Int Aquat Res (2022) 14:223-230
https://doi.org/10.22034/IAR.2022.1959159.1282

SHORT COMMUNICATION

Morphological and molecular characterization of two species of *Saprolegnia* isolated from a rainbow trout (*Oncorhynchus mykiss*) hatchery in Iran

Sina Mirmazloomi . Maryam Ghiasi . Seyedeh Ainaz Shirangi . Saltanat Najjar Lashgari . Ali Reza Khosravi

Received: 20 May 2022 / Accepted: 13 September 2022 / Published online: 18 September 2022

Abstract In the present study, we aimed to identify *Saprolegnia* spp. from infected rainbow trout eggs in a hatchery in the north (Mazandaran province) of Iran. Morphological identification was conducted by light microscopic observation of various life stages of *Saprolegnia*. For the molecular characterization, the internal transcribed spacer (ITS) region was amplified by using universal primers ITS 1 and ITS 4. Based on morphological and molecular findings, two *Saprolegnia* spp. have been identified. i.e., *Saprolegnia parasitica* (Isolate KMG3, MW819780) and *S. salmonis* (Isolates KMG1, MW819740 and KMG2, MW819707). It is worth mentioning that the latter - *S. salmonis* - is the first report in Iran. The phylogenetic tree inferred by a Neighbor-Joining model revealed that three representative isolates with a well-supported value together with the known isolates of *S. parasitica* and *S. salmonis* formed a well-defined clade. An in vitro experiment was conducted to study the growth of *S. salmonis* (KMG2) and *S. parasitica* (KMG3) at various incubation temperatures. Both species showed similar hyphal growth rates at 10 and 18°C after 3 days of incubation ($P > 0.05$).

Keywords Rainbow trout . *Saprolegnia parasitica* . *Saprolegnia salmonis* . Internal transcribed spacer (ITS) . Neighbour-joining model . Hyphal growth rate

Introduction

Members of the genus *Saprolegnia* are one of the main pathogens which are responsible for the 'typical water mold infection' or saprolegniasis of freshwater fish and their eggs (Ke et al. 2009; Noga 2010). Saprolegniasis causes annually great losses in the production of different freshwater fish hatcheries in the world (Mousavi et al. 2007; Shahbazian et al. 2010; Ghiasi et al. 2010; Eissa et al. 2013). High mortality rates of rainbow trout eggs in hatcheries in Iran have become widespread in recent years and shreds of evidence have shown that fungal infection may cause up to 50% of annual egg loss (Shahbazian et al. 2010). Besides

Sina Mirmazloomi . Maryam Ghiasi (✉)
Caspian Sea Ecology Research Center, Iranian Fisheries Research Organization, Agriculture Research Education and Extension Organization, P.O. Box: 961, Farah-Abad, Sari, Iran
e-mail: ghiasimaryam4@gmail.com

Seyedeh Ainaz Shirangi
Department of Biology, Faculty of Basic Science & Engineering, Gonbad Kavous University, Gonbad Kavous, Iran

Saltanat Najjar Lashgari
Cold-Water Fishes Research Center, Iranian Fisheries Science Research Organization, Agriculture Research Education and Extension Organization, Tonekabon, Iran

Ali Reza Khosravi
Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

adopting new strategies to manage the infection, there is an essential need to understand the phylogeny, taxonomy, and epidemiology of etiological agents (Magray et al. 2019; Elameen et al. 2021).

Saprolegniasis in fish eggs is featured by the presence of cotton-like tufts on them (Elameen et al. 2021). *Saprolegnia* spp. are capable of colonizing the surface of the dead eggs and suffocating the surrounding living eggs (Kitancharoen and Hatai 1996). Fish eggs are also thought to be killed by a direct adhesion mechanism followed by penetrating the chorionic membrane regulating the osmosis of the embryo (Eissa et al. 2013; Liu et al. 2014). The most important species in the genus *Saprolegnia* is *S. parasitica* (Shin et al. 2017). The species was found to be the most destructive pathogen that caused primary infections in both salmon eggs and fish (Shin et al. 2017; Elameen et al. 2021). However, other species such as *S. diclina*, *S. ferax*, *S. australis*, *S. monica*, and *S. salmonis* are also reported from different fishes and geographical areas (Hussein and Hatai 1999; Die´guez-Uribeondo et al. 2007; Ghiasi et al. 2010; Sandoval-Sierra et al. 2014; Liu et al. 2017).

Traditionally, fish pathogenic *Saprolegnia* spp. were identified based on some morphological features including the structure and shape of sporangia, mode of zoospore release, and the characteristics of their sexual structures (Kitancharoen et al. 1997). Due to some ambiguous results of these types of taxonomy, molecular methods, especially polymerase chain reaction (PCR) and partial sequencing of intsernal transcribed spacer ITS gene could be invaluable tools (Sarowar et al. 2014) and were effectively adopted by many authors to designate the species (Die´guez-Uribeondo et al. 2007; Sandoval-Sierra et al. 2014, Paul et al. 2015; Liu et al. 2017; Tedesco et al. 2021).

