European Journal of Biology and Biotechnology
Vol 5 | Issue 3 | May 2024
ISSN 2684-5199

RESEARCH ARTICLE

New Species of *Preussia* from Sedimentary Cost in Basrah Province, Iraq

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ABSTRACT

A novel undiscovered fungal species was obtained from brackish environments located in Al-Faw city, situated in the southern region of Basrah, Iraq, through phylogenetic assessments of the ITS and TEF1α genomic regions. Sediment sample was collected from the seacoast of Al-Faw region and cultured on Potato carrot agar (PCA) and Potato dextrose agar (PDA), then incubated at 25 °C for 14 days. It was ascertained that this species clustered within the genus *Preussia*. Subsequent in-depth examinations of its morphological and anatomical features corroborated its distinctiveness. This previously unknown species is introduced here as *P. aseelix*. One of its notable characteristics is the absence of a true fruiting body, which is replaced by an asexual state represented by pycnidia.

Keywords: Basrah, New species, *Preussia*, Sediment.

Submitted: March 12, 2024
Published: May 17, 2024

10.24018/ejbio.2024.5.3.510

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1. Introduction

Microorganisms adapted to halophilic environments flourish in the challenging conditions found in these habitats, having developed molecular and cellular mechanisms to manage factors like osmotic pressure and reduced water activity. Such environments are teeming with fungal species that remain unidentified, demonstrating a promising area for further exploration (Sayed *et al*., 2020; Wingfield *et al*., 2023).

Ascomycota is one of the largest fungal phyla and encompasses approximately 93,000 existing species (Bennett & Turgeon, 2017). Members of Ascomycota are ubiquitous and distributed across terrestrial, freshwater, and marine ecosystems (Bennett & Turgeon, 2016). Sexual reproduction in ascomycetes frequently occurs in response to adverse environmental conditions, leading to significant genetic diversity among species (Nieuwenhuis & James, 2016; Agrawal *et al*., 2018). The purpose of purging deleterious mutations and selecting advantageous mutations for adaptation to fluctuating environments (Otto & Lenormand, 2002).

Preussia (Sporormiaceae, Pleosporales, Dothideomycetes) was proposed by Fuckel (1866) to include species of ascomycetes with non-ostiole ascomycota, bitunicate asci and multi-celled ascospores with a germ slit in each cell and a gelatinous hyaline sheath, inhabiting mainly soil and fallen dead leaves, while anamorphs is usually represented by *Chrysosporium* spp. and *Phoma* spp. (Von Arx, 1973; Asgari & Zare, 2010; Crous *et al*., 2018; Lücking *et al*., 2020). Recently, the Index of Fungorum (2020) has listed 98 species of *Preussia*. In Iraq, many new species belonging to this genus were isolated, most of which were isolated from dung and plant debris (Guarro *et al*., 1997a, Guarro *et al*., 1997b, Abdullah *et al*., 1999) in addition, *Preussia dispersa* was also isolated from the marshes of southern Iraq by Abdullah *et al*. (2010).

2. Materials and Methods

2.1. Sample Collection

The sample of sedimentary soil was collected from the seacoast sediment of the Al-Faw region during August 2021, while the salinity and pH of the soil sample were calculated by salinometer (Lovibond, Germany) and pH meter (Adwa, Romany), respectively.

Two different types of media, Potato carrot agar (PCA) and Potato dextrose agar (PDA) (Himedia), were used for the isolation of fungus isolate by using the dilution method described by Wicklow and Whittingham (1974) culture media were incubated at 25 °C for two weeks. After incubation period, fungal growth was examined and purified then pure colonies were kept in PDA slant at 4 °C.

2.2. Identification Study

The fungal isolate was identified morphologically according to the appearance of colonies in addition to microscopic properties.

