish is becoming popular worldwide and fish worldwide trade is increasing, it is important that health organizations be aware of this disease, as it will be very interesting to investigate the life cycle of individual *Kudoa*, which is currently quite limited (Iwashita et al. 2013; Kasai et al. 2016b). The parasitized fish is not harmful for consumption after cooking, but infected fish may have an undesirable muscle texture (Zhou et al. 2009).

Since the work of Whipps et al. (2004) members of the families Pentacapsulidae, Hexacapsulidae and Septemcapsulidae were transferred to the genus *Kudoa* and these parasites are defined as Multivalvulida myxosporeans with four or more polar capsules and valves. Currently, there are approximately one hundred of recognized species included in this genus with a large geographical distribution and infecting a large variety of marine and estuarine fishes (Eiras et al. 2014; Suzuki et al. 2015).

Morphology of *Kudoa* spores is fundamental to species identification, but molecular characterization combined with morphometric data of mature spores, infected tissues preferences, host specificity and geographical distribution, enable more accurate identification of *Kudoa* species (Abdel-Ghaffar et al. 2016). So, despite the identification of the species is primarily based on the morphology and morphometry of the spores, may also include molecular characterization (Burger & Adlard, 2010; Eiras et al. 2014).

Some data about surveyed fish species

As previously mentioned, several *Kudoa* species infect the host's muscle and several times leaves fish unsuitable for commercialization and consumption. So, it is important to verify the presence and the levels of infection of this parasite, mainly in commercially important fish species. Tunas are very important fisheries species but, there are very few reports of the occurrence of *Kudoa* spores in tunas all over the world and as far as we know there are only two references of the occurrence of *Kudoa* spores in tunas fished in Atlantic Ocean (Kent et al. 2001; Griffin et al. 2014).

Tuna and tuna-like species are economically important and a significant source of food. They support one of the largest fisheries in the world in terms of landings and economic value with catches of more than 7 million tons (FAO, 2014). The principal target species of industrial tuna fisheries are albacore [*Thunnus alalunga*, (Bonnaterre, 1788)], bigeye [*Thunnus obesus*, (Lowe, 1839)], bluefin tuna [*Thunnus thynnus*, (Linnaeus, 1758)], skipjack [*Katsuwonus pelamis*, Linnaeus, (1758)] and yellowfin tuna [*Thunnus albacares*, (Bonnaterre, 1788)] with 72.4, 18.2 and 9.4 % of the 2013 catch coming from the Pacific Ocean, Indian Ocean and a combined of Atlantic Ocean and Mediterranean Sea respectively (Nikolic et al. 2016).

Tunas, belonging to the family Scombridae, occupy, in terms of volume, one of the largest habitats on the planet (Brill, 1996). Albacore is a cosmopolitan epipelagic and mesoplagic species inhabiting waters of all oceans. This tuna species can be found in surface waters with temperature ranges from 15.6ºC to 19.4ºC (Collette & Nauen, 1983). Bigeye tuna inhabit epipelagic and mesopelagic zones of tropical and subtropical waters with temperatures between 17ºC and 22ºC. This species is absent from Mediterranean Sea (Collette & Nauen, 1983; Fuller et al. 2015). Skipjack is an epipelagic species widely distributed from temperate to tropical areas, but it is absent from the Black Sea. It can be found in water temperatures between 20.5ºC to 26ºC (Collette & Nauen, 1983; Tanabe et al. 2001; Wang et al. 2018). The geographical distribution of these three commercially important tuna species is shown in figure 3.

