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New disease records for hatchery-reared sturgeon. II. Phaeohyphomycosis due to *Veronaea botryosa*

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ABSTRACT: A series of fungal cases in hatchery-reared juvenile and young adult Siberian sturgeon *Acipenser baerii* and white sturgeon *A. transmontanus* occurred at production facilities in Florida and California, USA, respectively. Affected fish exhibited abnormal orientation and/or buoyancy, emaciation, coelomic distension, exophthalmos, cutaneous erythema, and ulcerative skin and eye lesions. Necropsies revealed haemorrhage throughout the coelom, serosanguinous coelomic effusion and organomegaly with nodular or cystic lesions in multiple organs. Fungal hyphae were observed in 27 fish (24 *A. baerii* and 3 *A. transmontanus*) via microscopic examination of tissue wet mounts and on slides prepared from colonies grown on culture media. Histopathological examination of these infected tissues revealed extensive infiltration by melanised fungal hyphae that were recovered in culture. Phenotypic characteristics and sequencing of the fungal isolates with the use of the internal transcribed spacer region and 28S rRNA gene confirmed the aetiological agent as *Veronaea botryosa*. To our knowledge, this is the first documentation of *V. botryosa* infection in fish, although melanised fungi of the closely related genus *Exophiala* are well-known pathogens of freshwater and marine fishes.

KEY WORDS: *Acipenser baerii* · *Acipenser transmontanus* · Fungus · Aquaculture

INTRODUCTION

The Kingdom Fungi includes a wide variety of eukaryotic organisms that are known to cause disease in both plants and animals (Ainsworth 1976). To date, a limited number of truly primary fungal diseases have been well characterized in fish (Neish & Hughes 1980, Khoo 2000). Some 'fungal' pathogens known to infect fish are actually water molds (Class Oomycota) rather than true fungi (Noga 1993). Oomycetes such as *Saprolegnia, Aphanomyces*, and *Branchiomyces* spp. have been associated with significant disease in wild and cultured fish populations (Meyer & Robinson 1973, Bruno & Stamps 1987, Bly et al. 1992, Willoughby et al. 1995).

True fungi, which are morphologically and phylogenetically distinct from the Oomycetes, are known to cause disease in animals. Phaeohyphomycosis, which

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is the collective term for infection with melanised fungi that form hyphae in tissues (de Hoog & McGinnis 1987, Naggie & Perfect 2009), has been documented worldwide in invertebrates, fish, amphibians, reptiles, birds, and mammals including humans (Zeng et al. 2007, de Hoog et al. 2011, Seyedmousavi et al. 2013). Infections in humans are rare and typically affect immunocompromised individuals (Ayadi et al. 1995, Foulet et al. 1999, Matsushita et al. 2003, Sutton et al. 2004). Lesions are most commonly cutaneous or subcutaneous (Revankar 2006), although more severe cases of systemic infection have been reported (Revankar et al. 2002). Melanised fungal infections are being reported with increasing frequency in fish and have been documented in captive freshwater and marine teleosts and elasmobranchs including Atlantic salmon and cutthroat trout (family Salmonidae), channel catfish (Ictaluridae), cod (Gadidae), Japanese flounder (Paralichthyidae), King George whiting (Sillaginidae), leafy and weedy seadragons (Syngnathidae), and smooth dogfish (Triakidae) (Carmichael 1966, Fijan 1969, Blazer & Wolke 1979, Gaskins & Cheung 1986, Langdon & McDonald 1987, Reuter et al. 2003, Kurata et al. 2008, Nyaoke et al. 2009).

Sturgeon belong to the family Acipenseridae within the order Acipenseriformes. They have a holarctic distribution and are among the oldest ray-finned fish families, with fossils dating back to the Early Jurassic period (Gardiner 1966). Sturgeon species are among the largest and longest lived of all known fishes (Bemis et al. 1997, Berra 2007). For several centuries, sturgeon have been prized for their meat and caviar, but overfishing, habitat destruction, and pollution have severely compromised wild stocks of many species (Artyukhin 1997, Debus 1997, Baker & Borgeson 1999). As a result, all sturgeon species are listed as CITES Appendix I or II (see www.cites.org/eng/app/2012/E-2012-09-25.pdf) and are on the IUCN Red List of Threatened Species (Billard & Lecointre 2000, IUCN 2001). Due to fishery restrictions, catches of wild sturgeon have sharply decreased in the last 2 decades and are steadily being replaced with farmed fish raised for meat and caviar. Currently, aquaculture production of sturgeon occurs predominantly in China, followed by Europe, Russia, and the USA (Chebanov & Billard 2001, Bronzi et al. 2011).