2.3. Molecular Identification and Phylogenetic Analysis

Genomic DNA extraction of the X6 isolate, primers synthesis, PCR amplification, and sequencing were conducted at Macrogen Company, South Korea. The universal primers ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) were used to amplify the ITS gene encoding the 5.8S rRNA (ITS1-5.8SrRNA-ITS2 fragment) (White *et al*., 1990). For further identification TEF1α primers were used through amplification the translational elongation factor 1α (TEF1α) using primers EF1–1018F (5′-GAY TTC ATC AAG AAC ATG AT-3′) and EF1–1620R (5′-GAC GTT GAA DCC RAC RTT GTC-3′). Gradient PCR was applied ranging from (48 °C–68 °C). The best annealing Temp was 48 °C, as shown in Fig. 1. The sequence results were analysed by BLAST, Basic Local Alignment Search Tool, and compared with the database at NCBI, the National Centre for Biotechnology Information (Altschul *et al*., 1997). The sequences were deposited to Gen Bank for accession Number confirmation. Alignments for each data set were made by MEGA11 using the ClustalW algorithm and refined with MUSCLE. The alignment comprised sequences from isolated species and those representing various species within the genus of *Preussia*, sourced from the NCBI database. Sequence phylogeny was constructed employing Maximum Likelihood (ML) and the most suitable nucleotide substitution model determined by MEGA11. Models with the lowest Bayesian Information Criterion (BIC) scores are regarded as the most accurate descriptors of the substitution pattern. The bootstrap consensus tree was inferred from 1000 replicates. After genomic analysis, the sequence data of isolated endophytic fungi were submitted to GenBank for Accession Number verification.

2.4. Result of Phylogeny

The PCR amplification of the ITS region and TEF from isolate X6 produced PCR products measuring 500 and 567 base pairs (bp), respectively. For species identification, the sequences generated in this study were aligned against all sequences of well-documented *Preussia* species. The outcome of BLAST searching, indicating that the isolated species X6 has 88.58% similarity (ITS) with *Preussia* sp. (in: Fungi) isolate CK1259 (MH473918.1) and 96.72% similarity (TEF) with *Preussia* sp. (in: Fungi) strain SEED1 (KX894788.1). Thus, this isolated species was identified as a new species of *Preussia* genus and was named as *Preussia aseelix*. The gene bank accession numbers for X6 (ITS) and (TEF) were (OP214773) and (OP974691) respectively were confirmed and given by NCBI. This fungal species was reported first time in Basrah, IRAQ as the current study showed. Phylogenetic trees were constructed using DNA sequences from the isolated species in conjunction with reference strains. The resulting phylogenetic tree revealed a discernible relationship between the isolated species and the reference strains (Figs. 2A and 2B).

2.5. Physiological Assays

The physiological assays included measuring the growth at different temperatures (25 °C, 28 °C, and 37 °C) on PDA, as well as the test of cycloheximide resistance was evaluated.