Identification of *Saprolegnia* spp. in different regions of Iran has been carried out mostly based on morphological characteristics (Mousavi et al. 2007; Shahbazian et al. 2010; Khosravi et al. 2012). However, *S. parasitica* and *S. diclina* have been identified for the first time in Persian sturgeon (*Acipenser persicus*), Rainbow trout, and Caspian trout (*Salmo trutta caspius*) eggs by Random amplification of polymorphic DNA-Polymerase chain reaction (RAPD-PCR) (Ghiasi et al. 2010; 2014). Then, Masigol et al. (2020) characterized four *Saprolegnia* spp. including *S. parasitica*, *S. anisospora*, *S. ferax*, and *S. diclina* from Anzali lagoon (in the north of Iran) by morphological and molecular methods.

In the present study, we aimed to isolate and identify aquatic fungi from infected rainbow trout eggs by using some physiological and morphological characteristics as well as molecular phylogenetic analysis of ITS of the ribosomal DNA.

Materials and methods

Rainbow trout eggs with fungal infection were collected from a trout hatchery in Tonekabon (Mazandaran, Iran) from January to February 2020 to study the fungal species. The eggs were maintained in conditions similar to the hatchery system; placed in a tray with flow-through water at the temperature of 10 °C, but no prophylactic treatment was provided. The infected eggs were collected in screw cap bottles containing 50 ml sterile distilled water (SDW) which was supplemented with chloramphenicol (10 mg/L) to prevent bacterial contamination. Then, they were washed several times with SDW and inoculated in sterile plates of yeast extract glucose chloramphenicol (YGC) agar. Culture plates were incubated at 18°C for 5-6 days with regular daily inspection for any expected fungal growths. Harvested fungal colonies were purified and the slide culture technique was adopted on retrieved colonies for initial morphological identification (Hussein et al. 2001; Eissa et al. 2013).

For morphological identification, isolates were grown in Glucose Yeast (GY) broth (D(+)-glucose monohydrate 10 g, yeast 2 g, potassium dihydrogen orthophosphate (KH_2PO_4) 2.04 g, disodium hydrogen phosphate anhydrous (Na_2HPO_4) 0.596 g in 1 L of distilled water and autoclaving) (Ghiasi et al. 2010), for 2 d at 18 °C and allowed to sporulate. The isolates were assigned to the genus level according to the discharge of zoospores (Fig. 1d) (Sandoval-Sierra et al. 2014). Moreover, 5mm agar plugs with fungal hyphae were excised from the advancing edge of 6 d old colony and incubated with autoclaved hempseed and 5ml SDW in a glass petri dish at 18 and 5 °C for 4 w. The cultures were examined for producing of asexual (zoosporangia, gemmae) and sexual (oogonia and antheridia) organs once a week (Hussein and Hatai 1999). All chemicals used, unless otherwise stated, were obtained from Merck Company (Darmstadt, Germany).

The inoculum was prepared by cutting at the advancing edges of a young colony growing on SDA agar with a 6 mm diameter border. The different *Saprolegnia* isolates were inoculated on a fresh SDA agar dish.

