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Additional Novel Data on *Lignicolous ascomycetes* from Southern Iraqi Aquatic Environments

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ABSTRACT

Lignicolous aquatic fungi play an important role in the biodegradation of submerged woody substrates in diverse water bodies. In this study, ten lignicolous ascomycetous fungi were cultured from decaying plant materials submerged in aquatic habitats. These taxa were *Aniptodera megalospora*, *Aniptosporopsis lignatilis*, *Ascosacculus aquaticus*, *Halopyssothecium voraginesporum*, *Melanocarpus oblatus*, *Phaeonectriella lignicola*, *Preussia terricola*, *Pseudorhypophila marina*, *Trematosphaeria pertusa*, and *Trichocladium achrasporum*. All reported species are new records for Iraq. Brief morphological descriptions, illustrations, and notes on taxonomy, ecology, and global distribution are provided. Living cultures and/or dried specimens of all available species were deposited in the University of Basrah herbarium.

INTRODUCTION

The Shatt Al-Arab River is formed by the confluence of the Tigris and Euphrates Rivers near the city of Qurna in southern Iraq. It flows for approximately 190 km before entering the Arabian Gulf near the city of Faw. The river is strongly influenced by tidal movements originating in the Gulf, which propagate northward and regularly affect water levels and flow. The eastern part of the Hammar Marshes is connected to the Shatt Al-Arab. Water conditions range from fresh in the northern reaches near Qurna to increasingly saline (brackish) toward the southern estuary because of saltwater intrusion from the Arabian Gulf. In recent years, salinity has increased as freshwater inputs from the Tigris and Euphrates Rivers have declined, allowing Gulf water to penetrate farther upstream. These changes in the aquatic environment have altered biodiversity, including fungal biodiversity **(Al-Taie *et al.*, 2014; Al-Tememi *et al.*, 2015)**. Changes in the aquatic environment of Basrah Governorate, particularly salinity, are therefore likely to affect fungal community composition and warrant further ecological, taxonomic, physiological, and bioactivity studies. Lignicolous aquatic fungi include organisms occurring in marine, brackish, and freshwater habitats and play an essential role in the biodegradation of submerged plant material **(Hu *et al.*, 2013; Xu *et al.*, 2020)**. Aquatic fungi may occur as free-living organisms or in association with plants and animals **(Al-Ameen *et al.*, 2024; Mohammed & Al-Taie, 2024)**. In this region, aquatic environments have received less research attention than terrestrial environments, which have been examined in numerous studies aimed at identifying and classifying their fungal communities **(Al-Mishrey *et al.*, 2021; Ahmed *et al.*, 2023; Al-Hamdani & Al-Dossary, 2023; Muhsen & Al-Dossary, 2023)**. Nevertheless, aquatic environments in Basrah Governorate, southern Iraq, are abundant and diverse and support a rich variety of aquatic and riparian plants. **Abdullah (1983)** provided the first report of aquatic mycelial fungi from Iraq. Since then, numerous studies have been conducted, and the recorded species were summarized in an annotated checklist encompassing fungal taxa isolated from submerged plant detritus in Iraqi aquatic environments **(Al-Saadoon & Al-Dossary, 2014)**. Recent studies have documented fungi associated with aquatic invertebrates and water samples in Iraq, further demonstrating the diversity of fungal communities across regional aquatic habitats **(Al-Ameen *et al.*, 2024; Mohammed & Al-Taie, 2024)**. A total of 67 fungal species are currently known from Iraqi aquatic environments. Ascomycetes constitute the majority of the known species, whereas anamorphic states (hyphomycetes and coelomycetes) are less numerous. This study reports additional novel and noteworthy lignicolous species collected from different aquatic habitats in southern Iraq.

MATERIALS AND METHODS

Approximately 398 fragments of decomposed plant debris, including *Phragmites australis*, Typha sp., unidentified twigs, and floating or submerged timber, were collected randomly from three locations between September and November 2018: the eastern Hammar Marshes, Karmat Ali on the Shatt Al-Arab River, and Al-Faw District on the Arabian Gulf in Basrah, southern Iraq. Submerged samples were placed directly in collection bags and transported to the research laboratory for culturing or stored under refrigeration until examination. Ecological conditions at the sampling sites were as follows: salinity was 10.06‰ at the East Al-Hammar Marsh station, 14.33‰ at Karmat Ali, and 42.7‰ at Al-Faw District; mean temperature ranged from 17.73 to 29.9 °C; pH ranged from 7.85 to 8.98; and mean dissolved oxygen concentration ranged from 3.4 to 7.6 mg/L. The collected plant material was thoroughly rinsed with running water followed by sterile water and then incubated at 25°C on sterile, moistened filter paper in glass chambers. The segments were examined periodically under a dissecting microscope for fungal growth. Fungal cultures were obtained by isolating individual spores on potato carrot agar (PCA) supplemented with 50 mg/L chloramphenicol. Once growth was evident, a portion of the hyphae from each isolate was subcultured to obtain a pure culture. Species that did not grow in artificial culture were described on the basis of their characteristics on natural substrates. All recorded fungi were identified using relevant references, including **Kohlmeyer and Volkmann-Kohlmeyer (1991)**, **Hyde *et al.***

(1999), **Jones *et al.* (2009)**, **Guarro *et al.* (2012)**, **Tanaka *et al.* (2015)**, **Hyde *et al.* (2016)**, **Jones *et al.* (2017)** and **Samson (2022)**.

