Vol. 132: 125–134, 2019
https://doi.org/10.3354/dao03316

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published January 10

Morphological and molecular identification of a new *Kudoa thyrsites* isolate in Mediterranean silver scabbardfish *Lepidopus caudatus*

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ABSTRACT: Myxozoans of the genus *Kudoa* (Myxosporea, Multivalvulida) infect marine and estuarine fish species worldwide. Some *Kudoa* species are of concern to the seafood industry since they may generate macroscopic cysts in the fish host's musculature, or cause post mortem myoliquefaction, commonly known as 'soft flesh'. One of the economically most important species is *K. thyrsites*, a myoliquefactive myxosporean parasite that occurs in many wild and cultured marine fish species worldwide. Here we identified a *K. thyrsites* isolate as the causative agent of myoliquefaction in silver scabbardfish *Lepidopus caudatus* from the Alboran Sea (western Mediterranean Sea). For comparative and validation purposes, the morphological and molecular characteristics of the isolate were compared with fresh spores of a *K. thyrsites* isolate infecting Atlantic mackerel *Scomber scombrus* from the Norwegian Sea. Myxospores of both isolates shared a stellate appearance and contained 4 unequal pyriform polar capsules (1 large, 1 small and 2 intermediate). These morphological traits were consistent with all other previously described *K. thyrsites* isolates. Moreover, the small subunit rDNA sequences of the Mediterranean and Norwegian Sea isolates revealed 100% similarity, and matched 100% with *K. thyrsites* isolates previously recorded in myoliquefactive Atlantic mackerel from the North Sea and off southern England. The findings suggest that *K. thyrsites* is the primary cause of myoliquefaction in silver scabbardfish from the Alboran Sea. This report represents the first morphological and molecular characterization of *K. thyrsites* in the Mediterranean Sea. A set of new allometric characters is proposed as additional descriptors for more accurate and specific description of kudoid myxospores.

KEY WORDS: *Kudoa thyrsites* · Mediterranean Sea · 'Soft flesh' · Myoliquefaction · *Lepidopus caudatus* · Molecular identification · New morphological characters · Fish parasite

1. INTRODUCTION

Species of the genus *Kudoa* Meglitsch, 1947 (Myxozoa, Multivalvulida) are parasites of marine and estuarine fishes with a worldwide geographical distribution (Moran et al. 1999, Whipps & Kent 2006, Eiras et al. 2014, MacKenzie & Kalavati 2014). The typical life cycle of myxozoans involves annelids (e.g. oligochaetes and polychaetes) as invertebrate definitive hosts and fish as vertebrate intermediate hosts (Wolf & Markiw 1984, Shaw et al. 1997, Young 2002, Lom & Dyková 2006). Actinospores released from the annelid host infect fish through various entry sites, such as the skin, fins or gills (Yokoyama et al. 2012). However, the transmission pathways of *Kudoa* species, as well as the life cycle putatively involving an

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alternate invertebrate host and a fish infective stage, are still unresolved. The kudoids are defined by their myxospore life cycle stage that occurs in the vertebrate host (i.e. fish) (Lom 1987). The myxospores are characterized by 4 or more shell valves, each containing a single polar capsule (Lom & Dyková 2006).

In early studies, the identification of kudoids to the species level was based on morphology and morphometry of myxospores, i.e. shape and dimensions of the spore, polar capsules and polar filaments (Lom & Arthur 1989), and more recently on molecular analyses of the small (SSU) and large (LSU) ribosomal DNA (rDNA) subunits (Whipps & Kent 2006, Burger & Adlard 2010, 2011). A more accurate identification of *Kudoa* species can be achieved by integrating both morphological and molecular data, together with ecological and biological aspects such as tissue tropism, host specificity, pathogenicity and geographical distribution (Whipps et al. 2004, Adlard et al. 2005, Shin et al. 2016).

To date, more than 100 nominal *Kudoa* species have been described from many phylogenetically distant fish host species (Moran et al. 1999, Lom & Dyková 2006, Eiras et al. 2014, Sato & Kasai 2016). Most of these are histozoic parasites infecting the skeletal muscles of fish (Moran et al. 1999, Lom & Dyková 2006, Eiras et al. 2014), while others occur in the brain, heart, gills, kidney, ovary or intestines (Eiras et al. 2014).