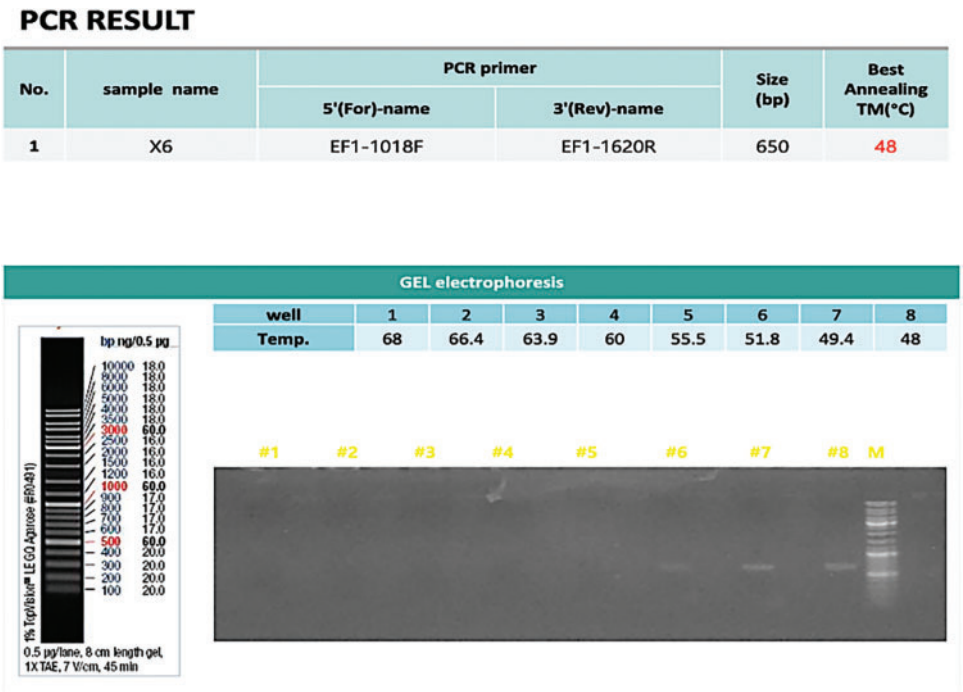

Fig. 1. The best annealing temperature for TEF1α region amplification.

