Aphanomyces invadans, the causal agent of Epizootic Ulcerative Syndrome, is a global threat to wild and farmed fish

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ABSTRACT

Aphanomyces invadans is a eukaryotic pathogen and the causative agent of Epizootic Ulcerative Syndrome (EUS) in fish and is responsible for mortalities of up to 100% in aquaculture. *A. invadans* was first discovered in Japan in 1971, and since then it has been found in Australia, North America, Southern African countries and Asia. Methods for the correct identification of *A. invadans* are well established now and involve PCR-based detection and microscopy. However, the pathogenesis of *A. invadans* is poorly understood. Environmental stress (mainly temperature) and the associated immunocompromised fish seem to induce infections of *A. invadans* and outbreaks of EUS. Understanding the process of infection in more depth is fundamental for the discovery of novel effective treatments to combat the disease. In this review, we discuss morphological characteristics of *A. invadans* and its pathogenicity as well as various approaches of treatment.

Keywords: *Aphanomyces invadans*, EUS, EGA, oomycete, aquaculture

1. INTRODUCTION

In times of a rapidly increasing world population, food resources and security become critically important and pathogens infecting crops and farmed animals are coming into focus in the aquaculture industry and in quaranteen departments. A major problem for the agri- as well as aquaculture industry are animal and plant pathogenic oomycetes, which are also responsible for the destruction of many natural host populations (van West, 2006). Oomycetes are eukaryotic pathogens that belong to the Stramenopile Kingdom and are thus genetically related to brown algae but are morphologically similar to fungi (Baldauf et al., 2000; Gleason et al., 2018). A common infection of freshwater and brackish fish species is Epizootic Ulcerative Syndrome (EUS), which is caused by the oomycete *Aphanomyces invadans*. This water mould threatens especially the aquaculture industry in the Asia-Pacific region. *A. invadans* was first described in 1971 during an outbreak in a freshwater fish farm in Japan (Egusa and Masuda, 1971; Oidtmann, 2012). Since then, outbreaks of EUS have become more frequent and disseminated worldwide including Asia, Australia, North America and more recently Africa (Fig. 1, Tab. 1).

A. invadans was originally declared as an invasive fungus called *Aphanomyces invaderis* (Willoughby et al., 1995) that was able to produce clinical signs of EUS (Lilley and Roberts, 1997). Later, it was referred to as *Aphanomyces piscicida* (causing mycotic granulomatosis), *Aphanomyces* sp (causing red spot disease (RSD)) (Lilley and Roberts, 1997) or simply called EUS-related Aphanomyces (ERA) (Lumanlam-Mayo et al., 1997). Recently, it was proposed to change the name of the disease from EUS to Epizootic granulomatous aphanomycosis (EGA) (Baldock et al., 2005; OIE, 2017). However, so far the term EUS seems to be preferred when (Kamilya and Baruah, 2014) describing the ulcerative disease caused by *A. invadans*. Together with *A. frigidophilus*, *A. astaci* and *A. stellatus* (Diéguez-Uribeondo et al., 2009) *A. invadans*, belongs to the genus *Aphanomyces* which is beside Saprolegnia the second group in the Saprolegniaceae family within the phylum Oomycota. Besides animal pathogens, this genus also contains plant parasitic and saprotrophic/opportunistic species (Fig. 2). Interestingly, not all isolates of *A. invadans* seem to be pathogenic on fish. A strain naturally occurring in water bodies in Malaysia neither induces an infection in the natural environment nor under laboratory conditions (Afzali et al., 2013).

So far eighty-seven different fish genera were reported to be affected by EUS (Kamilya and Baruah, 2014) with Chana and Puntius being affected the most (Kar, 2016; Lilley et al., 1992). Generally, bottom-dwelling fish like murrels (Chondar and Rao, 1996) or catfishes (Roberts et al., 1994) seem to be highly susceptible. While the size of the fish does not determine an EUS

outbreak (Cruz-lacierda and Shariff, 1995), younger fish seem to be more prone to EUS compared to adult fish (Gomo et al., 2016; Pagrut et al., 2017).

Recently, the genome of a fish pathogenic strain of *A. invadans* was completely sequenced by the Broad Institute. This allows us to mine for the genes for enzymes such as proteases, effector proteins and other proteins important in pathology (discussed in more detail below). As the global dissemination of *A. invadans* seems to be difficult to prevent, molecular research in this area, now facilitated through the availability of genome sequences, is forging ahead. Therefore, we summarise the current knowledge about this emerging oomycete, including in depth discussion of morphological characteristics, pathogenicity as well as various control strategies.

2. LIFE CYCLE OF *A. INVADANS*

A. invadans passes through the typical life stages of an oomycete without the sexual stage which is often rare or absent in animal-pathogenic oomycetes (Dick et al., 1999). In contrast, the asexual stage is distinctive for the genus *Aphanomyces*, which is characterized by the formation of biflagellate zoospores from clusters of primary cysts at hyphal tips (Diéguez-Uribeondo et al., 2009).