Living cultures and/or dried specimens, together with permanent slides, were deposited in the herbarium of the University of Basrah.

RESULTS AND DISCUSSION

Aniptodera megalospora **K. D. Hyde; W. H. Ho & K. L. Tsui, Mycoscience, 40: 165 (1999)** (Fig. 1)

Ascomata are globose, 120–300 µm in diameter, submerged to partially immersed, hyaline, becoming black at maturity, and provided with a long neck. Asci are clavate, thin-walled, 8-spored, and evanescent. Ascospores measure 55–82 × 10–12 µm, are bi- to triseriate, hyaline, cylindrical to fusiform, bicellular, comparatively thick-walled, possess polar appendages, and are not constricted at the septum.

Examined material: Dead stems of *Phragmites australis* submerged in the eastern Hammar Marsh, Basrah, southern Iraq, 22 November 2018.

Notes: This species was isolated from woody debris submerged in freshwater ecosystems in Brunei, Hong Kong, and Malaysia. Its ascospores are larger than those of other *Aniptodera* species **(Hyde *et al.*, 1999)**. This is the fifth species of the genus recorded in Iraq. *Aniptodera chesapeakensis*, *A. fusiformis*, *A. muritaniensis*, and *A. palmicola* were previously recorded from plant debris submerged in saline and freshwater environments in different parts of southern Iraq **(Al-Saadoon & Abdullah, 2001; Al-Saadoon & Al-Dossary, 2014)**. Although this species was originally recorded from a freshwater habitat, the present collection was obtained from an environment with a salinity of 18.85%. Transitions between habitats, including from marine to terrestrial environments and vice versa, are possible **(Amend *et al.*, 2019)**. Several genera occurring in freshwater and terrestrial environments, including *Didymella*, *Leptosphaeria*, *Phaeosphaeria*, *Savoryella*, and *Trematosphaeria*, also contain marine species, supporting the hypothesis of fungal transitions among habitats **(Jones *et al.*, 2009; Sakayaroj *et al.*, 2011)**.

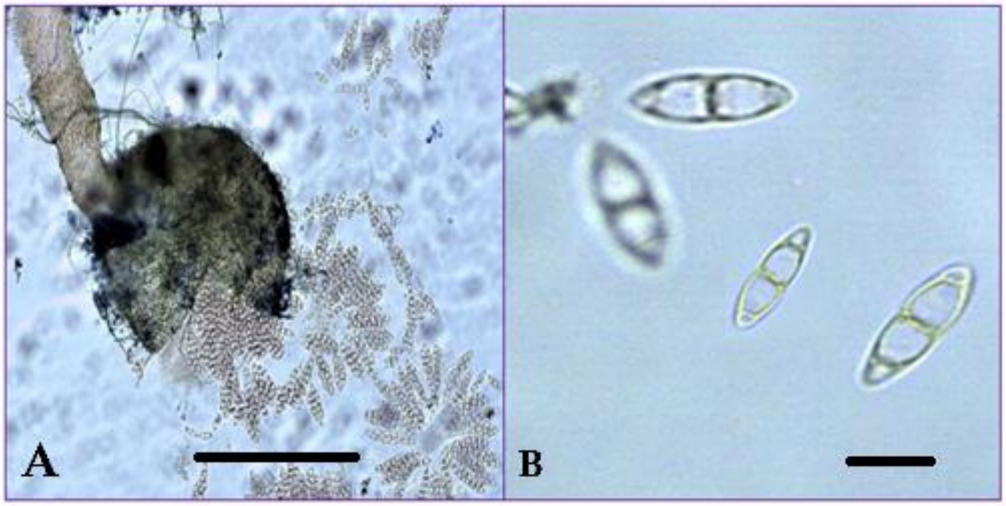

Fig. 1. *Aniptodera megalospora*. (A) Ascoma and asci; (B) ascospores.
Scale bars: A = 100 µm; B = 20 µm.

Aniptosporopsis lignatilis **(K.D. Hyde) K. L. Pang; C. L. Lu; W. T. Ju & E.B. G. Jones, Botanica Marina 60 (4): 459 (2017)** (Fig. 2)

Synonym: *Aniptodera lignatilis* K.D. Hyde, Aust. Syst. Bot. 5: 11 (1992).