The relatively slow maturation period and large size of sturgeon compared to other commonly cultured fish contribute to greater potential in intensive culture for problems such as poor water quality and increased risks of stress and disease (LaPatra et al. 1996, Savin et al. 2011). In particular, viral pathogens (e.g. adenovirus, alloherpesviruses, and iridoviruses) have negatively impacted sturgeon aquaculture and restoration efforts (Hedrick et al. 1985, 1990, 1991, LaPatra et al. 1994, Bauer et al. 2002, Kelley et al. 2005, Kurobe et al. 2008, 2010, 2011, Waltzek et al. 2014, this volume). Here we report infection with the melanised fungus *Veronaea botryosa* in cultured Siberian *Acipenser baerii* and white sturgeon *A. transmontanus*.

MATERIALS AND METHODS

Clinical history

Over a 7 yr period (2006 to 2012), juvenile and subadult Siberian sturgeon were presented to the University of Florida's Tropical Aquaculture Laboratory (TAL; Ruskin, FL) for diagnostic evaluation. All fish had been captive hatched and reared at the same production facility. Fish had been housed in 18 700 or 31 000 l recirculating indoor tanks supplied with degassed well water with a salinity of 0. Systems were aerated, oxygenated, and equipped with microscreen drum mechanical filtration and a moving bed bioreactor. Temperatures were maintained between 19 and 27°C and fish were fed a 45% protein/19% fat salmon feed (Nelson & Son's Silver Cup). The producer's common presenting complaint was increasing mortality. A similar production facility in California had reported several years of ongoing mortality in subadult white sturgeon associated with fungal infection. Three fish from the most recent outbreak were submitted to the California Animal Health and Food Safety Laboratory (CAHFS), San Bernardino, CA, for post-mortem examination. The submitted fish were 5 yr old females raised as described above except they had been housed in 300 000 l indoor freshwater recirculation tanks with temperatures maintained between 20 and 24°C and fed a 43% protein/14% fat sturgeon diet (EWOS, Bergen).

Ante-mortem skin, fin, and gill biopsies from Siberian sturgeon were obtained, after which fish were euthanized using 2.5 g l^{-1} tricaine methanesulphonate buffered with a 2:1 (w/w) ratio of sodium bicarbonate. In both the Florida and California cases, post-mortem examinations included gross and cytological (wet mount) examinations of internal and external tissues. Tissue samples including skin, gill, heart, liver, kidney, stomach, intestine, pancreas, spiral colon, gonad, brain, and eye were placed into 10% neutral buffered formalin for histopathological examination, and duplicate samples of tissues from Siberian sturgeon were placed into RNA*later*® solution (Invitrogen) for molecular analysis.

Bacterial and fungal identification

During necropsies of Siberian sturgeon, sterile swabs of brain, liver, spleen, posterior kidney, gas bladder, and grossly apparent lesions were inoculated onto trypticase soy agar plates with 5% sheep blood (blood agar) and incubated aerobically at 28°C. For the white sturgeon, cultures from liver, kidney, and gonad from two-thirds of affected fish were inoculated onto blood agar and Sabouraud dextrose agar plates and incubated anaerobically at 10°C and 25°C. A single fungal isolate from a Siberian (hereafter referred to as DI13-194) and white (DI13-193) sturgeon were submitted to the Fungus Testing Laboratory of The University of Texas Health Science Center at San Antonio (UTHSCSA), San Antonio, TX, for identification by a multi-phasic approach, employing phenotypic features and molecular characterization. At UTHSCSA, the 2 isolates were transferred to potato flake agar plates and incubated at 25, 30, and 35°C. Both the macroscopic morphology of the resulting colonies and the microscopic features were determined approximately 12 d post inoculation at 30°C.

Histopathology

Formalin-fixed tissues from Siberian sturgeon were submitted either to the University of South Florida College of Medicine, Department of Pathology and Cell Biology (Tampa, FL) or Histology Tech Services, Inc. (Gainesville, FL), where they were routinely processed, embedded in paraffin, sectioned, and mounted on glass slides. Tissue sections were stained with haematoxylin and eosin. Fixed tissues from white sturgeon were similarly processed at CAHFS. Based on preliminary necropsy findings, some slides were also stained with the Ziehl-Neelsen technique to screen for acid-fast bacteria or Grocott's methenamine silver (GMS) and Fontana-Masson stains to identify and describe fungi.