Aphanomyces spp. propagate asexually by the formation of zoosporangia comprising 30 to 50 primary zoospores which are released through a lateral evacuation tube into the environment (Hawke et al., 2003). Interestingly, isolates from the Philippines can have up to 2 lateral evacuation tubes per sporangium (Baruah et al., 2012) and isolates from Thailand have normally 4 tubes per sporangium (Callinan et al., 1995).

After release from sporangia, primary zoospores immediately encyst at the apical tip and form achlyoid clusters (Fig. 3). From these clusters secondary zoospore are released which are the main free-swimming stage (movie S1). Later, secondary zoospores transform into a germling by forming a germ tube, which eventually develops into mycelium (Olson et al., 1984; Willoughby et al., 1995). However, encysted zoospores of parasitic *Aphanomyces* species are capable of releasing a new zoospore generation instead of germinating (Diéguez-Uribeondo et al., 2009). This process is called repeated zoospore emergence (RZE) or polyplanetism (Diéguez-Uribeondo et al., 1994).

Mycelium of *Aphanomyces* species is cylindrical hyphoid and coenocytic. Hyphae in infected fish show only limited branching with a variable diameter of 6 up to 27 µm but are considerably

narrower under culture conditions (Roberts et al., 1993). At later stages of infection, *A. invadans* also produces vegetative hyphae, sporangia with primary zoospores and spore clusters (Diéguez-Uribeondo et al., 2004; Roberts et al., 1993). Sporulation of *A. invadans* occurs in waters of low salinity, between 0 and 8 psu (practical salinity unit 5 ‰), depending on species, with maximal sporulation at 25°C and no sporulation at 30°C (Kiryu et al., 2005).

3. PATHOGENICITY OF *A. INVADANS*

3.1 Clinical symptoms

Under normal conditions skin defence mechanism of fish are sufficient to prevent an infection with *A. invadans*. However, a suppression of the immune system can result in an outbreak of EUS. Various environmental determinants such as colonisation of the fish by other pathogens (viruses, bacteria or ectoparasites), a low pH or low oxygen concentrations in the water can affect primary defence mechanisms of fish (Kiryu et al., 2005). Under appropriate conditions, some *A. invadans* isolates are capable of acting as a primary pathogen (Sanaullah et al., 2001).

Infections of fish by *A. invadans* are initiated by attachment of motile zoospores. They are attracted to their host, attach to the damaged skin and form germination tubes that penetrate the skin. Hyphae invade deep into lower tissues resulting in extensive ulceration and destruction of the tissue (OIE, 2017, 2009). The typical signs of ulceration can be used as a presumptive diagnosis for EUS on affected fish (Bondad-Reantaso et al., 1992). The occurrence of skin lesions varies depending on the fish species as well as manifestation and ranges from crucial areas of intense inflammation and hyperaemia of the skin to deep ulcerations with uncovering of the lower muscle tissue (Huchzermeyer et al., 2018). Early lesions are characterized by haemorrhagic bullae with small foci of ulceration and are often observed on the lateral surface (Hawke et al., 2003; OIE, 2017; Pathiratne and Rajapakshe, 1998; Yadav et al., 2014). Concomitantly, scales are often protruding. In contrast, lesions in bluegills are characterised by proliferative tan areas on the skin which are associated with scale loss (Hawke et al., 2003). Mildly infected fish show only minor inflammation without external lesions but petechial haemorrhagic spots on body, mouth as well as anal fin and exophtalmia. In severe cases, swollen haemorrhagic areas, massive inflammation and large deep ulcerations in association with necrosis of the myotome can be observed (Pathiratne and Rajapakshe, 1998; Yadav et al., 2014). Ulcers appear as white areas on the skin of the fish with reddish centres that turn into complete red areas and later result in external haemorrhages and distended abdomens. As the disease progresses, eyes are protruding, the body becomes putrefactive and in some cases also the head is eroded, which results in the

death of fish due to the severity of the disease (Hawke et al., 2003; Podeti and Benarjee, 2017).

3.2 Pathology

An infection with *A. invadans* is histologically characterised by severe necrosis of the muscle tissue around the invading hyphae which later develop into enclosed granulomas (Wada et al., 1994). Whereas non-invasive aseptic hyphae with a smaller diameter are frequently observed at the surface of the site of infection (Huchzermeyer et al., 2018; Yadav et al., 2014). Migrating hyphae are observed along fascial layers and between myofibrils where they cause myconecrosis (Chinabut et al., 1995; Pradhan et al., 2008). The severe lesions attract large numbers of inflammatory cells which results in oedema and hyperplasia of gill lamella, but only marginal changes of internal organs (Bondad-Reantaso et al., 1992).

Most of the haematological data revealed that fish with EUS show a significant increase of white blood cells (especially neutrophils) due to a local inflammatory response (Pathiratne and Rajapakshe, 1998; Qureshi et al., 2001), while the number of red blood cells, the haemoglobin concentration and the haematocrit level are reduced because of the blood loss caused by the haemorrhagic lesions (Das and Das, 1993; Pathiratne and Rajapakshe, 1998; Podeti and Benarjee, 2017, 2015). The combination of an increase of neutrophils and a reduction of erythrocytes (including haematocrit and haemoglobin content) shows that *A. invadans*-infected fish suffer from anemic condition.