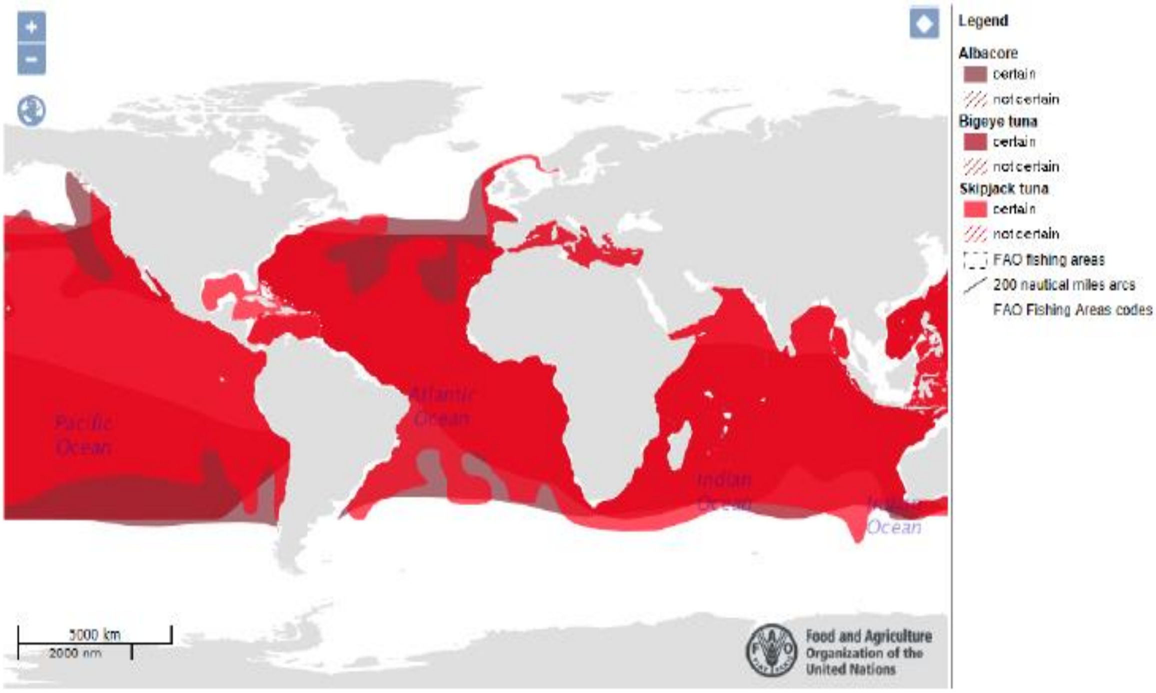

Fig.3 – Geographical distribution of *Thunnus alalunga*, *Thunnus obesus* and *Katsuwonus pelamis* (FAO, 2017a).

Being opportunistic predators, tunas essentially feed on fish, crustaceans and squids. Due to their size, adult tunas have predators such as sharks, billfishes and toothed whales (Collette & Nauen, 1983). They are capable of long migrations or movements and constituting one or two stocks in each ocean (Majkowski, 2007). Albacore present one of the largest fish migrations in the world (AZTI, 2004). In the North Atlantic, both juveniles and adults spend winter time in the central Atlantic area, but when spring comes, juveniles start a trophic migration that occurs until they reach maturity, to northeast Atlantic (AZTI, 2004; Nikolic et al. 2016). In summer and autumn, between April and September, the adults start reproductive migrations to spawning grounds, in the western part of North Atlantic (offshore Venezuela, Sargasso Sea and Gulf of Mexico) (AZTI, 2004; Nikolic et al. 2016). In autumn, albacore starts migrating back to the middle of the Atlantic through the south of Portugal, Canary, Madeira and Azores archipelagos (AZTI, 2004). Migrations of bigeye tuna is characterized by seasonal movements depending on age groups and the nature of migrations (IEO, 2006a). In the Eastern Atlantic, the principal breeding and nursery area of bigeye is in the Gulf of Guinea (IEO, 2006a; Gonzalez et al. 2008). The juveniles remain in this area until spring, when they begin to migrate towards the tropics. They migrate along the coast of Africa, some continue to the Azores, Madeira and Canary Islands or migrate from the interior of the Gulf of Guinea towards the Central Atlantic (IEO, 2006a). Adults feeding areas are placed at both northern and southern temperate areas and they also migrate to the equator to reproduce (IEO, 2006a; Gonzalez et al. 2008). During October to November, the specimens which had migrated to the archipelagos return south (IEO, 2006a). Skipjack's migrations are influenced by environmental conditions (temperature, salinity, nutrients etc.) In the Eastern Atlantic, migrations generally follow the coastline, both North-South, and South-North, in the Gulf of Guinea. Some specimens migrated from the equatorial zone in April, reaching Dakar, Madeira and the Canary Islands in July-August (IEO, 2006b). Transatlantic migrations also occurred in these three species (AZTI, 2004; IEO, 2006a; 2006b). These fish species are consumed worldwide, both fresh or canned and they are amongst the most widely traded seafood (Majkowski, 2007; Mullon et al. 2017; Brill et al. 2017).