Kudoa spp. are considered non-pathogenic parasites of fish. However, several species can generate macroscopic cysts in the muscle and may induce post mortem myoliquefaction (Alvarez-Pellitero & Sitjà-Bobadilla 1993, Moran et al. 1999, Eiras et al. 2014). The muscle degeneration, commonly known as 'soft flesh', 'milky flesh' or 'jelly flesh', may irreversibly reduce the quality of the fish fillet and the marketability of the fish product, resulting in economic losses to the seafood industry, as well as loss of consumer confidence (Moran et al. 1999, Lom & Dyková 2006, Levsen 2015). Furthermore, some human health issues (e.g. gastrointestinal disorders) associated with the consumption of raw fish products from wild and farmed fish infected by *Kudoa* spp. (e.g. *K. septempunctata*) have recently been reported (Kawai et al. 2012, Iwashita et al. 2013, Suzuki et al. 2015, Yahata et al. 2015).

One of the most conspicuous 'soft flesh'-inducing species is *K. thyrsites* (Gilchrist 1924), which infects the somatic and cardiac musculature of several wild and cultured marine fish species throughout temperate seas of the world (Moran et al. 1999, Whipps & Kent 2006). In contrast to the rather narrow fish host range of most *Kudoa* species, and many myxozoans in general (Moran et al. 1999, Whipps & Kent 2006, Burger et al. 2008, Yokoyama et al. 2012), *K. thyrsites* has been recorded from more than 30 phylogenetically comparatively distant teleost fish species. Moreover, infections with *K. thyrsites* have been reported from commercially important wild fish stocks such as Northeast Atlantic mackerel (Levsen et al. 2008), mahi-mahi *Coryphaena hippurus* off western Australia (Langdon 1991, Whipps et al. 2003) and snoek *Thyrsites atun* off South Africa (Henning et al. 2013), the latter being the type host species of *K. thyrsites* (Gilchrist 1924). *K. thyrsites* has also been documented in several important sea-reared fish species such as olive flounder *Paralichthys olivaceus* from Japanese waters (Yokoyama et al. 2004), coho salmon *Oncorhynchus kisutch* and Atlantic salmon *Salmo salar* in British Columbia, Canada (Whitaker & Kent 1991, St-Hilaire et al. 1998, Moran et al. 1999, Marshall et al. 2016), as well as other areas including Ireland, Chile and Australia (Palmer 1994, Munday et al. 1998, Lopez & Navarro 2000). For instance, *K. thyrsites* infections caused significant problems for the Atlantic salmon aquaculture industry in British Columbia, where the loss of revenue due to degraded fish products reached 50 million Canadian dollars in 2002 (Funk et al. 2007, Marshall et al. 2016).

Despite its almost cosmopolitan distribution (Moran et al. 1999, Whipps & Kent 2006, Henning et al. 2013, MacKenzie & Kalavati 2014), *K. thyrsites* has not been previously reported in fish from the Mediterranean Sea. Only a few reports of *Kudoa* sp. infections in fish from the Mediterranean Sea exist so far. A case of 'soft flesh' was observed in 1 swordfish *Xiphias gladius* caught off Sicily, caused by an unidentified *Kudoa* sp. (Gaglio et al. 2010). Pampoulie et al. (1999) described *K. camarguensis* as the causative agent of myoliquefaction in 2 gobiid fish caught in the lagoon waters of the River Rhone delta in southern France. Moreover, in the same geographical area, macroscopic pseudocysts of *K. lunata* were found in the muscle of 3 species of scaldfish (*Arnoglossus* spp.) (Lom et al. 1983). Along the Mediterranean North African coast, spores of *K. nova* were detected in whitish macroscopic pseudocysts embedded in the muscle of Atlantic horse mackerel *Trachurus trachurus* (Campbell 2005). Some other species infecting the ovaries (*K. azevedoi*, Mansour et al. 2013), spleen, intestine (*K. trifolia*, Holzer et al. 2006) and mesenteries (*K. unicapsula*, Yurakhno et al. 2007) of several different Mediterranean fish species have also been reported. However, none of the 'soft flesh'-

inducing *Kudoa* species so far reported from the Mediterranean Sea were genetically identified to species level.