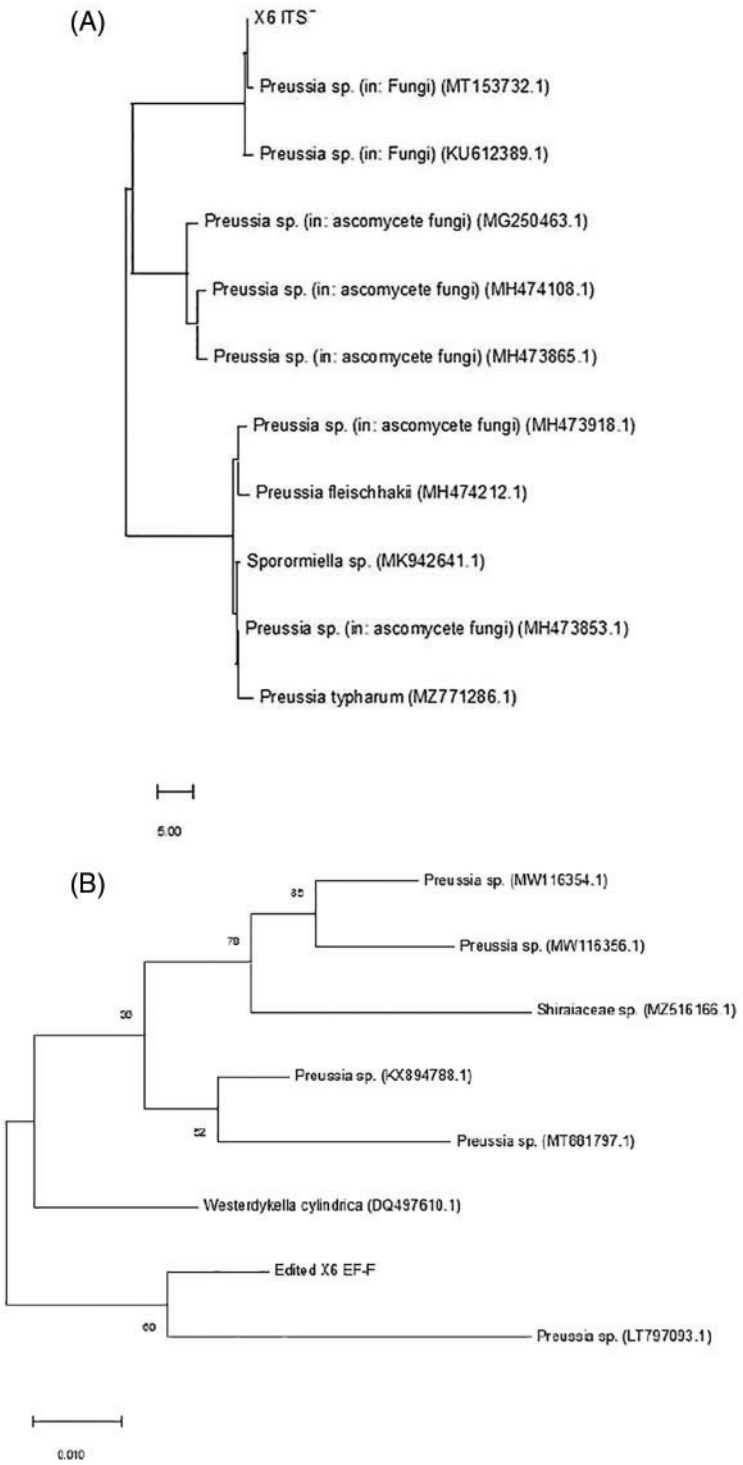

Fig. 2. Phylogenetic tree represents neighbour-joining analysis of (A) ITS domain and (B) TEF1α gene sequences depicting the relationships of the isolated fungi (isolate_X6) with closely related reference sequences of *Preussia* species retrieved from NCBI. Each numerical value represents the percentage of bootstrap samples, a total of 1000 samples, that support the internal branches with a confidence level of 50% or higher.

3. Results and Discussion

The present finding revealed the isolation of *Preussia aseelix* as a new species of the world from sediment samples collected from southern Iraq. The salinity of samples was reached 51.3 ppt, whereas the acidity recorded at 8.12, and the sediment temperature was 30 °C.

3.1. Growth Condition

The optimum temperature for growth was 25 °C, in spite of the growth was failed at 37 °C. On the other hand, the isolate showed resistance of cycloheximide.

3.2. Taxonomy

Based on morphological notes and outcomes of molecular information and phylogenetic analysis, we erect the new species as follows:

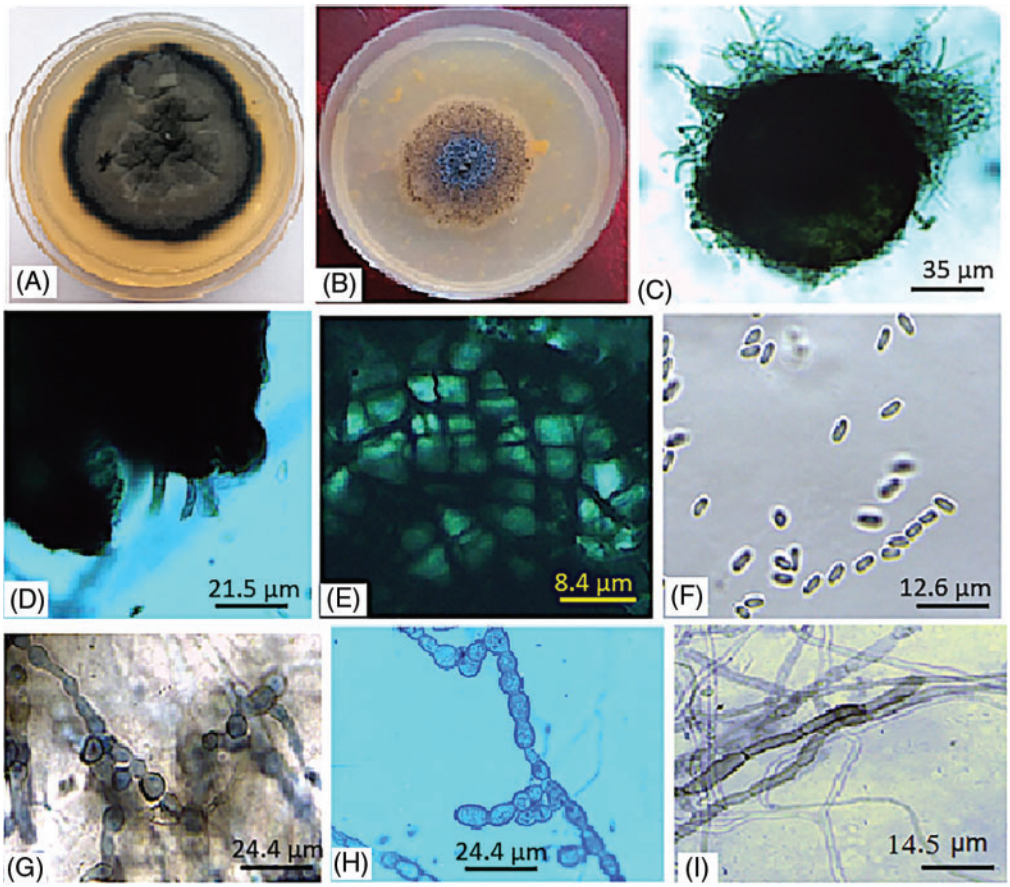

Fig. 3. The morphological feature of the isolated species, *Preuassia aseelix:* (A) Colony on PDA after 14 days; (B) Colony on PCA after 14 days; (C) pycnidial conidiomata; (D) Setae; (E) Pycnidial wall; (F) Conidia; (G,H) Chlamydospores; (I) Vegetative hyphae.

