Some zoosporic fungi of the district Bajaur, with nine new records from Pakistan

MUHAMMAD ABDUL HAQ
Department of Botany
University of Karachi, Karachi, Pakistan
and: Department of Botany,
Government Post Graduate College
Khar, Bajaur, Pakistan
E-mail: muhammad.abdulhaq@gmail.com

SALEEM SHAHZAD
Department of Agriculture and Agribusiness Management
University of Karachi
Karachi, Pakistan

SYEDA QAMARUNNISA
The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE)
University of Karachi
Karachi, Pakistan

ABDUL MUBEEN LODHI
Department of Plant Protection
Sindh Agriculture University
Tandojam, Pakistan

Accepted 14. April 2021.

ABDUL HAQ, M., SHAHZAD, S., QAMARUNNIS, S., LODHI, A. M., 2021: Some zoosporic fungi of the district Bajaur, with nine new records from Pakistan. – Österr. Z. Pilzk. 29: 99–115.

Key words: *Oomycota, Chromista, Saprolegniaceae*, *Olpidiopsidaceae*, *Blastocladiaceae,* water molds, ribosomal DNA. – New records. – Pakistan.

Zusammenfassung: Diese Arbeit ist Teil einer biodiversitäts- und biosystematischen Studie zoosporenbildender Pilze in Bajaur, dem neu entstandenen Distrikt der Provinz Khyber Pakhtunkhwa. Insgesamt 19 Isolate wurden morphologisch charakterisiert und bis auf eines konnten alle bis auf Artniveau identifiziert werden. Zehn Arten gehörten zu den *Saprolegniaceae* und je eine Art zu den *Olpidiopsidaceae* und *Blastocladiaceae*. Von diesen scheinen neun Taxa, nämlich *Brevilegnia minutandra, Leptolegnia caudata, Aphanomyces scaber, Saprolegnia australis, Saprolegnia* sp.*, Achlya orion, Calyptralegnia ripariensis, Olpidiopsis saprolegniae* var. *levis* und *Allomyces arbusculus,* neue Nachweise für Pakistan zu sein, *Dictyuchus monosporus, Saprolegnia ferax* und *Achlya bisexualis* werden zum ersten Mal aus diesem Distrikt gemeldet. *Saprolegnia australis* wird erstmals von einem wildlebenden Süßwasserfisch, nämlich *Paraschistura alepidota*, gemeldet. Die Pilze werden detailliert beschrieben und illustriert. Die rDNA ITS-Region von 15 Isolaten wurde amplifiziert und sequenziert. Die phylogenetische Beziehung zwischen den Gattungen der Familie *Saprolegniaceae* wird diskutiert.

Abstract: This work is part of a biodiversity and biosystematic study of zoosporic fungi of Bajaur, the newly merged district of Khyber Pakhtunkhwa Province. A total of 19 isolates were characterized morphologically and except one all were identified up to species level. Ten species belonged to *Saprolegniaceae* and one species to each of the *Olpidiopsidaceae* and *Blastocladiaceae*. Of these, nine taxa, *viz*. *Brevilegnia minutandra, Leptolegnia caudata, Aphanomyces scaber, Saprolegnia australis, Saprolegnia* sp., *Achlya orion*, *Calyptralegnia ripariensis Olpidiopsis saprolegniae* var. *levis* and *Allomyces arbusculus,* appeared to be new records for Pakistan, *Dictyuchus monosporus*, *Saprolegnia ferax* and *Achlya bisexualis* are reported for the first time for this district. *Saprolegnia australis* is reported for the first time from a wild freshwater fish, namely *Paraschistura alepidota*. The fungi are described in detail and illustrated. The rDNA ITS region of 15 isolates was amplified and sequenced. The phylogenetic relationship among the genera of the family *Saprolegniaceae* is discussed.

Fungi and fungus-like organisms are among the most diverse life forms on Earth. HAWKSWORTH (1991), estimated that there are 1.5 million fungi. However, the recent estimates based on high-throughput sequencing methods suggest 5.1 million fungal species (BLACKWELL 2011). Till the last quarter of the 20th century, all these organisms were kept in a polyphyletic assemblage under the kingdom *Fungi*. Except for the slime moulds, AINSWORTH (1973) kept the rest of zoosporic fungi in his subdivision '*Mastigomycotina*", with three classes: *Chytridiomycetes*, *Hyphochytridiomycetes* and *Oomycetes*. The last two of them are now kept along with diatoms and brown algae in kingdom *Straminipila* (ALEXOPOULOS & al. 1996, DICK 2001).

Saprolegniaceae is a family with 20 genera (DICK 2001) that are characterized by eucarpic coenocytic mycelial thalli, heterokont biflagellate zoospores and oogamous type of sexual reproduction. These organisms are usually found as saprobes in terrestrial and aquatic environments. According to oomycetes' taxonomists, the family is a polyphyletic assemblage and various amendments have been proposed to make it monophyletic (DICK & al. 1984, 1999; BEAKES & al. 2014). The family *Olpidiopsidaceae*, which DICK (2001) treated as *insertae sedis,* includes holocarpic endophytic obligate parasites. *Blastocladiaceae* (order *Blastocladiales*) was initially classified within phylum *Chytridiomycota*. However, based on molecular and zoospore ultrastructural characters, JAMES & al. (2006) demonstrated that it was not monophyletic with Phylum *Chytridiomycota* and created a new phylum *Blastocladiomycota*.

Zoosporic fungi are ubiquitous and most of them can be isolated from freshwater and soil with relative ease. They occur as saprotrophs on a wide variety of substrata, playing a key role in the ecosystem as decomposers of organic materials (MÜELLER & al. 2004). Several genera consist of important pathogens of fish (PAXTON & WILLOUGHBY 2000, CZECZUGA & al. 2002, CHAUHAN & al. 2012, MASTAN & al. 2012), midge eggs (MARTIN 1981), crustaceans (VENNERSTROM & al. 1998), mosquito larvae (SEYMOUR 1984, BISHT & al. 1996, SCHOLTE & al. 2004) and plants (WICKER & al. 2001, PETERS & GRAU 2002). Saprolegniasis of two carps caused by *Saprolegnia* and *Achlya* species has been reported from Pakistan (IQBAL & ASGHAR 2012). Cases of Epizootic Ulcerative Syndrome (EUS) in freshwater fish caused by *Aphanomyces invadens* and *Aphanomyces* sp. have also been reported from Pakistan (LILLEY & al. 1998, WADAHAR & al. 2012). The present study was aimed to study the diversity, taxonomy and phylogeny of zoosporic fungi in Bajaur.

Materials and methods

Isolation and purification: Samples of soil, freshwater (both lotic and lentic), aquatic insects, fish and algae were collected from various sites of the target area. *Oomycetes* were isolated using baiting techniques and direct inoculation of infected specimens. The media used for isolation and purification were PDA, CMA and GYPS, amended with antibiotics penicillin, streptomycin and nystatin (ATLAS 2005). Voucher cultures are kept at Pest and Disease Research Lab (PDRL), Department of Agriculture and Agribusness Management, University of Karachi, Karach, Sindh, Pakistan.

Identification: Based on the morphological characters, each isolate was identified using the manuals and keys provided by JOHNSON (1956), SEYMOUR (1970), KHULBE (2001) and JOHNSON (2002). For the majority of isolates, identification was also assisted by performing BLASTn search at National Center for Biotechnology Information (nih.gov). The current names and synonymy of the taxa were determined from the online databases *viz*., MycoBank (https://www.mycobank.org/) Index Fungorum (http://www.indexfungorum.org/) and Species Fungorum (http://www.speciesfungorum.org/).

DNA isolation and PCR: DNA was extracted following the protocol described by MÖLLER & al. (1992) using mycelium from 3-8 days old cultures grown on pea broth. The ITS region of nuclear DNA

(ITS1-5.8S-ITS2) was amplified using universal eukaryotic primers, UNup18S42 (BAKKEREN & al. 2000) and UN-lo28S22 (LÉVESQUE & DECOCK 2004) as forward and reverse primers, respectively. The 20µl PCR reaction mixture contained final concentration of 1X reaction buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM each of forward and reverse primers, 1U of Taq polymerase and 50–100 ng of template DNA. Thermo cycler program was 94 ºC for 4 min followed by 35 cycles of 94 ºC for 30 sec, 60 ºC for 35 sec, and 72 ºC for 90 sec. A final extension was made at 72 ºC for 8 min. To evaluate the quality of amplified DNA, visual quantification was made by comparison to the DNA ladder. For electrophoresis, 3 µl of amplified DNA was loaded on 1 % agarose gel. The amplified DNA was sent to Bioneer (DAEJEON 306-220, South Korea) for sequencing. The primers used for PCR were also used for sequencing. The sequences were submitted to GenBank (Tab. 1).

Tab. 1. The zoosporic fungi isolated and investigated in the present study.