The silver scabbardfish *Lepidopus caudatus* is a benthopelagic trichiurid, widely distributed throughout temperate oceans of the world (Parin 1986, Demestre et al. 1993). It generally inhabits the continental shelf and the upper slope, seasonally migrating in the water column (Demestre et al. 1993, Nakamura & Parin 1993, D'Onghia et al. 2000). In the Mediterranean region, especially Spain, Italy, Albania and Tunisia, *L. caudatus* is one of the commercially most important fish species intended for human consumption (Rosa et al. 2006, Iwamoto 2015). To date, little is known about the epidemiology of *Kudoa* sp. in *L. caudatus,* except of a single report of myoliquefactive *K. thyrsites* in this fish species, caught off South Africa (Henning et al. 2013).

The aim of the present study was to identify and characterize, by morphological and molecular means, a 'soft flesh'-inducing *Kudoa* species in silver scabbardfish caught in the Alboran Sea (western Mediterranean Sea). For comparative and validation purposes, the spore characteristics of this new isolate were compared with those of freshly obtained spores of a *K. thyrsites* isolate from Atlantic mackerel *Scomber scombrus* caught in the Norwegian Sea.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 35 silver scabbardfish, caught in October 2014 off the coast of Motril, Alboran Sea (36° 34′ N, 3° 30′ W), were parasitologically examined for anisakid nematodes as part of the EU Project PARASITE (GA no. 312068). The fish (n = 35; mean ± SD total length and weight: 122 ± 7.8 cm and 1468 ± 309 g), were obtained during regular commercial fishing operations. The samples were cool-stored on board and subsequently transported in a refrigerated truck (2°C) to the laboratory of the Parasitology Section at the Department of Public Health and Infectious Diseases of the Sapienza-University of Rome, Italy.

During inspection for parasites (approximately 48 h after the fish were caught), 2 of the silver scabbardfish showed abnormally soft and mushy texture of the muscle tissue comprising the fillets. The extent of muscle liquefaction in each fish was assessed by manual muscle texture testing and visual inspection of the muscle appearance, i.e. whether the basic segmental myomere structure was intact or not.

Two subsamples of soft muscle tissue were taken from each fillet of the affected fish. Following St-Hilaire et al. (1997), the samples were placed on glass slides, moistened with saline water and then minced with a scalpel blade. The resulting minced tissue was collected into plastic tubes and allowed to rest for 30 min, and then centrifuged. A small aliquot was pipetted from the bottom of each tube and used to prepare 2 wet smears of each sample. The slides were then examined using bright-light microscopy at 400× magnification. Whenever kudoid spores were detected, the wet smears were embedded and mounted in glycerine jelly (Crookham & Dapson 1991). For further morphological and molecular analyses, 10 Eppendorf tubes (1.5 ml) were filled with 'soft' muscle tissue of the 2 *Kudoa*-positive silver scabbardfish, and stored frozen at –20°C.

For comparative and validation purposes, the same inspection and analytical procedures were applied to 8 specimens of Atlantic mackerel showing muscle liquefaction after manual texture testing and visual inspection. The mackerel examined belonged to a batch of 916 specimens (mean total length and weight: 36 ± 2 cm, 434 ± 65 g) caught in September 2017 during a research cruise in the southern Norwegian Sea (approx. 64° 04′ N, 00° 40′ E).

2.2. Morphological analysis of spores

The measurements and the overall morphological analysis of the spores were performed using Nikon Digital Sight DS-L1 measuring tool software on multiple digital images obtained in an Olympus BX51 microscope at 1000× magnification. In total, 60 spores (30 in lateral and apical view, respectively) from each of the silver scabbardfish and Atlantic mackerel samples were measured in accordance with the morphometric characters commonly used to identify *Kudoa* species (Lom & Arthur 1989, Levsen et al. 2008). Morphometric characters include spore length, width and thickness, and length and width of the large, intermediate and small polar capsules (PCs) (Lom & Arthur 1989, Levsen et al. 2008) (Fig. 1). All measurements are given in µm, while the overall measuring uncertainty of the morphometric spore characters was set to 0.5 µm.