3.3 Effector proteins

A generally accepted model how oomycetes successfully establish an infection of their host is the secretion of so called effector proteins. Effector proteins are released during the infection and they target host factors to overcome host defence mechanisms or they adapt the host metabolism for the pathogen's benefit. Recently, an *A. invadans* genome (strain NJM9701) was sequenced (Aphanomyces WGS initiative, Broad Institute (broadinstitute.org)) including its mitochondrial genome (Makkonen et al., 2016) with Illumina sequencing technology (Genbank Accession PRJNA188082). Initial analysis using FungiDB (http://fungidb.org/fungidb/) shows that the genome has a good repertoire of effector proteins that are predicted to interact with various host processes (Tab. 2). As expected the effector repertoire shows more similarities to the fish-pathogenic *S. parasitica* than to the plant-pathogenic *P. infestans*. As in *S. parasitica*, in *A. invadans*, the so called RxLR and CRN effectors are absent and seem to be unique to the plant pathogenic *Phytophthora* species. In line with a previous report on active proteases of *A. invadans* secreted into the culture filtrate is the high number of predicted proteases in the genome (Majeed et al., 2017). Interestingly,

the *A. invadans* genome lacks disintegrins as well as haemolysin-E and codes for fewer Ricin-like proteins, when compared to *S. parasitica*.

4. IDENTIFICATION OF *A. INVADANS*

In the past it was very challenging to identify *Aphanomyces* sp. correctly because of the difficult procedures for isolation and conditions for growth in culture (Hawke et al., 2003). These days, beside clinical signs of EUS infected fish, molecular methods are also used to identify *Aphanomyces* sp.

5.1 PCR-based

A very sensitive and specific technique is ITS sequencing (Internal Transcribes Spacer) which is widely used for the molecular identification and differentiation of oomycetes from species to genus level (Gozlan et al., 2014; Hart, 1997; Huchzermeyer et al., 2018; Leclerc et al., 2000; J. Lilley et al., 2003; J. H. Lilley et al., 2003; Phadee et al., 2004; Robideau et al., 2011; Takuma et al., 2010; Vandersea et al., 2006). The three main lineages of *A. invadans* (Fig. 2) can be also distinguished by ITS sequencing (Diéguez-Uribeondo et al., 2009).

In addition, restriction fragment length polymorphisms (RFLPs) and sequences of ribosomal DNA (rDNA) were used to distinguish *A. invadans* from other species including saprolegniacaea (Lilley et al., 2003).

Recently, also the mitochondrial gene cytochrome c oxidase subunit I (COI) was used for the identification of *Aphanomyces* species. The mitochondrial genome was sequenced recently and found to be 49,061 basepairs long and has a large inverted repeat region of about 12kb (Makkonen et al., 2016). Comparison of ITS and COI sequences of 23 oomycete genera including *Aphanomyces* sp. revealed, that both are suitable for the identification of oomycetes but in some cases COI was more discriminative than ITS at the species level. However, COI sequences are not conserved throughout all *Aphanomyces* species (Robideau et al., 2011).

5.2 Microscopy-based

Mycelia-like structures and zoospores of *A. invadans* can be detected by *in-situ* hybridisation (FISH) (OIE, 2009; Vandersea et al., 2006) or immunofluorescent staining on histological sections of infected fish (Devaraja et al., 2004; Miles et al., 2003).

5.3 Immunological methods

Various immunological methods were established to reliably identify *A. invadans* with a very high sensitivity (7 µg/ml, dilutions up to 10^{-11}) such as FTA (Monoclonal antibody-based flow-through immunoassay, (Adil et al., 2013)) or immunoblots (Ganapathi et al., 2008; Miles et al., 2003).

5. CAUSATIVE AGENT OF EUS

Zoospores of *A. invadans* from infected tissues can be captured with a baiting method (Afzali et al., 2013; Kiziewicz et al., 2011). Although *A. invadans* is the causative agent of EUS (Huchzermeyer and Van der Waal, 2012), also other pathogenic viruses (mostly rhabdovirus), bacteria (mainly *Aeromonas hydrophila*) or parasitic protozoans are routinely co-isolated from *A. invadans*-infected fish (Tab. 3). However, no evidence currently exists confirming that these organisms are causally contributing to EUS disease (John and George, 2012). Parasitic infections possibly induce stress in fish and thereby might predisposing them to infection (Subasinghe, 1993).

