First description of spontaneous granulomatous aerocystitis by *Phoma herbarum* in a wild greater amberjack (*Seriola dumerili* Risso, 1810)

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*Phoma herbarum* Westend (family Didymellaceae) is a saprophytic fungus mostly recognized as a plant pathogen (Aveskamp, Gruyter, & Crous, 2008; Bennett, Ponder, & Garcia-Diaz, 2018; Kumla, Suwannarach, & Lumyong, 2016; Neumann Brebaum & Boland, 1999). With a ubiquitous distribution, *P. herbarum* can be isolated from vegetable debris, inorganic material, water sources, humans and animals (Boerema, 1964; Boerema, Gruyter, Noordeloos, & Hamers, 2004).

In fish, *P. herbarum* has been reported to act as a facultative pathogen, causing a chronic progressive and lethal visceral mycosis. Previous reports detailed the infection in coho salmon (*Oncorhynchus kisutch* Walbaum), chinook salmon (*O. tshawytscha* Walbaum) and rainbow trout (*O. mykiss* Walbaum) in hatcheries in the Northwest, Midwest United States and Alaska (Boerema et al., 2004; Burton, Meyers, Starkey, & Follett, 2004; Faisal, Elsayed, Fitzgerald, Silva, & Mendoza, 2007; Ross, Yasutake, & Leek, 1975). Moreover, a visceral mycosis with similar features was also found in farmed ayu (*Plecoglossus altivelis altivelis* Temminck & Schlegel) in Japan, caused by an unidentified species of the genus *Phoma* (Hatai, Fujimaki, Egusa, & Jo, 1986).

Infections by *P. herbarum* in fish were characterized by lethargy and erratic swimming, for example swimming on their sides or in a vertical position, and resting on their sides or on the bottom, as infection progresses (Faisal et al., 2007; Ross et al., 1975). Physical changes included swollen and haemorrhagic vents, areas of muscle softening, petechiae along the lateral and ventral body surfaces and exophthalmia (Buller, 2014; Faisal et al., 2007; Ross et al., 1975). Evaluation of the internal organs revealed the presence of white caseous necrotic masses and congestive walls in the swim bladder. Adhesions between the swim bladder and gastrointestinal tract, stomach distension by yellowish fluids and haemorrhagic areas in the kidneys and adjacent musculature were also reported (Faisal et al., 2007; Ross et al., 1975). Histopathology showed abundant septate hyphae in the lumen of the swim bladder, extensive inflammatory infiltrate and degenerated necrotized and sloughed cells from the organ walls. Based on the anatomopathological observations, the swim bladder appears to be the most affected organ by *P. herbarum* (Burton et al., 2004; Faisal et al., 2007; Hatai et al., 1986; Roberts, 2012; Ross et al., 1975).

The exact portal of entry for *P. herbarum* in fish is still unclear. An often suggested theory is that ingested conidia, from feed or surface water, could pass through the pneumatic duct from the digestive tract to the swim bladder (Burton et al., 2004; Faisal et al., 2007; Ochiai, Kodera, Kon, Miyazaki, & Kubota, 1977). Nonetheless, the proposed theory was based on the reports of *P. herbarum* spontaneous infections exclusively in species that maintain the pneumatic duct—physostomous species (Burton et al., 2004; Faisal et al., 2007; Ochiai et al., 1977; Ross et al., 1975).

Greater Amberjack (*Seriola dumerili* Risso, 1810; family Carangidae) is a marine fish commonly found over offshore chasms, drop-offs or rocky outcrops (Harris, Wyanski, White, Mikell, & Eyo, 2007; Smith-Väniz, Pina Amargos, Brown, Curtis, & Williams, 2015).
in the Atlantic and Indo-Pacific Oceans (Andaloro & Pipitone, 1997; Harris et al., 2007; Sley, Taieb, Jarboui, Ghorbel, & Bouain, 2016). As an opportunistic predator, it preys both on benthic and on pelagic species, either close to the surface or close to the bottom (Andaloro & Pipitone, 1997; Jerez & Vassalo-Agius, 2016; Smith-Vaniz et al., 2015). Whilst being a popular species in commercial and recreational fisheries, greater amberjack has also been recently introduced for aquaculture diversification (Jerez et al., 2018; Zupa et al., 2017). Hence, knowledge on pathologies affecting this species is of extreme importance in order to avoid the transmission of diseases, a major bottleneck in fish production and performance, and economic losses.

