Morphological and ITS-Based Phylogenetic Characterization of Truncospora ochroleuca: A New Record from Northwestern Tunisia

Zouhour OUALI

ouali.zzouhour@gmail.com

Banque Nationale de Gènes

Imed SBISSI

University of Gabes, Institute of Arid Regions of Médenine

Giuseppe VENTURELLA

University of Palermo

Maria Letizia GARGANO

University of Bari Aldo Moro

Atef JAOUANI

Tunis El Manar University

Research Article

Keywords: Truncospora, North Africa polypore, poroid fungi, phylogenetic analyses

Posted Date: June 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9541666/v1

Additional Declarations: No competing interests reported.

Abstract

Truncospora ochroleuca is a polypore species belonging to the family Polyporaceae (Polyporales), previously reported from Europe, Asia, Africa, and the Americas. In this study, we report the first record of *T. ochroleuca* from northwestern Tunisia, where it was collected on a living *Pinus pinea* L. tree in a subhumid forest ecosystem. The species was characterized using macro- and micromorphological features, cultural characteristics, and molecular phylogenetic analyses based on the internal transcribed spacer (ITS) rDNA region. Morphologically, the Tunisian specimen exhibited typical diagnostic traits of *T. ochroleuca*, including small ungulate basidiomata, a di- to trimitic hyphal system with dextrinoid skeletal hyphae, and thick-walled, ellipsoid, strongly dextrinoid basidiospores.

Phylogenetic analyses (MP, ML, and Bayesian inference) confirmed the placement of the specimen within the monophyletic *Truncospora* clade, clearly distinct from *Perenniporia* sensu stricto. The Tunisian sequence showed 99.45% similarity to a French strain of *T. ochroleuca*.

This finding expands the known distribution of the species in the Mediterranean region and highlights the underexplored diversity of polypore fungi in North Africa. Further multilocus phylogenetic studies are recommended to better resolve species boundaries within the genus *Truncospora*.

Introduction

Truncospora was originally proposed as a genus by Pilát (1941). It is distinguished by small, pileate basidiocarps; a di- to trimitic hyphal system with clamped generative hyphae; dextrinoid and cyanophilous skeletal hyphae; and hyaline, thick-walled, truncate, ellipsoid, and strongly dextrinoid basidiospores (Zhao and Cui 2013). In 1953, Pilát validly published the genus with two species: *Truncospora oboensis* Decock and *T. ochroleuca* (Berk.) Pilát.

Ryvarden (1972) subsequently regarded *Truncospora* as a synonym of *Perenniporia* Murrill due to their morphological similarities (Decock 2011; Li et al. 2018; Zhao et al. 2013). However, later phylogenetic analyses demonstrated that *Truncospora* represents a genetically distinct, monophyletic lineage within the “core polyporoid clade,” clearly separated from *Perenniporia* (Robledo et al. 2009; Zhao and Cui 2013; Ji et al. 2023).

The genus *Truncospora* includes both temperate and tropical species distributed globally, with records from Africa (Decock 2011; Ryvarden and Johansen 1980), the Americas (Zhao et al. 2016), Asia (Zhao and Cui 2013), and Europe (Spirin et al. 2015). Several species within the genus remain insufficiently studied. Among the known taxa, *T. ochroleuca*, a member of the order Polyporales and family Polyporaceae, is primarily associated with living hardwoods and only rarely occurs on conifers.

T. ochroleuca is considered a tropical to subtropical species and remains very rare in Europe. It was first recorded in Portugal (Pinto-Lope, 1953), then in Spain and France (Jahn, 1972; 1973), where it grows on variety of living hardwood hosts and rarely on conifers (Reid, 1975). In Europe, it is mainly associated

with the Mediterranean region and its characteristic hosts (Bernicchia and Gorjón, 2019). However, its distribution extends beyond the Mediterranean, with additional records reported along the coasts of southern England and Wales (Ryvarden and Melo, 2017).