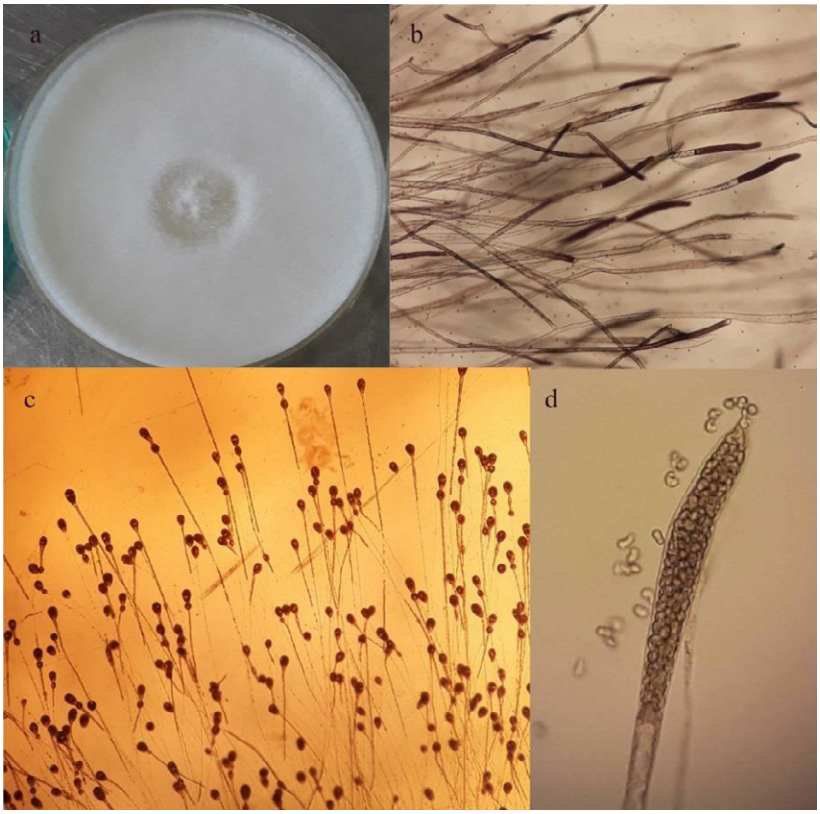

Fig. 1 Morphological characteristics of *Saprolegnia* spp. (a) Full growth of the *S. parasitica* (KMG3) on SDA. (b) Presence of abundant zoosporangia and (c) gemmae in chains in hemp seed culture of *S. salmonis* (KMG2). (d) Saprolegnoid type of zoospore discharge in *S. parasitica* (KMG3).

The colony hyphal radial growth rates were determined after 3 d of incubation at 10, 18, and 30°C (Ghiasi et al. 2010).

For molecular identification, a small piece of mycelium was removed from the purified YGC agar plates of *Saprolegnia* spp. and the genomic DNA was extracted by using a DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instruction (Caruana et al. 2012). DNA concentration was measured at 260 nm and stored at −20 °C. The 750 base pair segment of the ITS region of ribosomal DNA was amplified by using 5′-TCCGTAGGTGAACCTGCGG-3′ (ITS1) and 5′-TCCTCCGCTTATTGATATGC-3′ (ITS4) primers (White et al. 1990) as follows: 3 min at 95°C, followed by 35 cycles of 45 s at 93°C, 60 s at 58°C and 90 s at 72°C. PCR product was then purified by using GF-1 PCR Clean-up, and electrophoresis was done on 2% agarose gel. The nucleotide sequences of purified PCR products were analyzed with an automated Sanger dideoxy fluorescent nucleotide assay using an ABI 3730XL DNA Analyzer (Erfanmanesh et al. 2019). For confirmation, resulted sequences including the region spanning the 5.8S rDNA and the two ITS1 and ITS2 were compared with other ITS regions in the gene bank database by using the Basic Local Alignment Search Tool (nucleotide BLAST).

The phylogenetic tree was inferred by a Neighbor-Joining model. The bootstrap consensus tree which was inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed. The percentage in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Evolutionary analysis was conducted in Molecular Evolutionary Genetics Analysis (MEGA, version 7.0,) (Kumar et al. 2016). *Aphanomyces invadans* was chosen as the outgroup.

The differences between hyphal radial growth rates of *Saprolegnia* spp., at different temperatures, were compared by using the independent t-test. Differences between treated and controls were evaluated at a p-value < 0.05. The analysis was performed by using the SPSS software package v. 15 for Windows (Microsoft Corporation, USA).

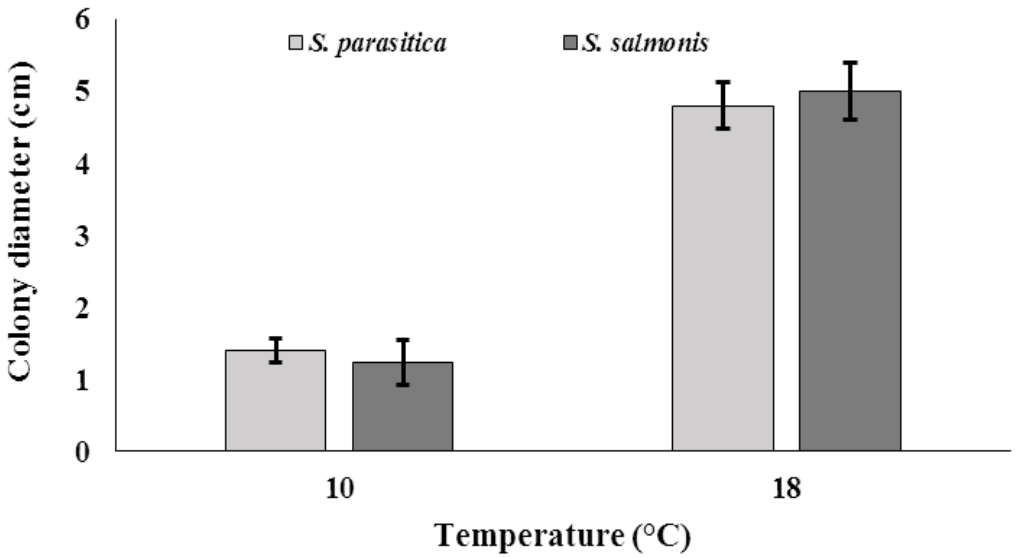

Fig. 2 Schematic diagram based on colony diameter (cm) *Saprolegnia parasitica* (KMG3), and *S. salmonis* (KMG2) after 3 days of incubation at 10 and 18°C