To the best of our knowledge, we present in this paper, the first description of a spontaneous granulomatous acerocystitis caused by *P. herbarum* in greater amberjack, a wild marine physiologist.

A female adult greater amberjack, 27 kg weight and 112 and 132 cm, furcal and total length, respectively, was captured alive by local fishermen in La Santa, the north-west Lanzarote, Canary Islands (Spain 29°06′40″N 13°40′00″W), in early autumn. It was submitted in October 2016 to the Division of Infectious Diseases and Ichthyopathology, Institute for Animal Health and Food Safety (IUSA), University of Las Palmas de Gran Canaria, Canary Islands, Spain.

A standard necropsy for finfish, according to Meyers (2009), was performed, and tissue samples were collected for histopathological analysis.

Tissue samples from the swim bladder, anterior and posterior kidney, liver, stomach, intestine, heart, gills and gonads were fixed in 10% phosphate-buffered formalin solution, embedded in paraffin wax, sectioned at four-μm sections and stained with haematoxylin and eosin (H&E), Gram, Ziehl–Neelsen, Gomori’s Methenamine Silver (GMS) and Periodic Acid–Schiff (PAS), according to the protocols detailed in Bancroft, Layton, and Suvarna (2013).

Formalin-fixed paraffin-embedded samples from the swim bladder were submitted to Instituto Valenciano de Microbiología for fungal identification by PCR. DNA was extracted using DNeasy® Blood and Tissue Kit (Qiagen). PCR was performed using MyTaq™ HS Mix (Bioline, UK). Primers used for fungal amplification were the ones described by White, Bruns, Lee, and Taylor (1990). The initial sequence obtained from 264 base pairs after the Basic Local Alignment Search Tool (BLAST) analysis provided 99%–100% identities for several species of *Phoma* spp., *Leptosphaeria* spp., *Epicoccus* spp. and *Dothidea* spp. To confirm the identification, an analysis was performed with specific primers of *Phoma herbarum* for the MAT gene (accession: AY748945.1).

External examination showed no abnormalities or lesions. Gross findings in the internal organs were confined to the swim bladder. It presented a complete loss of morphology and generalized thickening and opacity of the wall with markedly increased size (36 mm × 16 mm) and diminished to obliterated lumen. Multiple, often coalescing, translucent to whitish variable-sized (20–80 mm) fluid-filled cystic lesions replaced the normal structure (Figure 1a,b). Irregular tan to dark brown, gritty and raised nodules, ranging from 7 to 20 mm, were also observed amidst the cystic lesions (Figure 1b). Both cystic and gritty nodules were well-demarcated and separated by smooth, thick bands of fibrous connective tissue. Multifocal haemorrhagic foci were noticed, with particular emphasis on the caudal aspect of the organ.

Histological examination of the swim bladder revealed multiple variable-sized cysts, lined by one up to twelve layers’ thick of attenuated to cuboidal epithelial cells and distended by clear spaces or filled with eosinophilic homogenous proteinaceous fluid (Figure 2a). The remaining tissue was replaced by numerous often coalescent, variable-sized granulomas with a core of necrotic tissue, often pigmented and mineralized, surrounded by epithelioid macrophages, foreign body-type multinucleated giant cells and lymphocytes. Granulomas and cystic lesions were separated by prominent variable thick bands of vascularized loose connective tissue containing scant and disperse small aggregates of lymphocytes and macrophages (Figure 2b). Frequently, within the granulomas, large numbers of filamentous irregular branching septate hyphae, 3–6 μm width, with rare bulbous swellings, were observed (Figure 2c) and highlighted with GMS (Figure 2d) and PAS.

