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Short communication

First Report of *Enteromyxum leei* (Myxozoa) in the Black Sea in a Potential Reservoir Host *Chromis chromis*

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ABSTRACT—Damselfish *Chromis chromis* collected from the Black Sea coasts of Sinop, Turkey, were examined for myxosporeans in June and July 2013. One of 25 healthy fish and 2 dead fish had infections with *Enteromyxum leei*. Developing plasmodia and fully developed spores were numerously observed in the intestine and gall bladder of infected fish. This finding suggests that *E. leei* can develop normally in this fish species. Absence of fish farms close to the sampling site suggests that the parasite was not originated from farmed fish. This is the first report of *E. leei* in the Black Sea.

Key words: *Enteromyxum leei*, *Chromis chromis*, Black Sea, Turkey, reservoir host

During a parasitological survey of the damselfish *Chromis chromis* from the Black Sea coasts of Sinop, Turkey, where there is no aquaculture facilities nearby, in the months June and July 2013, and from a research facility in the Faculty of Fisheries and Aquatic Sciences in June 2013, a myxosporean parasite was encountered. Numerous young plasmodia and spores were observed in the gall bladder and intestine of heavily infected 2 dead fish, previously collected from the Black Sea, held in an aquarium and only in the intestine of slightly infected one of 25 wild fish. Fresh parasites were examined with an Olympus BX51 microscope using phase-contrast, and photographed by DP-25 digital camera using data-processing software DP2-BSW.

In the gall bladders, numerous young plasmodia with immature spores were observed and vegetative stages were disporic plasmodia (Fig. 1A). Plasmodia (n = 20) measured 17.6 μm (14.7–20.0 μm) in length and 15.5 μm (14.0–17.3 μm) in width.

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Typical mature spores in the intestine and gall bladder of infected fish were in an arcuate, almost semicircular shape (Fig. 1B–D). Polar capsules were elongated, tapering to their distal ends, open at one side of the spore, diverging at an angle of about 90°. Polar filaments coiled 7 times on average (range 6–8). Spore and polar capsule dimensions are provided in Table 1. Based on the overall morphology and spore dimentions, the parasite was identified as a myxozoan, *Enteromyxum leei*.

The phylum Myxozoa is composed entirely of endoparasites, including some that cause diseases which substantial impact on aquaculture and fisheries around the world (Kent *et al*., 2001). Myxosporean infection occurs in a wide range of both marine and freshwater fish species. Some reviews have stressed the importance of those species that are associated with pathology in mariculture (Alvarez-Pellitero and Sitjà-Bobadilla, 1993; Alvarez-Pellitero *et al*., 1995) and in freshwater farming (El-Matbouli *et al*., 1992). *Enteromyxum leei* is certainly one species of such concern. To our knowledge, the genus *Enteromyxum* includes 3 species of histozoic and enteric myxozoan parasites in marine cultured fish: the aforementioned *E. leei* (formerly *Myxidium leei*) from sea breams, with wide host range, *Enteromyxum scophthalmi* from cultured turbots, *Scopthalmus maximus* (Palenzuela *et al*., 2002) and *Enteromyxum fugu* (formerly *Myxidium fugu*) from cultured tiger puffer, *Takifugu rubripes* (Yanagida *et al*., 2004).

A wide host range of *E. leei*, seems rather unusual among the Myxozoa (Padrós *et al*., 2001). It also seems rather unusual that the gall bladder was heavily infected not only by developing stages but also mature spores of *E. leei* in the present study as it was recorded only by Le Breton and Marques (1995). This suggests that the gall bladder is the original habitat for *E. leei*.