Various allometric relationships were assessed with respect to their usefulness as additional specific morphological descriptors of kudoid myxospores (see Table 1). Thus, the ratios between spore width 1 (W1) and width 2 (W2), thickness 1 (T1) and thickness 2 (T2), length of the large PC (LPCL) and length of the

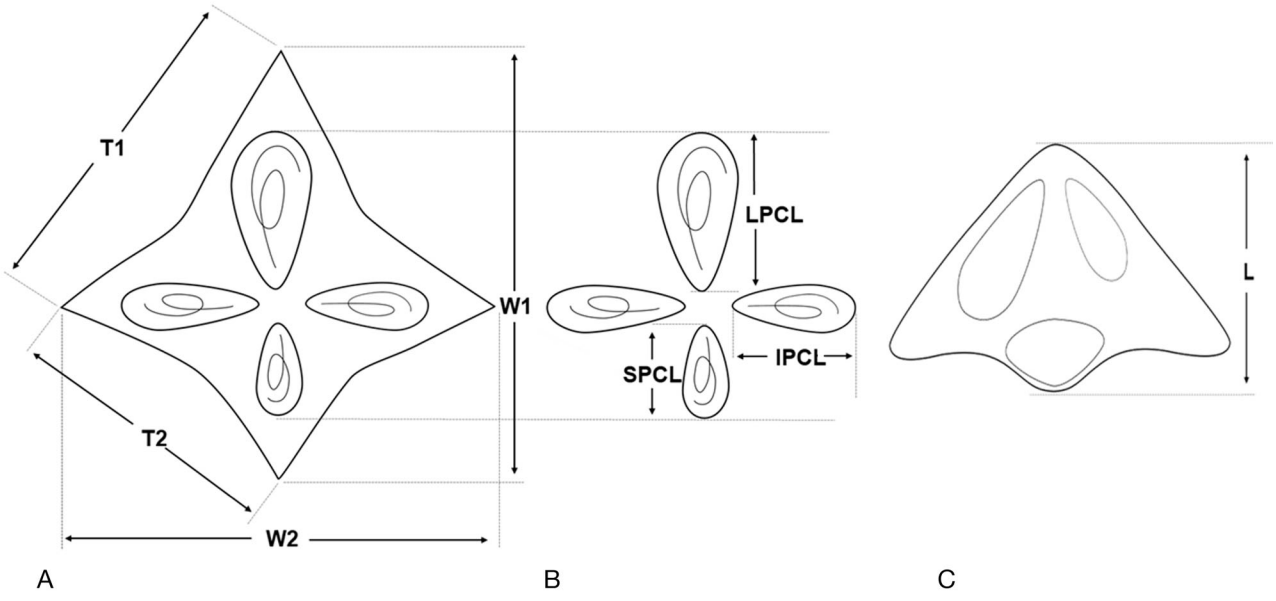

Fig. 1. Schematic drawings for the morphological characterization of kudoid spores. (A) Apical view of spore. W1 (W2): width 1 (2), T1 (T2): thickness 1 (2). (B) Measurements of the polar capsules (PC). LPCL: length of the large PC; IPCL: length of the intermediate PC; SPCL: length of the small PC. (C) Lateral view of spore. L: length

intermediate PC (IPCL), as well as the ratio between the length of the large PC (LPCL) and the length of the small PC (SPCL) were calculated (see Table 1).

Measurements of morphological characteristics of *Kudoa* sp. spores obtained from silver scabbardfish and Atlantic mackerel were statistically compared using Student's *t*-test. A Shapiro-Wilk test was performed to assess if the data were normally distributed (95% confidence level). Equal variances were assessed graphically. Subsequently, measurement data were transformed into base-10 logarithmic scale to achieve normality. All statistical analyses were performed using Statistica v13.1. The level of statistical significance was set to 95% ($p < 0.05$).

Finally, the morphological appearance and morphometric data of the present *Kudoa* sp. myxospores were compared with the published data of *K. thyrsites* isolates described from various fish host species and localities (see Table S1 in the Supplement at www.int-res.com/articles/suppl/d132p125_supp.pdf).