In this study, we report the first occurrence of *T. ochroleuca* from northwestern Tunisia. The specimen was collected from a living *Pinus pinea* L. tree and is characterized through morphological and phylogenetic analyses.

Materials and Methods

Site Description and Sampling Procedure

The specimen was collected in northwestern Tunisia, in the dense and spiny forest of the Nefza region (NEF) (Béja Governorate (BE)), specifically in Bellif 1 forest (BEL1) (37°05'50" N; 9°01'56.3" E; 302 m a.s.l.). The area belongs to the subhumid bioclimatic stage according to Emberger (1955), with annual rainfall ranging from 800 to 1000 mm, and mean maximum and minimum temperatures of 36°C and 6.6°C, respectively.

Soil type (calcareous clay) was determined using data from the National Research Institute of Rural Engineering, Water and Forests (Address Street Hédi El Karray El Menzah IV, 1004 Tunis, BP 10 Ariana 2080 Tunisia). Soil pH (6.6) was measured according to ISO standards (1994).

The sporophore was collected from a living *Pinus pinea* L. tree. Photographs were taken in situ prior to careful removal of the sporophore. After macroscopic examination, part of the specimen was air-dried at room temperature and deposited in the herbarium of the Laboratory of Mycology, National Gene Bank of Tunisia (NGBT) under the inventory code BE_NEF_BEL1_N440. The remaining material was immediately lyophilized (Unicryo MC4L, UniEquip GmbH, Germany) and stored for subsequent analyses.

The original strain of *T. ochroleuca* was isolated from internal basidiome tissues on Potato Dextrose Agar (PDA) and purified under laboratory conditions. Living cultures are preserved at the Laboratory of Mycology (NGBT), with a duplicate deposited at the Laboratory of Environmental Sciences and Sustainable Development (LASED).

Macromorphological characteristics were recorded from fresh sporophores in the field. Micromorphological features, including hyphal system structure and basidiospore characteristics, were examined from dried specimens. Microscopic observations were conducted on slide preparations stained with Cotton Blue, following Zhao et al. (2015). Sections were analyzed using a Leica DLMB light microscope. Species nomenclature follows Index Fungorum (http://www.index-fungorum.org/Names/Names.asp).

DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted using the FavorPrep™ Plant Genomic DNA Mini Kit according to the manufacturer's protocol. The internal transcribed spacer (ITS) region was amplified using the primer pair ITS1/ITS4 (Vilgalys and Hester 1990; White et al. 1990). PCR amplification was performed as described by Ouali et al. (2018). PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (New England *BioLabs*). Sequencing reactions were carried out using the same primers on an Applied Biosystems 3130 DNA Analyzer at the Laboratory of Microorganisms and Active Biomolecules (LMBA-FST), Faculty of Sciences of Tunis, Tunisia. The obtained ITS rDNA sequence was compared with sequences available in GenBank using the BLAST algorithm. The newly generated sequence was deposited in GenBank under accession number PV476753.

Phylogenetic Analysis (dup: abstract ?)

The ITS sequence obtained from the Tunisian specimen was analyzed together with reference sequences retrieved from GenBank. Multiple sequence alignments were performed using ClustalW (Thompson et al. 1997). The best-fit nucleotide substitution model was selected using ModelTest-NG (Darriba et al., 2020) based on the Akaike Information Criterion (AIC). All characters were treated as equally weighted and unordered, and gaps were considered as missing data. Phylogenetic trees were reconstructed using Maximum Parsimony (MP) in PAUP* 4.0b10 (Swofford, 2002) and Maximum Likelihood (ML) in RAxML (Stamatakis 2014). Node support was evaluated by bootstrap analysis with 1000 replicates. *Heterobasidion annosum* (KC492906) and *Stereum hirsutum* (AB733150) were used as outgroup taxa.

Bayesian inference was conducted using MrBayes 3.2.9 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003). Two independent runs were performed for 400,000 Markov Chain Monte Carlo (MCMC) generations under default settings. The average standard deviation of split frequencies (ASDSF) was below 0.01 at the end of the analysis, indicating convergence.

