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Association of Oomycetes *Aphanomyces invadans* with Freshwater fishes

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Introduction

In the past 30 years, there have been outbreaks of one with skin ulcerations associated disease, characterized histopathologically by a granulomatous and necrotizing myositis and dermatitis, in freshwater and Brackish water fish in much of India (Pradhan et al., 2007). The diseases were independently named differently given: mycotic granulomatosis (MG) in Japan, red spot disease (RSD) in Australia, and epizootic ulcerative syndrome (EUS) in Southeast Asia (Lilley et al., 1998. Catap and Munday, 2002). Some authors pointed out similarities of the isolated Aphanomyces spp. regarding morphology and cultivation (Wada et al., 1994) and similarities in the histopathological one Picture of the disease (Fraser et al., 1992) and suspected one individual species as causative infectious agents. Recent research confirmed that in all of them the pathogenic oomycete *Aphanomyces invadans* is involved (Callinan et al., 1995; Lilley and Roberts, 1997; Lilley et al., 1997). The context of another, on the east coast of the United States occurring ulcerative disease, ulcerative called mycosis (UM), with the EUS it was long unclear. The isolated there *Aphanomyces* spp. have since been confirmed as *Aphanomyces invadans* (Blazer et al., 2002).

Occurrence and distribution

Mycotic granulomatosis (MG)

The first case of an EUS-like clinical picture was clear given by Lilley et al. (1998) with farmed Ayu (*Plecoglossus altivelis*) in Oita Prefecture, Japan (Egusa and Masuda, 1971). A characteristic granulomatous host reaction to invasive emerged penetrating hyphae and the name of the disease mycotic given granulomatosis. It quickly spread to other prefectures and attacked various fish species (Miyazaki and Egusa, 1972).

Red spot disease (RSD)

A skin ulcer associated and red spot disease in brackish water fish in Queensland, Australia reported (Mckenzie and Hall, 1976). The disease spread and concerned fresh and brackish water fish in coastal rivers in New South Wales (Rodgers and Burke, 1981; Callinan et al., 1989) and the North of New Guinea (Rodgers and Burke, 1981), Northern Territory (Pearce, 1990) and Western Australia (Callinan, 1994).

Epizootic ulcerative syndrome (EUS)

Following the outbreaks of Mycotic granulomatosis and red spot disease, disease spread westward through Asia, affected by skin ulcerations and high mortalities was marked in numerous fresh and brackish water fish. In 18 Countries in the Asia-Pacific region have been

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affected by Epizootic ulcerative syndrome outbreaks reported, but not all can be safely assigned to the Epizootic ulcerative syndrome as not all Cases a histopathological examination or the isolation of *Aphanomyces invadans* (Lilley et al., 1998).

Shaheen et al. (1999) isolated *Aphanomyces* spp. of Mullets (*Mugil cephalus* and *Liza ramada*) with skin ulcerations. The Animals came from an earth pond on the western bank of the Suez Canal. The Isolates showed those typical of *Aphanomyces invadans* Growth characteristics. A pathohistological examination of the diseased fish was not reported.

Ulcerative mycosis (UM)

The ulcerative mycosis was first diagnosed in *Brevoortia tyrannus* in the estuary of the Pamlico River in North Carolina observed. The disease reached to an extent of an epizootic (Dykstra et al., 1986). Investigations in the subsequent years showed that the Ulcerative mycosis was a widespread Disease at Menhaden in Florida, North Carolina, and Virginia (Dykstra et al., 1989). Other fish species were also infected affected (Noga et al., 1991), but the prevalence was clearly lower than the Menhaden herrings (Levine et al., 1990). The first Cases in freshwater confirmed as *Ulcerative mycosis* among blues Sunfish (*Lepomis macrochirus*) and spotted ones Fork catfish (*Ictalurus punctatus*) (Blazer et al., 2002). Hawke et al. (2003) reported on the occurrence of UM in spotted catfish, Blue sunfish and black catfish (*Ameiurus melas*) from fish ponds in southeast Louisiana.

Etiology

The causative agent of Epizootic ulcerative syndrome is the oomycete *Aphanomyces invadans* (Lilley et al., 1997). A diverse selection of other microorganisms was isolated from Epizootic ulcerative syndrome infected fish (Burke & Rodgers, 1981; Callinan & Keep, 1989; Kanchanakhan, 1996; Blazer et al., 1999; Mastan & Qureshi, 2001). The sick fish, especially those with Skin ulcerations, susceptible to opportunistic infections are pathogens, it is especially in the case of long-standing diseases difficult to pinpoint the primary cause. True, some of these Pathogens must, however, contribute significantly to the course of the disease differentiated from the causative infectious agent *Aphanomyces invadans* (Lilley et al., 1998).