Isolate/ voucher#	Species	Source	Bait	Locality	Coordinates	Alt. (m)	GenBank. Accession #
274_AH	*Achlya bisexualis*	water	hemp seed	Nawagai	34° 39’ 56.8” N 71° 17’ 36.2” E	952	KP663630
324_AH	*A. bisexualis*	soil	hemp seed	Khar Stream	34° 45’ 07.6” N 71° 32’ 58.4” E	829	KP663631
331_AH	*A. bisexualis*	water	hemp seed	Bagh A-rang	34° 42’ 30.5” N 71° 39’ 37.2” E	1035	KP663632
332_AH	*A. bisexualis*	soil	hemp seed	Gul Bela	34° 51’ 50” N 71° 31’ 29.1” E	947	MG309758
335_AH	*A. orion*	water	hemp seed	Gul Bela	34° 51’50” N 71° 31’29.1” E	947	-
436_AH	*Allomyces arbusculus*	soil	hemp seed	Kotki	34.747331° N 71.360422° E	1097	-
263_AH	*Aphanomyces scaber*	water	hemp seed	Inam Khwaro	34° 46’ 06.6” N 71° 29’ 32.2” E	936	KP663634
264_AH	*A. scaber*	water	insect	-	34° 46’ 6.6” N 71° 29’ 32.2” E	936	KP663635
295_AH	*Brevilegnia minutandra*	damp soil	hemp seed	GPGC. Khar	34° 45’ 19.8” N 71° 32’ 37.8” E	834	KM061378
341_AH	*Calyptralegnia pariense*	water	hemp seed	Main stream Khar	34° 45’ 7.6” N 71° 32’ 58.4” E	829	-
278A_AH	*Dictyuchus monosporus*	damp soil	hemp seed	Bandari	34° 53’ 1.4” N 71° 23’ 40.6” E	1382	KJ470883
278B_AH	*D. monosporus*	water	seasame eed	Khar Stream	34° 45’ 7.6” N 71° 32’ 58.4” E	829	KP663638
267_AH	*Leptolegnia caudata*	*Chara* sp.	mosquito larvae	Dandokai	34° 52’ 9.3” N 71° 31’ 9.1” E	971	KP752054
266_AH	*L. caudata*	*Chara* sp.	mosquito larvae	Dandokai	34° 52’ 9.3” N 71° 31’ 9.1” E	971	KJ486651
431_AH	*Olpidiopsis saprolegniae* var. *levis*	*S. ferax*	hemp seed	Khar Stream	34° 45’ 7.6” N 71° 32’ 58.4” E	829	-
325_AH	*Saprolegnia australis*	water	hemp seed	Halki Char	34° 45’ 56.7” N 71° 18’ 53” E	1345	KP663629
303_AH	*S. australis*	*S. alepidota*	direct inc.	Khar Stream	34° 45’ 7.6” N 71° 32’ 58.4” E	829	KP663628
326_AH	*S. ferax*	algae	direct inc.	Khar Stream	34° 45’ 7.6” N 71° 32’ 58.4” E	829	KP663636
265_AH	Saprolegniaceae sp.	*Chara* sp.	direct inoc.	Khar Stream	34° 45’ 7.6” N 71° 32’ 58.4” E	829	KP663637

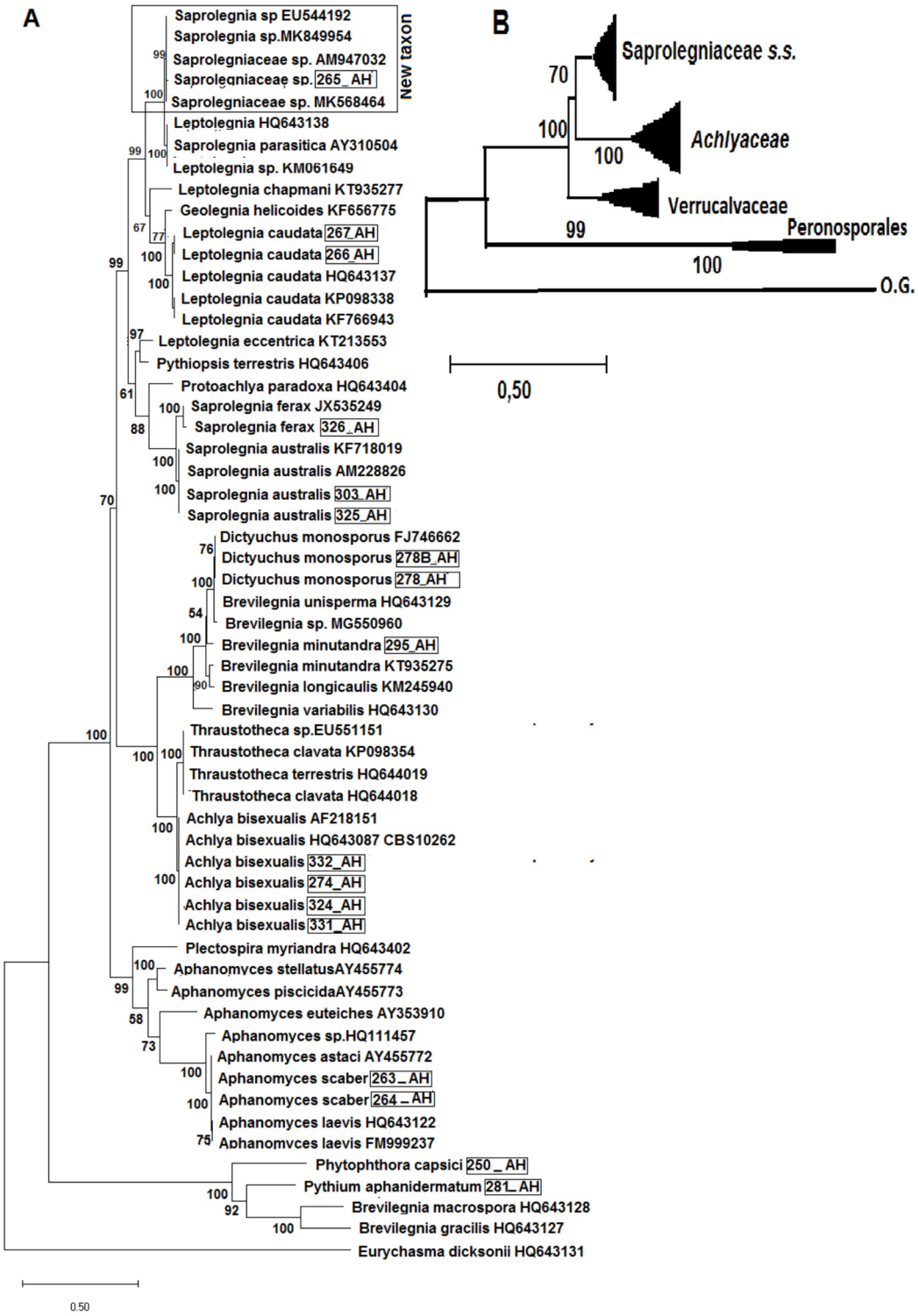

Fig. 1. Maximum Likelihood analysis of the *Saprolegniales* ITS sequences. Evolutionary history inferred by the Maximum Likelihood method and Hasegawa-Kishino-Yano model. Tree with the highest log likelihood (-11023.01) shown. Percentage of trees in which the associated taxa clustered together shown next to the branches. Initial tree(s) for the heuristic search obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.3400)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 15.63% sites). Tree drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 58 nucleotide sequences with a total of 928 positions in the final dataset. *A* Original tree. *B* Condensed tree.

Phylogenetic analysis: Phylogenetic analyses were conducted using dataset of ITS rDNA sequences of the 15 isolates from this study and closely related published sequences deposited in GenBank. The sequences were aligned with Clustalw program, available in MEGAX (KUMAR & al. 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Hasegawa-Kishino-Yano model (HASEGAWA & al. 1985). The robustness was improved in terms of bootstrap proportions (FELSENSTEIN 1985) with 500 replicates.

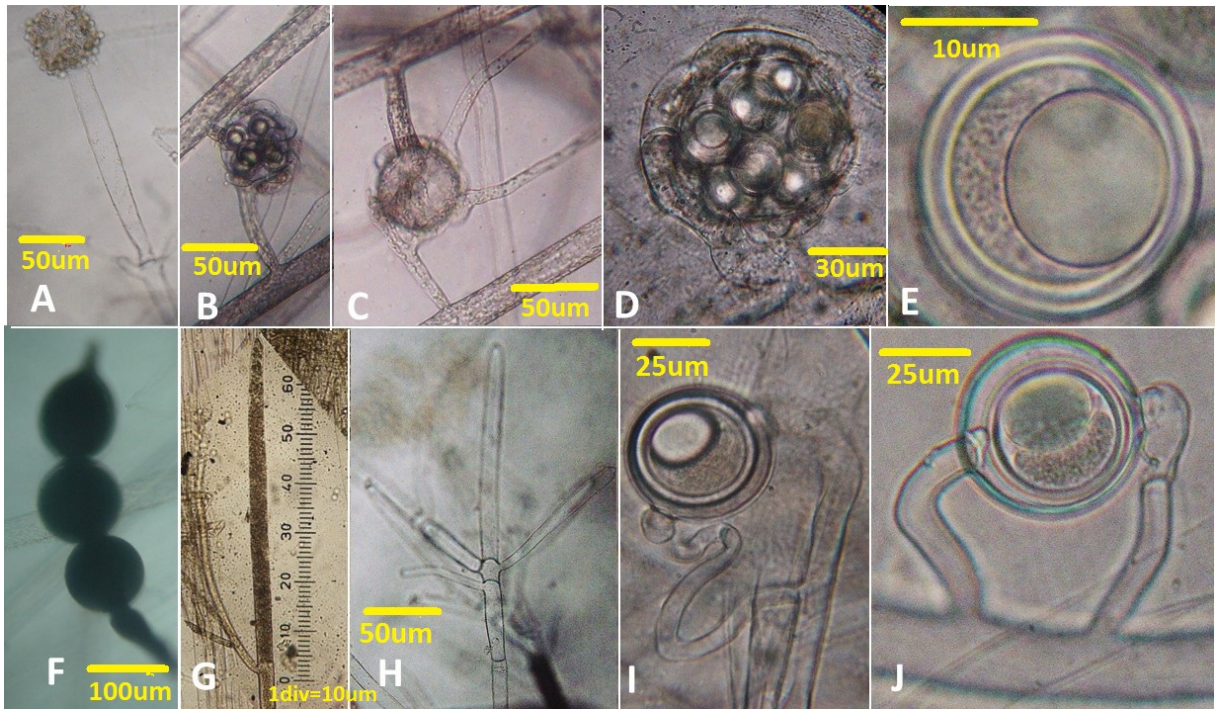

Fig. 2. *A–F Achlya bisexualis*, *A* Sporangium with zoospores discharge, *B–D* oogonia with diclinous antheridial attachment, *E* eccentric oospore, *F* gemmae in chain. *G-J Achlya orion*, *G, H* zoosporangia, *I, J* oogonia with monoclinous antheridia.