Tunas are an important seasonal fisheries resource in Madeira, often comprising around half of total landings (Gouveia et al. 2017). Bigeye tuna were the most important tuna caught in the region in the last decades, with landings generally superior of those of albacore and skipjack, surpassing in some years the sum of the landings of these two last species (Gouveia et al. 2001, 2017). Albacore, who is also fished in variable amounts, reach its catches peak in 2014 (Hermida & Delgado, 2016; Gouveia et al. 2017). These three main tuna species present a marked seasonal pattern in the region. The catches of skipjack occur mainly in late summer and autumn and the ones of bigeye tuna and albacore in spring months. In recent years tuna catches have remained relatively stable (around an average of 2.400 tonnes) in Madeira archipelago (Gouveia et al. 2017).

To the best of our knowledge a survey of *Kudoa* spores in blue whiting [*Micromesistius poutassou*, (Risso, 1827)] from Portuguese EEZ was never been conducted. So, we decided to do a kudoid survey in the muscle of this species too.

Blue whiting is a small oceanic and benthopelagic fish belonging to the family Gadidae, abundant along North Atlantic continental slopes. It is distributed from Barents Sea South through the Eastern Norwegian Sea, around and off Iceland, through the Eastern Atlantic, in the Western Mediterranean Sea and south along the African coast. It also occurs around Southern Greenland, Southeast Canada and Northeastern coast of the USA. (Fig. 4) (Cohen et al. 1990; Silva et al. 1997).

Fig.4 – Geographical distribution of *Micromesistius poutassou* (FAO, 2017b).

The diet is mostly based on crustaceans, specially copepods, euphasids and larvae of decapods (Silva et al. 1997; Cabral & Murta, 2000). The spawning occurs in the spring and summer in the area to the west of British Isles. After spawning, the adults go on a feeding migration upon the Norwegian Sea and return in the autumn to the spawning grounds. The main spawning grounds are UK islands, Portugal, Bay of Biscay, Faeroes, Norway and Iceland, above the continental shelf (Cohen et al. 1990; Silva et al. 1997).

The exploitation of blue whiting from the whole Northeast Atlantic are around half a million tonnes a year (Silva et al. 1997).

Blue whiting is marketed fresh and frozen, and part of the catches used in the fishmeal industry. However, it can also be used in the production of higher valued products such as surimi (Cohen et al. 1990; Silva et al. 1997).

As referred before there are no studies about *Kudoa* parasitosis in fishes of Madeira archipelago. So, since we had the great opportunity to have muscle samples of three commercially important tuna species, albacore (*T. alalunga*), bigeye (*T. obesus*) and skipjack (*K. pelamis*) from Madeira, we did a survey of *Kudoa* spores in these three tuna species from Madeira Portuguese EEZ sub-area. Additionally, and for the same reasons, a survey on this parasite was conducted in blue whiting (*M. poutassou*) from continental Portuguese EEZ.

2. Materials and Methods

Sampling

Twenty-two albacore, thirty bigeye and thirty skipjack were captured in Madeira archipelago between May 2016 and September 2017. Parameters such as landing date, total length (cm), fork length (cm), weight (kg) and sex were determined in each fish. From each tuna specimen a portion of dorsal muscle just behind the head were collected and frozen at -20ºC until examination.

Additionally, a total of thirty blue whiting were acquired from a local fish market in Matosinhos, Portugal mainland, between January and February 2018. In each fish total length (cm), fork length (cm) and weight (g) were measured. The gonads were also examined and, whenever possible, the sex determined following the guidelines of the sexual maturation scale of Esper et al. (2000).

The consistency of the musculature of *M. poutassou* was evaluated consistent or soft and after, muscle samples were collected from three different regions: anterior, median and posterior.