6. EUS OUTBREAKS

6.1 Outbreaks in nature

Since the defence system of fish has to be supressed in order to be infected by *A. invadans*, a major focus of research lies on the environmental conditions triggering an EUS outbreak. Under laboratory conditions the optimal growth temperature for *A. invadans* is between 19-22 °C, while under natural conditions *A. invadans* seems to be more robust (Hawke et al., 2003). Some EUS outbreaks are associated with heavy rainfall and flood events that can cause a sudden drop in temperature from 20°C to 10-15 °C (Bondad-Reantaso et al., 1992; Choongo et al., 2009; Go et al., 2012; Hawke et al., 2003; Lilley and Roberts, 1997). Since fish are poikilothermic organisms and do not control their body temperature, a decrease of the environmental temperature automatically results also in a drop of the body temperature (Ernst et al., 2016). Indeed, it was shown in infected snakeheads (*Channa striatus*) that the inflammatory cell response is reduced with a decrease in temperature which likely delays the inflammatory response of the fish to an infection (Catap and Munday, 2002; Chinabut et al., 1995). In addition, a reduced temperature also favours the sporulation of *A. invadans* (Lumanlam-Mayo et al., 1997). However, a series of EUS outbreaks was reported in relation to a temperature increase in Southeastern Louisiana (Hawke et al., 2003) and India (Pradhan et al., 2014). It was observed that hyphae grow actively outside the body on the surface around lesions at higher temperatures, while they invade deeper into fish tissues at 20 °C (Kar, 2016).

Most EUS outbreaks occur in slightly acidic water (pH 6.0-7.0) on sandy soil with low levels of alkalinity, hardness and chlorides (Choongo et al., 2009; Hawke et al., 2003). Generally, *A. invadans* is quite resistant to environmental stress, such as high salinity or variable temperatures and can also survive in high alkaline condition and harder water (Das and Das, 1993; Pradhan et al., 2014). In contrast, extremely high or low pH values cause damage to fish tissues, especially the gill and the epidermis which reduces the resistance to potential infections (Svobodova et al., 1993; Thampuran et al., 1995).

To understand EUS it is important to have a model system ready which allows to copy an infection of a fish by *A. invadans* under controlled laboratory conditions. Zoospores of *A. invadans* are produced by incubating mycelium in starvation medium. The isolated zoospores, transmitted by injection or immersion, will attach to the host and initiate the infection and reproduction process (Kiryu et al., 2002). Artificial infections of fish by injection show clinical signs comparable to natural infections, including localised swelling and reddening. On day 4 scale loss and ulceration of lower musculature appear which develop into deep ulceration as the disease progresses (Afzali et al., 2015). Histopathological features of moribund and dead fish were also the same as for fish with EUS collected from the wild (Pradhan et al., 2010).

6.1 Global trade and spread of EUS

Globalisation and the involved global trading is a major amplifier for the dissemination of pathogens worldwide. Thus, by increasing import activities EUS is also transmitted to EUS free countries such as Europe (EFSA Panel on Animal Health and Welfare, 2011). Around 4000 freshwater species from farms and 1400 wild-caught marine species of ornamental fish or pet fish are traded internationally from tropical countries per annum. (Whittington and Chong, 2007).

Skin lesion infections of ornamental fishes were reported in several pet shops in Germany which were confirmed by PCR to be caused by *A. invadans*. The infected fish were Blue gourami (*Trichogaster trichopterus*) from Thailand, Cardinal tetra (*Paxelrodi*) from Brazil and Dwarf gourami (*Colisa lalia*) from Vietnam. The results are quite alarming as the traded fish comes with a certificate from the country of origin stating EUS-free (El-Matbouli et al., 2014).

An outbreak of an *A. invadans* infection in Japan was also found in the pet fish Dwarf gourami (*Colisa lalia*) imported from Singapore (Hatai et al., 1994; Wada et al., 1994) and Golden gourami (Hanjavanit et al., 1997). All reported fish have shown the typical clinical signs of EUS including haemorrhagic, dermal swelling and scale loss.

A similar case was also described for the *Pseudorasbora parva* (topmouth gud-geon) carrying *Sphaerothecum destruens* whereby *S. destruens* was able to invade natural river system in more than 30 countries (Gozlan et al., 2010).

7. TREATMENT STRATEGIES

Since *A. invadans* has a major impact on the aquaculture industry especially in Asian countries, a lot of research is done in the area of treatment of EUS and how to prevent infections with *A. invadans*. Unfortunately, the treatment options are limited at present.

7.1 Chemicals and antibiotics

Treatment with Malachite green (which has been banned, worldwide) and formalin are to date the most effective ways to prevent EUS because mycelium as well as zoospores of *A. invadans* are killed at very low concentrations (Afzali and Wong, 2017; Campbell et al., 2001; Lilley and Inglis, 1997) but are hazardous to farm workers and the environment (Srivastava et al., 2004). Also exposure of the fish to H_2O_2 and 20% NaCl for 1 hour were shown to be capable to kill *A. invadans* isolates (Lilley and Inglis, 1997).

Another treatment strategy is the application of chitosan into fish food. A challenge of fish with *A. invadans* fed with and without chitosan supplemented food resulted in a significantly lower cumulative mortality of chitosan-treated fish (Shanthi Mari et al., 2014).

Also ZnO_3 nanoparticles and ClO_2 have shown promising toxiticity against *A. invadans* but no challenge experiments were performed yet (Borisutpeth et al., 2010; Shaalan et al., 2017).