Ascomata are hyaline to cream, globose to subglobose, submerged to partially immersed or superficial, and 160–400 µm in diameter. Asci are 8-spored, clavate, unitunicate, and 120–160 × 25–39 µm. Ascospores measure 37–60 × 11–17 µm, are hyaline, relatively thick-walled, fusiform, bicellular, not constricted at the septum, and possess bipolar filamentous appendages. Material examined: Dead stems of *Typha domingensis* submerged in the eastern Hammar Marsh, Basrah, southern Iraq, 22 November 2018.

Notes: This species was first isolated from freshwater in Australia under the name *Aniptodera lignatilis* **(Hyde, 1992)**. It was subsequently transferred to the genus *Aniptosporopsis* as *Aniptosporopsis lignatilis* on the basis of phylogenetic analysis **(Jones *et al.*, 2017)**. The species was later recovered from Hong Kong, Malaysia, Mauritius, the Philippines, Seychelles, South Africa, and Taiwan **(Hyde *et al.*, 1999; Pang *et al.*, 2011)**. The present collection was isolated from an environment with a salinity of 10.85%. This is the first record of the genus *Aniptosporopsis* in Iraq.

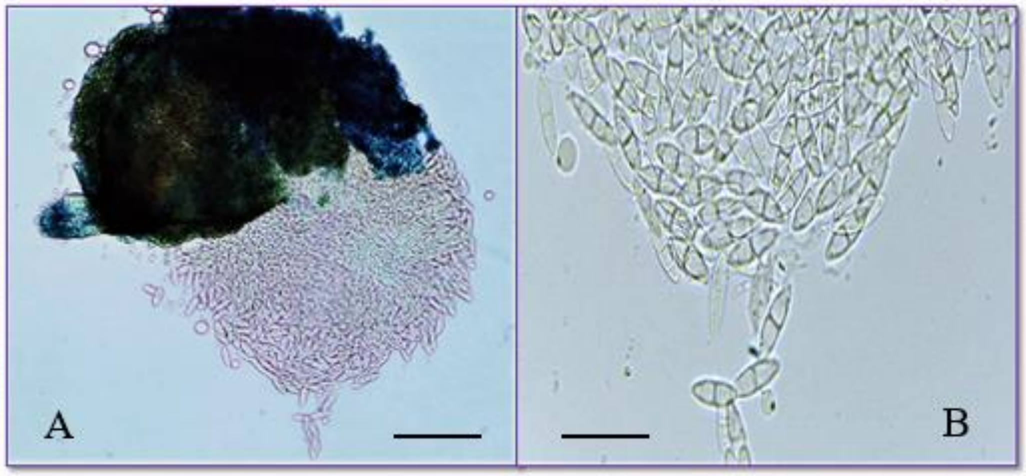

Fig. 2. *Aniptosporopsis lignatilis*. (A) Ascoma and asci; (B) asci and ascospores.
Scale bars: A = 100 µm; B = 20 µm.

***Ascosacculus aquaticus* (K. D. Hyde) J. Campb; J. L. Anderson & Shearer, Mycologia 95 (3): 545 (2003)** (Fig. 3)

Synonym: *Halosarpheia aquatica* K.D. Hyde, Australian Systematic Botany 5(4): 407 (1992)

Ascomata are 140–200 µm in diameter, subglobose to pyriform, immersed or semi-immersed, brown, and possess a long neck. Asci measure 30–50 × 10–20 µm, are thin-walled, and 8-spored. Ascospores measure 20–30 × 7–9 µm, are hyaline, fusiform to cylindrical, thin-walled, bicellular, somewhat constricted at the septum, and possess polar filiform appendages.

Examined material: Dead stems of *Phragmites australis* immersed in the eastern Hammar Marsh, Basrah, southern Iraq, 10 September 2018.

Notes: The genus contains two species **(Kirk *et al.*, 2008)**. This species was originally recorded on plant material submerged in Australian rivers **(Hyde, 1992)**, and was subsequently isolated from Costa Rica **(Campbell *et al.*, 2003)** and from grasses submerged in freshwater heavily polluted with organic matter in Tabasco, Mexico **(Rosique-Gil *et al.*, 2008)**. *Halosarpheia aquatica* was transferred to the genus *Ascosacculus*; both genera include freshwater fungi **(Campbell *et al.*, 2003; Jones *et al.*, 2009)**.

The ascospores in the Mexican collection were smaller (22–32 × 7–8 µm) than those of the Australian type material (33.5–64 × 7–10 µm) **(Campbell *et al.*, 2003; Rosique-Gil *et al.*, 2008)**.

The present material is highly consistent with the description of the Mexican specimen, particularly in ascospore dimensions. The current isolate was recovered from an environment with a salinity of 10.6%. This is the first record of the genus *Ascosacculus* in Iraq.