In the last decades, significant problems due to myxosporean infection in Mediterranean mariculture have emerged (Le Breton and Marques, 1995; Athanassopoulou *et al*., 1998, 1999; Rigos *et al*., 1999) and *E. leei* is often implicated in significant losses in cultured sharp snout seabream (*Diplodus puntazzo*) and gilthead seabream (*Sparus aurata*) (Athanassopoulou *et al*., 1999). The impact of the disease is not limited to direct mortality, but also to weight loss, poor conversion efficiency, delayed growth and reduced marketability (Golomazou *et al*., 2004, Palenzuela, 2006; Rigos and Katharios, 2010). Since the first description of *Enteromyxum leei* as *Myxidium leei* in cultured gilthead sea bream *Sparus aurata* from southern Cyprus (Diamant *et al*., 1994), the parasite has been reported along the entire Mediterranean basin, the Red Sea and Japan and to date >46 fish species reported to be susceptible to this parasite (Estensoro *et al*., 2010). Padrós *et al*. (2001) reported *E. leei* infections in the

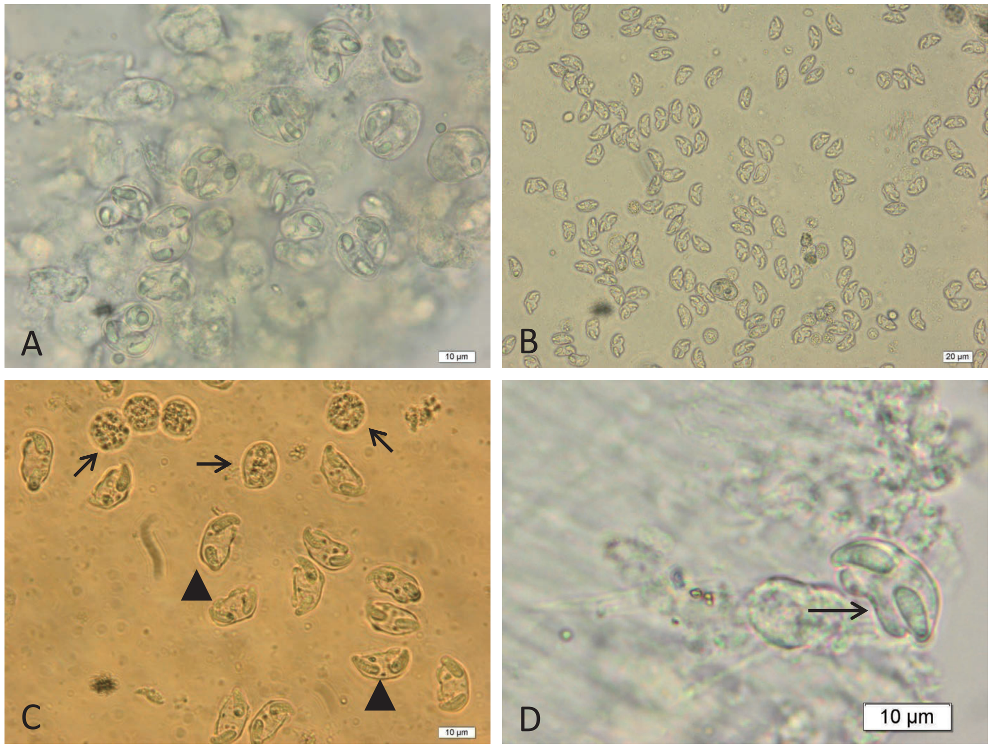

Fig. 1. Different developmental stages of *Enteromyxum leei* in the gall bladder and intestine of *Chromis chromis*. **A.** Numerous disporic plasmodia containing immature spores in the gall bladder, **B.** Numerous spores in the intestine, **C.** Several developing plasmodia (arrows) and mature spores (arrowheads) in the intestine, **D.** Fully developed spore (arrow) with prominent polar capsules and characteristic spore shape.

Table 1. Comparison of morphometrical data from different reports of *Enteromyxum leei*