![FIGURE 1](a) Lateral view of the swim bladder with multiple cystic lesions and haemorrhagic foci (Cr—cranial, Ca—caudal, V—ventral, D—dorsal). (b) Inner aspect of the swim bladder. Numerous cystic structures (arrow) admixed with multiple variable-sized tan to dark brown solid nodules (•) [Colour figure can be viewed at wileyonlinelibrary.com]
Tissue samples from other organs did not reveal the presence of fungal infection and no other pathological changes were observed, apart from a mild infection with nematodes (Anisakidae) in the stomach lumen, without associated lesions.

The detection and sequencing of fungal DNA allowed the identification of the species as Phoma herbarum in the swim bladder samples of the present study.

Phoma herbarum is a saprophytic fungus with a ubiquitous distribution (Boerema et al., 2004). In fish, it has been reported to act as a facultative pathogen, causing a chronic progressive and lethal visceral mycosis, being the swim bladder the most affected organ (Burton et al., 2004; Faisal et al., 2007; Roberts, 2012; Ross et al., 1975).

The swim bladder is a hydrostatic organ whose primary function is to regulate buoyancy (Bruno, Noguera, & Poppe, 2013; Genten, Terwinghe, & Danguy, 2009; Helfman, Collette, Facey, & Bowen, 2009). Two primary types of swim bladder are currently recognized. In physostomous species (e.g., Salmonidae), the swim bladder is connected to the oesophagus by the pneumatic duct. The pneumatic duct favours the entrance of gas to the swim bladder by swallowing air at the surface and passing it down the gut (Bone & Moore, 2008; Bruno et al., 2013). In contrast, in physoclist species (e.g., Carangidae), the connection between the swim bladder and the digestive tract ceases to exist during the embryonic development and the filling and emptying mechanisms of the swim bladder are done by diffusion with the bloodstream (Bruno et al., 2013; Genten et al., 2009; Hughes, Rowland, Stewart, & Gill, 2016).

Prior reports of visceral mycosis in fish by P. herbarum appear to be restricted to hatchery-reared fish of the family Salmonidae in freshwater (Faisal et al., 2007; Ross et al., 1975; Wood et al., 1968). Because swim bladder was the most affected organ, it was hypothesized that infection could have resulted from the passage of the fungus from the digestive tract, after ingestion of food or detritus, to the swim bladder, through the pneumatic duct (Burton et al., 2004; Faisal et al., 2007; Hatai et al., 1986). In physoclist species, this theory would not apply due to the absence of the pneumatic duct (Bruno et al., 2013). Transmission by inhalation, enabled during the swim-up stage, was another postulated route of infection. It was suggested that aerosolized conidia from plants with P. herbarum deposited in the water surface could be inhaled during gas exchange and pass to the swim bladder (Wood et al., 1968; Burton et al., 2004). Again, the absence of the pneumatic duct undermines this theory in physoclist species. However, transmission may occur during gas exchange. When oxygen dissolved in the surrounding water enters the bloodstream via the efferent arterioles in gills, it goes through the dorsal aorta, reaching the swim bladder. Ross et al. (1975) and Faisal et al. (2007) reported vasculitis and fungal invasion of the dorsal aorta. In addition, it was suggested by Camus, Berliner, Hyatt, Hatcher, and Clauss (2014), in a case of aerocystitis by Exophiala xenobiotica, that the swim bladder infection could be the result of a vascular invasion at an undetermined primary site, through haematogenous seeding of the gas gland capillary rete. The fact that greater amberjack often preys close to the surface, where aerosolized conidia from plants with P. herbarum deposit (Andaloro & Pipitone, 1997), would make it particularly susceptible to an infection by this route. However, we cannot further hypothesize on this portal of entry, as dorsal aorta was not sampled in the present case. Recently, it was reported that, in some species of Carangidae (e.g., S. dumerili, S. lalandi), the swim bladder may not be completely sealed (Hughes et al., 2016). Hughes et al. (2016) detailed a specialized anatomical structure, consisting of a membranous opening, dorsal in the swim bladder, which led to a flattened tube that bifurcated around the vertebral...
columns and exited via a small hole in the pharyngocleithral membrane underneath each operculum. This connection could be considered as an important portal of entry of *P. herbarum* in species with this feature. In experimental infections with *P. herbarum*, other portals of entry apart from oral (Burton et al., 2004; Easa, Hatem, Sakr, & Refai, 1984; Ross et al., 1975) and airborne transmission (Burton et al., 2004; Ross et al., 1975) were considered, such as bath immersion (Ali, Hashem, & Al-Salahy, 2011), intraperitoneal injection (Burton et al., 2004; Easa et al., 1984), subcutaneous injection (Ali et al., 2011; Easa et al., 1984) and swabbing on scarified gills (Easa et al., 1984). Overall, the highest mortalities were obtained by intraperitoneal and subcutaneous injection (Ali et al., 2011; Burton et al., 2004; Easa et al., 1984) and in a less extent, by oral transmission (Easa et al., 1984; Ross et al., 1975), airborne transmission (Ross et al., 1975) and bath exposure (Ali et al., 2011). However, results were not conclusive due to differences between studies with regard to dosage, exposition time, water conditions and environment.