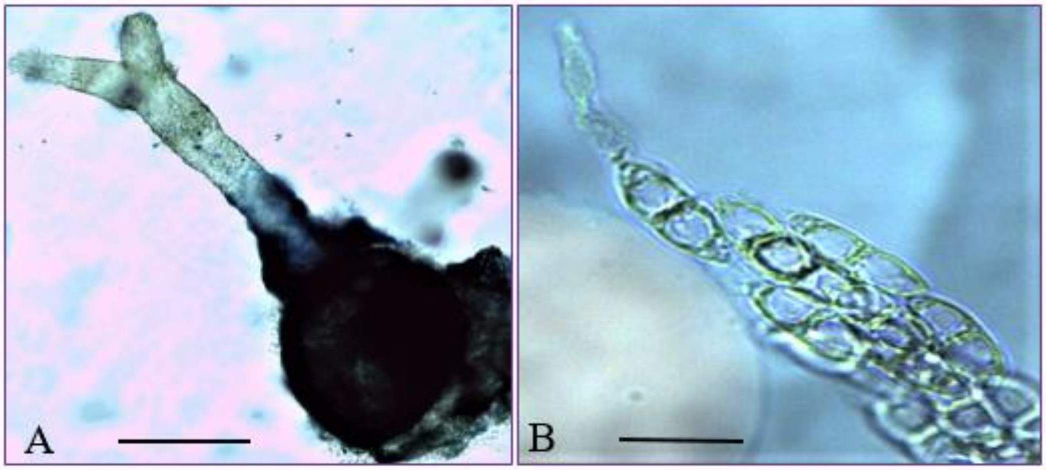

Fig. 3. *Ascosacculus aquaticus*. (A) Ascoma with a long neck; (B) ascus with ascospores.

Scale bars: A = 100 µm, B = 20 µm.

***Halopyssothecium voraginesporum* (Abdel-Wahab; Bahkali & E.B.G. Jones) M.S. Calabon; K.D. Hyde & E.B. G. Jones, Mycological Progress 20:716 (2021)** (Fig. 4)

Basionym: *Lentithecium voraginesporum* Abdel-Wahab, Bahkali & E.B.G. Jones, Fungal Diversity 80:53(2016)

Colonies on PCA grew slowly, reaching 4 cm in 10 days at 25°C. They were brown to dark brown with a well-defined margin. Ascomata are globose to subglobose, submerged, and 125–215 µm in diameter. Asci measure 38–52 × 8–10 µm, are 8-spored, bitunicate, and clavate. Ascospores measure 12–22 × 3–6 µm, are elliptical, biseriate, brownish-yellow to dark brown, rounded at the ends, and uniseptate, with the upper cell larger and wider than the lower cell. The anamorph was not determined.

Examined material: Dead stems of *Phragmites australis* immersed in marine water at Al-Faw, Arabian Gulf, Basrah, southern Iraq, 8 October 2018.

Notes: *Lentithecium voraginesporum* was first reported from dead stems of *Phragmites australis* submerged in the Arabian Gulf, Saudi Arabia **(Abdel-Wahab *et al.*, 2016)**. The species was subsequently transferred to *Halobyssothecium* as the new combination *Halobyssothecium voraginesporum* on the basis of ITS, SSU, LSU, and TEF1-α sequence data and morphological characteristics **(Calabon *et al.*, 2021)**. Brown ascospores, a rough surface, and the absence of a gelatinous sheath are characteristic of this species **(Hyde *et al.*, 2016; Su *et al.*, 2016; Calabon *et al.*, 2021)**.

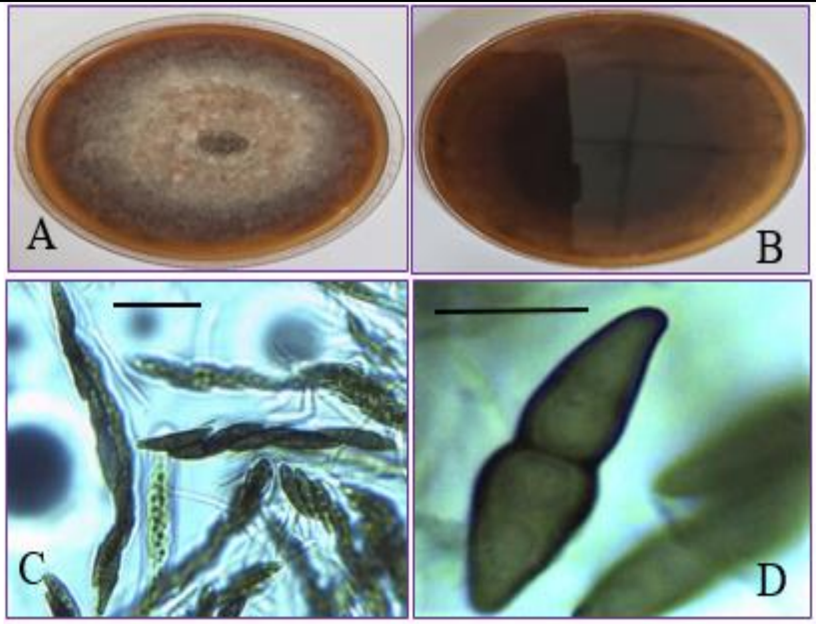

Fig. 4. *Halopyssothecium voraginesporum*. (A) Obverse view of the colony on PCA; (B) reverse view of the colony; (C) asci; (D) ascospore.