Results

Morphological Analysis

The collected specimen grows on wood and developed a small sporophore with a soft and spongy texture when fresh. The sporophore was attached by a narrow base, applanate to ungulate (hoof-shaped), measuring up to 5 cm in width, projecting 3 cm horizontally from the substrate, and approximately 0.5 cm thick (Fig. 1(a)).

The upper surface is glabrous to slightly velvety, white to cream-ochraceous, and distinctly zonate, with pale yellowish-brown to pale purplish-brown concentric bands. The margin is thick, rounded, slightly lobed, and lighter in color (Fig. 1(b)). The pore surface is white to pale cream, with circular pores clearly visible to the naked eye (Fig. 1(c)). When dry, the sporophore became woody and hard, displaying straw to wood coloration. The context showed a single or weakly stratified tube layer (Fig. 1(d)). These

macromorphological features are consistent with previous descriptions of *T. ochroleuca* (Zambrano-Forero 2023). This record adds a new polypore species to the fungal inventories previously reported from Tunisia (Ouali et al. 2018, 2020, 2025).

Microscopically, the specimen exhibited a di- to trimitic hyphal system. Generative hyphae are clamped, and skeletal hyphae were thick-walled (up to 4.5 µm wide), dextrinoid, and cyanophilous (Fig. 2(a, b)). Basidiospores measured on average 14.38 × 8.75 µm, are ellipsoid, hyaline, thick-walled, smooth, and strongly dextrinoid (Fig. 2(c, d)), in agreement with the diagnostic features reported by Zhao and Cui (2013).

Cultural Characteristics

On Potato Dextrose Agar (PDA) at 25°C, *T. ochroleuca* exhibited very slow radial growth, reaching maximum mycelial development after 25–30 days of incubation. The culture showed complete but slow radial colonization, forming a dense and compact mycelium with a hard appearance. A noticeable discoloration of the culture medium (browning of the medium under the colony) was observed during growth (Fig. 3), suggesting significant enzymatic activity typical of wood-degrading fungi.

Phylogenetic Analysis

Phylogenetic analysis based on ITS rDNA sequences revealed two well-supported clades corresponding to the genera *Perenniporia* and *Truncospora*. The newly generated ITS sequence was aligned with 42 reference sequences of *Truncospora* and *Perenniporia* retrieved from previous studies (Zhao and Cui 2013; Zhao et al. 2013). *Stereum hirsutum* (Willd.) Pers. (AB733150) and *Heterobasidion annosum* (Fr.) Bref. (KC492906) were used as outgroup taxa (Fig. 4).

PCR amplification of the ITS region yielded a fragment of approximately 582 base pairs. The final alignment comprised 44 sequences and 485 characters, of which 397 were conserved, 24 were parsimony-informative, and 64 were variable.

Comparative analysis of ITS sequences from *T. ochroleuca* specimens originating from East Asia (China) and Europe (Czech Republic and France) revealed clear sequence divergence among geographic populations (Fig. 4), supporting observations reported by Spirin et al. (2015).

The Tunisian specimen clustered within the *Truncospora* clade and showed 99.45% sequence identity with *T. ochroleuca* strain MUCL 46106 (GQ355957) from France (Fig. 4).

Although the internal transcribed spacer (ITS) region is widely accepted as the primary DNA barcode for fungi (Schoch et al. 2012), the high sequence similarity among *Truncospora* species and among geographically distinct populations of *T. ochroleuca* limits its discriminatory power. Given the close similarity of rDNA sequences within the genus, analysis of additional molecular markers would be valuable to improve species delimitation and clarify phylogenetic relationships (Zhao and Cui 2013).