No sufficiently effective antibiotics have been described to kill *A. invadans*. Streptomycin had the strongest effect and reduced *A. invadans* growth by 70-100 % at a concentration of 500ppm (Lilley and Inglis, 1997).

7.2 Plant extracts

Interestingly, extracts of a whole range of various plants were successfully tested against *A. invadans* (Tab. 4). But also single compounds from *Azadirachta indica*, *Ocimum sanctum*, *Curcuma longa* like azadirachtin, camphor, and curcumin, respectively have been shown to successfully protect fish against an infection with *A. invadans* when injected intramuscularly or used in fish food (Harikrishnan et al., 2009). Another compound (eugenol) extracted from clove oil was very effective against *A. invadans*. However, eugenol is also very toxic for salmonids and to a lesser extent to cyprinids (Hussein et al., 2000).

7.3 Immunostimulants

Immunostimulants are substances that stimulate the immune system by inducing its activation or increasing its activity of any of its components and therefore a potential way to counterbalance immunosuppression by stress or decreased environmental temperatures.

Vitamin C was shown to have an immunostimulative effect because it increased the total number of white blood cells which improves the handling of stress by fish, including improved

resistance against infections and faster recovery from stress situations (Innocent et al., 2011). The commercial immunostimulant Salar-bec comprising several vitamins reduces the spread of mycelium on the fish body and induces the formation of granulomata to limit spread of the infection on the fish (Miles et al., 2001), which are a main characteristic of EUS resistant fish species (Chinabut et al., 1995; Wada et al., 1996).

Also the addition of fish oil as a source of lipid to fish diets can reduce the effect of a temperature drop on the immune response by improving the membrane fluidity (Ernst et al., 2016).

Ethanol extracts of *Solanum nigrum* can be also immunostimulative and thereby preventing fish disease and reduce the mortality by up to 20 % (Abu et al., 2017) while water-based extracts did not show any effect (Haniffa et al., 2011).

Recently, the metal-based compound Zeolite was shown to enhance the immunological response and disease resistance against *A. invadans* as well as growth of the fish (Jawahar et al., 2016)

7.4 Vaccination

Vaccination of fish against *A. invadans* had thus far only limited success. Vaccination trials have been based on protein extracts made from *A. invadans* hyphae (Saikia and Kamilya, 2012). In this study, vaccination was done in combination with an adjuvant which significantly increases the antibody production and activates components of the innate immunity. Although a reduction in mortality was demonstrated in challenged fish, it was not statistically significant.

8. FUTURE PERSPECTIVES

In the last 5 decades *A. invadans* is disseminating increasingly faster and is now found worldwide, which is probably due to the globalisation and maybe even climate change (Fig. 1). While significant progress was made regarding diagnostics of *A. invadans*, limited perspectives for control strategies exist at present.

It is clear from the research reviewed that treatment using various plant extract, chemicals and antibiotics were widely tested, but most of them are only applicable under controlled laboratory conditons in small scales. In addition, the utilisation of drugs and antibiotics in aquaculture is highly regulated to avoid safety risks to the public and the environment and to prevent the development of resistant strains of pathogens. Therefore, farmers are often left without any reliable preventive plans.

Biotechnology and molecular techniques have proven to deliver successful tools to control diseases (Alvarez et al., 2004) but not for *A. invadans* as of yet. The genome of *A. invadans*

has been sequenced (Aphanomyces WGS initiative, Broad Institute (broadinstitute.org)) and will form the starting point for future studies towards understanding the biology of *A. invadans*. Now genome editing and gene silencing or mutagenesis systems such as RNAi or CRISPR/Cas are urgently required to functionally characterise genes. We anticipate that detailed molecular studies of the host-pathogen interaction will ultimately provide new targets for the development of novel therapeutics. However, it is extremely important that agricultural scientists take the threat of the spread of Aphanomyces seriously and that industry and governments provide adequate funding for research into preventative measures.

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Tab. 1 EUS outbreaks worldwide including their first year of occurrence (see also Fig. 1).

Country	Year of occurrence	Reference
Japan	1971	(Egusa and Masuda, 1971)
Australia	1972; 2001	(Boys et al., 2012; McKenzie and Hall, 1976)
Papua New Guinea	1975-1976; 1982-1983	(Coates et al., 1989; Haines, 1983)
Singapore	1977, 1997	(Hanjavanit et al., 1997; Roberts et al., 1986)
Malaysia	1979-1980	(Jothy, 1981; Shariff and Law, 1980)
Thailand	1981	(Tonguthai, 1985; Ulcerative Fish Disease Committee, 1983)
China	1982	(Lian, 1990)
Vietnam	1983	(Xuan, 1990)
Lao PDR	1983	(Lilley et al., 1992)
Myanmar	1984	(Lilley et al., 1992)
Cambodia	1984	(Lilley et al., 1992)
Philippines	1985	(Llobrera and Gacutan, 1987; Mines and Baluyot, 1986)
Sri Lanka	1987	(Costa and Wijeyaratne, 1989)
Bangladesh	1988	(Barua, 1994; Roberts et al., 1989)
Hong Kong	1988	(Wilson and Lo, 1992)
Nepal	1989	(Shrestha, 1994)
Bhutan	1989	(Phillips, 1989)
India	1989; 1991	(Das and Das, 1993)
Indonesia	1990	(Rukyani, 1994)
Pakistan	1996	(Kanchanakhan, 1996)
USA	1980;1997; 1998	(Blazer et al., 1999; Dykstra et al., 1986; Hargis, 1985; Sosa et al., 2007)
Africa	2006;2007;2010;2012; 2014	(Andrew et al., 2008; EFSA Panel on Animal Health and Welfare, 2011; FAO, 2009; Gomo et al., 2016; Huchzermeyer et al., 2018; Huchzermeyer and Van der Waal, 2012)
Iraq	2007-2008	(EFSA Panel on Animal Health and Welfare, 2011)
Canada	2010	(EFSA Panel on Animal Health and Welfare, 2011)