Scale bars: C and D = 10 µm.

Melanocarpus oblatus **Guarro & v.d. Aa, Persoonia 13 (3): 270 (1987)** (Fig. 5)

Colonies on PCA developed slowly, reaching 4 cm in 7 days at 25°C, and produced red and yellowish-white secretions. Ascomata are globose, 200–300 µm in diameter, submerged or semi-immersed, and covered with yellow hyphae. Asci are cylindrical, 8-spored, 40–45 × 10–12 µm, and evanescent. Ascospores are smooth, 6–8 × 6–7 µm, oblate, spherical in surface view and elliptical in lateral view; at maturity, they are dark brown and possess a distinct basal germ pore.

Examined material: Wood submerged in marine water in Al-Faw District, Arabian Gulf, Basrah, southern Iraq, 8 October 2018.

Notes: This species was first reported from the Upper Volta region of Africa as a single strain **(Arx *et al.*, 1987)** and was subsequently described and illustrated by **Guarro *et al.* (1996)**. *Melanocarpus oblatus* is mesophilic, unlike *M. albomyces*, which is highly thermophilic. **Guarro *et al.* (1996)** recorded *M. thermophilus* as a new combination. It had been described by **Abdullah and Al-Bader (1992)** as a new thermophilic variety recovered from soil in Mosul Forest, northern Iraq, under the genus *Thielavia* [*Thielavia minuta* (Cain) Malloch & Cain var. thermophila Abdullah and Al-Bader]. More recently, *Melanocarpus thermophilus* was transferred to the genus *Parvomelanocarpus* as *Parvomelanocarpus thermophilus* **(Wang *et al.*, 2022)**. The present strain of *M. oblatus* may represent the second record worldwide after strain CBS 775.85 described by **Arx *et al.* (1987)**.

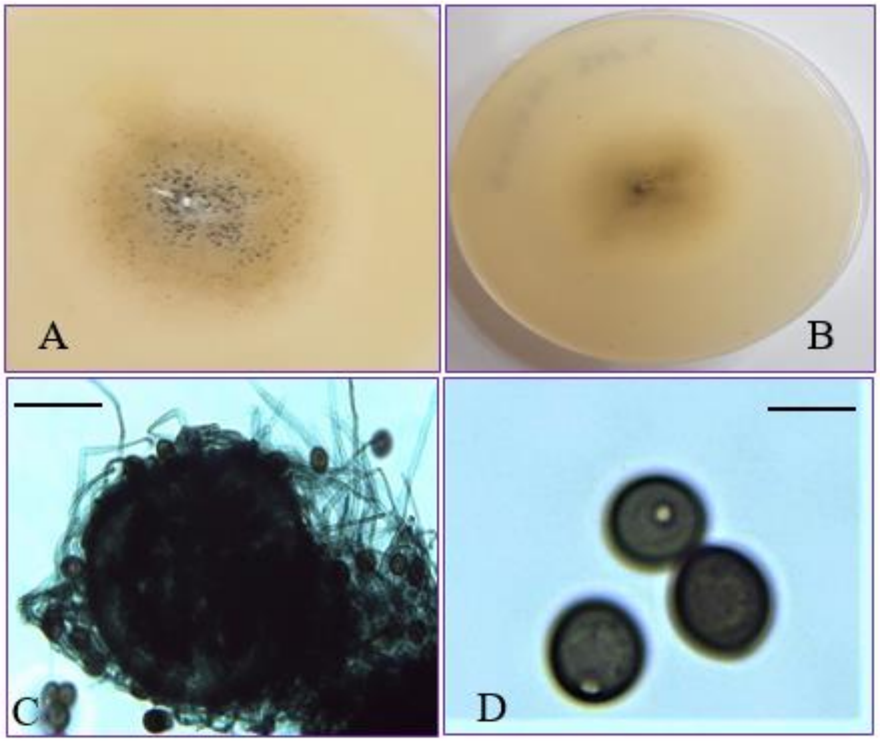

Fig. 5. *Melanocarpus oblatus*. (A) Obverse view of the colony on PCA; (B) reverse view of the colony; (C) ascoma; (D) ascospores.

Scale bars: C = 100 µm, D = 10 µm.

***Phaeonectriella lignicola* R.A. Eaton et E.BG. Jones, Nova Hedwigia 19 (3-4): 779 (1971)** (Fig. 6)

The ascoma has a neck measuring 100–317 × 46–63 µm; ascomata are 100–350 × 200–575 µm, globose to subglobose, submerged, hyaline to brown, and possess an ostiole. Asci are 8-spored, thin-walled, and 100–417 × 22–35 µm. Ascospores measure 26–30 × 9.5–11 µm, are ellipsoidal to fusiform, hyaline to light brown, bicellular, thin-walled, and somewhat constricted at the septum.