Parasitological examination

The method used to detect *Kudoa* spores were that one proposed by Saraiva et al. (2017). In tuna species it was used 1 gram of the dorsal muscle of each fish and in specimens of *M. poutassou*, 1 gram of muscle from each region (anterior, median and posterior) of each fish. The muscle was placed on a lip of a petri dish, moistened with 5 ml of phosphate buffered saline (PBS), macerated with a scalpel and squashed with the basis of the petri dish. Firstly, the squashed muscle was observed under a stereomicroscope for possible detection of cysts. After, the liquid was squeezed out into a centrifuge tube and allowing the spore suspension to settle during some minutes. After, 3 drops (~ 25µl) were pipetted from the bottom of the tube to a microscope slide and a cover with a coverslip. Slides were observed under a Differential Interference Contrast (DIC) microscope at 400x magnification. Detected kudoid spores were photographed with an integrated camera on the microscope and measured according to Burger & Adlard (2010). Prevalence, mean intensity and mean abundance were determined according to Bush et al. (1997).

Samples from positive results were preserved in ethanol 90% (v/v) to subsequent molecular characterization and phylogenetic analysis.

Data analysis

Data analyses were carried out using IBM SPSS statistics software.

Parasite levels among the three regions of *M. poutassou* were compared using non – parametric tests for related samples. Cochran test was used for occurrence comparisons and Friedman's analysis of variance by ranks for abundance comparisons, followed by multiple comparisons whenever significant differences were detected. For all tests, statistical significance was accepted when $p < 0.05$.

3. Results

The size (total and fork length and weight) and sex of tuna examined are shown in table 1.

Table 1 – Mean values and standard deviations of total length (cm), fork length (cm), weight (Kg) and sex (%) of females (F), males (M) and not determined (ND) from T. alalunga, T. obesus and K. pelamis.

	Total length (cm)	**Fork length (cm)**	**Weight (Kg)**	**Sex (%)**
	Mean±sd	**Mean±sd**	**Mean±sd**	**F M ND**
T. alalunga (n=22)	95.1±4.7	87.9±3.8	15.0±2.3	55 – 45 - 0
T. obesus (n=30)	85.3±8.9	77.6±9.5	10.7±3.4	43 – 47 - 10
K. pelamis (n=30)	53.6±3.3	50.6±3.1	2.9±0.6	67 – 33 - 0

From all examined tunas no cyst or pseudocyst was evident macroscopically in muscles, a *Kudoa* spore was detected in only one bigeye tuna.

The spore was stellate in apical view and have four pyriform capsules of unequal size (Fig.5). Measurements are shown in table 2.

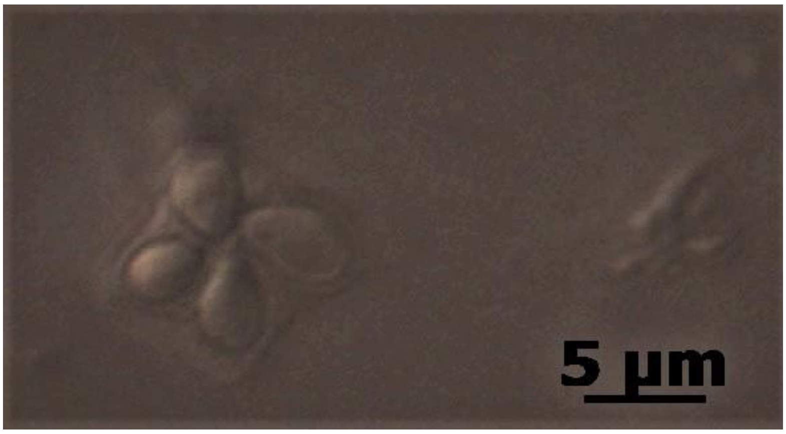

Fig. 5 – Spore of *Kudoa* sp. in apical view from *T. obesus*.

Table 2 – Measurements (in µm) of the spore detected in the muscle of *T. obesus* (width, W; thickness, T and length, L).

Spore			**Large polar capsule**		**Small polar capsule**	
W	T	L	L	W	L	W
-	7.39	-	4.93	2.73	2.96	2.50

Blue whiting from Portuguese coast were examined and their length and weight determined (table 3). From the blue whiting examined 27% were females, 23% males and in the remaining 50% undetermined. Only four specimens presented soft musculature.

Table 3. Mean values and standard deviations of total length (cm), fork length (cm) and weight (g) from *M. poutassou*.