DNA extraction and PCR

Molecular identification of the fungus in infected tissues and cultures from the Siberian and white sturgeon was performed at the UTHSCSA and at University of Florida's Wildlife and Aquatic Animal Veterinary Disease Laboratory (WAVDL). At UTHSCSA, fungal genomic DNA was extracted from hyphae (isolates DI13-193 and DI13-194) recovered from a 24 h potato dextrose agar plate incubated at 30°C using Prepman Ultra reagent (Applied Biosystems) as specified by the manufacturer. The PCR was performed in 50 µl reaction volumes containing Triple Master *Taq* DNA polymerase (Fisher Scientific), dNTPs, and primers at concentrations specified by the manufacturer's instructions. All PCR amplifications were performed in a commercial thermal cycler with an initial denaturation step at 95°C for 10 min; 30 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and elongation at 72°C for 1 min; and a final elongation step at 72°C for 10 min. PCRs targeted the D1/D2 region of the fungal 28S rRNA gene (NL1/NL4; O'Donnell 1993) and the internal transcribed spacer (ITS) region between the fungal 18S and 28S rRNA genes (ITS1/ITS4; White et al. 1990; Table 1).

At WAVDL, DNA was extracted from tissues preserved in RNA*later*®, from fungal cultures, or from 50 µm sections of formalin-fixed, paraffin-embedded (FFPE) tissues with the MAXWELL® 16 DNA Purification Kit (Promega) as specified by the manufacturer. Reaction volumes were 20 µl and consisted of 0.1 µl of Platinum *Taq* DNA Polymerase (Invitrogen), 2.0 µl of 10× PCR Buffer, 0.8 µl of 50 mM $MgCl_2$, 0.4 µl of 10 mM dNTPs, 1.0 µl of 20 µM of forward and reverse primers, 11.7 µl of molecular grade water, and 3 µl (or up to 100 ng) of DNA template. The ITS region was amplified using primers ITS86/ITS4 (Lott et al. 1998, White et al. 1990; Table 1). Thermal cycler conditions consisted of an initial denaturation of 5 min at 94°C; 36 cycles of denaturation at 94°C for

Table 1. Primer pairs used to amplify fragments of the D1/D2 region of the fungal 28S rRNA gene and the internal transcribed spacer (ITS) region between the fungal 18S and 28S rRNA genes. Amplicon size does not include primer sequences

Target gene	Primer name	Sequence (5′–3′)	Amplicon size (bp)
28S	NL-1	GCA TAT CAA TAA GCG GAG GAA AAG	574
	NL-4	GGT CCG TGT TTC AAG ACG G	
ITS	ITS1	TCC GTA GGT GAA CCT GCG G	596[a], 598[b]
	ITS4	TCC TCC GCT TAT TGA TAT GC	
	ITS86	GTG AAT CAT CGA ATC TTT GAA C	284
	ITS4	TCC TCC GCT TAT TGA TAT GC	
	SF58	GCC TGT TCG AGC GTC ATT AT	116
	SF213	CAG TAC GTG TCC CGT GAA GA	

[a]Fungal isolate DI13-194 from *Acipenser baerii*
[b]Fungal isolate DI13-193 from *A. transmontanus*

1 min, annealing at 46°C for 1 min, and extension at 72°C for 1 min; and a final elongation step at 72°C for 10 min. A specific primer pair (SF58/SF213), based on the sequence obtained with the ITS86/ITS4 primers, was designed to amplify a shorter region of the fungal ITS when working from FFPE tissues. The master mix and thermal cycling protocol were the same as that of the ITS86/ITS4 protocol except that the annealing temperature was 50°C and the number of cycles was increased to 40.

Sequencing and BLASTN analysis

After electrophoresis in 1% agarose gels, bands were extracted and purified using a QIAquick gel extraction kit (Qiagen). Molecular identification conducted at the UTHSCSA was performed via Sanger sequencing as previously described by White et al. (1990). Purified DNA samples from WAVDL were submitted to the University of Florida's Interdisciplinary Center for Biotechnology Research for Sanger sequencing on an ABI 3130 DNA sequencer (Applied Biosystems). Sequences were aligned and primer sequences were removed using CLC Main Workbench 5.7.1 (www.clcbio.com). General BLASTN searches (www.ncbi.nlm.nih.gov/blast/Blast.cgi) of the 28S and ITS region sequences were then performed (Altschul et al. 1997). The output from the BLAST searches was sorted on maximum identity and was considered significant at a cutoff of ≥97% identity and a query coverage of ≥90%.

RESULTS

Clinical signs and gross pathology

Submitted fish ranged from 1 to 10 kg in weight and 0.4 to 1.4 m in total length. Clinical signs ranged from mild to severe, and most fish displayed varying combinations of emaciation, abnormal buoyancy and/or inappropriate orientation in the water (most commonly upside down at the surface). Gross lesions in these sturgeon included coelomic distension, ulcerative lesions on the body, face and/or eyes (Fig. 1A), unilateral or bilateral exophthalmos, and cutaneous erythema, particularly on the ventrum, fins, and perioral region (Fig. 1B).