Nomenclature

Hatai et al. (1977) found an oomycete belonging to Genus *Aphanomyces* from Ayu suffering from *Mycotic granulomatosis*, named as *Aphanomyces piscicida*. For the *Aphanomyces* sp. isolate obtained from the culture of a fish infected with EUS was isolated in Thailand (Roberts et al., 1993) and the name of *Aphanomyces invadans* suggested by Willoughby et al. (1995). According to Khan et al. (1998), the Organism listed in the Index of Fungi as *Aphanomyces invadans* since 1997 (David & Kirk, 1997).

Taxonomy

Due to morphological features, the Oomycetes have been around for many years included in Fungi. However, molecular genetic studies showed that they are closely related to algae, but relationship with fungi does not exist (Leipe et al., 1994; Baldauf et al., 2000; Dick, 2001). Other names for this group of organisms are Peronosporomycetes or Phycomycetes (DICK, 2001).

Domain: Eukaryota
Kingdom: Chromista
Phylum: Oomycota
Class: Oomycetes
Order: Saprolegniales
Family: Leptolegniaceae
Genus: Aphanomyces

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Species: *Aphanomyces invadans*

Classification of *Aphanomyces invadans* according to David & Kirk, 1997

Aphanomyces invadans strains were found along with closely related ones Oomycetes using the following molecular biological methods characterizes: - Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Callinan et al., 1995; Lilley et al., 1997) - Random amplification of polymorphic DNA (RAPD) (Johnson et al., 2004) - Western blot (Lilley et al., 1997) - Pyrolysis mass spectrometry (PyMS) (Lilley et al., 2001) Restriction Fragment Length Polymorphism (RFLP) (Lilley et al., 2003) - amplification of the DNA region coding for the ribosomal RNA, subsequent sequencing and comparison of the sequences obtained (Lilley et al., 2003). These studies revealed a high level of genetic Homogeneity of the *Aphanomyces invadans* strains found in a single clonal lineage are to be considered. *Aphanomyces astaci*, the Cancer plague pathogen, turned out to be the closest relative (Lilley et al., 2003).

Morphology, Physiology and Development

The Oomycetes were morphological characterized in the past assigned to the Fungi and their morphological structures using terms applicable to describe fungi. The hyphae-like structures of the pathogen is called hyphae and the hyphae-like network is called mycelium. The hyphae of *Aphanomyces invadans* are broad, unseptate with rounded ends and have a diameter of 11.7 to 16.7 µm, while in laboratory culture they have a diameter of 8.3 µm in newly formed hyphae (Willoughby et al., 1995). This morphology of the mycelium is related to the substrate where the organism is located (Willoughby, 1995). The organism shows very slow growth of 4 mm per day at 24 °C on agar plate (Vishwanath et al., 1998) and grows at temperatures from 5 to 36 ° C and a salinity of up to 1% NaCl (Lilley et al., 1998). Willoughby & Chinabut (1996) reported the difficulty in maintaining *Aphanomyces invadans* in the laboratory without the host organism. The release of toxic or enzymatic substances for infecting the host can cause damage to *Aphanomyces invadans*.

Lilley & Roberts (1997) compared those from *Epizootic ulcerative syndrome (EUS Red spot disease (RSD)* and *Mycotic granulomatosis (MG)* infected fish isolated strains of *Aphanomyces invadans* with various saprophytic *Aphanomyces* spp. from Thailand regarding their cultural requirements and characterize to determine differences. There were none among the *Aphanomyces invadans* strains significant differences among each other. They showed optimal growth on glucose-peptone-yeast agar at 26°C to 30 °C and died at 37 °C, grew very slowly at all temperatures and were unable to certain culture media such as Sabouraud Dextrose Agar, Commeal Agar and Malt extract agar to grow. The growth characteristics of the saprophytic *Aphanomyces* spp. were variable within the group, but at all temperatures including 37 °C faster growth than *Aphanomyces invadans* with an optimum growth at 34 to 38 °C.

Inside the sporangia, at the terminal ends of the hyphae arise and the diameter is nearly 10 µm (Willoughby et al., 1995), the cytoplasm splits into a series of elongated units, the primary spores that are typical of the Aphanomyces genus are of achlyoid type and spore are released outside (Scott, 1961) and spore are forms in bunch. Typically, there are four spore, consisting of primary spore with a 6.7 to 10 µm in diameter, produced by each sporangium (Roberts et al., 1993).