Conclusion

This study reports *T. ochroleuca* for the first time in Tunisia, thereby expanding the known distribution of the species and contributing to the growing inventory of macrofungi in Northern Tunisian forests. The finding underscores the high yet still underexplored diversity of polypores in North Africa. ITS-based phylogenetic analyses confirm the monophyly of *Truncospora* and its clear separation from *Perenniporia sensu stricto*, supporting previous molecular studies. Although *T. ochroleuca* clusters closely with *T. macrospora*, morphological differences, particularly the presence of a dark brown crust and larger basidiospores (14.38 × 8.75 µm) in *T. macrospora*, allow reliable species delimitation.

Given the limited molecular data currently available from Mediterranean and North African regions, further multilocus phylogenetic studies are necessary to better resolve species boundaries and to clarify the biogeographic patterns of *T. ochroleuca* in the Mediterranean basin.

Declarations

Author Contribution

Z.O wrote the main manuscript text and prepared figures 1,2 and 3.I.S written the result part of phylogeny and prepared figure 4.All authors reviewed the manuscript.

References

1. Bernicchia A, Gorjón SP. 2019. Polypores of Mediterranean Region; S.P. Publishers. Bologna: Italy, in Press.
2. Cha I E, Rouchka EC. 2005. Comparison of Current BLAST Software on Nucleotide Sequences. Proc IPDPS (Conf). 4;19:8. doi: 10.1109/IPDPS.2005.145. PMID: 21243090; PMCID: PMC3021256.
3. Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T. 2020. ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Mol. Boil. Evol*. 37(1):291–294.
4. Decock 2011. "Studies in *Perenniporia* s. l. (Polyporaceae): African Taxa VII. *Truncospora oboensis* sp. nov. an undescribed Species from High Elevation Cloud Forest of São Tome. *Cryptogam. Mycol.* 32(4):383–390. https://doi.org/10.7872/crym.v32.iss4.2011.383.
5. Emberger L. 1955. Une classification biogeographique des climats. *Recl Trav Lab Bot Geol Zoo*. 7:3–43.
6. Jahn H. 1972/73. Neue europäische Funde von *Perenniporia ochroleuca* (Berk.) Ryv. *Westf. Pilzbr*. 9:68–72.
7. Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP. 2001. Bayesian inference of phylogeny and ITS impact on evolutionary biology. *Sci.* 294(5550):2310–2314.
8. ISO. 1994. Qualité du sol détermination du pH. NF ISO 10390 Novembre1994.

9. Ji X, Sun Y-F, Wu D-M, Gao N, Cui B-K. 2023. An Updated Phylogenetic Assessment and Taxonomic Revision of *Perenniporia* sensu lato (Polyporales, Basidiomycota). *J. Fungi.* 9(2):173. https://doi.org/10.3390/jof9020173.
10. Li HJ, Zhou M, Si J. 2018. *Perenniporia punctata* sp. nov. (Polyporales, Basidiomycota), a new species discovered from China. *Phytotaxa*. 360(1):54–60.
11. Ouali Z, Compagno R, Sbissi I, Gargano ML, Rhaiem A, Ben Naceur M, Venturella G, Jaouani A. 2018. A preliminary check-list of macromycetes in northern Tunisia. *Plant Biosyst*. 152(1):31–58.
12. Ouali Z, Boudagga S, Sbissi I, Calvo R, Venturella G, Jaouani A, Gargano M. L. 2020. Updated checklist of macromycetes of Tunisia. *Plant Biosyst*. 155(4):691–699. (10.1080/11263504.2020.1779838).
13. Ouali Z, Sbissi I, Venturella G, Gargano M. L., Jaouani, A. 2025. Contribution to the Northern Tunisia macromycetes updated checklist. *Plant* Biosyst. 159(4):1–9. https://doi.org/10.1080/11263504.2025.2513340.
14. Pilát A. 1941. Atlas des Champignons de l'Europe, III: Polyporaceae I (in French). Prague. p. 365.
15. Pilát A. 1953. "Hymenomycetes novi vel minus cogniti Cechoslovakiae. II". *Acta Mus. Nat Pra*. 9B(2): 1–109.
16. Pinto-Lopes J. 1953. Polyporaceae de Portugal (excepto resupinadas). *Revta Fac. Ci. Univ. Lisb*. II (C), 2, 157–238.
17. Reid DA. 1975. Notes on some Jugoslav fungi. *Acta Bot. Croat*. 34:133–13.
18. Robledo GL, Amalfi M, Castillo G, Rajchenberg M, Decock C. 2009. "*Perenniporiella chaquenia* sp. nov. and further notes on *Perenniporiella* and its relationships with *Perenniporia* (Poriales, Basidiomycota)". *Mycol*. 101(5):657–673. doi:10.3852/08-040.
19. Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinform.* 19(12):1572–1574.
20. Ryvarden L. 1972. "Studies in the Aphyllophorales of the Canary Islands with a note on the genus *Perenniporia*". *Nord. J. Bot.* 19(2):139–144.
21. Ryvarden L, Melo I. 2017. Poroid Fungi of Europe. 2nd ed. Oslo, Norway: Fungiflora.
22. Ryvarden L, Johansen I. 1980. A preliminary polypore flora of East Africa. Fungiflora, Oslo, Norway. 636 pp.
23. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinform*. 30(9):1312–1313. doi:10.1093/bioinformatics/btu033.
24. Swofford DL. 2002. PAUP: phylogenetic analysis using parsimony (and other methods), Version 4.0b10. Washington DC: Laboratory of Molecular Systematic, Smithsonian Institute.
25. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*. 25(24):4876–4882.

26. Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol*. 172(8):4238–4246.
27. White TJ, Bruns TD, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky J, White TJ, editors. PCR protocols: a guide to methods and applications. New York (NY): Academic Press; p. 315–322.
28. Zambrano-Forero CJ, Dávila-Giraldo LR, Motato-Vásquez V, Villanueva PX, Rondón-Barragán IS, Murillo-Arango W. 2023. Diversity and distribution of macrofungi (Ascomycota and Basidiomycota) in Tolima, a Department of the Colombian Andes: an annotated checklist. *Biodivers. Data J*. 11: e104307.
29. Zhao CL, Cui BK. 2013. "*Truncospora macrospora* sp. nov. (Polyporales) from Southwest China based on morphological and molecular data". *Phytotaxa*. 87(2):30–38.
30. Zhao CL, Cui BK, Dai Y.C. 2013. "New species and phylogeny of *Perenniporia* based on morphological and molecular characters". *Fungal Divers*. 58(1):47–60. doi:10.1007/s13225-012-0177-6.
31. Zhao CL, Cui BK, Song J, Dai YC. 2015. Fragiliporiaceae, a new family of Polyporales (Basidiomycota). *Fungal Divers*. 70:115–126. http://dx.doi.org/10.1007/s13225-014-0299-0.