Organomegaly, petechiae, and ecchymoses were present in the liver, spleen, and anterior and/or posterior kidney (Fig. 1C). Affected organs contained 1 to multiple nodules and fluid-filled cysts. The coelomic cavities of some fish also contained small to large volumes of serosanguinous to opaque fluid (Fig. 1D), and multiple fish had distended gas bladders. Serosae of the stomach, intestines, and spiral colon of all 3 white sturgeon were irregularly raised. Green plaques were found loosely attached to the serosal surfaces of coelomic organs in 2 Siberian sturgeon (Fig. 1E), and plaques were also found in the gas bladder lumen and ventricular chamber of the heart in a single fish (Fig. 1F).

Microscopic pathology

Cytological examination of freshly (wet) mounted tissues by squash preparation or touch impressions revealed low to high numbers of slender, septate, melanised fungal hyphae in samples from the viscera of 16 of 27 fish (Table 2). Infected tissues included heart, kidney, liver, spleen, stomach, intestine, and gonad (Fig. 2). The green plaques observed during necropsy contained high numbers of melanised hyphae.

Histologic examinations performed on 5 Siberian and 3 white sturgeon revealed low to high numbers of fungal hyphae infiltrating multiple tissues including skin, muscle, liver, spleen, anterior and posterior kidney, intestine, and heart, with evidence of fungal angioinvasion (i.e. penetration of the blood vessel wall) in multiple tissues (Fig. 3). Although some infected tissues showed only minor microscopic changes, other infected tissues had multifocal and coalescing granulomas containing hyphae (e.g. the kidney) or a mixed inflammatory cell infiltrate comprised of macrophages (including multinucleated giant cells in some cases), granulocytes, and lymphocytes together with necrosis (e.g. liver).

Bacterial and fungal identification

No bacterial species was consistently isolated from either white or Siberian sturgeon. Gram-negative bacteria, including *Aeromonas* sp. and *Shewanella putrefaciens*, occasionally grew from lesions.

Tissue swabs from 21 of 27 affected fish streaked on trypticase soy agar with 5% sheep blood and on Lowenstein-Jensen media incubated at 28°C (*Acipenser baerii* at TAL) or on blood agar and Sabouraud dextrose agar plates at 25°C (*A. transmontanus* at CAHFS) readily grew fungal colonies within 24 to 72 h (Table 2). The fungal isolate DI13-193 from California white sturgeon and isolate DI13-194 from