Results

The systematics of *Saprolegniaceae*

The ML phylogenetic analysis (Fig. 1), based on ITS region of the rDNA gene, not only shows the DNA molecular genetic affinities of the taxa from this study with the taxa submitted in the GenBank but also gives a picture of the phylogenetic relationships among the genera of the *Saprolegniaceae sensu lato*. The three distinct clades in the phylogenetic tree corroborate a rather recent proposal of BEAKES & al. (2014) for splitting *Saprolegniaceae* into three families: *Verrucalvaceae*, *Achlyaceae* and *Saprolegniaceae* s.str. The rationale for the family *Leptolegniaceae* (DICK & al. 1999, DICK 2001) could not be confirmed at least in this analysis since *Leptolegnia* nested within the genera having the saprolegnoid mode of zoospore discharge. The respective positions of *L. eccenterica* and *Geolegnia helicoides* in the tree indicate, that the genus *Leptolegnia* is paraphyletic. This paraphyly has been also reported by STECIOW & al. (2013) and Rocha & al. (2018). A redefinition of these taxa is needed. As *L. caudata* shares the geolignoid zoospore discharge with *G. helecoides* (Fig. 3B), the transfer of the later taxon to *Leptolegnia* is hereby recommended. This analysis is in agreement with the

view of SORENSON (1968), that *Brevilegnia macrospora* and *B. gracilis* might not be part of the Saprolegnialean galaxy.

Species list

Achlya bisexualis COKER & A. COUCH, In COKER, J. Elisha Mitchell Sci. Soc. 42: 207, pl. 27. 1927. – Fig. 2 (A–F)

Description: Dioecious. Mycelium dense near the substratum. Hyphae slender to stout, branched; 20–50 µm wide. Zoosporangia abundant, fusiform to cylindrical, renewed sympodially or in basipetal succession; 120–450 × 25–40 µm. Discharge achlyoid. Primary zoospores 10–12 µm in diam. Gemmae abundant, spherical, dolioform, obovate, obpyriform or short cylindrical; thin-walled, terminal, intercalary, single or catenate; 70–150 µm in diam. Oogonia lateral, sometimes terminal, rarely intercalary, spherical or obpyriform, occasionally oval, rarely dolioform; (40–)55–70(–120) µm. Oogonial wall pitted under the antheridial cell attachment. Oogonial stalk 1–10 times the diameter of oogonium. Oospores eccentric, rarely maturing; (2–)5–12(–20) per oogonium and not filling it; 23–30 µm in diameter. Antheridia diclinous, slender irregularly branched, wrapping around the oogonium, persisting.

Comments: Three species of the genus *Achlya*, namely *A. bisexualis, A. heterosexualis*, and *A. ambisexualis* are dioecious. *Achlya heterosexualis* differs from our isolates by having monoclinous and androgynous antheridia along with diclinous antheridia. *Achlya ambisexualis* differs from our isolates by its characteristics features that oospores fill the whole oogonium. Also, the number of oospores per oogonium is relatively higher in *Achlya ambisexualis* than *Achlya bisexualis.* The ITS sequences of our four isolates (Tab. 1) were 100 % identical and showed 100 % similarity with *A. ambisexualis* accessions. However, slight variation in growth rate existed among them. Isolate 331_AH was slower growing than the other three isolates. This species has been previously reported from Sindh, Pakistan (LODHI 2007) whereas, it is reported for the first time from Bajaur.

Achlya orion CokEr & COUCH, J. Elisha Mitchell Scient. Soc. 36: 100 (1920). – Fig. 2 (G–J)

Description: Monoecious. Principal hyphae stout, branched, 10–55 µm wide. Zoosporangia cylindrical, fusiform, renewed sympodially or in basipetal succession; 85–568 × 30–55 µm. Zoospore discharge and behaviour achlyoid; encysted zoospores 10–12 µm in diam. Gemmae sparse, cylindrical, intercalary. Oogonia abundant, lateral, spherical, smooth, 39–50 µm in diam., wall pitted under regions of antheridial cell attachment, stalk 2–5(–10) times longer than the diameter of oogonium, mostly curved down. Oospores eccentric, spherical, mostly one, rarely 2–5 per oogonium, 32–40 µm in diam, wall 2–4 µm thick.

Achlya orion can be identified by its spherical oogonia containing mostly one oospore and by a recurved oogonial stalk. Antheridial branches mostly monoclinous, rarely androgynous. However, in our isolate, the antheridia were only monoclinous. It is reported for the first time from Pakistan.

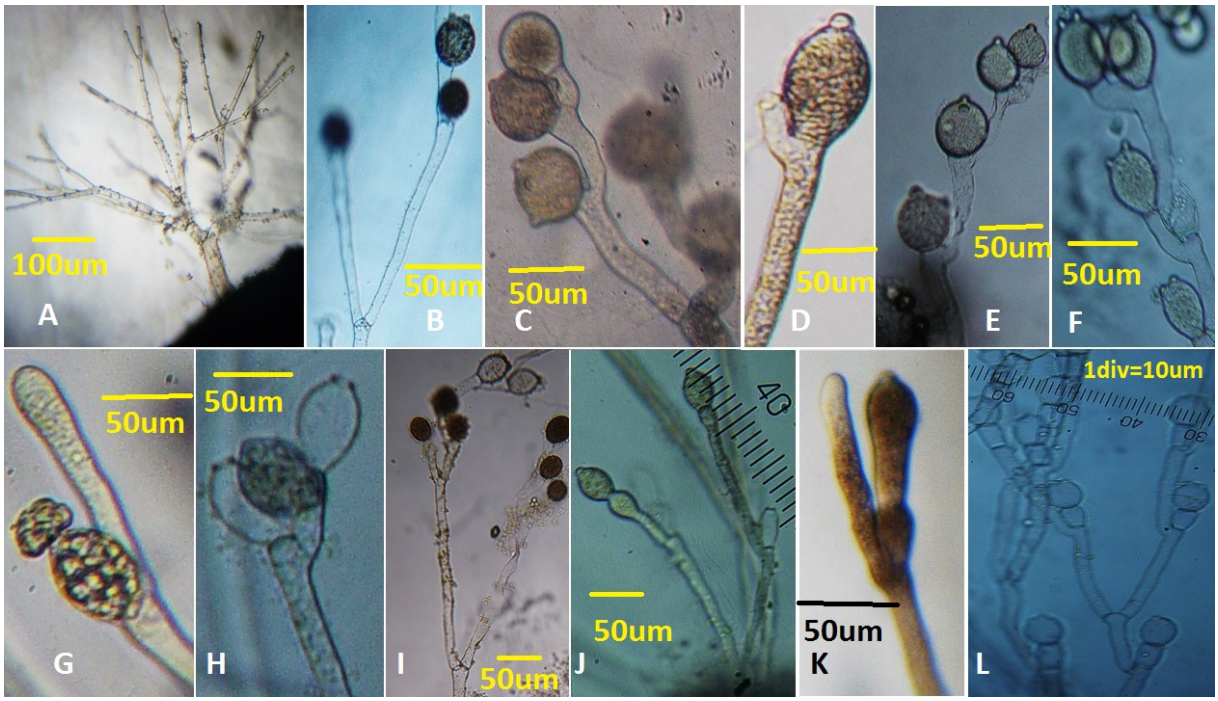

Fig. 3. *Allomyces arbusculus*, *A* Germling producing dichotomously branched thallus from a trunk-like basal cell, *B–F* branches of thallus showing sporangia with up to three papillae, *G* formation and release of zoospores, *H* branch with primary and secondary sporangia, *I* branches with resting sporangia, *J–L* branches with hypogynous gametangia.

Allomyces arbusculus E. J. BUTLER [as 'arbuscula'], Ann. Bot., Lond. 25: 1027 (1911). – Fig. 3 (A–L)
=*Allomyces arbusculus* f. *dichotomus* (COKER & F. A. GRANT) KANOUSE, Amer. J. Bot. 14: 303 (1927)
=*Allomyces arbusculus* var. *minor* R. EMERS., Lloydia 4: 136 (1941)
=*Septocladia dichotoma* COKER & F. A. GRANT, J. Elisha Mitchell Scient. Soc. 37: 180 (1922)

Description: Principal hyphae stout, dichotomously branched, pseudoseptate, 10–60 µm in diam. at base, basal joints 85–700 µm long, central joints up to 700 µm long; tips blunt, hyaline; Sporophytes producing terminal, ellipsoid or ovoid, thin-walled zoosporangia with rounded apex, sometimes catenulate, 45–80 × 23–50 µm with 1–4 releasing papillae; encysted zoospores 9–12 µm in diam.; resting sporangia abundant, 45–80 × 35–60 µm; sex organs abundant, in pairs; female gametangia terminal, dome-shaped, hyaline or grayish, 45–80 × 35–60 µm, male gametangia hypogynous, smaller, 35–60 × 30–45 µm.