Results

Visual inspection of the cultured SDA plates has exposed the prominent growth of mold colonies in both species. The colonies were represented as cysts of whitish cottony long hairs. The morphological features of the isolates were observed by optical microscopy. Both species were characterized by extensive and dense mycelium. They showed the appearance feature of branched coenocytic hyphae along with masses of immature and mature sporangia (zoospore discharge was saprolegnoid) as well as the abundance of gemmae, often in chains, which are indicative of asexual reproduction. The isolates did not show any sexual reproduction in this study (Fig.1).

The hyphal radial growth rates of two isolates (KMG3, and KMG2) were compared at different temperatures. At 10 and 18 °C, all of the isolates grew similarly, and no significant differences were found between them ($P > 0.05$) (Fig. 2). However, none of the isolates were able to grow at 30 °C (Fig. 2).

The aligned rDNA ITS matrix comprised 654 characters. The nucleotide sequence of three representative isolates was submitted to Gene Bank. BLAST alignment demonstrated that two isolated specimens, MW819740 and MW819707, were 99.59 and 100% similar to the type strain *S. salmonis* (AY647193), respectively, and the other one (MW819780) was 99.32% similar to the type strain *S. parasitica* (AY455776) (Table 1). The phylogenetic analysis using the neighbor-joining method showed that the amplified specimens of *S. parasitica* and *S. salmonis* were grouped with the known specimens of these species from other studies. They belonged to a well-supported monophyletic group showing similar evolutionary relationship with other specimens of these species (Fig 3). Also, the sequence of *S. parasitica* in this study was evolutionarily separated from *S. parasitica* specimens in Anzali lagoon (Iran) and Scotland (Fig. 3). Furthermore, the obtained specimens from this study were isolated from other groups belonging to *S. hypogyna*, *S. ferax*, and other *Saprelegnia* spp. in other locations.

Discussion

Tonekabon is an important center for the production of rainbow trout eyed egg, fry, and fingerling in the north of Iran. For this reason, we chose this center as the source of *Saprolegnia* strains causing saprolegniasis in rainbow trout in the north of Iran. In this study, the isolates KMG 1-3 from infected eggs of rainbow trout showed no sexual reproduction, and the asexual characters were featured with an abundance of gemmae in chains or catenulate shapes. Sterility is a common feature of many isolates obtained from fish lesions and infected eggs (Ke et al. 2009; Vega-Ramírez et al. 2013; Shaheen et al. 2015; Tandel et al. 2021). How-

Table 1 The list of the recovered *Saprolegnia* isolates with their Gene bank accession number

Isolates	Scientific name	Host	Accession number
KMG1	*S. salmonis*	Rainbow trout eggs	MW819707
KMG2	*S. salmonis*	Rainbow trout eggs	MW819740
KMG3	*S. parasitica*	Rainbow trout eggs	MW819780