2.3. Molecular analysis

Molecular identification of *Kudoa* sp. was carried out on samples of liquefied muscle collected from 2 silver scabbardfish caught in the Alboran Sea and from 2 Atlantic mackerel fished in the Norwegian Sea. *Kudoa* sp. DNA was extracted from 40 mg of muscle tissue, using the DNeasy® Blood and Tissue Kit (Qiagen). The SSU rDNA (18S rDNA) was amplified using the primer pair K.thyr18Sfor_3 (5′-GGT CAT ATG CTC GTC TCA AAG-3′) and K.thyr18Srev_3 (5′-TCG GTC AAG ACA ATT TAA CCG-3′) (Levsen 2015). Polymerase chain reaction (PCR) was carried out in a 50 µl volume containing 100 ng template DNA, 1 µl of each primer (10 mM), 2 µl of $MgSO_4$ (50 mM) (Invitrogen), 5 µl of 10× high-fidelity buffer (Invitrogen), 4 µl of dNTPs (10 mM) (Invitrogen), 0.2 µl Platinum *Taq* DNA polymerase (Invitrogen) and MilliQ water. The PCR reaction was run with the following conditions: 95°C for 5 min (initial denaturation), then 35 cycles of 95°C for 30 s (denaturation), 55°C for 30 s (annealing) and 72°C for 1 min (extension), followed by a final post-amplification at 72°C for 7 min.

All PCR products were purified using the QIAquick® PCR purification kit (Qiagen) and sequenced with an Automated Sanger DNA Sequencer using BigDye™ Terminator v3.1 mix (Thermo Fisher Scientific) at the DNA Sequencing Laboratories of the University of Bergen, Norway. Sequencing was performed in both directions to ensure reading accuracy. All SSU rDNA sequences obtained were aligned in Clustal X version 2.0 software (Larkin et al. 2007) and analysed in GenBank (BLAST, www.ncbi.nlm.nih.gov/BLAST), in comparison with other kudoid sequences previously deposited.

3. RESULTS

3.1. Epidemiological data

Two out of 35 silver scabbardfish caught in the Alboran Sea showed signs of abnormally soft and mushy texture upon arrival at the laboratory, approximately 48 h post catch and stored at 2°C during transport. Total length and total weight of the 2 affected fish was 135 and 121 cm, and 1778 and 1500 g, respectively. Microscopic analysis of liquefied muscles of the affected specimens revealed the presence of numerous myxospores throughout the fish flesh, subsequently identified as *Kudoa* sp. spores. The Atlantic mackerel samples consisted of 916 fish, of which 8 showed clear signs of muscle liquefaction. Subsequent microscopy of muscle samples from each of the affected mackerel revealed the presence of numerous *Kudoa* sp. spores. Thus, the relative occurrence of post mortem myoliquefactive kudoosis ('soft flesh') was 5.7% in silver scabbardfish and 0.9% in Atlantic mackerel.

3.2. Morphological and morphometric characteristics of spores

The spores obtained from silver scabbardfish (Fig. 2A) and Atlantic mackerel (Fig. 2B) showed similar morphological appearance. In apical view, mature spores were stellate, with 4 unequal pyriform PCs, each within thin-walled valves (Fig. 2). The *Kudoa* sp. spores from both fish species were characterized by 1 large, 1 small and 2 intermediate PCs, directed apically to one another (Fig. 2). Single-coiled polar filaments were sometimes visible within each PC (Fig. 2). In lateral view, spores were subconical in shape, with posterior apices of valves forming lanceolate processes.

In accordance with the morphological characteristics described by Egusa (1986), Kent et al. (1994), Canning & Okamura (2004) and Lom & Dyková (2006), all present spore samples were assigned to the genus *Kudoa*. The morphometric and allometric data (mean ± SD, range) of spores isolated from silver scabbardfish and Atlantic mackerel, are given in Table 1.

Statistical analysis confirmed that there was no significant morphometric variation between the 2 host isolates of *Kudoa* sp. The overall morphology, together with the morphometric spore measurements, showed close similarity with isolates of *K. thyrsites* described in the literature (Table S1).