Comments: The genus *Allomyces* comprises nine species, which are saprotrophic and have a wide geographical distribution (KIRK & al. 2008). It exhibits an isomorphic alternation of generation, bearing gametophytic (haploid) and sporophytic (diploid) thalli in its life cycle. *Allomyces arbusculus*, which was described from India (BUTLER 1911) has been reported from many parts of the world (WOLF 1941). It is the first ever report of any *Allomyces* species from Pakistan.

Aphanomyces scaber DE BARRY, Jahrb Wiss. Bot. 2: 178 (1860). – Fig. 4 (A–D)
Description: Hyphae delicate and less branched, up to 10 µm wide. Sporangia filamentous, straight, unbranched, 91–350 × 7–11 µm. Spores dimorphic. Primary spores cy-

lindrical to oval in a single row in the sporangium, encysting at the exit orifice in achlyoid fashion, becoming spherical; 7–10 µm in diam., releasing the secondary zoospore through a pore. Gemmae absent. Oogonia spherical, 20–25(–30) µm in diam. Oogonial wall unpitted, with short blunt papilla; 3 × 2 µm. Oospores single, very rarely two per oogonium; 18–21 µm in diam., centric. Oospore wall 1–1.5 µm thick. Antheridia absent or androgynous.

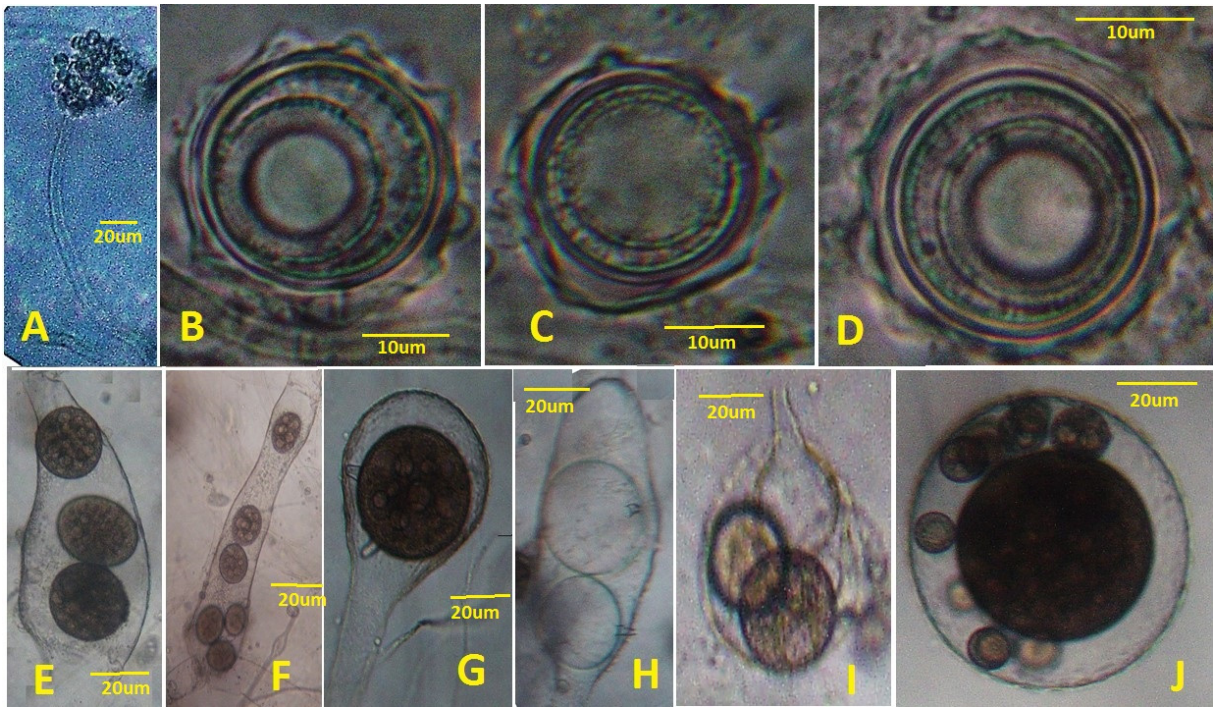

Fig. 4. *A–D Aphanomyces scaber, A* filamentous sporangia with zoospores discharge, *B–D* oogonia with single oospore. *E–J Olpidiopsis saprolegniae* var. *levis*, *E–I* sporangia, *J* resting spore with compagnion cells.

Comments: *Aphanomyces* is a relatively diverse genus in the group, with a total of 47 taxa (www.mycobank.org). Sexual structures were found in 264_AH, isolated from an unknown insect, while 263_AH isolated on hemp seed from the same site, remained asexual. Following the key to the species provided by JOHNSON & al. (2002), 264_AH was identified as *A. scaber*. Both isolates were 100 % identical with respect of their DNA sequence. The accessions of *A. astaci* and *A. laevis* have 99 % similarity in ITS sequence (Fig. 1). However, they differ in morphology; the first lacks sexual structure while the second has smooth walled oogonia. *Aphanomyces keratinophilus, A. invadens* and an unidentified *Aphanomyces* species have been previously reported from Pakistan (LILLEY & al. 1998, HUSSAIN & al. 2010, WADAHAR & al. 2012) and this is the first report of *A. scaber* from Pakistan.

Brevilegnia minutandra HOHNK, Veröff. Inst. Meeresf., Bremerhaven 1: 27. 1952. – Fig. 5 (A–E)

=*Brevilegnia unisperma* var. *montana* COKER & BRAXTON ex COCKER, J. Elisha Mitchell Sci. Soc. 42: 213, pl. 30, figs. 1–9, 1927

=*Brevilegnia unisperma* var. *delica* COCKER & ELEXENDER ex COKER, J. Elisha Mitchell Sci. Soc. 42: 214 pl. 31. 1927.

=*Dityuchus missouriensis* var. *moruzii* TOMA, Rev. Romaine Biol., Ser. Bot., 15: 253 pls. 4, 6. 1970.
=*Brevilegnia montana* (COKER & BRAXTON) JOHNSON, Mycologia 69(2): 289, figs. 1-8.1977.

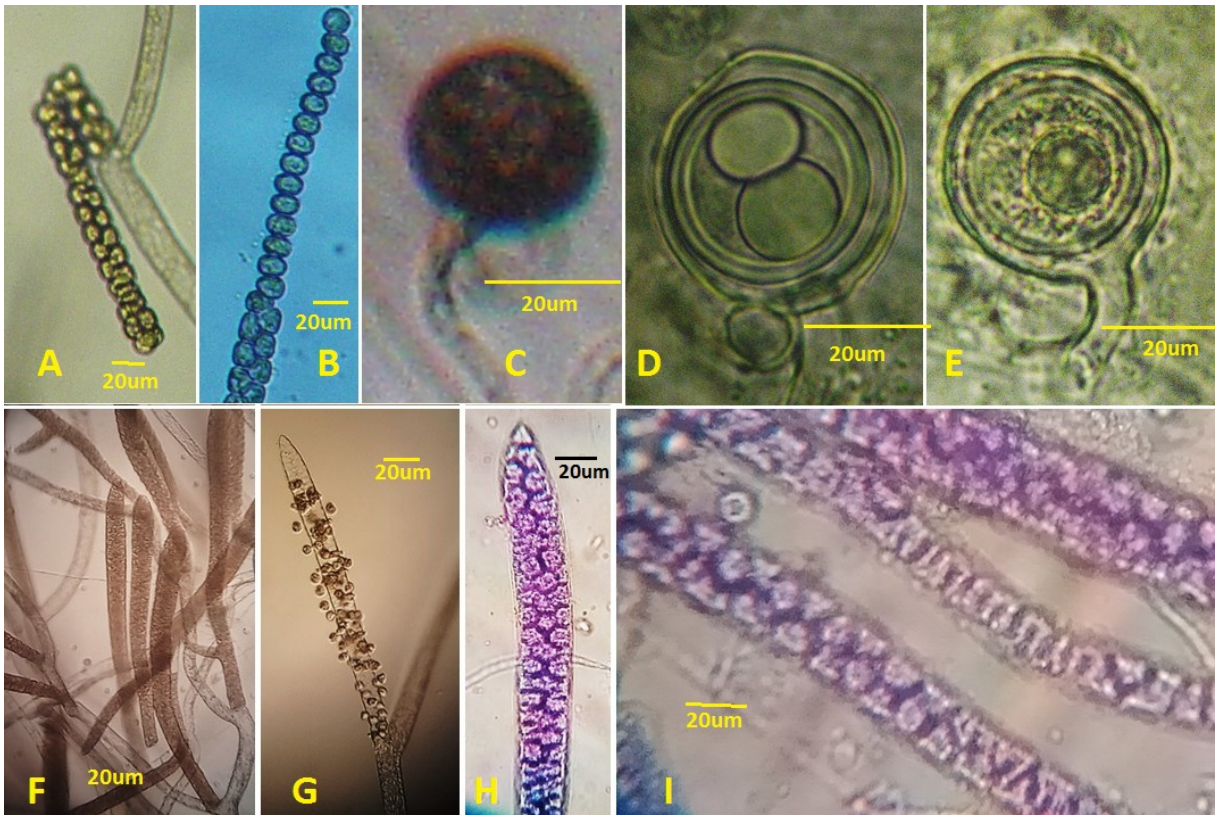

Fig. 5. *A–E Brevilegnia minutandra, A, B* sporangia, *C* oogonium with androgynous antheridium, *D, E* oogonia with oospore. *F–I Dictyuchus monosporus* sporangia showing encysted zoospores.