Figures

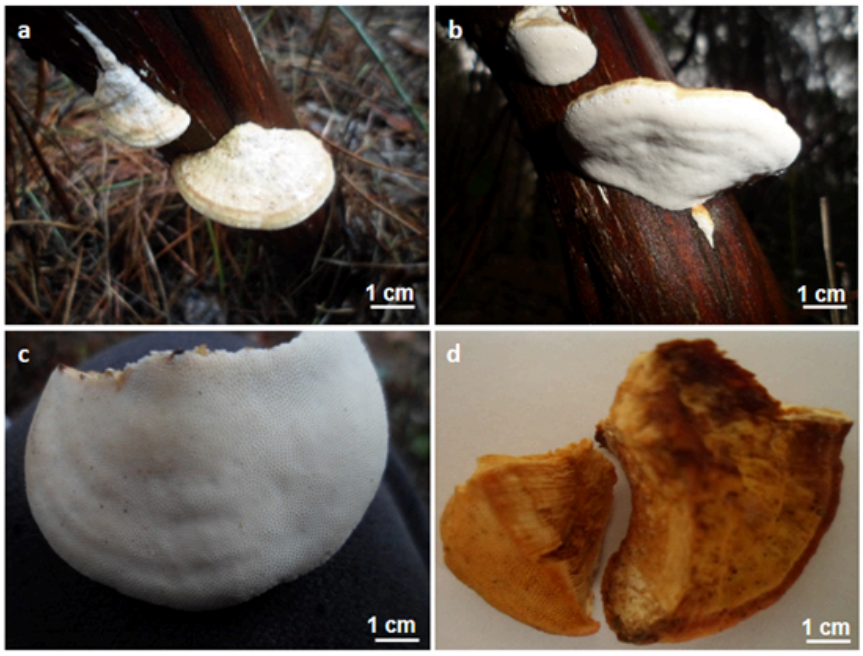

Figure 1

Macroscopical characters of *Truncospora ochroleuca* (a) Forme of fresh sporophore on living tree of *Pinus pinea* L. (b) Detail of sporophore margin (c) Lower surface of sporophore and detail of pores (d) Forme of dried sporophore

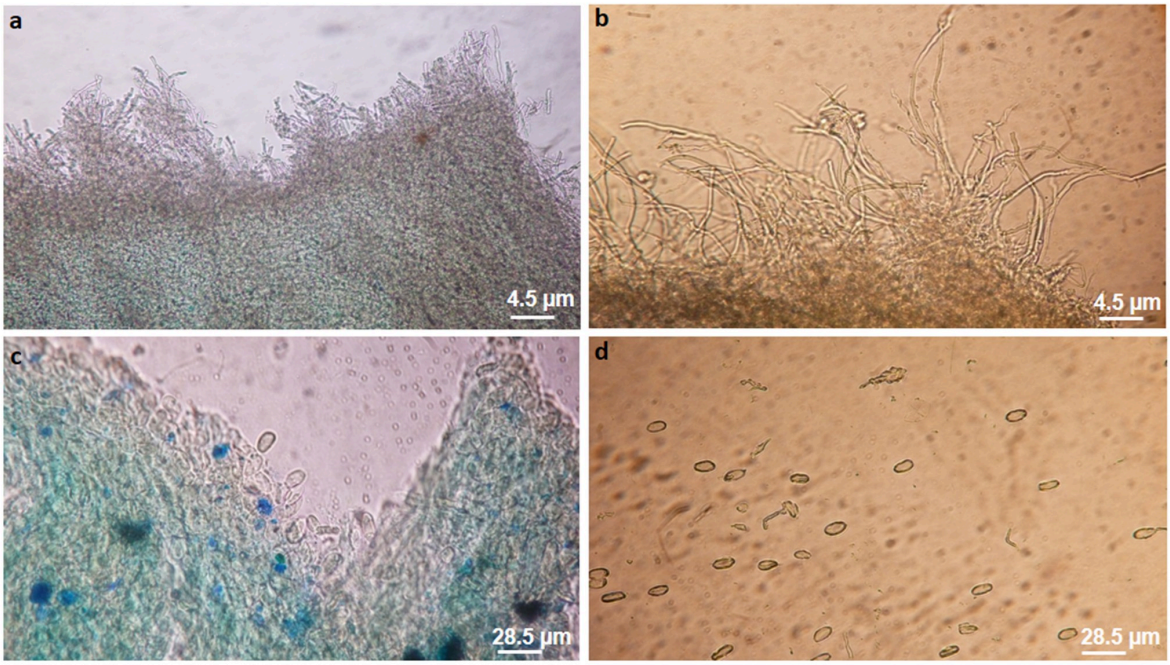

Figure 2

Microscopic structures of *Truncospora ochroleuca* (a) Abundant and generative system hyphae (bar, 4.5 µm) (b) Thick-walled contextual hyphae (bar, 4.5 µm); (c and d) Thick-walled ellipsoid basidiospores (bar, 28.5 µm)