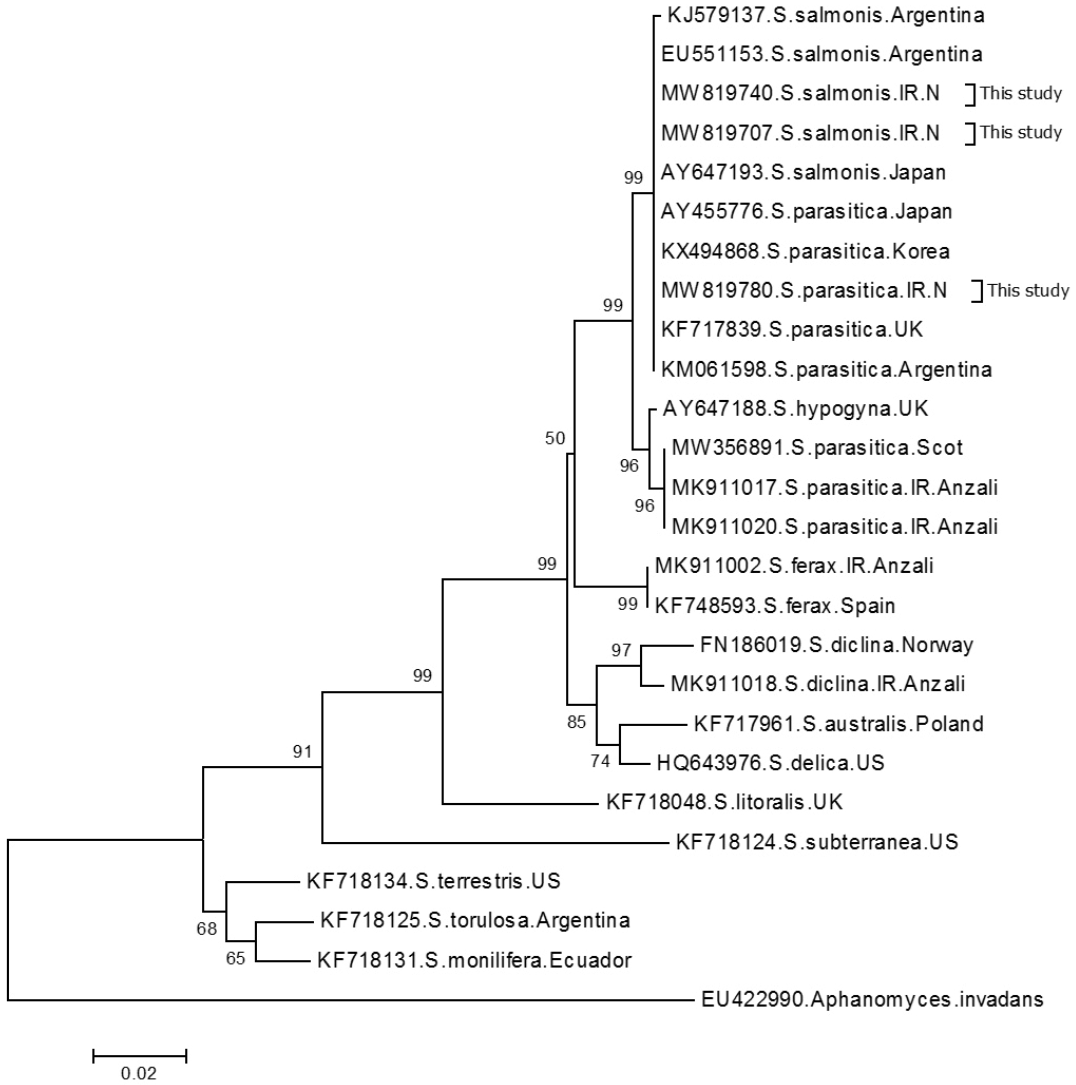

Fig. 3 Phylogenetic tree of *Saprolegnia* isolated from infected rainbow trout eggs in this study based on the sequences of internal transcribed spacer (ITS) rDNA. This phylogenetic tree was constructed with the Neighbor-Joining method using MEGA version 7 and boot strapped 1000 times to assess the reliability of data. Numbers next to the branches indicate bootstrap values and scale bar represent evolutionary distance. Sequences were compared with the *Aphanomyces invadans* as outgroup of phylogenetic analysis.

ever, based on sequence analysis of the ITS region of the rDNA, the isolates KMG1 and 2 were identified as *S. salmonis* and the isolate KMG3 was detected as *S. parasitica*. Contrary to our findings (unattainable sexual reproduction after 28 days of incubation for all isolates), sexual reproduction has been reported by some researchers in both *S. salmonis* (Hussein and Hatai 1999; Liu et al. 2017) and *S. parasitica* (Stueland et al. 2005; Eissa et al. 2013). Environmental variables could influence the growth, reproduction, and intensity of water mold infections (Hussein et al. 2001). Therefore, using molecular tools such as PCR together with partial sequencing of the ITS gene was one of the most important choices for species designation in the genus *Saprolegnia* (Eissa et al. 2013).

As shown by the phylogram (Fig. 3), the strains KMG1-3 formed a well-defined clade, which was as same as *Saprolegnia* strains obtained from fish lesions in the Republic of Korea (KX494868) (Shin et al. 2017) and Japan (EU551153, AY647193, AY455776) (Ke et al. 2009) congruent with previous studies (Die´guez-Uribeondo et al. 2007; Elameen et al. 2021). However, we also found that this clade comprises isolates from water (KF717839) (Sandoval-Sierra et al. 2014) in agreement with the previous study (Die´-guez-Uribeondo et al. 2007). The *S. hypogyna* strain (AY647188) formed a very well-supported sister clade (96%) and corresponded to Clade Ia in Die´guez-Uribeondo et al. (2007). Interestingly, *S. parasitica* recovered from rotten leaves in Anzali lagoon, Iran (MK911017 and MK911020) (Masigol et al. 2020) fell into another branch apart from ours. Clustering of the animal pathogenic oomycete species in a group aside from plant pathogenic/saprotroph was also recorded by other authors (Die´guez-Uribeondo et al. 2007; Sarowar et al. 2019). Plant pathogenic/saprotroph oomycetes carry genes encoding a large array of enzymes to degrade the plant cell wall (mainly composed of pectin), including polygalacturonase, pectin, and pectate lyases, whereas, these appear to be absent in the animal pathogenic oomycetes (Jiang et al. 2013). Moreover, unlike the isolate KMG3, the isolates MK911017 and MK911020 did not produce gemmae in the hemp seed culture (Masigol et al. 2020), a characteristic that might be associated with pathogenicity, and could be used as a criterion for classification of the animal pathogenic *Saprolegnia* spp. (Yuasa and Hatai 1995).