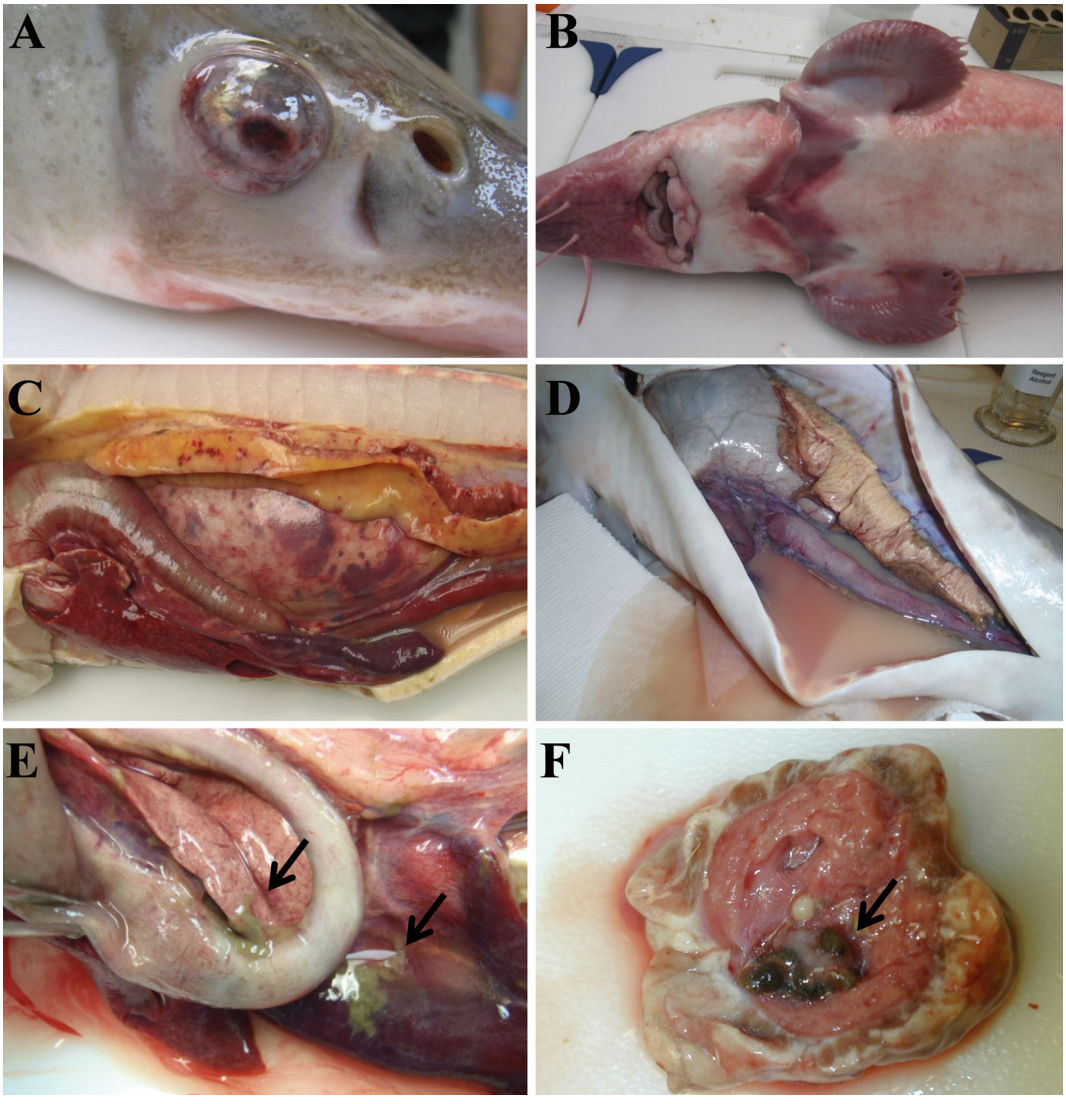

Fig. 1. Gross (A, B) external and (C–F) internal pathology of *Acipenser baerii* infected with *Veronaea botryosa.* (A) An ulcer is present in the cornea of the right eye. (B) Severe erythema is present over the ventral skin and fins; erythema is present over the ventral skin of the body. (C) Petechial and ecchymotic haemorrhages are present in the viscera, along with haemorrhage or congestion in the serosa of the spiral valve. (D) Marked opaque blood-tinged coelomic effusion. (E) Multiple green fungal plaques (arrows) are loosely adherent to serosal surfaces of liver, spleen, and intestine. (F) A fungal plaque (arrow) in the ventricular chamber of the heart

Florida Siberian sturgeon were identified as *Veronaea botryosa* by microscopic features and molecular characterization (see below). Colonies were velvety to woolly and were olivaceous-brown with a black reverse (Fig. 4A). Both isolates failed to grow at 35°C, unlike a human isolate reported by Sutton et al. (2004). Microscopic features included brown, straight to slightly-flexuous conidiophores, often darker in the apical, geniculate, conidiogenous region, producing mostly 2-celled, smooth, hyaline to pale brown conidia with rounded apices and truncate bases (Fig. 4B). DI13-193 and DI13-194 have been deposited into the University of Alberta Microfungus Collection under accession numbers UAMH 11818 and UAMH 11819, respectively.

Sequencing and BLASTN analysis

After primer removal, the Siberian sturgeon isolate DI13-194 produced an amplicon with 99.8% sequence identity (595/596) to *V. botryosa* isolated from an olive (reference sequence NR_103593), and the white sturgeon isolate DI13-193 was identical

Table 2. Case summaries for 24 Siberian sturgeon *Acipenser baerii* and 3 white sturgeon *A. transmontanus* infected with *Veronaea botryosa* during cases from 2006 to 2012. Although histology was performed on all fish, a representative subset of 8 fish were examined for the purposes of this manuscript and the remainder are designated 'NE' (not examined). FFPE: formalin fixed, paraffin embedded; NA: not applicable

Fish ID	Species	Histology performed	Fungus present		PCR		
			Wet mount	Culture	Sample type	Primer pair(s)	ID
2006							
1	*A. baerii*	+	–	–	FFPE	SF58/SF213	*Veronaea*
2	*A. baerii*	NE	–	–	FFPE	SF58/SF213	PCR neg.
2008							
3	*A. baerii*	NE	+	–	NE	NA	NA
4	*A. baerii*	NE	–	–	FFPE	SF58/SF213	*Veronaea*
5	*A. baerii*	NE	–	–	FFPE	SF58/SF213	*Veronaea*
6	*A. baerii*	NE	–	+	FFPE	SF58/SF213	PCR neg.
7	*A. baerii*	NE	–	+	FFPE	SF58/SF213	PCR neg.
8	*A. baerii*	NE	–	+	FFPE	SF58/SF213	PCR neg.
9	*A. baerii*	+	–	+	FFPE	SF58/SF213	*Veronaea*
10	*A. baerii*	+	–	+	FFPE	SF58/SF213	*Veronaea*
2009							
11	*A. baerii*	+	+	+[a]	Culture	NL-1/NL-4, ITS1/ITS4	*Veronaea*
12	*A. baerii*	+	+	+	FFPE	SF58/SF213	*Veronaea*
2011							
13	*A. baerii*	NE	–	+	Culture	ITS86/ITS4	*Veronaea*
14	*A. baerii*	NE	–	+	NE	NA	NA
15	*A. baerii*	NE	+	+	NE	NA	NA
2012							
16	*A. baerii*	NE	+	+	Culture	ITS86/ITS4	*Veronaea*
17	*A. baerii*	NE	+	+	RNA*later*	ITS86/ITS4	*Veronaea*
18	*A. baerii*	NE	+	+	RNA*later*	ITS86/ITS4	*Veronaea*
19	*A. baerii*	NE	+	+	RNA*later*	ITS86/ITS4	*Veronaea*
20	*A. baerii*	NE	+	–	RNA*later*	ITS86/ITS4	*Veronaea*
21	*A. baerii*	NE	+	+	Culture	ITS86/ITS4	*Veronaea*
22	*A. baerii*	NE	+	+	Culture	ITS86/ITS4	*Veronaea*
23	*A. baerii*	NE	+	+	Culture	ITS86/ITS4	*Veronaea*
24	*A. baerii*	NE	+	+	Culture	ITS86/ITS4	*Veronaea*
25	*A. transmontanus*	+	+	+[a]	Culture	NL-1/NL-4, ITS1/ITS4	*Veronaea*
26	*A. transmontanus*	+	+	+	NE	NA	NA
27	*A. transmontanus*	+	+	+	NE	NA	NA