Tab. 2 Repertoire of effector proteins of *A. invadans* (FungiDB).

Gene families	*A. invadans*	*S. parasitica*	*P.infestans*
RXLR	0	1	596
Crinkler	0	0	452
NPP1-like proteins	0	0	67
Elicitin and elicitin like	12	32	86
Cutinase	0	0	4
Pectin methyl esterases	1842	1955	1641
Glycosyl hydrolase	526	748	760
Pectate lyases	0	2	49
Polygalaturonases			
PAN	41	53	27
CBEL	0	0	4
Ricin	23	80	8
Gal-Lectin-binding	5193	6594	5452
Jacalin-like lectin	52	120	61
Jacalin-like	2	4	15
Legume-like lectin	52	120	61
Legume-like	3	3	3
Disintegrin	0	22	0
Protease inhibitors,all	182	209	317
Serine protease	54	47	65
Metalloprotease	37	39	38
Cysteine protease	67	81	59
ABC transporter, all	108	147	161
Kinases	35	39	34
Notch protein	12	21	1
Haemolysin E	0	5	0

Tab. 3 Pathogens co-isolated with *A. invadans* from fish with EUS.

	Pathogen	Infected fish	Country	References
Viruses				
Rhabdoviruses	Ulcerative disease rhabdovirus (UDRV)	Striped snakeheads and freshwater eel	Northern Thailand and Myanmar	(Frerichs et al., 1989)
	Snakehead rhabdovirus (SHRV)	Snakehead	Thailand	(Ahne et al., 1988)
	Rhabdovirus (different to UDRV)		Sri Lanka Philippines	(Frerichs et al., 1989; Lilley and Frerichs, 1994; Lio-Po et al., 2000)
Birnaviruses	Sand goby virus (SGV)	Ulcerated sand goby	Thailand	(Hedrick et al., 1986)
	snakehead fish virus	Snakehead fish	Thailand	(Saitanu et al., 1986)
	IPNV	Diseased snakeheads and eye-spot barb	Thailand, Myanmar, Lao PDR	(Wattanavijarn et al., 1988)
		Spleen of ulcerated snakehead	Singapore	(Subramanian et al., 1993)
Reoviruses	Snakehead reovirus (SKRV)	Diseased snakehead	Thailand	(Roberts et al., 1994)
Ranaviruses		Largemouth bass	Guangdong Province, China	(Deng et al., 2011)
Bacteria				
	Aeromonas hydrophila	Ulcerated snakehead, Ulcerated murrel, Ulcerated carp	Philippines,India, Pakistan	(Bondad-Reantaso et al., 1992; Chandrakanthi et al., 2000; Dhanaraj et al., 2008; Iqbal et al., 1998; Podeti and Benarjee, 2017; Rab et al., 2001; Saha and Pal, 2000; Thampuran et al., 1995)
	Staphylococcus aureus	Ulcerated Murrel	India	(Podeti and Benarjee, 2017)
	Pseudomonas aeruginosa	Ulcerated murrel, Ulcerated carp	India, Pakistan	(Dhanaraj et al., 2008; Podeti and Benarjee, 2017; Rab et al., 2001; Saha and Pal, 2000; Thampuran et al., 1995)
	Salmonella salmonicida	Ulcerated Murrel	India	(Podeti and Benarjee, 2017)
	Flexibacter columnaris	Ulcerated snakehead	Philippines	(Bondad-Reantaso et al., 1992)
	Streptococcus group Q1	Ulcerated mrigal carp	India	(Sharma and Sihag, 2013)
	Shigella spp	Ulcerated mrigal carp	India	(Sharma and Sihag, 2013)
	Streptococcus faecalis	Ulcerated mrigal carp	India	(Sharma and Sihag, 2013)
	Cellobiosococcus sciuri	Ulcerated mrigal carp	India	(Sharma and Sihag, 2013)
	Micrococcus luteus	Ulcerated mrigal carp, Puntius sp, Mystus sp	India	(Saha and Pal, 2000; Sharma and Sihag, 2013; Thampuran et al., 1995)
	Vibrio sp	Ulcerated murrel, Puntius sp, Mystus sp	India	(Dhanaraj et al., 2008; Saha and Pal, 2000)