Description: Monoecious. Mycelium dense, hyphae slender, branched. Sporangia abundant, straight, renewed sympodially;70–1200 × 12–40 µm. Spores monomorphic, in 1–3 rows, discharge brevilegnoid, 10–15 µm in diameter. Gemmae not observed. Oogonia lateral, spherical, sometimes ovate; 15–27 µm (av.24.3µm). Oogonial wall unpitted and smooth. Length of oogonial stalk 3–6 times the diameter of oogonium. Oospores eccentric, one per oogonium; 13–23 (av. 18.9) µm in diam. Oospore wall up to 3 µm thick. Antheridial branches rarely produced and usually absent in most of the colonies, when present, androgynous and extremely short. Antheridial cell when present extremely small; fertilization tubes not observed.

Comments: *Brevilegnia minutandra* has similarity in sporangial morphology with *B. unisperma* var. *unisperma,* however, the later has predominantly irregular or ornamented oogonial wall. *B. bispora* differs by having achlyoid primary and secondary sporangia. Figure 1 shows the ITS sequence relationship of 295_AH with accessions presently available in GenBank. *B. longicaulis* differs from *B. minuteandra* by having diclinous antheridia. *B. unisperma* var. *delica* has clavate, fusiform, and cylindrical sporangia, with brevilegnoid, dictyucoid and achlyoid spore release. *B. longicaulis* has strictly diclinous antheridia. *B. variabilis* has dictyucoid rarely achlyoid discharge and larger oogonial size (Inaba & Tokumasu, 2002). Previously unnamed *Brevilegnia* sp. has been reported from Bajaur and District Bhimber, Azad Kashmir (ABDUL HAQ &

SHAHZAD 1998, HUSSAIN & al. 2010), while this is the first report of *B. minutandra* from Pakistan.

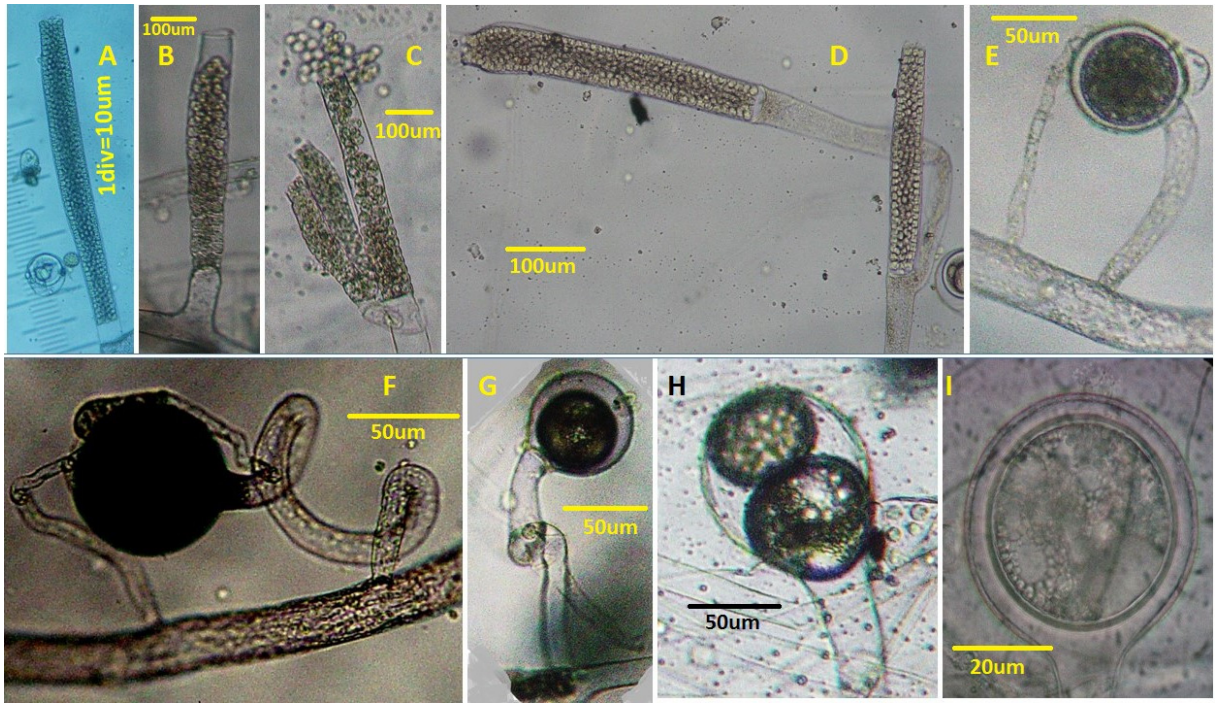

Fig. 6. *Calyptralegnia riparіensis, A–D* Zoosporangia*, E, F* oogonia with monoclinous antheridia, *G* oogonium with single oospore, *H* oogonium with two oospores, *I* oospore.

Calyptralegnia riparіensis HÖHNK, Veröff. Inst. Meeresf., Bremerhaven 2: 232, figs. 1–7. 1953. – Fig. 6 (A–I)

Description: Monoecious. Hyphae stout; less to moderately branched. Sporangia fusiform, cylindrical, or clavate; renewed sympodially in basipetalous fashion; sometime in subumbellate cyme; (150–)190–580(–645) × 35–75 µm. Spores monomorphic, discharged apically through the rupture of sporangium wall or at the release of a calyptra-like portion of the wall; behavior after sporangium dehiscence calyptralegnoid. Primary spore cysts angular; 11–13 µm. Gemmae absent. Oogonia lateral, obpyriform or globose and with a straight or slightly bent neck; (35–)62–78(–112) µm. Oogonial wall unpitted and smooth. Oogonial stalks 2–4 times longer than oogonial diameter, slender; sometimes twisted or coiled, unbranched. Oospores often abortive, subeccentric, spherical or oval; 1–2 per oogonium; (25–)45–65(–95) µm. Oospore wall 2–4 µm thick. Antheridial branches mostly monoclinous. Antheridial cells clavate and laterally appressed.

Comments: *Calyptralegnia* is among the less studied genera of oomycetes. Presently, it is represented by three species: *C. achlyoides, C. basraensis* and *C. riparіensis* (DICK 2013). The description of *C. basraensis,* which has been described from Iraq, could not be accessed and hence not compared, however, the description of the other two species is given by SEYMOUR & PADGETT (2002). Due to similarity in most of the characteristics, it is hard to differentiate *C. riparіensis* from *C. achlyoides.* The oospores in *C. riparіensis* are eccentric while they are subcenteric in *C. achlyoides*. Antheridial branches when present, are monoclinous in *C. riparіensis* and androgynous, occasionally or infrequently diclinous in *C. achlyoides. Calyptralegnia riparіensis* is reported for the first time from Pakistan.

Dictyuchus monosporus LEITGEB, Jahrb. Wiss. Bot. 7:374, pls. 22, 23. 1869–70. – Fig. 5. (F–I)
= *Dictyuchus anomalus* NAGAI, J. Fac. Agaric. Hokkaido Imp. Univ. 32: 28, pl.7, figs. 1–6.
= *Dictyuchus missouriensis* COUCH, J. Elisha Mitchell Sci. Soc. 46: 227, pl. 15. 1931.

Description: Dioecious. Mycelium slender to stout, sparingly branched; 12–43.5 µm wide at the base. Zoosporangia abundant, cylindrical, unbranched or branched, often disarticulating; renewed sympodially, rarely basipetal; 70–845 × 12–40 µm. Zoospore discharge and behaviour dictyucoid. Zoospores in 2–5 rows, encysted within the zoosporangium, secondary zoospores liberated separately through individual pores leaving the walls of primary zoospores in the zoosporangium and giving it a net-like appearance: encysted zoospores 9–11 µm in diam. Gemmae and oogonia not observed.

Comments: The species level identification is difficult in the dioecious forms of *Dictyuchus*. Both our isolates were dioecious, as they did not produce any sexual structures. COKER & METHEWS (1937) listed *D. carpophorus* and *D. sterile* along with *D. anomalus* as synonyms of *D. monosporus.* The sequences that showed >99 % sequence similarity to our isolates in BLAST search have been included in the cladogram (Fig. 1). This appears to be the first record of this species from District Bajaur.

Leptolegnia caudata DE BARRY, Bot. Zeitung (Berlin) 46: 631, pl. 9 Fig. 5. 1888. – Fig. 7 (A–H)

Description: Mycelium delicate, less dense, submerged, hyphae slender, sparingly branched. Sporangia filamentous, straight, unbranched, rarely branched; 180–1000 × 12–18 µm. Discharge leptolegnoid with exit orifice often situated near the substratum, rarely geolegnoid. Spores dimorphic, in a single row; 13–26 × 11–16 µm. Gemmae rare. Oogonia lateral, spherical to subspherical; (30–)38–49(–55) µm. Oogonial wall unpitted, smooth, slightly protruding in a beak-like manner at juncture with antheridial cell. Oogonial stalk 1–4 times the oogonial diameter. Oospores subcenteric, spherical, single per oogonium, pinkish, usually filling the oogonial cavity, sometime not maturing; (27–)35–46(–49) µm. Antheridial branches diclinous, rarely monoclinous, slender, branched or unbranched. Antheridial cells clavate.