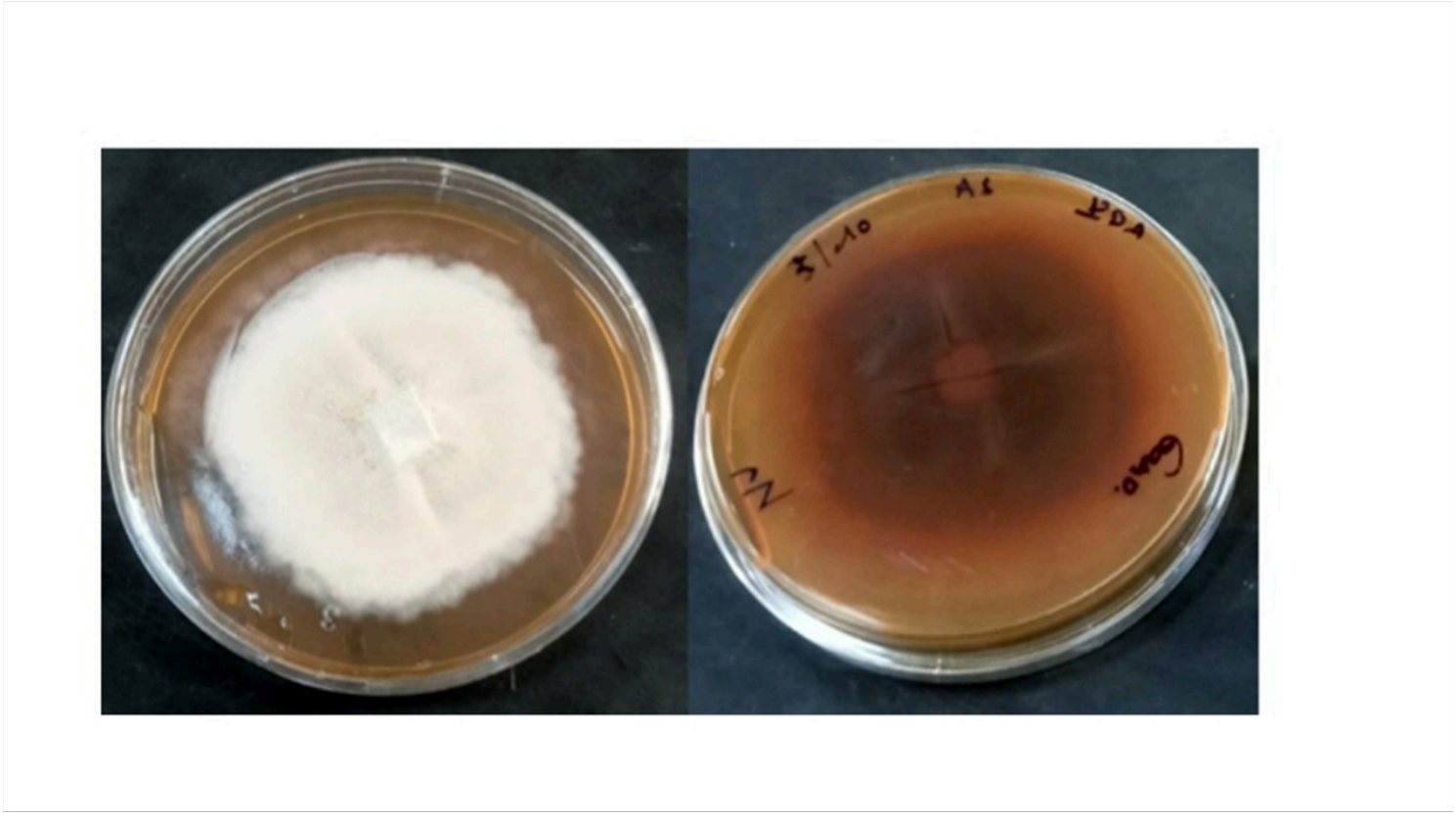

Figure 3

Mycelium grown of *Truncospora ochroleuca* on potato dextrose agar medium showing a dense and hard-appearing mycelium