Examined material: Dead stems of *Phragmites australis* immersed in the eastern Hammar Marsh, Basrah, southern Iraq, 10 September 2018.

Notes: This species has been reported from submerged plant material in the United Kingdom and Mauritius **(Hyde *et al.*, 1999)**. The present collection lacked appendages, consistent with the original species description **(Eaton & Jones, 1971)**. **Jones (1995)** used this character to distinguish *Phaeonectriella* from the genus *Aniptodera*.

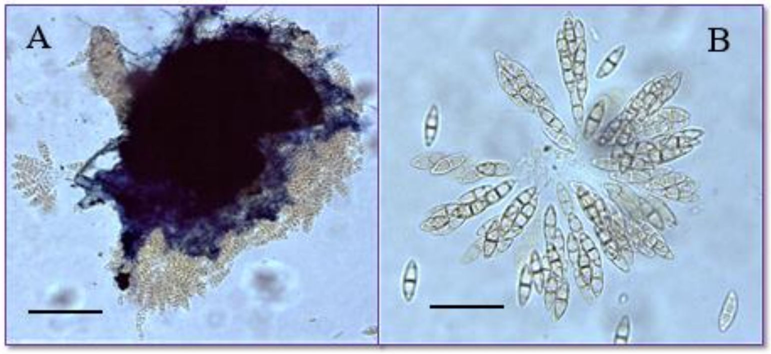

Fig. 6. *Phaeonectriella lignicola*. (A) Ascoma and asci; (B) asci and ascospores.

Scale bars: A = 100 µm, B = 20 µm.

***Preussia terricola* Cain, Canadian Journal of Botany, 39 (7): 1641 (1961)** (Fig. 7)

Colonies on PCA grew moderately, reaching 5 cm in 10 days at 25°C. Ascomata are 150–350 µm in diameter, globose to subglobose, and dark brown. Asci are cylindrical and measure 75–120 × 12–22 µm. Ascospores measure 26–32 × 5–5.5 µm, are dark brown and 4-celled, with cells approximately equal in size and shape; the longitudinal germ slit is parallel to the ascospore or slightly oblique, and no sheath is present.

Examined material: Dead stem of *Phragmites australis* submerged in the eastern Hammar Marsh, Basrah, southern Iraq, 10 September 2018.

Notes: Most species in this genus have been isolated from herbivore dung **(Chang & Wang, 2009)**, although many have also been isolated from soil, wood, and dead plant debris and have occasionally been reported as endophytes **(Guarro *et al.*, 1997a; Mouchacca, 2004; Kruys, 2015)**. *Preussia terricola* has been isolated from soil, plants, and animal dung **(Kruys & Wedin, 2009; Guarro *et al.*, 2012)**. Several *Preussia* species produce bioactive secondary metabolites, particularly ceussomerins, that have demonstrated antibacterial activity **(Chang & Wang, 2009; Mapperson *et al.*, 2014)**.

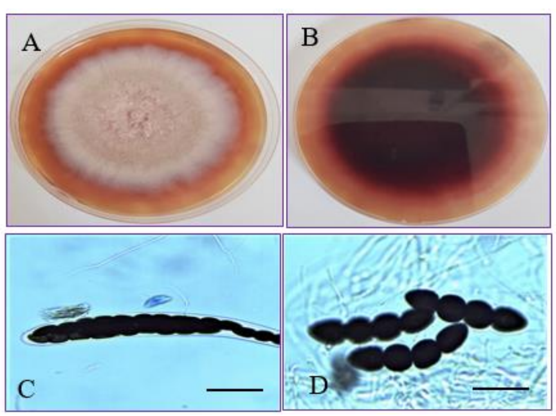

Fig. 7. *Preussia terricola*. (A) Obverse view of the colony on PCA; (B) reverse view of the colony; (C) ascus; (D) ascospores.

Scale bars: C and D = 20 µm.

Pseudorhypophila marina **(Furuya & Udagawa) Y. Marin & Stchigel, Journal of Fungi 7 (3, no.181): 10 (2021)** (Fig. 8)

Basionym: *Zopfiella marina* Furuya & Udagawa, Journal of Japanese Botany 50: 249 (1975).

Synonym: *Zopfiella submersa* Guarro, Al-Saadoon; Gene & Abdullah, Mycologia 89 : 958 (1997)

Colonies on PCA expanded rapidly, reaching 7–8 cm in 7 days at 25°C. Colonies were light brown, with a brown to light-brown reverse. Ascomata are globose, brown to black, and covered with septate, branched hyphae. Asci are clavate, shortly stipitate, evanescent, and 8-spored. Ascospores are elliptical and 1-septate; the upper cell measures 13–22 × 10–15 µm, is brown, has a truncate base, and bears an apical germ pore, whereas the lower cell measures 5–8 × 3–5 µm and is hyaline, cylindrical, and thin-walled. Conidia are borne along peg-like structures.