[a]Fungal morphologic identification performed at the University of Texas Health Science Center at San Antonio (Texas, USA) for 2 isolates: DI13-194 from *A. baerii* (2009) and DI13-193 from *A. transmontanus* (2012)

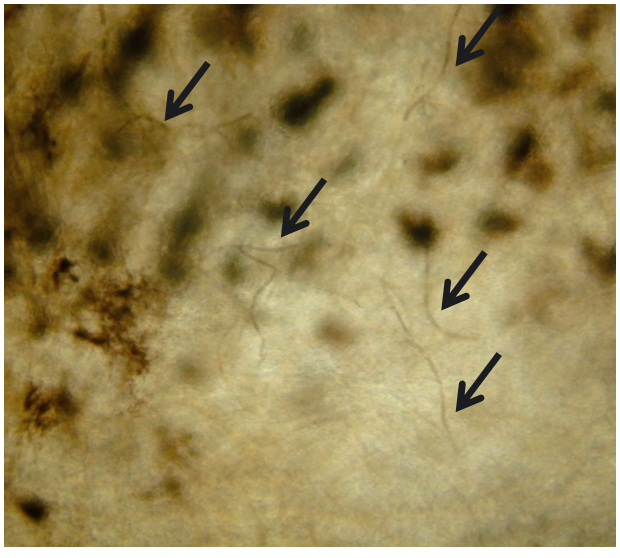

Fig. 2. Wet mount of a sample from the liver of a Siberian sturgeon *Acipenser baerii* infected with *Veronaea botryosa*. Low numbers of slender fungal hyphae are present (arrows)

(598/598) to *V. botryosa* isolated from a human patient (GenBank accession AB369905). These Siberian and white sturgeon isolates produced amplicons with 99.3% sequence identity (594/598) when compared to each other. Amplicons from the D1/D2 region of the 28S rRNA gene were identical to each other in the 2 sturgeon isolates (574/574) and also identical to *V. botryosa* isolated from soil and rotten wood (GenBank accession numbers AB245483 and AB245484, respectively).

At WAVDL, 10 Siberian sturgeon fungus cultures or infected tissue samples were tested with primers ITS86/ITS4, and all were found to be identical (284/