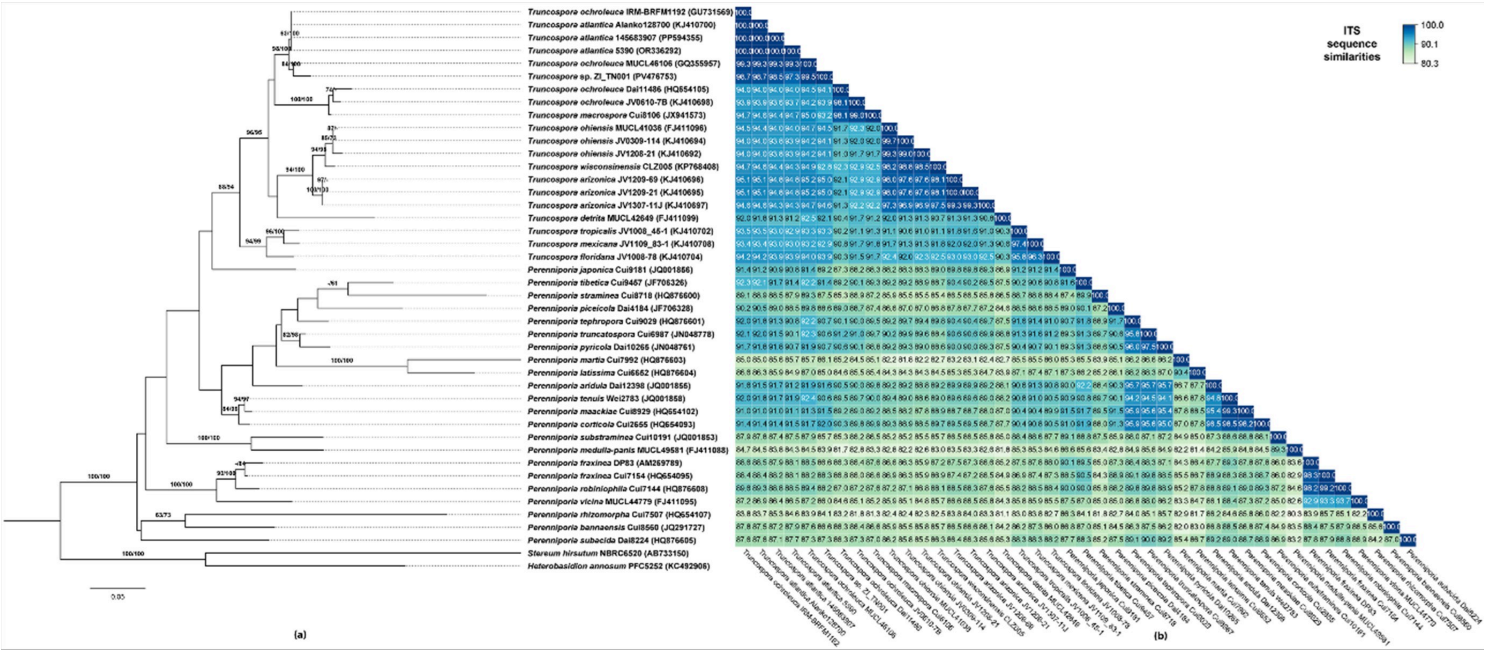

Figure 4

(a) ML phylogenetic tree showing the relationships between strains ZI_Tn001 and closely related *Truncospora* species based on ITS rDNA sequences. The tree was constructed using the Trn+I+G4 model and is midpoint rooted. Branch lengths represent the number of nucleotide substitutions per site. Bootstrap support values above 60% are indicated at the nodes, with ML values on the left and MP values on the right. *Heterobasidion annosum* PFC5252 and *Stereum hirsutum* NBRC6520 were used as the outgroup. The scale bar represents the average number of substitutions per site. (b) Heatmap showing pairwise ITS rDNA gene sequence similarities among the species