	Escherichia coli	Ulcerated murrel, Ulcerated snakehead	India	(Dhanaraj et al., 2008; Thampuran et al., 1995)
	Enterobacteriaceae	Ulcerated snakehead, Ulcerated murrel	Philippines, India	(Bondad-Reantaso et al., 1992; Dhanaraj et al., 2008)
	Bacillus sp	Ulcerated snakehead, Puntius sp, Mystus sp	India	(Saha and Pal, 2000)
	Moraxella sp	Ulcerated snakehead, Puntius sp, Mystus sp	India	(Saha and Pal, 2000)
Parasites				
Protozoans	*Chilodonella* sp., *Costia* sp., *Epistylis* sp., *Glossatella* sp., *Ichthyophthirius* sp., *Scyphidia* sp., *Trichodina* spp			(Bondad-Reantaso et al., 1992; Callinan et al., 1997; Reungprach et al., 1983; Subasinghe, 1993)
Metazoan	*Dactylogyrus* sp., *Gyrodactylus* sp.			(Reungprach et al., 1983)
Myxosporeans	*Henneguya* sp. and *Thelohania* sp.			(Callinan et al., 1997)
Monogeneans				(Callinan et al., 1997)
Ecto-parasite	*Lernaea* sp, *Argulus* sp	Ulcerated carp	Pakistan	(Callinan et al., 1997; Rab et al., 2001)
Fungus				
	Aspergillus sp	Infected Murrel	India	(Dhanaraj et al., 2008)
Oomycete				
	Saprolegnia spp.	Ulcerated carp	Pakistan	(Rab et al., 2001)

Tab. 4 Plant extracts effective against *A. invadans* growth.

Genus	Species	Reference
Herb	*Piper betle*	(Borisutpeth et al., 2009)
	Mammea siamensis	(Borisutpeth et al., 2009)
	Tamarindus indica	(Borisutpeth et al., 2009)
	Rosa spp	(Borisutpeth et al., 2009)
	Psidium guajava	(Borisutpeth et al., 2009; Campbell et al., 2001)
	Azadirachta indica	(Harikrishnan et al., 2010, 2009, 2005)
	Solanum nigrum	(Haniffa et al., 2011)
	Cassia fistula	(Borisutpeth et al., 2014)
	Rauvolfia tetraphylla	(Yogeshwari et al., 2015)
Mangrove plant	*Sonneratia alba*	(Afzali and Wong, 2017)

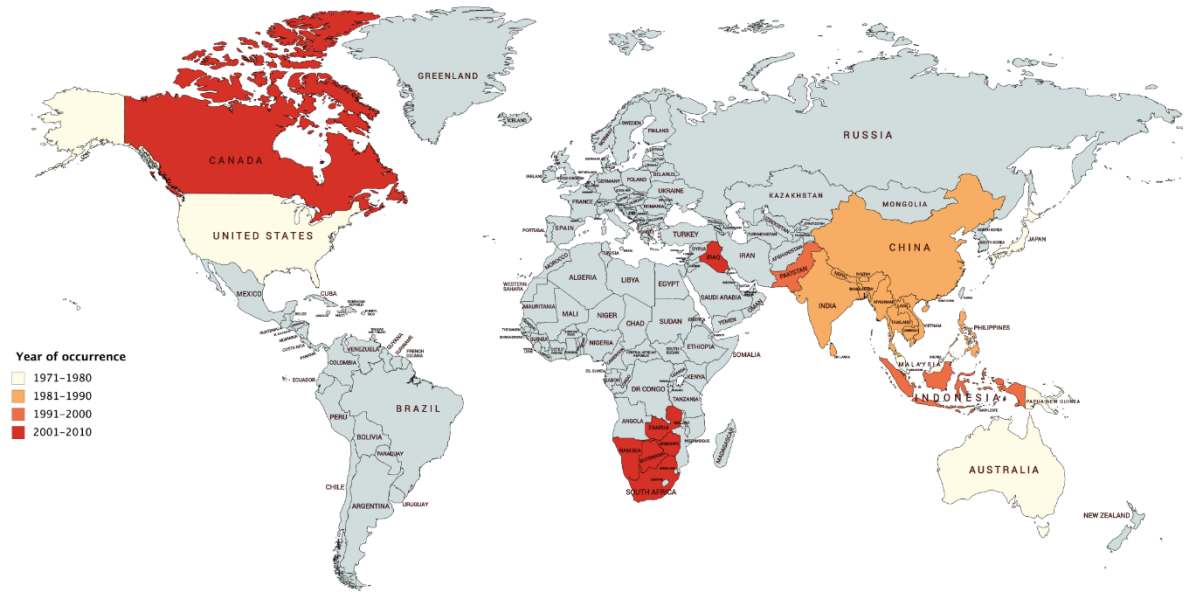

Fig. 1 First occurence of EUS outbreaks in fresh water fish worldwide. (ee also Tab. 1) Countries are labelled in colours according to the decade of their first *A. invadans* infection outbreak. The outbreaks started in the South-East of Asia and are now disseminating to all continents likely due to globall trading.