The motile spore are equipped with two flagella, 6 µm in size kidney-shaped secondary spores are formed at a temperature of 22 ° C within 12 hours of the development of the sporangia from the primary spore are released. Under certain environmental conditions or in the presence of a host or substrate, the secondary pore encysts and germinated by means of a germ tube to form new hyphae (Lilley et al., 1998). In the presence of nutrient-containing substances can come out

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Secondary cysts, in turn, create new zoospores (Lilley et al., 1999). This phenomenon, which occurs in some Oomycetes and in which several Tertiary generations of zoospores formed from the secondary spore cyst is called polyplanetism. It is used as an adaptation to the Parasitism is seen in some members of the genus Aphanomyces (Cerenius & Söderhall, 1985).

Species identification by the most important representatives of fish pathogens Oomycetes, the species within the genera Aphanomyces, Achlya and Saprolegnia, occurs through sexual structures. These are usually missing in fish parasitic species and are also found in *Aphanomyces invadans* unknown (Lilley et al., 1998). Alderman & Polglase (1988) lack sexual structures as a more common phenomenon pathogenic members of the Oomycetes.

Susceptible fish species

The lists of fish species affected in EUS outbreaks include well over 100 species (Tonguthai, 1985; Frerichs et al., 1989). The skin and muscles of fish can only be used in a limited way different injuries and infections react. The appearance of Skin ulcers in a species of fish during an EUS outbreak does not mean per se that the species suffers from EUS and is therefore susceptible (Frerichs et al., 1989). The fact that a pathology-based diagnosis of EUS many examinations were not carried out before 1994, makes it probable that at least some reports are misleading (Roberts et al., 1994; Lilley et al., 2002).

The disease was not found in obligatory marine fish species (Roberts et al., 1994; Lilley et al., 2002). Species reported is said that they were not affected in EUS outbreaks are the carp (*Cyprinus carpio*), tilapia (*Oreochromis mossambicus and niloticus*) and the Milkfish (Chanos chanos) (Costa & Wijeyaratne, 1989; Ahmed & Rab, 1995; Lilley et al., 1998) Labeo rohita (Yogeshwari et al., 2015). In experimentally infected carp (Wada et al., 1996) and tilapia (Khan et al., 1998) failed to find clinical Symptoms are evoked.

Infection

The natural infection cycle is still not fully understood in EUS infected organism or have died from the disease No formation of sporangia has yet been observed in fish. When examining the skin ulcerations of diseased fish with the mycelium of the pathogen was clearly visible, but apart from the terminal hyphae tips deep in the fish tissue died (Roberts et al., 1993). The question therefore arises as to how a direct horizontal transmission of Fish to fish takes place. It was pointed out the possibility that the fish infectious through zoospores were produced in the aquatic environment (Thompson et al., 1997).

The infectious stage of the pathogen is the free-swimming zoospore. In The EUS was able to perform numerous attempts using intramuscular Zoospore injection (Chinabut et al., 1995; Wada et al., 1996; Lilley & Roberts, 1997; Khan et al., 1998; Kiryu et al., 2002) and in a few Felling through zoospore exposure in the form of a spore bath (Lilley, 2001; Kiryu et al., 2003) in susceptible fish species. The free-swimming zoospores show fish slime and Fish skin of susceptible and unresponsive fish species, but also against various sugar compounds and amino acids with the exception of arginine, there is positive chemotaxis (Sihalath, 1999). At Investigations of EUS lesions in dwarf threadfish (*Colisa lalia*) were the pathological changes in the caused by the pathogen

Skeletal muscles most pronounced and increased in the direction of the internal Organs down (Wada et al., 1994). Other authors reported this as well (Noga et al., 1988; Callinan et al., 1989; Roberts et al., 1993). It is suggested that the pathogen was on the external body surface and penetrated into the host, here the primary lesion established and from there hyphae advanced in the direction of the abdominal cavity. Lilley et al. (1998) considered *Aphanomyces invadans* to be secondary pathogen, that entered in the form of a damaged or missing epidermis needed.

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Using electron microscopic, studies have shown that zoospores adhere to a attach intact epidermis, forming a germ tube and the epithelium could penetrate. However, the creation resulted a portal of entry in the form of mechanical damage to the Mucus layer and the epidermis or intramuscular injection of Zoospores lead to a significantly higher prevalence and mortality. Investigations into the minimum infectious dose resulted in Menhaden herrings (*Brevoortia tyrannus*) that a single injected zoospore was capable of producing a infection that leads to death (Kiryu et al., 2003).

Conclusion

In the past 5 decades, *A. invadans* are spreading ever faster and are now found around the world, probably because of globalisation, and perhaps even because of climate change. There are currently very limited prospects of control strategies, even though considerable progress has been made in diagnosis of *A. invadans*.

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