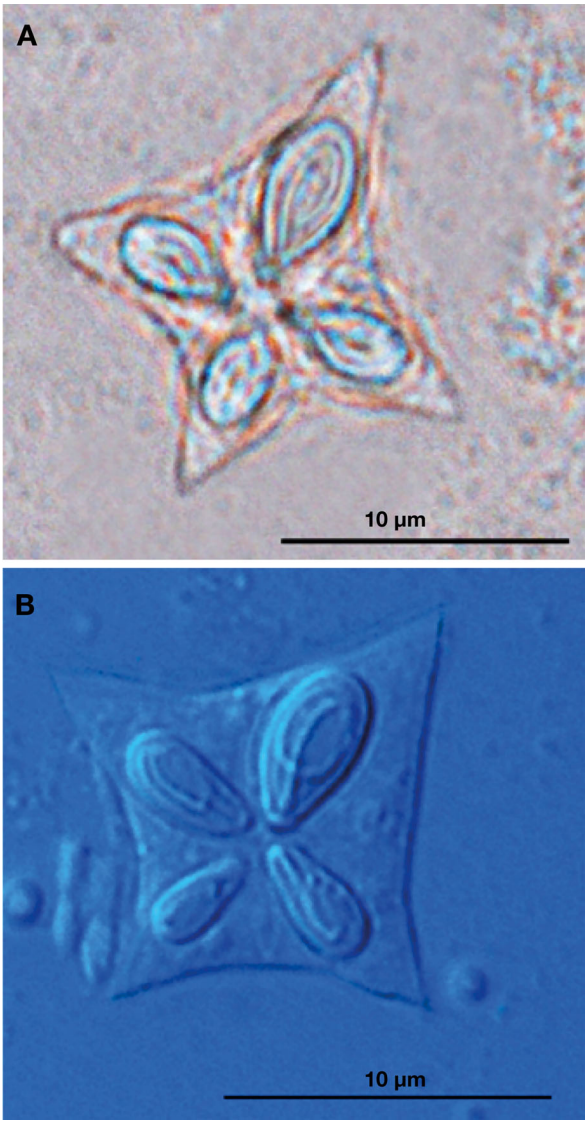

Fig. 2. Comparison between mature spores of 2 isolates of *Kudoa thyrsites* in apical view. (A) *K. thyrsites* in *Lepidopus caudatus* from the Alboran Sea. (B) *K. thyrsites* in *Scomber scombrus* from the Norwegian Sea

3.3. Molecular identification

SSU rDNA sequences (1200 bp in length) were obtained from *Kudoa* spores sampled from liquefied muscle of the examined fish specimens. The alignment by Clustal X of the SSU rDNA sequences from silver scabbardfish (GenBank accession no. MH899080), showed 100% match with the corresponding sequence of the *Kudoa* sp. isolate from Atlantic mackerel (GenBank accession no. MH899081).

Moreover, the latter sequences matched 100% (over 1200 bp) with the SSU rDNA sequence of

Table 1. Comparative spore morphometry of *Kudoa thyrsites* isolates from silver scabbardfish *Lepidopus caudatus* and Atlantic mackerel *Scomber scombrus* from the Alboran Sea and the Norwegian Sea, respectively. Sixty spores were measured for each parameter and fish host species. Measurements (mean, SD and range) are given in µm. Morphological character definitions are given in Fig. 1. TL (TW): total length (width) of myoliquefactive fish

Morphometrical characteristic or ratio	Locality and host species: Alboran Sea *Lepidopus caudatus* N = 2 (TL: 135 and 121.0 cm; TW: 1778 and 1500 g)			Norwegian Sea *Scomber scombrus* N = 8 (mean TL: 37 cm; mean TW: 436.5 g)		
	Mean	SD	(Range)	Mean	SD	(Range)
W1	15.5	1.0	(13.0–17.5)	15.5	1.5	(13.0–18.5)
W2	14.5	1.0	(12.0–17.0)	14.5	1.5	(11.5–17.5)
T1	12.0	1.0	(9.5–14.5)	12.0	1.5	(9.5–17.5)
T2	10.0	1.0	(8.5–12.5)	9.5	1.0	(7.0–11.5)
L	7.5	1.0	(6.5–11.0)	7.0	1.0	(5.5–8.0)
LPCL	5.0	0.5	(4.0–6.5)	5.5	1.0	(4.5–7.5)
IPCL[a]	3.5	1.0	(2.5–6.0)	4.0	1.0	(2.5–5.5)
SPCL	3.0	0.5	(1.5–4.5)	3.0	0.5	(2.0–4.0)
W1:W2	1.1	0.1	(1.0–1.2)	1.1	0.1	(0.9–1.2)
T1:T2	1.2	0.2	(0.9–1.6)	1.2	0.2	(1.0–1.6)
LPCL:IPCL	1.4	0.3	(0.7–1.8)	1.4	0.2	(1.2–2.0)
LPCL:SPCL	1.8	0.3	(1.1–3)	1.9	0.3	(1.5–2.7)