Based on several authors, *S. australis, S. delica*, and *S. diclina* were found to be the major causes of fungal infection in the eggs and embryonic stages of salmonid fishes (Hussein et al. 2001; Sandoval-Sierra et al. 2014; Magray et al. 2021). However, our results are more in line with previous studies (Mousavi et al. 2007, Shahbazian et al. 2010, Khosravi et al. 2012; Ghiasi et al. 2014), which found that *S. parasitica* was the most frequently identified species associated with egg infections in Iran. Such discrepancies could be due to local environmental factors that may influence the prevalence of a particular species (Hussein et al. 2001; Shahbazian et al. 2010).

The occurrence of saprolegniasis in hatcheries may be related to water quality and temperature (Hussein et al. 2001). Our results showed that *S. salmonis* (KMG2) and *S. parasitica* (KMG3) could grow at 10 and 18 °C with a similar radial growth rates ($P > 0.05$), but no growth was observed at 30 °C (Fig 2). The observed similar temperature tolerance in two isolates may be due to the high genetic similarity as well as the source of isolation (rainbow trout hatchery). This finding was confirmed by Kitancharoen et al. (1996), who demonstrated that the temperature tolerance of the fungi is related to the source from which they were isolated.

Conclusion

Overall, the current study contributes to the first species-level morphological and molecular identification of *S. salmonis* from rainbow trout eggs in Iran. The isolates together with *S. parasitica* isolate belonged to a well-defined phylogenetic group and shared similar morphological and physiological characteristics. Further studies are needed to evaluate the degree of pathogenicity of such species.

Competing Interest The authors declare that they have no conflict of interest.

Authors Contribution Experimental design: Sina Mirmazloomi, Maryam Ghiasi, and Ali Reza Khosravi. Performing the experiments: Sina Mirmazloomi and Maryam Ghiasi. Data curation: Sina Mirmazloomi, Maryam Ghiasi, and Seyedeh Ainaz Shirangi. Writing the paper: Sina Mirmazloomi, Maryam Ghiasi, and Seyedeh Ainaz Shirangi. Obtained permission to conduct fieldwork and specimens' collection: Sina Mirmazloomi, Maryam Ghiasi, and Saltanat Najjar Lashgari.

Acknowledgements This work was performed at Caspian Sea Ecology Research Center, Sari, Iran and Faculty of Veterinary Medicine, University of Tehran, Iran. Also, we would like to thank Dr. Iraj Ashrafi Tamai for his technical assistance in molecular works.

References

Caruana S, Yoon GH, Freeman MA, Mackie JA, Shinn AP (2012) The efficacy of selected plant extracts and bioflavonoids in controlling infections of *Saprolegnia australis* (Saprolegniales; Oomycetes). Aquaculture 358:146-154. https://doi.org/10.1016/j.aquaculture.2012.06.035

Diéguez-Uribeondo J, Fregeneda-Grandes JM, Cerenius L, Pe´rez-Iniesta E, Aller-Gancedo JM, Teresa Telleri´a M, Soderhall K, Martın MP (2007) Re-evaluation of the enigmatic species complex *Saprolegnia diclina–Saprolegnia parasitica* based on morphological, physiological and molecular data. Fungal Genet Biol 44(7):585-601. https://doi.org/10.1016/j.fgb.2007.02.010

Eissa AE, Abdelsalam M, Tharwat N, Zaki M (2013) Detection of *Saprolegnia parasitica* in eggs of angelfish *Pterophyllum scalare* (Cuvier–Valenciennes) with a history of decreased hatchability. Int J Vet Sci Med 1(1):7-14. https://doi.org/10.1016/j.ijvsm.2013.04.001

Elameen A, Stueland S, Kristensen R, Fristad RF, Vrålstad T, Skaar I (2021) Genetic analyses of *Saprolegnia* strains isolated from salmonid fish of different geographic origin document the connection between pathogenicity and molecular diversity. JoF 7(9):713. https://doi.org/10.3390/jof7090713

Erfanmanesh A, Beikzadeh B, Mohseni FA, Nikaein D, Mohajerfar T (2019) Ulcerative dermatitis in barramundi due to coinfection with Streptococcus iniae and Shewanella algae. Dis Aquat Org 134(2):89-97. https://doi.org/10.3354/dao03363

Ghiasi M, Khosravi AR, Soltani M, Binaii M, Shokri H, Tootian Z, Rostamibashman M, EbrahimzadehMosavi H (2010) Characterization of *Saprolegnia* isolates from Persian sturgeon (*Acipencer persicus*) eggs based on physiological and molecular data. J mycol méd 20(1):1-7. https://doi.org/10.1016/j.mycmed.2009.11.005