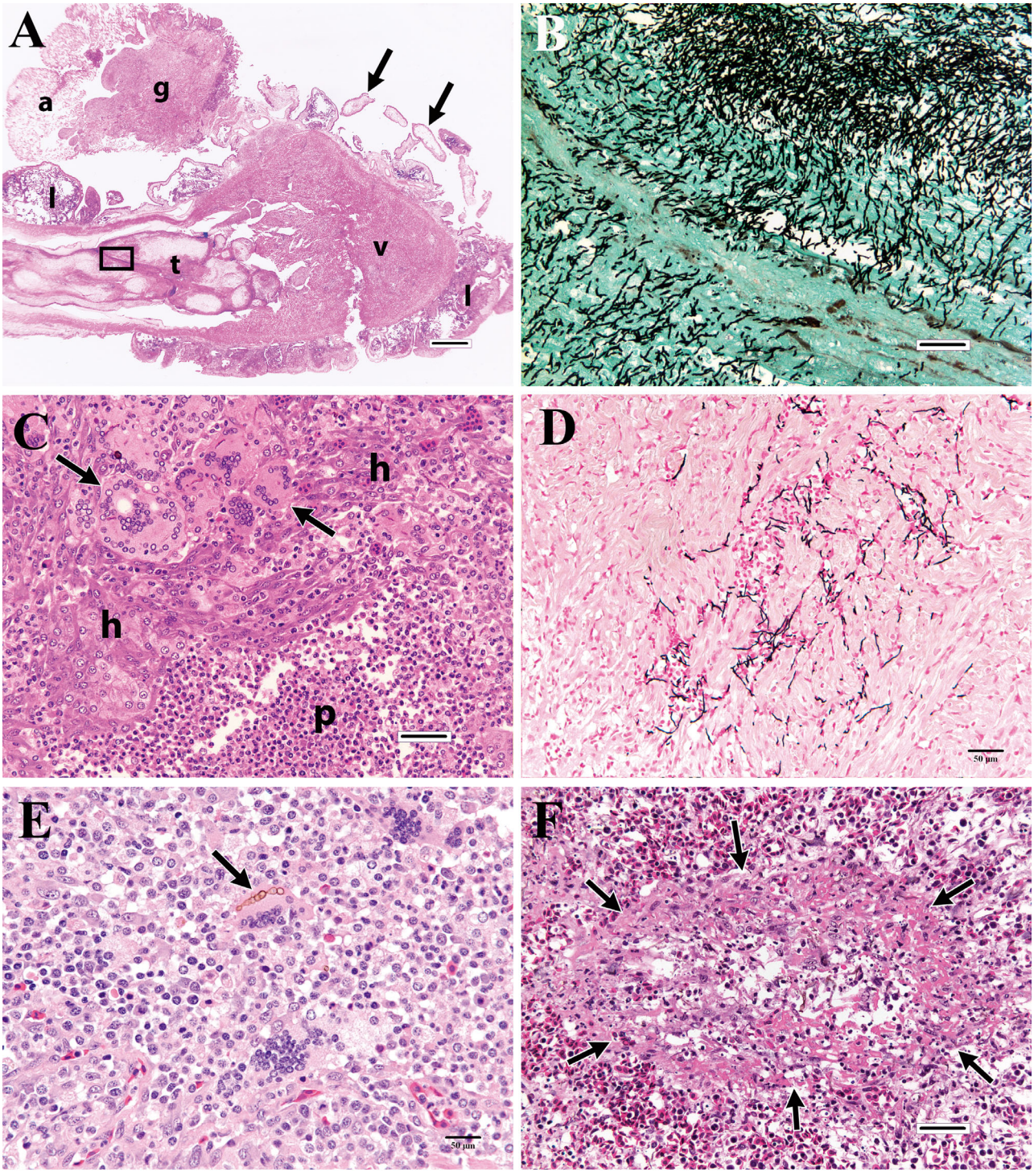

Fig. 3. Histopathologic findings in *Veronaea botryosa* infections of Siberian sturgeon *Acipenser baerii*. (A) The ventricle (v) of the heart contains a massive thrombus (t) within its chamber, and granulomatous inflammation (g) occupies much of the atrium (a). Papillary proliferation of the mesothelium (arrows) along the surface of the epicardial lymphomyeloid tissue (l) represents an additional chronic inflammatory response to infection. Boxed area corresponds to panel B. (B) Cardiac thrombus stained using Grocott's methenamine silver technique to highlight dense mats of fungal hyphae. (C) Mycotic hepatitis featuring disrupted, regenerating liver tubules (h), multinucleated giant cell macrophages (arrows), and an adjacent area of necrosis with pyogranulomatous inflammation (p). (D) Fontana-Masson stain used to demonstrate melanised fungal hyphae invading the ventricular myocardium. (E) A multinucleated giant cell in a focus of granulomatous nephritis contains a pigmented *V. botryosa* hypha (arrow). (F) Fungal angioinvasion and necrotizing vasculitis involving a splenic blood vessel (arrows). Scale bars = (A) 1 mm, (B–F) 50 µm

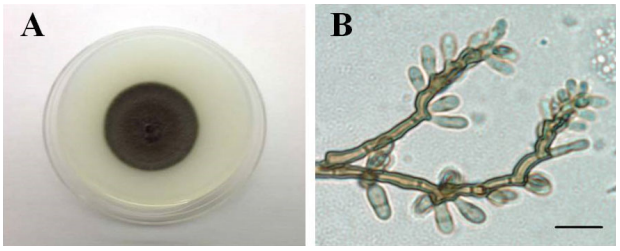

Fig. 4. (A) Colony of *Veronaea botryosa* after 11 d at 30°C on potato flake agar plate. (B) Microscopic colonial morphology of *V. botryosa*, showing slender, septate hyphae with conidiophores producing mostly 2-celled, smooth, hyaline to pale brown conidia. Scale bar = 10 µm

284) to the aforementioned ITS sequence generated at UTHSCSA from Siberian sturgeon isolate DI13-194. An additional 6 out of 10 Siberian sturgeon FFPE samples tested with primers SF58/SF213 also yielded ITS sequences identical (116/116) to Siberian sturgeon isolate DI13-194. Sequences for isolates DI13-193 and DI13-194 have been deposited into GenBank under accession numbers KF772207 and KF772206 (fungal ITS region), and KF772208 and KF772205 (28S rRNA gene), respectively.