Examined material: Dead stems of *Phragmites australis* immersed in the eastern Hammar Marsh, Basrah, southern Iraq, 10 September 2018.

Notes: *Triangularia mangenotii* and three species formerly placed in *Zopfiella* (*Z. marina*, *Z. pilifera*, and *Z. submersa*) were incorporated into *Pseudorhypophila* on the basis of combined ITS, LSU, rpb2, and β-tubulin sequence data, and the new genus was placed in the family Novicularisporaceae **(Harms *et al.*, 2021)**. *Zopfiella submersa* was introduced as a new species by **Guarro *et al.* (1997b)** and compared with several members of *Zopfiella*, including *Z. marina*, which was first described by **Furuya and Udagawa (1975)**. **Harms *et al.* (2021)** considered these species to represent the same taxon and proposed their synonymy on the basis of morphological and phylogenetic similarity. *Z. marina* develops an asexual morph and ascospores with an apical pore and was isolated from marine mud at a depth of 120 m and 500 km from the coast of China **(Furuya & Udagawa, 1975)** in contrast to *Z. submersa*, which was isolated from submerged dead culms of *Arundo donax* and *Phragmites* sp. in the Euphrates River, Iraq **(Guarro *et al.*, 1997b)**,

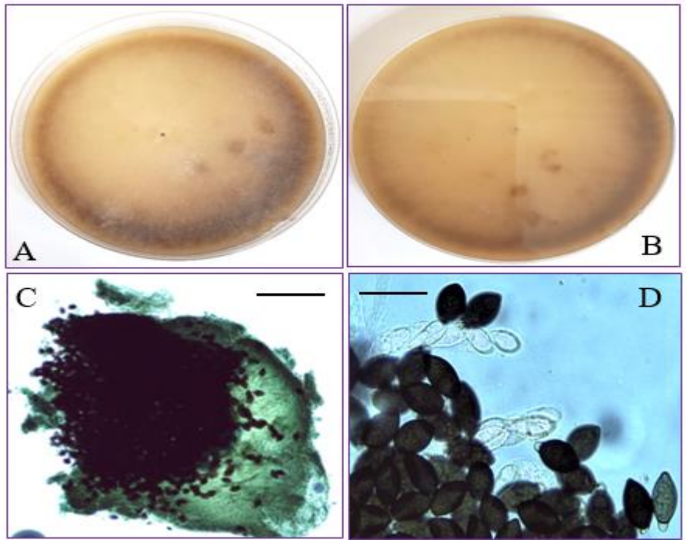

Fig. 8. *Pseudorhypophila marina*. (A) Obverse view of the colony on PCA; (B) reverse view of the colony; (C) ascoma and ascospores; (D) asci and ascospores; (E) ascospores.

Scale bars: C = 50 µm, D and E = 10 µm.

***Trematosphaeria* p ertusa (Pers.) Fuckel, J.b., Nassauischen Vereins Naturk. 23-24:162 (1870)** (Fig. 9)

At 25°C, colonies on PCA grew moderately, reaching 4 cm in 10 days; colonies were raised and woolly, with a dark-grey surface and dark reverse. Ascomata measure 280–380 × 200–350 µm, are submerged to partially submerged, aggregate or solitary, globose to clavate, black, and possess a neck. Asci measure 70–100 × 12–15 µm, are clavate to cylindrical, 8-spored, and biseriate. Ascospores measure 22–27 × 6–8 µm, are ellipsoidal, dark brown, uniseptate and occasionally triseptate, somewhat curved, and constricted at the septum.

Examined material: Dead stems of *Phragmites australis* immersed in the Shatt Al-Arab River near the Al-Karma River, Basrah, southern Iraq, 24 October 2018.

Notes: According to **Suetrong *et al.* (2011)** and **Zhang *et al.* (2012)**, *Trematosphaeria pertusa* is a terrestrial fungus capable of surviving in freshwater environments. In Europe, it has been isolated from decomposing wood and bark of *Fraxinus excelsior* and from wood of *Platanus* submerged in water **(Zhang *et al.*, 2012)**. It has also been isolated from woody branches submerged in rivers and ponds in Japan **(Tanaka *et al.*, 2015)**. Multigene phylogenetic analysis recently assigned *Madurella grisea* to the genus *Trematosphaeria*; *T. grisea* has been isolated from human infections, water, and some foods **(Ahmed *et al.*, 2014)**. Compared to strains described by **Zhang *et al.* (2008)**, isolates reported in the current study had smaller asci and ascospores consistent with those reported by **Tanaka *et al.* (2015)**.