[a]Combined measurements (mean) of the 2 intermediate polar capsules of *K. thyrsites* spore

the previously identified *K. thyrsites* isolate from Atlantic mackerel caught in the North Sea (GenBank accession no. EU154349, Levsen et al. 2008), as well as the SSU rDNA sequence of another *K. thyrsites* isolate from Atlantic mackerel caught off southern England (GenBank accession no. AY542482, Whipps & Kent 2006). On the other hand, the present *K. thyrsites* SSU rDNA sequences obtained from silver scabbardfish and Atlantic mackerel shared 99% similarity with *K. thyrsites* sequences of the same gene recorded from other geographical areas and deposited in GenBank, i.e. South Africa (accession nos. AY542481, AY941819, Whipps & Kent 2006; and AY078430, Whipps et al. 2003), eastern Australia (accession no. AY152747, Whipps et al. 2003), British Columbia (accession no. AF031412, Hervio et al. 1997) and Japan (accession nos. LC128644, LC128645, Kasai et al. 2016; and AY382607, Yokoyama & Itoh 2005).

4. DISCUSSION

Post mortem liquefaction of fish muscle tissue, commonly referred to as 'soft flesh', is a phenomenon primarily associated with *Kudoa* spp. infections, as well as some other species of multivalvulid myxosporeans (Moran et al. 1999). To date, only a few cases of 'soft flesh'-inducing *Kudoa* species have been reported from fish caught in the Mediterranean Sea (Pampoulie et al. 1999, Gaglio et al. 2010). However, none of the *Kudoa* species was identified or described molecularly.

In the present study, a case of post mortem myoliquefactive kudoosis in 2 silver scabbardfish from the Alboran Sea is reported. Based on overall spore morphology and molecular analysis, the causative species was identified as *K. thyrsites* (Gilchrist 1924). The finding represents the first molecular and morphological identification of *K. thyrsites* in fish from the Mediterranean Sea. For comparative and validation purposes, the morphological and molecular characteristics of the present *K. thyrsites* isolate from the Mediterranean Sea were compared with equally processed and analysed fresh spores of a *K. thyrsites* isolate infecting Atlantic mackerel caught in the Norwegian Sea.

The overall morphological characteristics of the spores from liquefied muscle tissue of the silver scabbardfish samples were in close agreement with those of the *K. thyrsites* isolate from Atlantic mackerel (Fig. 2), as well as with other *K. thyrsites* isolates previously described from different fish host species and geographical areas (Table S1).

Despite the similarities in overall shape and appearance, it appears from the literature that various spore measurements, as well as some other specific characteristics, differ considerably between the *K. thyrsites* isolates described to date (Table S1). For instance, the presence of unequally sized PCs (1 large, 1 small and 2 intermediate) in both *K. thyrsites* isolates presently analysed (Fig. 2), has only been reported in a few other studies of this species, including the original description (Gilchrist 1924, Langdon 1991, Munday et al. 1998, Levsen et al. 2008). Additionally, the number of coils of the polar filament varies considerably between different reports (i.e. 1–3.5 coils) (Gilchrist 1924, Kabata & Whitaker 1981, Langdon 1991, Whipps et al. 2003, Levsen et al. 2008), thus rendering it a less reliable taxonomic character (Whipps et al. 2003). Spore width is another important morphological character of *K. thyrsites* that seems to vary widely among isolates, ranging from 12.0 µm in spores isolated from

South African snoek *Thyrsites atun* to 16.7 µm in spores obtained from Pacific hake *Merluccius productus* caught off Pacific North America (Whipps & Kent 2006).