Comments: Ten *Leptolegnia* species are present in MycoBank and Index Fungorum. JOHNSON et al. (2002) provided a key and descriptions for five species. *Leptolegnia caudata* is characterized by extension of the oogonial wall into a short beak-like structure at point of attachment to the antheridial cell. It could be differentiated from *L. chapmanii* and *L. eccentrica* by subcentric instead of eccentric oospores. *Leptolegnia chapmanii* also has oogonial wall ornamentations which are absent in *L. caudata*. We analyzed two isolates: 266_AH, isolated from mosquito larva, and 297_AH, from the same site on hempseed; the former could form both sexual and asexual structures, while the later failed to produce oogonia. Their ITS sequences were 100 % identical to each other. Figure 1 shows the phylogenic relation of our isolates of *L. caudata* to the taxa in GenBank. These isolates form a distinct clade that contains several unidentified isolates. It is the first report of *L. caudata* from Pakistan.

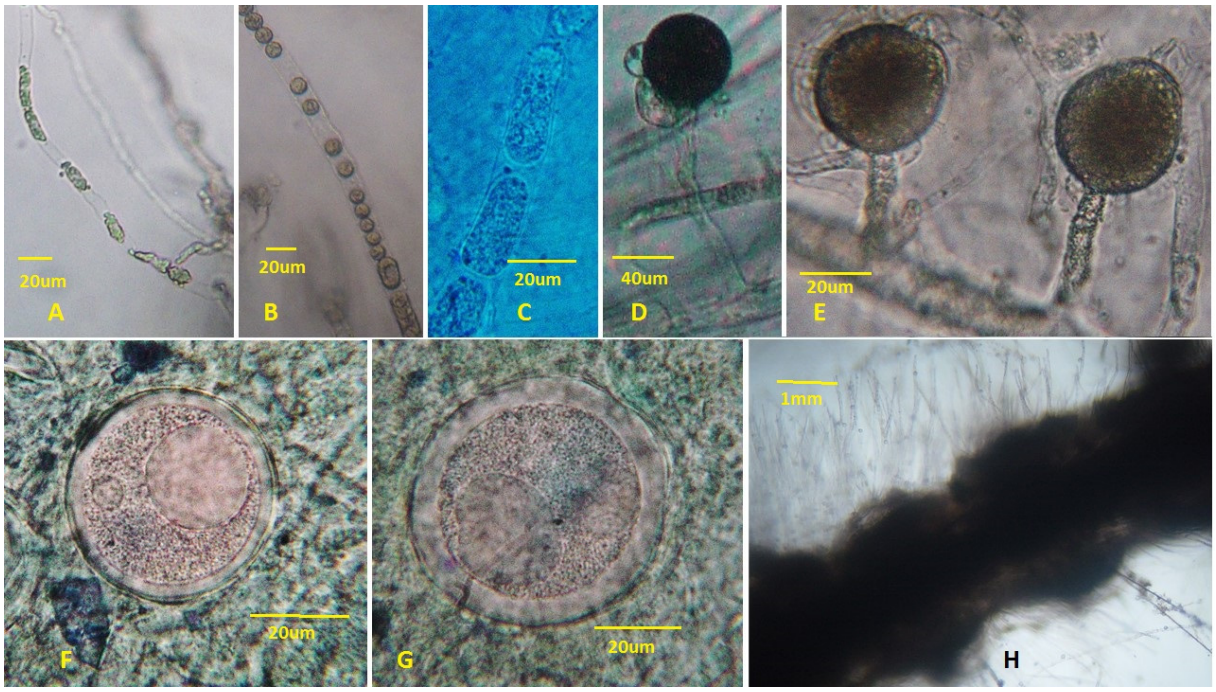

Fig. 7. *Leptolegnia caudata*, *A* leptolegnoid sporangial discharge, *B–C* geolegnoid sporangial discharge, *D, E* oogonia with antheridia, *F, G* oogonia and oospores, *H* infected mosquito larva.

Olpidiopsis saprolegniae* var. *levis COKER, The Saprolegniaceae: 185, 1923. – Fig. 4 (E–J)

Description: Thallus holocarpic, endobiotic. Zoosporangia one to many in terminal, occasionally intercalary swellings of the host hyphae, mostly spherical, ovoid or ellipsoidal; 35–190 µm in diam. (when ellipsoidal 33–46 × 12–25 µm). Wall smooth, colourless. Discharge tubes 1–3, cylindrical, 12–18 µm in length. Zoospores 2–4 µm long. Resting spores spherical 35–60 µm in diam. Wall thick and smooth, brownish. Companion cells one to many, 20–28 µm in diam., walls smooth, colourless.

Comments: Three varieties of *Olpidiopsis saprolegniae, viz. O. saprolegniae* var. *indica, O. saprolegniae* var. *levis* and *O. saprolegniae* var. *saprolegniae* have been reported from India (KHULBE 2001). The mycoparasite we recorded on *Saprolegnia ferax*, is *O. saprolegniae* var. *levis* as it has smooth walled resting spores, while the other two varieties have resting spores with spines or short ornamentations. It is the first report of the genus *Olpidiopsis* from Pakistan.

Saprolegnia australis ELLIOTT, New Zealand J. Bot. 6: 103. figs. 2, 4 c–f. 1968. – Fig. 8 (A–E)

Description: Dioecious. Mycelium dense, hyphae slender to stout; 12–40 µm wide. Sporangia cylindrical or clavate, internally proliferating; 40–450 × 20–35 µm. Discharge saprolegnioid. Zoospores dimorphic, encysted zoospores 10–12 µm in diam. Gemmae abundant with variable shape and size. Oogonia terminal or intercalary, sometimes lateral or sessile; spherical, cylindrical or fusiform; (32–)40–70(–110) µm. Oogonial wall pitted or smooth, stalk 1–3 times the diameter of the oogonium. Oospores subcentric, 4–30 per oogonium: 15–25 µm in diam. Antheridial branches diclinous, rarely monoclinous

Comments: *Saprolegnia australis,* which has predominantly subcentric oospores, differs from *S. diclina,* whose oospores are predominately centric. Morphologically, our isolates have a very close resemblance to *S. australis* as described by SEYMOUR (1970) and JOHNSON & al. (2002). The ITS sequence of both our isolates showed 100 % similarity with *S. australis* accessions KF748610, KF748604 and AM947034. It is the first report of *S. australis* from Pakistan. Isolate 303_AH was obtained from a freshwater fish, *Paraschistura alepidota,* which was floating dead on the surface of water.

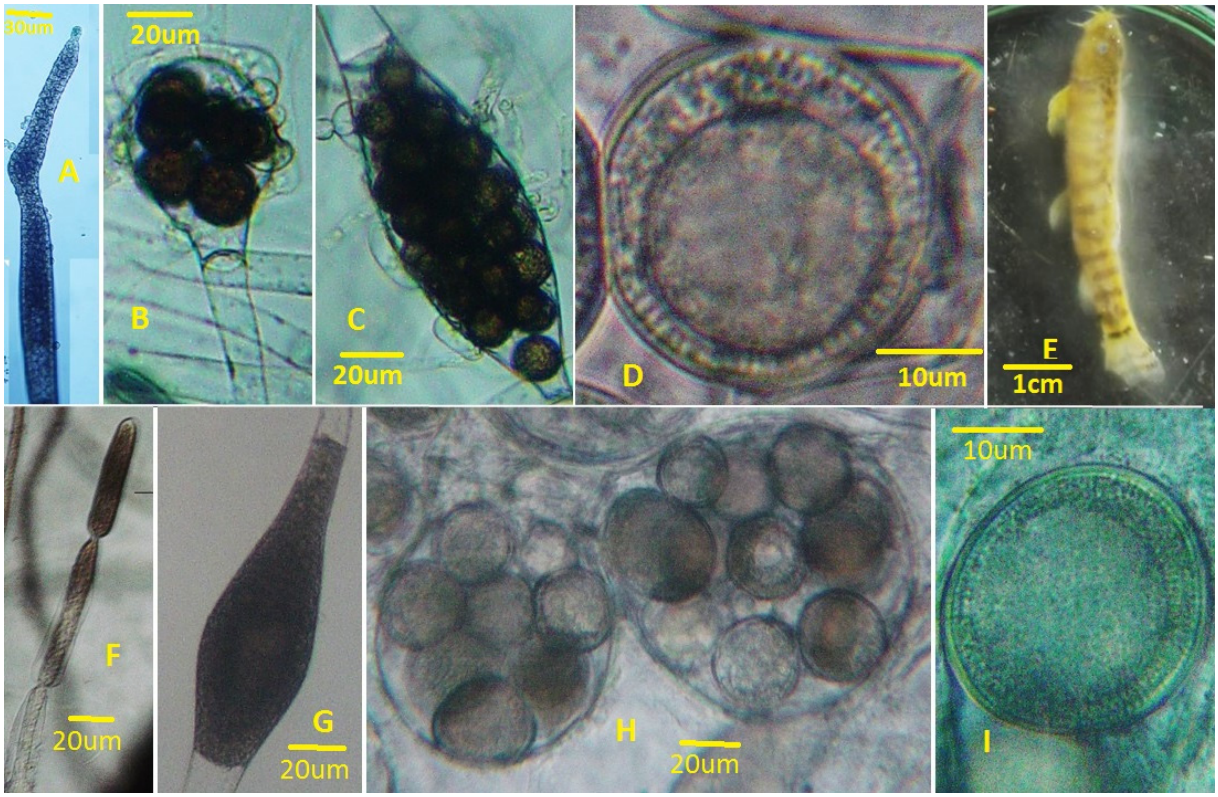

Fig. 8. *Saprolegnia australis*, *A* sporangium, *B* terminal oogonium with attached antheridia, *C* intercalary oogonium, *D* subcentric oospore, *E* infected fish (*Paraschistura alepidota*). *F-I Saprolegnia ferax*, *F* proliferating sporangium, *G* intercalary gemmae, *H* multi-sporous oogonium, *I* subcentric oospore.