Ghiasi M, Khosravi AR, Soltani M, Sharifpour I, Binaii M, EbrahimzadehMosavi H, Bahonar A (2014) Evaluation of physiological aspects and molecular identification of *Saprolegnia* isolates from rainbow trout (*Oncorhynchus mykiss*) and Caspian trout (*Salmo trutta caspius*) eggs based on RAPD–PCR. Iran Sci Fish J 22:82-92

Hussein MM, Hatai K (1999) *Saprolegnia salmonis* sp. nov. isolated from sockeye salmon, *Onchrhynchus nerka*. Mycoscience 40(5):387-391. https://doi:10.1007/bf02464392

Hussein MM, Hatai K, Nomura T (2001) Saprolegniosis in salmonids and their eggs in Japan. J wildl Dis 37(1):204-207. https://doi.org/10.7589/0090-3558-37.1.204

Jiang RH, De Bruijn I, Haas BJ, Belmonte R, Löbach L, Christie J, Van den Ackerveken G, Bottin A, Bulone V, Díaz-Moreno SM, Dumas B, Fan L, Gaulin F, Govers F, Grenville-Briggs LJ, Horner NR, Levin JZ, Mammella M, Meijer HJG, Morris P, Nusbaum C, Oome S, Phillips AJ, Van Rooyen D, Rzeszutek E, Saraiva M, Secombes CJ, Seidl MF, Snel B, Stassen JHM, Sykes S, Tripathy S, Van den Berg H, Vega-Arreguin JC, Wawra S, Young SK, Zeng Q, Dieguez-Uribeondo J, Russ C, Tyler BM, Van West P (2013) Distinctive expansion of potential virulence genes in the genome of the oomycete fish pathogen *Saprolegnia parasitica*. PLoS genetics 9(6):e1003272. https://doi.org/10.1371/journal.pgen.1003272

Ke XL, Wang JG, Gu ZM, Li M, Gong XN (2009) Morphological and molecular phylogenetic analysis of two *Saprolegnia* sp (Oomycetes) isolated from silver crucian carp and zebra fish. Mycol Res 113(5):637-644. https://doi.org/10.1016/j.mycres.2009.01.008

Khosravi AR, Shokri H, Sharifrohani M, Mousavi HE, Moosavi Z (2012) Evaluation of the antifungal activity of *Zataria multiflora*, *Geranium herbarium*, and *Eucalyptus camaldolensis* essential oils on *Saprolegnia parasitica*–infected rainbow trout (*Oncorhynchus mykiss*) eggs. Foodborne Pathog Dis 9(7):674-679. https://doi.org/10.1089/fpd.2011.1086

Kitancharoen N, Hatai K (1996) Experimental infection of *Saprolegnia* spp. in rainbow trout eggs. Fish Pathol 31(1):49-50. https://doi.org/10.3147/jsfp.31.49

Kitancharoen N, Hatai K, Yamamoto A (1997) Aquatic fungi developing on eggs of salmonids. J Aquat Anim Health 9(4):314-316. https://doi.org/10.1577/1548-8667(1997)009<0314:AFDOEO>2.3.CO;2

Kitancharoen N, Yuasa K, Hatai K (1996) Effects of pH and temperature on growth of *Saprolegnia diclina* and *S. parasitica* isolated from various sources. Mycoscience 37(4):385-390. https://doi.org/10.1007/BF02460994

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870-1874. https://doi.org/10.1093/molbev/msw054

Liu Y, De Bruijn I, Jack ALH, Drynan K, Van den Berg AH, Thoen E, Sandoval-Sierra V, Skaar I, Van West P, Diéguez-Uribeondo J, Van der Voort M, Mendes R, Mazzola M, Raaijmakers JM (2014) Deciphering microbial landscapes of fish eggs to mitigate emerging diseases. ISME J 8(10):2002-2014. https://doi.org/10.1038/ismej.2014.44

Liu S, Song P, Ou R, Fang W, Lin M, Ruan J, Yang X, Hu K (2017) Sequence analysis and typing of *Saprolegnia* strains isolated from freshwater fish from Southern Chinese regions. Aquac Fish 2(5):227-233. https://doi.org/10.1016/j.aaf.2017.09.002

Magray AR, Lone SA, Ganai BA, Ahmad F, Dar GJ, Dar JS, Rehman S (2019) Comprehensive, classical and molecular characterization methods of *Saprolegnia* (Oomycota; Stramnipila), an important fungal pathogen of fish. Fungal Biol Rev 33(3-4):166-179. https://doi.org/10.1016/j.fbr.2018.12.001

Magray AR, Lone SA, Ganai BA, Ahmad F, Hafeez S (2021) The first detection and in vivo pathogenicity characterization of *Saprolegnia delica* from Kashmir Himalayas. Aquaculture 542:736876. https://doi.org/10.1016/j.aquaculture.2021.736876