	Mean ± sd
Total length (cm)	19.4±1.0
Fork length (cm)	17.7±0.9
Weight (g)	41.41±9.57

Kudoa spores were detected in 60%, 70% and 73% of fish muscle from the anterior, median and posterior regions, respectively. The intensity and abundance of the infection also increase from the anterior to the posterior regions (table 4). However, no significant differences were detected in the occurrence of parasites in the different regions (p = 0.236). There are significant differences in abundance between regions (p = 0.040). There are no significant differences between the abundance of *Kudoa* spores between the anterior and median regions, and between the median and posterior regions, but significant differences were detected between the anterior and posterior regions.

Table 4 – Infection levels (prevalence (%), mean intensity and mean abundance) of anterior, median and posterior region from *M. poutassou*. Similar letters mean no differences between regions, different letters indicate significant differences.

	Anterior region	**Middle region**	**Posterior region**
Prevalence (%)	60	70	73
Mean intensity ± sd	29.11±42.74	40.86±62.71	63.27±93.77
(min-máx)	(1-144)	(1-232)	(1-306)
Mean abundance ± sd	17.47±35.11	28.60±55.45	46.40±84.72
(min-máx)	(0-144)	(0-232)	(0-306)
	a	ab	b

In apical view, kudoid spores were stellate and presented four pyriform polar capsules of different size (Fig. 6). In side view, spores were pyramidal (Fig. 7). Spore measurements are shown in table 5.

Table 5 - Spore measurement in apical and side view (in µm) from the muscle of *M. poutassou* (width, W; thickness, T and length, L).

	Apical View							**Side View**			
	Spore			**Large polar capsule**		**Small polar capsule**		**Large polar capsule**		**Small polar capsule**	
	W	T	L	L	W	L	W	L	W	L	W
Mean	15.17	8.25	8.42	4.65	3.18	3.66	2.48	6.12	3.16	4.97	2.63
Sd	0.90	0.67	0.53	0.48	0.25	0.51	0.30	0.61	0.30	0.41	0.23
Minimum	13.98	7.45	7.35	3.64	2.50	3.07	1.25	5.27	2.50	3.98	2.35
Maximum	17.75	10.84	9.00	6.32	3.64	5.89	3.00	6.86	3.63	5.63	3.11
n	30	30	15	35	35	35	35	13	13	12	12

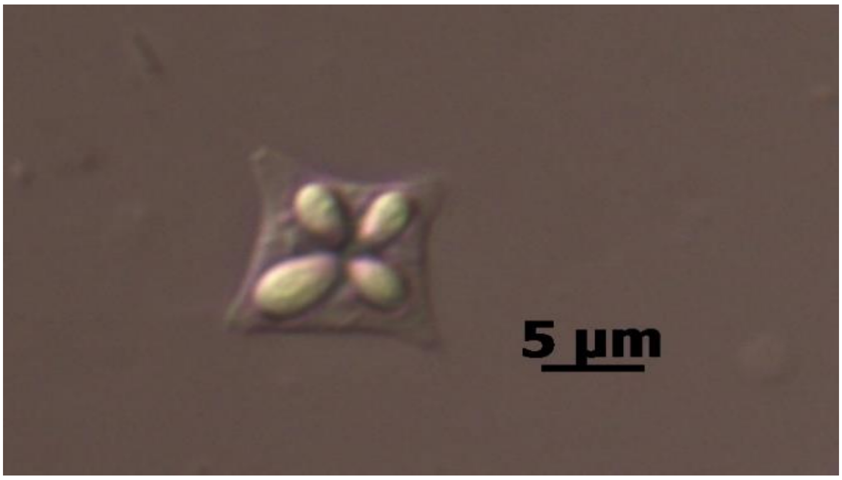

Fig. 6 – Spore of *Kudoa* sp. from *M. poutassou* in apical view.