Saprolegnia ferax (GRUITH.) KÜTZ., Phycol. General.: 157 (1843). – Fig. 8 (F–I)
≡*Conferva ferax* GRUITH., Nova Acta Acad. Caes. Leop.-Carol. 10: 445 (1821)
= *Saprolegnia ferax* var. *esocina* (MAURIZIO) Cejp, Fl. ČSR, Oomycet. 1: 246 (1959)
=*Saprolegnia esocina* MAURIZIO, Pringsheims Jahrb. Wissenschaftl. Bot. 30: 107 (1896)
= *Saprolegnia molluscorum* NEES in CARUS, Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur., 11: 513 (1823)

Description: Dioecious. Hyphae stout, 15–45 µm wide, branched. Zoosporangia abundant; cylindrical, clavate, or fusiform, occasionally spherical to subspherical internally proliferating, rarely renewed in basipetalous or cymose manner; 20–60 × 70–620 µm, Discharge saprolegnoid. Zoospore dimorphic, encysted zoospores 10–12 µm in diam. Gemmae variable in size, shape and position. Oogonia lateral, terminal or intercalary, spherical, obpyriform, sometimes obovate or clavate, rarely cylindrical; (30–)40–70(–120) µm. Oogonial stalk 1–3 times the diameter of oogonium. Oogonial wall generally pitted, rarely unpitted. Oospores centric or subcentric, spherical; (15–)20–30(–

35) µm, 6–20 per oogonium. Antheridial branches if present monoclinous or androgynous, rarely diclinous. Antheridial cell tubular to clavate, laterally applied to oogonium.

Comments: Intraspecific variation in morphology is very high among different isolates of *S. ferax*. SEYMOUR (1970) listed 29 synonyms of it, as its many isolates have been reported as new species. It is characterized by large, pitted oogonia, centric or subcentric oospores, and predominantly androgynous or monoclinous antheridia. In oogonial structure, *S. ferax* resembles *S. diclina*, however, the later has strictly diclinous antheridia. Many accessions of *S. ferax* showed 99–100 % similarity in BLAST search. Previously, it was reported from Sindh province of Pakistan (LODHI 2007). This is the first report of the species from Bajaur.

***Saprolegniaceae* sp.**

Mycelium dense. Hyphae slender, 8–12 µm wide, less to moderately branched. Sporangia, oogonia and gemmae not observed.

Comments: 265_AH was isolated from a *Chara* filament directly plated on agar medium. The hyphal width and overall appearance resembled to that of *Aphanomyces*. In BLAST search it showed 100 % sequence similarity with four GenBank accessions: *Saprolegnia* sp. (EU544192) isolated from *Daphnia* (WOLINSKA & al. 2008), *Saprolegniaceae* sp. (AM947032) from cray fish '*Astacus astacus*' (VRÅLSTAD & al. 2009), *Saprolegniaceae* sp. (MK568464) from freshwater gammarid amphipods (KESTRUP & al. 2011) and *Saprolegnia* sp. (MK849954) from salmonids (SAROWAR & al. 2019). The four unnamed animal pathogenic isolates and 295_AH make a distinct subclade with high boot strap support in *Leptolegnia* clade (highlighted in Fig. 1) and certainly, they represent a new taxon. *Saprolegnia parasitica* (CBS 540.67 AY310504) isolated from cray fish (OIDTMANN & al. 2004) which showed 98 % similarity to our isolate, appears to be a mis-identification. Two accessions of *Leptolegnia* sp., *viz.* KM061649 and HQ643138, were 97 and 98 % similar, respectively. In the absence of distinct morphological characters, the present isolate could not be assigned to a specific genus and thus is named temporarily as *Saprolegniaceae* sp. following KESTRUP et al. (2011).

Discussion

Although *Oomycetes* received much attention in terms of the diseases they cause, only few other details of their taxanomy and diversity are known. Recently some studies on this group have been conducted in Brasil (NASCIMENTO & PIRES-ZOTTARELLI 2012), Lithuania (MARKOVSKAJA 2007), Egypt (ALI 2007) and Sindh in Pakistan (LODHI 2007). Out of 20,000 estimated *Oomycetes* (ROSSMAN 1994), only 1,500 (DICK 2001) have been described. The gap is even wider as far as Pakistan is concerned where only 7–8 % of the known oomycetes have been reported (AHMAD & al. 1997, ABDUL HAQ & SHAHZAD 1998, LODHI 2007). This gap between the known and estimated number of oomycetes could be narrowed, if far-flung areas like Bajaur, are brought under such studies. The efficiency of these studies can be enhanced with the incorporation of DNA genetic analysis.

Although this study is by no means a comprehensive one, still it resulted in nine new records from Pakistan, including some fish parasites. The isolation of *Leptolegnia caudata* from mosquito larvae is a good sign and it may be used as a bio-control agent

against mosquitos. Such studies can be made more efficient and robust if researchers in this field are provided with access to modern techniques.

We would like to thank the administrations and workers of the Centralized Science Laboratory, University of Karachi, and the Department of Plant Protection Malir Halt, Karachi, for their help. We also thank Dr. ISHRAT JAMEEL of KIBGE and Mr. AMINULUL HAQ of GPGC, Khar Bajaur, for their help in this endeavor. In last but not the least, I would like to thank Dr. Irmgard Krisai-Greilhuber, Department of Botany and Biodiversity Research University of Vienna for her valuble suggestions for the improvement of this manuscript.

References

ABDUL-HAQ, M., SHAHZAD, S., 1998: *Oomycetes* from soil of Bajour Agency, FATA, Pakistan. – Pakistan J. Bot. **30**: 305–306.

AHMAD, S., IQBAL, S. H., KHALID, A. N., 1997: Fungi of Pakistan. – Sultan Ahmad Mycological Society of Pakistan. Department of Botany, University of the Punjab, Lahore, Pakistan.

AINSWORTH, G. C., 1973: Introduction and keys to higher taxa. – The fungi, IV A: 1–7.

ALEXOPOULOS, C. J., MIMS, C. W., BLACKWELL, M., 1996: Introductory mycology. 4th edn. – New York: Wiley.

ALI, E. H., 2007: Biodiversity of zoosporic fungi in polluted water drainages across Niles' Delta region, Lower Egypt. – Acta Mycol. **42**(1): 99–111.

ATLAS, R. M., 2005: Handbook of media for environmental microbiology. – Boca Raton: CRC press.

BAKKEREN, G., KRONSTAD, J. W., LÉVESQUE, C. A., 2000: Comparison of AFLP fingerprints and ITS sequences as phylogenetic markers in *Ustilaginomycetes*. – Mycologia **92**(3): 510–521.

BEAKES, G. W., HONDA, D., THINES, M., 2014: Systematics of the *Straminipila*: *Labyrinthulomycota*, *Hyphochytriomycota*, and *Oomycota*. – In: MCLAUGHLIN, D. J., SPATAFORA, J. W., (Eds.): The Mycota VII Part A Systematics and Evolution, 2nd edn, pp. 39–97. – Berlin, Heidelberg: Springer.

BISHT, G. S., JOSHI, C., KHULBE, R. D., 1996: Watermolds: potential biological control agents of malaria vector *Anopheles culicifacies*. – Curr. Sci. **70**(5): 393–395.

BLACKWELL, M., 2011: The Fungi: 1, 2, 3… 5.1 million species? – Amer. J. Bot. **98**(3): 426–438.

BUTLER, E. J., 1911: On *Allomyces*, a new aquatic fungus. – Ann. Bot. **25**(100): 1023–1035.

CHAUHAN, R., KAUR, P., SHARMA, S., 2012: Pathogenicity of some species of *Achlya* and *Saprolegnia* on Indian Major carps viz. *Catla catla*, *Cirrihinus mrigala* and *Labeorohita*. – J. Environ. Sci. Comp. Sci. Eng. Technol. **1**(3): 422–428.

COKER, W. C., METHEWS, V. D., 1937: *Saprolegniales*. – North Amer. Fl. **2**(1): 15–67.

CZECZUGA, B., KIZIEWICZ, B., DANILKIEWICZ, Z., 2002: Zoosporic fungi growing on the specimens of certain fish species recently introduced to Polish waters. – Acta Ichthyol. Piscator. **2(**32): 117–125.

DICK M.W., 2001. The *Peronosporomycetes*. – In MCLAUGHLIN, D. J., SPATAFORA, J. W., (Eds.): The Mycota VII Part A Systematics and Evolution, pp. 39–72. – Berlin, Heidelberg: Springer.

DICK, M. W., 2013: Straminipilous Fungi: systematics of the *Peronosporomycetes* including accounts of the marine straminipilous protists, the plasmodiophorids and similar organisms. – Springer Science & Business Media. Doi 10.1007/978-94-015-9733-3

DICK, M. W., WONG, P. T., CLARK, G., 1984: The identity of the oomycete causing ‘Kikuyu Yellows’, with a reclassification of the downy mildews. – Bot. J. Linn. Soc. **89**(2): 171–197.