Masigol H, Khodaparast SA, Mostowfizadeh-Ghalamfarsa R, Rojas-Jimenez K, Woodhouse JN, Neubauer D, Grossart HP (2020) Taxonomical and functional diversity of Saprolegniales in Anzali lagoon, Iran. Aquat Ecol 54(1):323-336. https://doi.org/10.1007/s10452-019-09745-w

Mousavi H, Hoosseinifard SM, Khosravi AR, Soltani M, Yosefian M (2007) Isolation and identification of parasite and saprophyte fungi from fungal affected eggs of the rainbow trout (*Oncorhynchus mykiss*) in Mazandaran province. J Vet Res 62:163-168

Noga EJ (2010) Fish disease: diagnosis and treatment. John Wiley and Sons, Iowa, USA

Paul Y, Leung WL, Hintz WE (2015) Species composition of the genus *Saprolegnia* in fin fish aquaculture environments, as determined by nucleotide sequence analysis of the nuclear rDNA ITS regions. Fungal biol 119(1):27-43. https://doi.org/10.1016/j.funbio.2014.10.006

Sandoval-Sierra JV, Latif-Eugenin F, Martín MP, Zaror L, Diéguez-Uribeondo J (2014) *Saprolegnia* species affecting the salmonid aquaculture in Chile and their associations with fish developmental stage. Aquaculture 434:462-469. https://doi.org/10.1016/j.aquaculture.2014.09.005

Sarowar MN, Saraiva M, Jessop CN, Lilje O, Gleason FH, Pieter VW (2014) Infection strategies of pathogenic oomycetes in fish. In: Jones EG, Hyde KD, Pang KL (ed) Freshwater fungi and fungal-like organisms, Walter de Gruyter GmbH and Co KG, p 217-243

Sarowar MN, Hossain MJ, Nasrin T, Naznin T, Hossain Z, Rahman MM (2019) Molecular identification of oomycete species affecting aquaculture in Bangladesh. Aquac Fish 4(3):105-113. https://doi.org/10.1016/j.aaf.2018.12.003

Shahbazian N, Ebrahimzadeh MH, Soltani M, Khosravi AR, Mirzargar S, Sharifpour I (2010) Fungal contamination in rainbow trout eggs in Kermanshah province propagations with emphasis on saprolegniaceae. Iran J Fish Sci 9:151-160

Shaheen AA, El Asely AM, Latif AAE, Moustafa MMA, Elsaied HE (2015). Saprolegniosis in goldfish (*Carassius auratus*) associated with novel strain; molecular characterization and electron scanning. EJ Aquac (ISSN: 2090-7877)

Shin S, Kulatunga DCM, Dananjaya SHS, Nikapitiya C, Lee J, De Zoysa M (2017) *Saprolegnia parasitica* isolated from rainbow

trout in Korea: characterization, anti-*Saprolegnia* activity and host pathogen interaction in zebrafish disease model. Mycobiology 45(4):297-311. https://doi.org/10.5941/MYCO.2017.45.4.297

Stueland S, Hatai K, Skaar I (2005) Morphological and physiological characteristics of *Saprolegnia* spp. strains pathogenic to Atlantic salmon, *Salmo salar* L. J Fish Dis 28(8):445-453. https://doi.org/10.1111/j.1365-2761.2005.00635.x

Tandel RS, Dash P, Bhat RAH, Sharma P, Kalingapuram K, Dubey M, Sarma D (2021) Morphological and molecular characterization of *Saprolegnia* spp. from Himalayan snow trout, *Schizothorax richardsonii*: A case study report. Aquaculture 531:735824. https://doi.org/10.1016/j.aquaculture.2020.735824

Tedesco P, Saraiva M, Sandoval-Sierra JV, Fioravanti ML, Morandi B, Diéguez-Uribeondo J, Van West P, Galuppi R (2021) Evaluation of potential transfer of the pathogen *Saprolegnia parasitica* between farmed salmonids and wild fish. Pathogens 10(8):926. https://doi.org/10.3390/pathogens10080926

Vega-Ramírez MT, Moreno-Lafont MC, Valenzuela R, Cervantes-Olivares R, Aller-Gancedo JM, Fregeneda-Grandes JM, Damas-Aguilar JL, García-Flores V, López-Santiago R (2013) New records of saprolegniaceae isolated from rainbow trout, from their eggs, and water in a fish farm from the State of México. Rev Mex Biodivers 84(2):637-649. https://doi.org/10.7550/rmb.28627

White TJ, Bruns T, Lee SJWT, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1):315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Yuasa K, Hatai K (1995) Relationship between pathogenicity of *Saprolegnia* spp. isolates to rainbow trout and their biological characteristics. Fish Pathol 30(2):101-106. https://doi.org/10.3147/jsfp.30.101

Publisher's Note