DISCUSSION

This series of cases, occurring in hatchery-reared white and Siberian sturgeon, confirmed melanised fungal infections via histology, culture, and/or wet mounts. Infections were characterized histologically by melanised fungal hyphae surrounded by mild to severe inflammation and necrosis. All fungal isolates and infected tissues that were frozen or preserved in RNA*later*® tested positive for *Veronaea botryosa* by PCR and sequencing, whereas just over half of the FFPE samples tested positive. The negative results on some of the FFPE samples may have been due to DNA degradation that is commonly associated with holding tissues in fixative for more than 24 h (Inoue et al. 1996).

To our knowledge, this is the first report of *V. botryosa* infection in a poikilothermic vertebrate. *V. botryosa* belongs to a small genus of widely distributed but poorly understood saprobic fungi typically found in soil and on plant materials (Arzanlou et al. 2007). Phylogenetically, *V. botryosa* groups with the *salmonis* clade of melanised fungi in the genus *Exophiala*, which cause infections in wild and captive populations of fish, amphibians, and invertebrates (de Hoog et al. 2011).

Although the ecology and epidemiology of this environmental fungus remain obscure, it has been reported in human infections worldwide (Matsushita et al. 2003, Sutton et al. 2004, Chen et al. 2006). However, the mode of transmission including the zoonotic potential of *V. botryosa* is not well understood (Badali et al. 2013). *V. botryosa* is generally considered more likely than *Exophiala* to cause disseminated disease in humans because most isolates cultured from human infections grow at incubation temperatures as high as 35 to 37°C (Matsushita et al. 2003, Sutton et al. 2004, de Hoog et al. 2011, Bonifaz et al. 2013). However, the zoonotic risk of the strains reported in this study may be reduced because they failed to grow on selective media at 35°C.

Repeated cases in 2 sturgeon species at 2 geographically distant production facilities raise the question of whether *V. botryosa* is more widespread within North American sturgeon aquaculture facilities. Discussions with facility personnel confirmed that there had been no contact between the 2 locations, and thus sturgeon in these facilities acquired the disease independently. Although the route of entry of *V. botryosa* into the sturgeon facilities remains unknown, potential sources include those observed in previously documented *Exophiala* or *Veronaea* infections, such as water, soil, and contaminated feed (Richards et al. 1978, Nyaoke et al. 2009, Badali et al. 2013).

Successful control of systemic fungal infections in fish has thus far proven difficult given the ubiquitous nature of fungi and a poor understanding of fungal ecology and disease epidemiology (e.g. growth requirements in aquatic environments, transmission dynamics), not to mention an equally poor understanding of the factors that sustain and determine the immunologic health of fish under aquaculture conditions. Furthermore, therapeutic options for fungal diseases in fish are limited, as no systemic antifungal drugs are currently approved for use in aquaculture. Coinfections with other pathogens, such as viruses, may predispose sturgeon to fungal infections and complicate treatment. Several of the Siberian and white sturgeon presented in this report had Gram-negative bacterial growth on cultures from aseptically collected tissue samples; however, no bacteria were noted on histopathological examination, and broad spectrum antibiotic treatments did not curb mortality at the Florida hatchery (data not shown).

Because the use of systemic antifungal therapies in food fish is currently impermissible, alternative methods of reducing fungal populations and improv-

ing host immunity by manipulating the aquatic environment merit future investigation. The exact environmental tolerances (e.g. temperature and salinity) of these sturgeon *V. botryosa* isolates are currently unknown. A better understanding of the effects that these parameters may have on growth and virulence of this fungus may allow for improved infection control through minor environmental modifications. Likewise, husbandry conditions such as water temperature and stocking densities may affect sturgeon susceptibility to fungal infections. Challenge experiments in sturgeon with white sturgeon iridovirus and acipenserid herpesvirus 2 have correlated increased stocking densities with higher mortality (Georgiadis et al. 2000). Other studies have reported increased mortality and secondary bacterial infection rates associated with elevated water temperatures (LaPatra et al. 1996). Thus, *V. botryosa* outbreaks may possibly be mitigated through the manipulation of water temperature and stocking densities. Given the importance of melanised fungal infections in a variety of fish taxa, increased knowledge of aquatic fungal ecology and disease epidemiology may also be beneficial in controlling infections of managed stocks reared for food, conservation programmes or display at public aquaria.

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