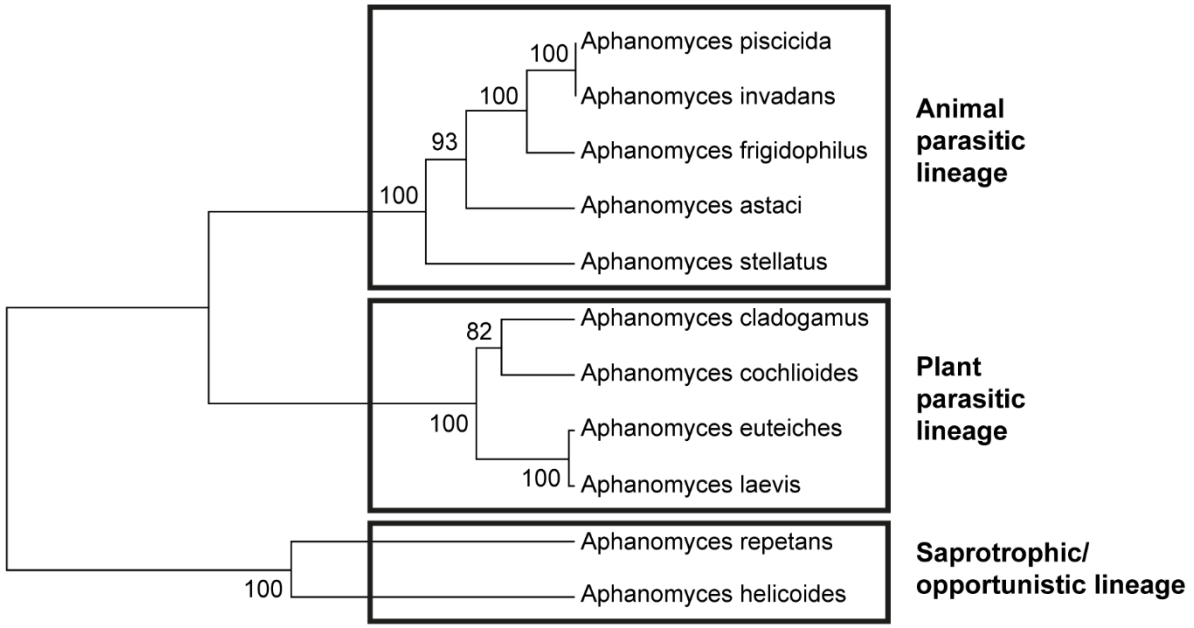

Fig. 2 Different lineages of *Aphanomyces* sp. infecting different groups of hosts. Phylogenetic tree is based on 5.8S ribosomal RNA gene from *A. piscicida* (AY283640), *A. invadans* (DQ403202), *A. frigidophilus* (AY647192), *A. astaci* (AY310501), *A. stellatus* (AY683888), *A. cladogamus* (AY353918), *A. cochlioides* (AY353911), *A. euteiches* (AY353901), *A. laevis* (AY283646), *A. repetans* (AY683889) and *A.helicoides* (AY310496). Phylogenetic tree was created with MEGA 7.0 and phylogeny was tested with the Bootstrap method (1000 bootstrap).

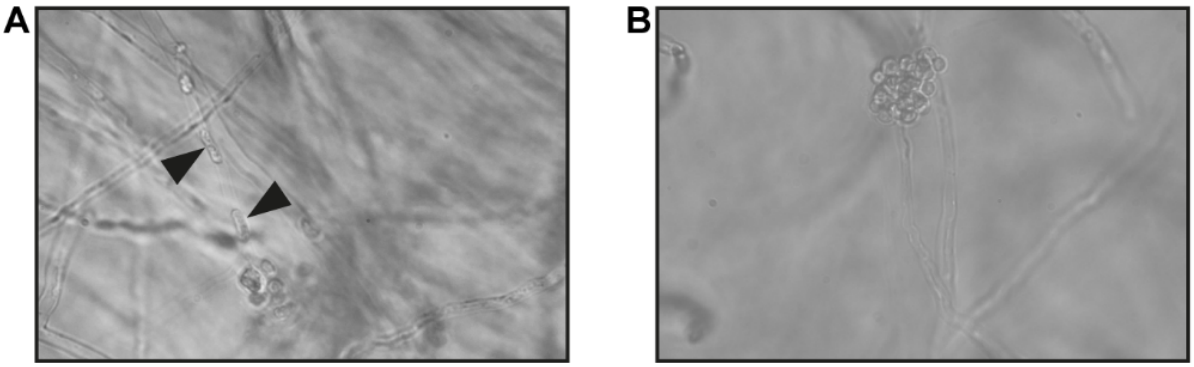

Fig. 3 Formation of achloid clusters by encysted primary zoospores at the hyphal tip. Similar to *Aphanomyces salsuginosus* also primary zoospores of *A. invadans* are transported in the hyphae (A, arrowheads) to form clusters at the hyphal tip from where secondary zoospores are released (B).

SUPPLEMENT

Movie S1. Formation of secondary zoospores of *A. invadans* from clusters of primary cysts at hyphal tips.