Despite being a ubiquitous fungus, few spontaneous infections by *P. herbarum* in fish have been reported. This allegedly low incidence supports the idea that the pathogenic effect of *P. herbarum* depends greatly on the immune status of its host (Faisal et al., 2007). In an experimental transmission of *P. herbarum* to chinook salmon, Burton et al. (2004) reported that survival rates tended to be higher under optimal environmental conditions. As already acknowledged, intrinsic factors such as species, genetic background, age, reproductive status and nutritional condition may have a negative impact in the immune system. Similarly, extrinsic factors, such as seasonality and temperature, handling, density and pollution, could also affect negatively the activity of the immune system (Magnadóttir, 2006; Plumb & Hanson, 2011; Voronin, 2014).

In previously reported infections with *P. herbarum*, fry and fingerlings were the most affected fish. These observations are in line with the idea that young fish are inherently more susceptible to diseases as they have not yet acquired natural resistance (Noga, 2010; Plumb & Hanson, 2011). Also, being poikilothermic animals, temperature significantly affects their metabolism, especially concerning immunity (Noga, 2010). A decrease in water temperature is reported to suppress the immune response. Fish from temperate and colder climates are particularly susceptible to infectious diseases during spring and autumn, when changes in water temperature are more abrupt (Noga, 2010; Plumb & Hanson, 2011). Stressful events, such as water temperature (Ross et al., 1975) and salinity changes (Hatai et al., 1986) or handling (Easa et al., 1984; Faisal et al., 2007; Hatai et al., 1986), were also described prior to the development of the infections by *P. herbarum*. However, the aforementioned cases of spontaneous infections by *P. herbarum* occurred in hatchery-reared species, under a controlled environment.

The present report concerns a wild adult greater amberjack, captured alive. In a wild environment, the number of stressors that may result in increased risk of infections is vast. Several factors could have prompted the infection. Reproductive status is known to modulate immunity, especially during the spawning season, when natural resistance of adult fish is reduced, as their energy is diverted into reproductive activities (Plumb & Hanson, 2011). Latent or opportunistic pathogens may also have the potential to cause immunosuppression, enabling the development of a secondary infection. In our case, the mild gastric nematode infection observed may have played a role in the suppression of the immune system, thus prompting the infection by *P. herbarum*. Also, a sudden change in water temperature could negatively affect the immune system. It is important to remark that *P. herbarum* optimal temperature for growth is between 20°C and 25°C (Boerema, 1964) and that temperatures in the eastern Atlantic Ocean usually hover around 20°C and 23°C from June to September. Nevertheless, it is important to bear in mind that *P. herbarum* is characterized by causing a chronic progressive disease and it is not possible to determine the definite role played by these stressors at the time of onset of the infection.

In summary, previous reports of spontaneous visceral mycosis by *P. herbarum* were limited to freshwater farmed species with a physostomous swim bladder configuration. To the best of our knowledge, this is the first report of a granulomatous aerocystitis by *P. herbarum* in a wild greater amberjack, a marine fish with a physoclistous swim bladder.

The importance of this finding lies mostly on the efforts that have been made for the past years to introduce greater amberjack in aquaculture. As *P. herbarum* has been associated with great losses in hatcheries (Faisal et al., 2007; Ross et al., 1975), it is essential to better identify potential diseases that may represent a bottleneck to fish production and performance, in order to be able to prevent or treat them and avoid economic losses.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interests.

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