Fish species	Spore		Polar capsule		Reference
	Width	Length	Width	Length	
Sparus aurata	6.9 (5.6–7.8)	14.7 (13.2–15.2)	3.2 (2.8–3.8)	7.4 (6.2–8.8)	Diamant *et al*. (1994)
Sciaenops ocellatus	7.4 (7.0–8.7)	17.5 (15.5–19.5)	3.8 (3.3–4.5)	8.4 (7.0–9.8)	Diamant (1998)
Oreochromis mossambicus	9.0 (8.0–10.6)	17.8 (16.5–18.9)	3.3 (3.0–3.5)	8.9 (8.3–10.0)	Diamant *et al*. (2006)
Diplodus puntazzo	5.0–7.0	15.0–19.0	2.5–4.0	6.5–9.0	Le Breton and Marques (1995)
Pagrus major	5.0–7.0	15.0–19.0	2.5–4.0	6.5–9.0	Le Breton and Marques (1995)
Diplodus sargus	6.3–6.9	14.4–17.3	2.3–3.5	6.9–8.1	Padrós *et al*. (2001)
Takifugu rubripes	7.9–11.0	15.9–19.1	2.1–3.6	6.1–7.2	Yanagida *et al*. (2004)
Cheilinus undulatus	6.3–9.7	12.9–16.9	3.2–5.9	5.1–9.0	Katharios *et al*. (2011)
Sparisoma cretense	8.29 (8.91–10.88)	17.07 (16.0–18.35)	4.17 (3.39–5.50)	8.29 (6.62–9.99)	Katharios *et al*. (2014)
Chromis chromis	7.3 (6.7–7.7)	14.7 (13.3–16.0)	2.7 (2.6–2.8)	6.4 (6.0–6.6)	This study

Average values (μm) are given with range values in parentheses.

exhibition and quarantine tanks of aquarium-reared fishes including *C. chromis* previously captured from NE Spanish Mediterranean coast.

The current knowledge on the myxozoan life cycle suggests an indirect cycle with an alternate myxosporean form in fish and an actinosporean form in oligochetes (Özer and Wootten, 2000; Özer *et al*., 2002). While the alternate host and the actinosporean form of *E. leei* have not yet been identified, Diamant (1997) has demonstrated experimentally a direct fish to fish transmission in sea bream by cohabitation, ingestion of infected intestinal mucosa and by waterborne contamination without the participation of an alternate host. This is important when considering the spread of *E. leei* in the Mediteranean because direct transmission might accelerate the spread of this parasite.

The damselfish *Chromis chromis* is a member of Pomacentridae and distributed in the eastern Atlantic, Mediterranean Sea, Baltic Sea and the Black Sea (Quignard and Pras, 1986; Zaitsev and Alexandrov, 1998; Dulcic, 2005). It prefers living in schools in mid-water, above or near rocky reefs, or above sea grass meadows, mainly at a depth of 3–35 m (Jardas, 1996). Despite its widespread distribultion, studies on the myxosporean parasites of this fish species is very limited (Lubat *et al*., 1989; Padrós *et al*., 2001). Absence of aquaculture facilities close to the sampling site of *C. chromis* in this study suggests that the parasite was not originated from farmed fish but from natural environment.

It is well-known that *E. leei* is one of the most serious pathogens for *S. aurata* and *D. puntazzo* and also represents a serious threat to other sparid fishes that have culture potential. It was experimentally shown by Sitjà-Bobadilla *et al*. (2007) that sea bass *Dicentrarchus labrax* could be a host for this parasite and considering its recent farming in the Turkish coasts of Black Sea, it might be a factor influencing negatively the growth of this fish species in the future. *Diplodus puntazzo* is also present in the Black Sea including Sinop coasts where this study was conducted and it should be kept in mind that *E. leei* may cause negative effects when this fish is cultured commercially in the Black Sea. Moreover, *C. chromis* is known to survive and reproduce in the Black Sea and has potential for aquarium trade. Thus, it might also be a threat for transmission of this parasite to other ornamental and native fish species inhabited in the same environment. Its spontaneous transmission between fish in nature as a direct fish-to-fish transmission among some fish species have already been achieved by cohabitation, ingestion of infected intestinal mucosa and by waterborne contamination. Thus, more research studies are warranted to evaluate this assumption - as well as studying this parasite as an emerging disease agent in the Black Sea near Turkey.

In conclusion, we reported the presence of an emerging parasite *E. leei* in an entirely new geographical region, the Black Sea, for the first time as well as its first presence in *C. chromis* in the Black Sea. This parasite is also a new record for Turkish fauna. The present study suggests that *C. chromis* is one of the natural hosts for *E. leei*.

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