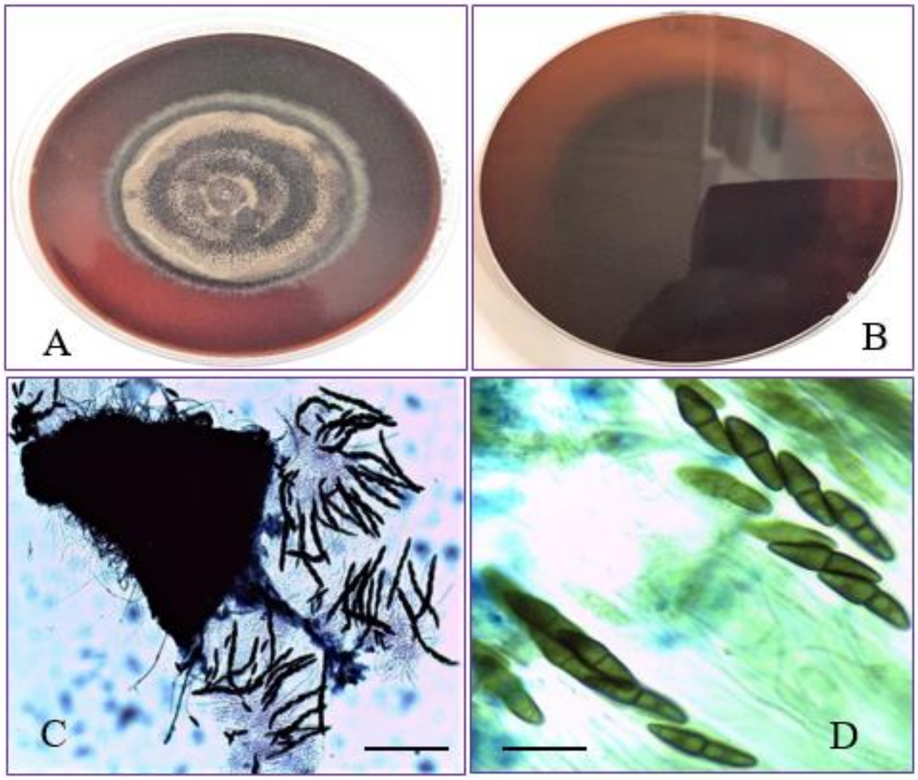

Fig. 9. *Trematosphaeria pertusa*. (A) Obverse view of the colony on PCA; (B) reverse view of the colony; (C) ascoma and asci; (D) ascospores.

Scale bars: C = 200 µm, D = 20 µm.

***Trichocladium achrasporum* (Meyers & R.T. Moore) M. Dixon ex Shearer & J.L. Crane, Mycologia 63 (2): 244 (1971)** (Fig. 10).

At 25°C, colonies on PCA grew moderately, reaching 6 cm in 10 days, and were black. Conidiophores are poorly differentiated, short, simple, straight, and smooth. Conidia measure 20–30 × 10–20 µm, are dark brown and 2–3-septate, and show only slight constriction at the septa. No teleomorph was recorded for this isolate.

Examined material: Submerged wood and dead *Typha domingensis* stems from the eastern Hammar Marsh, Basrah, southern Iraq, 10 October 2018.

Notes: This species was recorded as the asexual stage of *Halosphaeria mediosetigera* isolated from wood immersed in marine water **(Shearer & Crane, 1977; Jones *et al.*, 1984)**. Subsequent research, based on morphological and molecular characteristics, transferred this species to the genus *Halosphaeriopsis* **(Sakayaroj *et al.*, 2005; Jones *et al.*, 2009)**. In both its sexual and asexual forms, the fungus is marine and can tolerate high salt concentrations **(Shearer & Crane, 1977)**. Marine fungi tolerate high salinity through sophisticated physiological, genetic, and structural mechanisms that maintain cellular water and ionic balance. These mechanisms include the production and accumulation of osmotically active organic compounds, such as glycerol, within the cytoplasm to balance external osmotic pressure and prevent water loss and loss of cell cohesion; ion homeostasis; efflux pumps and membrane transporters; and activation of genetic and signaling pathways, among other mechanisms **(Gunde-Cimerman *et al.*, 2018; Jones *et al.*, 2022; Fernando *et al.*, 2023)**. In the present study, the species was isolated from *Typha domingensis* and submerged wood in the Hammar Marsh, where salinity reached 10.6%.

Fig. 10. *Trichocladium achrasporum*. Conidia and conidiophores.

Scale bar: = 20 µm.

CONCLUSION

Lignicolous fungi are an essential component of aquatic microbial communities. The aquatic environments of southern Iraq likely contain many fungal species that remain undocumented. The present study provides evidence that several species known from marine habitats can also occur in lower-salinity aquatic habitats in this region, although broader ecological sampling is required to determine the extent and direction of habitat transitions. Continued taxonomic and ecological surveys are therefore needed to document the remaining fungal diversity of southern Iraqi aquatic environments.

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