The intraspecific variability in spore dimensions appears to be relatively high in Myxosporea (Diamant et al. 2005). For example, observations in several other *Kudoa* species (e.g. *K. gunterae, K. hypoepicardialis, K. islandica, K. iwatai, K. shiomitsui, K. whippsi*), as well as some other Myxozoa (e.g. *Enteromyxum leei*) indicate that spore size apparently depends on developmental stage, geographical locality or fish host species (Padrós et al. 2001, Blaylock et al. 2004, Diamant et al. 2005, Burger & Adlard 2010, Kristmundsson & Freeman 2014). Other technical factors (e.g. heterogeneity of measuring techniques adopted by different authors, 3-dimensionality of the spores), may introduce uncertainty as to measurement accuracy. Measuring a curved 3-dimensional spore with a standard microscope has obvious limitations since only 1 focal plane can be imaged at a given point. Being a result of a projection, the image of the spore could therefore become considerably distorted, increasing the uncertainty associated with a measurement (Prabhat et al. 2004). The measuring uncertainty may increase even more when focussing on the smallest characters of a spore. Thus, although the width of the PCs is commonly reported as a specific character in *Kudoa* spp. spore descriptions, the accuracy of this measurement obtained here was considered not informative, and is thus not reported.

In light of these issues, we propose a set of additional morphometric characters, based on calculated allometric ratios of various spore dimensions, which can be useful when used together with conventional measurements. Thus, the presently considered ratios (W1:W2, T1:T2, LPCL:IPCL and LPCL:SPCL) were virtually identical in both *K. thyrsites* isolates examined here, i.e. they did not differ significantly. This observation suggests that these allometric ratios are consistent between the 2 isolates and can therefore be used as supplementary descriptors in species or isolates (e.g. *K. thyrsites*) in which spore size varies widely. In fact, since allometric ratios are derived relative values, they are unaffected by the variability of morphometrical spore dimensions.

The molecular identification of the present *Kudoa* isolates infecting silver scabbardfish and Atlantic mackerel was consistent with the morphological identification, thus confirming *K. thyrsites* as the primary cause of post mortem myoliquefaction in both fish species. The SSU rDNA sequences (1200 bp) of the *K. thyrsites* samples from silver scabbardfish (GenBank accession no. MH899080) were 100% identical to SSU rDNA sequences of *K. thyrsites* samples from Atlantic mackerel from the Norwegian Sea (GenBank accession no. MH899081). A complete match (100%) was also observed when the sequences of both isolates were aligned with published sequences of *K. thyrsites* isolates from Atlantic mackerel caught in the North Sea (GenBank accession no. EU154349, Levsen et al. 2008) and off the southern coast of England (GenBank accession no: AY542482, Whipps & Kent 2006). The results indicate that the 4 *K. thyrsites* isolates identified from geographically different waters in Europe (i.e. Alboran Sea, Norwegian Sea, North Sea and southern coast of England) are genetically indistinguishable at the locus examined (i.e. SSU rDNA).

The present finding suggests that the geographical distribution of *K. thyrsites* includes the western Mediterranean Sea. Furthermore, the Alboran Sea is considered an oceanographic transition zone between the Atlantic Ocean and the Mediterranean Sea, retaining some oceanographic characteristics more similar to the Atlantic basin than to the Mediterranean Sea (Tintore et al. 1988). From this area, several cases of fish and marine mammals infected with parasites of oceanic waters (e.g. the nematode *Anisakis simplex* s.s. and the monogenean *Heteraxinoides atlanticus*) of the Northeast Atlantic Ocean have been reported (MacKenzie et al. 2008, Mattiucci et al. 2018).

The apparently low host specificity of *K. thyrsites* and its wide geographical distribution render this parasite a potential loss factor in wild-catch fisheries and marine fish cultures in most temperate and subtropical seas and coastal areas. However, due to the lack of knowledge about the ecology, life cycle and transmission pathways of *K. thyrsites*, it is difficult to evaluate the boundaries of its geographical range and its actual distribution in the Mediterranean Sea. Thus, further research is required to assess the possible economic consequences for the fishing industry inflicted by myoliquefactive *K. thyrsites* infections in *L. caudatus,* as well as other *K. thyrsites*-susceptible and commercially exploited Mediterranean fish species.

Acknowledgements. We thank Hui-Shan Tung for her assistance and help with the molecular analyses. Stefania Billera's contribution in drawing the images was also highly appreciated. The study was partly supported by the European Union's Seventh Framework Program for Research, Technological Development and Demonstration, project

'Parasite risk assessment with integrated tools in EU fish production value chains (PARASITE)', Grant agreement (GA) no. 312068.

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Editorial responsibility: Dieter Steinhagen, Hannover, Germany

Submitted: May 17, 2018; Accepted: November 2, 2018
Proofs received from author(s): December 23, 2018