DICK, M. W., VICK, M. C., GIBBINGS, J. G., HEDDERSON, T. A., LOPEZ LASTRA, C. C., 1999: 18S rDNA for species of *Leptolegnia* and other *Peronosporomycetes*: justification for the subclass taxa *Saprolegniomycetidae* and *Peronosporomycetidae* and division of the *Saprolegniaceae sensu lato* into the *Leptolegniaceae* and *Saprolegniaceae*. – Mycol. Res. **103**(9): 1119–1125.

FELSENSTEIN, J., 1985: Confidence limits on phylogenies: an approach using the bootstrap. – Evolution **39**(4): 783–791.

HAWKSWORTH, D. L., 1991: The fungal dimension of biodiversity: magnitude, significance, and conservation. – Mycol. Res. **95**(6): 641–655.

HASEGAWA, M., KISHINO, H., YANO, T. A., 1985: Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. – J. Molec. Evol. **22**(2): 160–174.

HUSSAIN, T., ISHTIAQ, M. C., HUSSAIN, A., MEHMOOD, T., SULTANA, K., ASHRAF, M., 2010: Incidence of *Fungi* in Water Springs of Samahni Valley, District Bhimber, Azad Kashmir, Pakistan. – Int. J. Biol. **2**(2): 94–101.

INABA, S., TOKUMASU, S., 2002: The genus *Brevilegnia* (*Saprolegniales*, *Oomycetes*) in Japan. – Mycosci. **43**(1): 59–66.

IQBAL, Z., ASGHAR, M., 2012: Saprolegniasis in two commercially important carps. – Pakistan J. Zool. **44**(2): 591–595.

JAMES, T. Y., LETCHER, P. M., LONGCORE, J. E., MOZLEY-STANDRIDGE, S. E., PORTER, D., POWELL, M. J., ... VILGALYS, R., 2006: A molecular phylogeny of the flagellated fungi (*Chytridiomycota*) and description of a new phylum (*Blastocladiomycota*). – Mycologia **98**(6): 860–871.

JOHNSON, T. W., 1956: The genus *Achlya*: morphology and taxonomy. – Ann Arbor: The University of Michigan Press.

JOHNSON Jr., T. W., 2002: Biology and the systematics of the *Saprolegniaceae*. http://dl. uncw. edu/digilib/biology/fungi/taxonomy% 20and% 20systematics/padgett% 20book/.

KESTRUP, Å. M., THOMAS, S. H., VAN RENSBURG, K., RICCIARDI, A., DUFFY, M. A., 2011: Differential infection of exotic and native freshwater amphipods by a parasitic water mold in the St. Lawrence River. – Biol. Invasions **13**(3): 769–779.

KHULBE, R. D., 2001*:* A manual of aquatic fungi: *Chytridiomycetes* & *Oomycetes*. – Delhi: Daya Books.

KIRK, P. M., CANNON, P. F., MINTER, D. W., STALPERS, J. A., 2008: Dictionary of the *Fungi*. 10th edn. –Wallingford: CABI.

KUMAR, S., STECHER, G., LI, M., KNYAZ, C., TAMURA, K., 2018: MEGA X: molecular evolutionary genetics analysis across computing platforms. – Molec. Biol. Evol. **35**(6): 1547–1549.

LEVESQUE, C.A., DE COCK, A.W., 2004: Molecular phylogeny and taxonomy of the genus *Pythium*. – Mycol. Res. **108**(12): 1363–1383.

LILLEY, J. H., CALLINAN, R. B., CHINABUT, S., KANCHANAKHAN, S., MACRAE, I. H., PHILLIPS, M. J., 1998: Epizootic ulcerative syndrome (EUS) technical handbook. – Bangkok: The Aquatic Animal Health Research Institute (AAHRI).

LODHI, A. M., 2007: Taxonomic studies on oomycetous *Fungi* from Sindh. – Doctoral Dissertation, University of Karachi, Karachi.

MARANO, A. V., PIRES-ZOTTARELLI, C. L. A., BARRERA, M. D., STECIOW, M. M., GLEASON, F. H., 2011: Diversity, role in decomposition, and succession of zoosporic fungi and straminipiles on submerged decaying leaves in a woodland stream. – Hydrobiol. **659**(1): 93–109.

MARKOVSKAJA, S., 2007: The genus *Aphanomyces (Leptolegniaceae, Peronosporomycetes)* in Lithuania. – Bot. Lithuanica **13**(4): 237-244.

MARTIN, W. W., 1981: *Couchia circumplexa*, a water mold parasitic in midge eggs. – Mycologia **73**(6): 1143–1157.

MASTAN, S. A., REDDY, M. R. K., LAKSHMI, D. S., 2012: Oomycete infections in freshwater fishes. – Int. J. Fisheries Aquacult. **4**(9): 186–190.

MÖLLER, E. M., BAHNWEG, G., SANDERMANN, H., GEIGER, H. H., 1992: A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. – Nucl. Acids Res. **20**(22): 6115.

MUELLER, G. BILLS, M., G. F., FOSTER, M. S., 2004: Biodiversity of *Fungi*: inventory and monitoring methods. – Burlington: Elsevier Academic Press.

NASCIMENTO, C. D. A., PIRES-ZOTTARELLI, C. L. A., 2012: Diversidade de fungos zoospóricos da Reserva Biológica de Mogi Guaçu, estado de São Paulo, Brasil. – Rodriguésia **63**(3): 587–611.

OIDTMANN, B., SCHAEFERS, N., CERENIUS, L., SÖDERHÄLL, K., HOFFMANN, R. W., 2004: Detection of genomic DNA of the crayfish plague fungus *Aphanomyces astaci* (Oomycete) in clinical samples by PCR. – – Veterinary Microbiol. **100**(3–4): 269–282.

PAXTON, C. G. M., WILLOUGHBY, L. G., 2000: Resistance of perch eggs to attack by aquatic fungi. – J. Fish Biol. **57**(3): 562–570.

PETERS, R. D., GRAU, C. R., 2002: Inoculation with nonpathogenic *Fusarium solani* increases severity of pea root rot caused by *Aphanomyces euteiches*. – Pl. Disease **86**(4): 411–414.

ROCHA, S. C., LOPEZ-LASTRA, C. C., MARANO, A. V., DE SOUZA, J. I., RUEDA-PÁRAMO, M. E., PIRES-ZOTTARELLI, C. L., 2018: New phylogenetic insights into *Saprolegniales* (*Oomycota, Straminipila*) based upon studies of specimens isolated from Brazil and Argentina. – Mycol. Prog. **17**(6): 691–700.

ROSSMAN, A. Y., 1994: A strategy for an all-taxa inventory of fungal diversity. – In PENG, C.-I., CHEN, C. H., (Eds.): Biodiversity and terrestrial ecosystems, 169–194. Monograph Series No. 14. – Taipei: Institute of Botany, Academia Sinica.

SAROWAR, M. N., CUSACK, R., DUSTON, J., 2019: *Saprolegnia* molecular phylogeny among farmed teleosts in Nova Scotia, Canada. – J. Fish Diseases **42**(12): 1745–1760.

SEYMOUR, R. L., 1984: *Leptolegnia chapmanii*, an oomycete pathogen of mosquito larvae. – Mycologia **76**: 670–674.

SEYMOUR, R. L., 1970: The genus *Saprolegnia*. – Nova Hedwigia **19**(1, 2): 1–124.

SEYMOUR, R. L., PADGETT, D. E., 2002: Biology and systematics of the *Saprolegniaceae*. – http://www.ilumina-dlib. org.

SCHOLTE, E. J., KNOLS, B. G., SAMSON, R. A., TAKKEN, W., 2004: Entomopathogenic fungi for mosquito control: a review. – J. Insect Sci. **4**(1): 1–24.

SORENSON, W. G., 1968: Notes on *Brevilegnia gracilis* and *Brevilegnia macrospora*. – Mycologia **60**(1): 193–196.

STECIOW, M. M., LARA, E., PILLONEL, A., PELIZZA, S. A., LESTANI, E. A., ROSSI, G. C., BELBAHRI, L., 2013: Incipient loss of flagella in the genus *Geolegnia*: the emergence of a new clade within *Leptolegnia*? – IMA Fungus **4**(2): 169–175.

VRÅLSTAD, T., KNUTSEN, A.K., TENGS, T., HOLST-JENSEN, A., 2009: A quantitative TaqMan® MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague Aphanomyces astaci. – Veterinary Microbiol. **137**(1–2): 146–155.

VENNERSTRÖM, P., SÖDERHÄLL, K., CERENIUS, L., 1998: The origin of two crayfish plague (*Aphanomyces astaci*) epizootics in Finland on noble crayfish, *Astacus astacus*. – Ann. Zool. Fenn. **35**: 43–46.

WADAHAR, G., CHANDIO, M., MAHAR, M., 2012: Epizootic Ulcerative Syndrome (EUS) In freshwater fishes infected by fungal genera *Aphanomyces* and *Alternaria*, Sindh, Pakistan. – Sindh Univ. Res. J. – SURJ (Sci. Ser.) **44**(4).

WICKER, E., HULLE, M., ROUXEL, F., 2001: Pathogenic characteristics of isolates of *Aphanomyces euteiches* from pea in France. – Pl. Pathol. (Oxford) **50**: 433–442

WOLF, F. T., 1941: A contribution to the life history and geographic distribution of the genus *Allomyces*. – Mycologia **33**(2): 158–173.

WOLINSKA, J., KING, K. C., VIGNEUX, F., LIVELY, C. M., 2008: Virulence, cultivating conditions, and phylogenetic analyses of oomycete parasites in *Daphnia*. – Parasitology **135**(14): 1667–1678.