- *Preussia aseelix* Almusa, Al-Maqtoofi, Alrubayae, and Altooma, sp.nov.
- *Etymology:* Named based on the first name of the author.
- Teleomorph undetermined. Anamorph coelemycetous. Vegetative hyphae subhyaline, thick walled, septate, branched, smooth walled (2.4–3.8 µm × 10.5–13 µm) wide. Conidiomata pycnidial, superficial or partly immersed, dark brown to black, globose to ellipsoidal or pyriform, solitary or aggregated 12.2–17 µm, with a narrow ostiole, pycnidial wall composed of hyaline to brown cells (2.6–4.3 µm × 2.6–4.7 µm). The external appearance of the pycidial wall is often coated with setae that seen as ramify scattered and straight, soft, varied from light to dark brown, septate (2.3–2.42 µm × 9.3–12.5 µm), with sharpened apex. Conidiophores are reduced to conidiogenous cells. Conidiogenous cells phialidic, hyaline, smooth, ampuliform to doliiform, conidia unicellular, hyaline, ampuliform, cylindrical, straight or slightly curved, aseptate, occasionally allantoids, often biguttulate, 1.15–1.84 µm × 2.9–4 µm. Chlamydospores globose to subglobose, unicellular, arranged intercalary or terminal, occasionally polygonal, brown to olivaceous in color, always found in the chain (6.7–8.2 × 7.3–11 µm).
- *Culture characteristics:* colonies on PDA attaining 30–40 mm diameter in two weeks at 25 °C, downy, floccose, grey becoming dark, deeply immersed into the agar, crinkled, raised in the centre and flat at the edges, with regular margins, reverse yellowish brown. Meanwhile, colonies on PCA with a diameter of 30–35 mm in two weeks at 25 °C, forming concentring rings composed of pycnidia, flat with buff shades at the edges, margins regular, and reverse of the same colour (Fig. 3).

Similarly, a new species of *Preuassia* named *Preussia mediterranea* was recorded from an endophyte of different typical Mediterranean plant species, which was described and illustrated based on molecular and morphological features. The new species *Preussia mediterranea resembles P. australis, P. africana and P. similis* from a morphological point of view, but appeared closer to *P. minima and P. isabellae* by molecular analysis (Arenal *et al.,* 2007).

Interestingly, the present isolate resembles another new species, *Preuassia aegilopis*, that was isolated as an endophytic species from seeds of Aegilops umbellulata in Iran by not having a real fruiting body and being limited to the asexual phase only but differed with its morphologically and genetically (Mehrabi *et al.,* 2022).

Finally, the current investigation showed a description of novel *Preussia* species depending on morphological features and phylogenetic analysis which drawn from sequence analysis of the ITS region that does not give convincing results, while TEF1α region confirms the identification of new species.

Acknowledgment

We would like to express our special thanks to our instructor, Prof. Abdullah Al-Saadoon, for his benefit information in the classification field of fungi.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

References

Abdullah, S. K., Al-Dossari, M. N., & Al-Imara, F. J. (2010). Mycobiota of surface sediments in marshes of Southern Iraq. *Marsh Bulletin*, *5*, 14–26.

Abdullah, S. K., Al-Saadoon, A. H., & Guarro, J. (1999). New and interesting coprophilous ascomycetes from Iraq. *Nova Hedwigia*, *69*, 211–216.

Agrawal, S., Adholeya, A., Barrow, C. J., & Deshmukh, S. K. (2018). Marine fungi: An untapped bioresource for future cosmeceuticals. *Phytochemistry Letters*, *23*, 15–20. https://doi.org/10.1016/j.phytol.2017.11.003.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., *et al.* (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, *25*, 3389–3402. https://doi.org/10.1093/nar/25.17.3389.