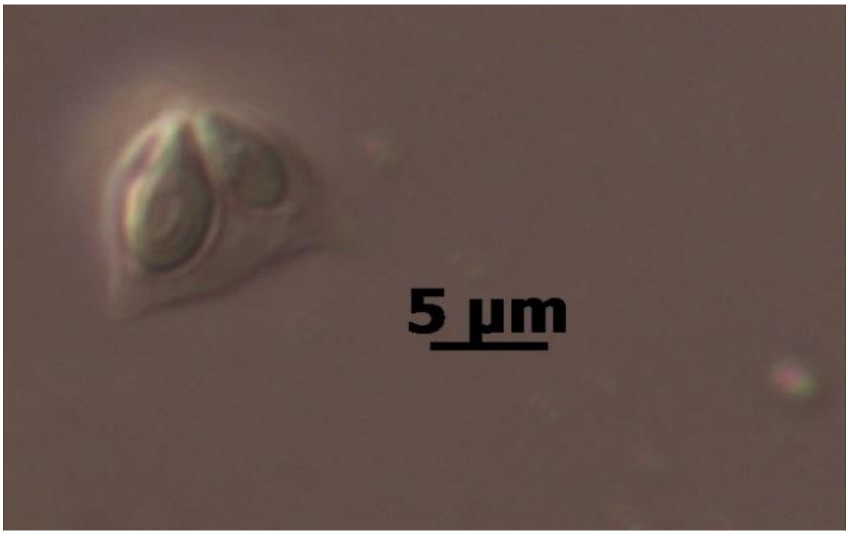

Fig. 7 – Spore of *Kudoa* sp. from *M. poutassou* in side view.

4. Discussion

As far as we know, this is the first report of *Kudoa* sp. in *T. obesus* from Madeira archipelago. There are reports of the occurrence of *K. neothunni* in the muscle of *T. obesus*, but also in *T. albacares*, *T. thynnus*, *T. orientalis* and *T. tonggol* from Pacific Ocean (Arai & Matsumoto, 1953; Abe & Maehara, 2013; Li et al. 2013; Kasai et al. 2017). *K. neothunni* spores have six valves and six polar capsules. *K.* thunni and *K. crumena*, described in *T. atlanticus* and *T. albacares*, respectively, were the only species reported in Atlantic Ocean, although, *K. thunni* was also reported in the muscle of *T. alalunga* from Pacific Ocean (Kent et al. 2001; Matsukane et al. 2011; Griffin et al. 2014). Both species are quadrate members of the Kudoidae with four polar capsules. Still in Pacific Ocean, *K. prunusi* was described in the brain of *T. orientalis* and *K. hexapunctata* in the muscle of *T. orientalis* and *T. tonggol* (Meng et al. 2011; Yokoyama et al. 2014b; Kasai et al. 2017). These species have five and six polar capsules respectively. From what has been said the detected spore could belong to *K. thunn*i or *K. crumena* species. However, in opposition of these two species in the detected spore there is not four uniform polar capsules being possible to be a species not identify yet. Most studies are needed in order to check this assumption.

Tunas, being pelagic fishes, feed in the water column, mostly on fish, crustaceans and squids (Collete & Nauen, 1983). The known myxosporean life cycle involve annelid worms that inhabit the substrate (Kent et al. 2001). *Kudoa* life cycle is very limited, not being fully described, but annelids are also suspected to be an alternative host in a possible two host life cycle (Cruz et al. 2003; Iwashita et al. 2013; Kasai et al. 2016b). So, since albacore, bigeye and skipjack do not feed on these organisms at least in deep oceanic environment as it is the case of Madeira archipelago, this may be an explanation to the absence of *Kudoa* spores in these species. Reproductive migrations patterns of these three tuna species occurs close to the continental shelves along the coast (Collette & Nauen, 1983), and probability during this time tunas can be infected with myxosporidians. Parasites have been used as biological tags to study migrations and distinguish stocks (Mele et al. 2010; Nikolic et al. 2016), so if our assumptions are correct, these parasites can be used to tagged tunas around the world and use as a tool to improve our knowledge in tuna stock discrimination and migrations.

Along the Portuguese coast, *Kudoa* spores has been reported in commercially important species (Gilman & Eiras, 1998; Cruz et al. 2003, 2011; Campbell, 2005).

However, as far as we know this is the first report of *Kudoa* spores in *M. poutassou*. The high prevalence of *Kudoa* infection and the soft flesh present in some specimens, demonstrate the impact of these parasites on product quality and economic value of *M. poutassou* of Portugal. A deeper characterization of this parasites including its molecular studies is being conducted. With this work it is improved the knowledge of the occurrence of kudoids spores in several economically important fish species from Northeast Atlantic Ocean.

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