Arenal, F., Platas, G., & Pelaez, F. (2007). A new endophytic species of *Preussia* (Sporormiaceae) inferred from morphological observations and molecular phylogenetic analysis. *Fungal Diversity*, *25*, 1–17.

Asgari, B., & Zare, R. (2010). Two new species of *Preussia* from Iran. *Nova Hedwigia*, *90*, 533–548. https://doi.org/10.1127/0029-5035/2010/0090-0533.

Bennett, R. J., & Turgeon, B. G. (2016). Fungal sex: The Ascomycota. *Microbiology Spectrum*, *4*, 10–1128. https://doi.org/10.1128/microbiolspec.funk-0005-2016.

Bennett, R. J., & Turgeon, B. G. (2017). Fungal sex: The Ascomycota. *The Fungal Kingdom*.

Crous, P. W., Schumacher, R. K., Wingfield, M. J., Akulov, A., Denman, S., Roux, J., *et al.* (2018). New and interesting fungi. 1. *Fungal Systematics and Evolution*, *1*, 169–215. https://doi.org/10.3114/fuse.2018.01.08.

Fuckel, L. (1866). Fungi Rhenani exsiccati. Cent. 12–17. *Hedwigia*, *5*, 14–30.

Guarro, J., Abdullah, S. K., GenÉ, J., & Al-Saadoon, A. H. (1997a). A new species of *Preussia* from submerged plant debris. *Mycological Research*, *101*, 305–308. https://doi.org/10.1017/S0953756296002638.

Guarro, J., Al-Saadoon, A., & Abdullah, S. (1997b). Two new coprophilous species of *Preussia* (Ascomycota) from Iraq. *Nova Hedwigia*, *64*, 177–184. https://doi.org/10.1127/nova.hedwigia/64/1997/177.

Index of Fungorum (2020). http://www.indexfungorum.org/Names/Names.asp [Accessed November 2020].

Lücking, R., Aime, M. C., Robbertse, B., Miller, A. N., Ariyawansa, H. A., Aoki, T., *et al.* (2020). Unambiguous identification of fungi: Where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus*, *11*, 14. https://doi.org/10.1186/s43008-020-00033-z.

Mehrabi, M., Asgari, B., & Zare, R. (2022). Novel endophytic species of Coniochaeta and *Preussia* from the botanical tribe Triticeae in Iran. *Nova Hedwigia*, *114*, 141–169. https://doi.org/10.1127/nova_hedwigia/2022/0668.

Nieuwenhuis, B. P., & James, T. Y. (2016). The frequency of sex in fungi. *Philosophical Transactions of the Royal Society B Biological Sciences*, *371*, 1–12. https://doi.org/10.1098/rstb.2015.0540.

Otto, S. P., & Lenormand, T. (2002). Resolving the paradox of sex and recombination. *Nature Reviews Genetics*, *3*, 252–261. https://doi.org/10.1038/nrg761.

Sayed, A. M., Hassan, M. H. A., Alhadrami, H. A., Hassan, H. M., Goodfellow, M., & Rateb, M. E. (2020). Extreme environments: Microbiology leading to specialized metabolites. *Journal of Applied Microbiology*, *128*, 630–657. https://doi.org/10.1111/jam.14386.

Von Arx, J. (1973). Ostiolate and nonostiolate Pyrenomycetes. *Proc Koninkl Nederl Akad Wet, Ser C*, 289–296.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, *18*, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.

Wicklow, D. T., & Whittingham, W. F. (1974). Soil microfungal changes among the profiles of disturbed conifer-hardwood forests. *Ecology*, *55*, 3–16. https://doi.org/10.2307/1934613.

Wingfield, L. K., Jitprasitporn, N., & Che-Alee, N. (2023). Isolation and characterization of halophilic and halotolerant fungi from man-made solar salterns in Pattani Province, Thailand. *PLoS One*, *18*, e0281623. https://doi.org/10.1371/journal.pone.0281623.