Mycosphere 13(2): 188–206 (2022) www.mycosphere.org ISSN 2077 7019
Article – Special issue
Doi 10.5943/mycosphere/si/1f/6

Fossil *Tetraploa* redefinition and potential contribution of dark pigments for the preservation of its spores in the fossil record

Nuñez Otaño NB[1*], Bianchinotti MV[2], Romero IC[3,4], Perez Pincheira E[1,5], Saxena RK[6] and Saparrat MCN[7*]

[1]*Laboratorio de Geología de Llanuras (CICyTTP-Prov.ER-UADER-CONICET). Facultad de Ciencia y Tecnología, Universidad Autónoma de Entre Ríos, Sede Diamante, 3105, Argentina*
[2]*CERZOS-UNS, CONICET-CCT Bahía Blanca, Camino La Carrindanga km 7, Bahía Blanca, Buenos Aires, B8000FWB, Argentina*
[3]*Department of Physics, Earth Science, and Space Systems Engineering, Morehead State University, Morehead, KY, USA*
[4]*Center for Tropical Paleoecology and Archaeology, Smithsonian Tropical Research Institute, Ciudad de Panama, Panama*
[5]*Cátedra de Palinología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. 64 N°3 B1904DZA La Plata, Buenos Aires, Argentina*
[6]*Birbal Sahni Institute of Palaeosciences, 53 University Road, Lucknow–226007, India*
[7]*Instituto de Fisiología Vegetal (INFIVE), Universidad Nacional de La Plata-CCT-La Plata-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Diag. 113 y 61, CC 327, 1900, La Plata, Argentina*

Nuñez Otaño NB, Bianchinotti MV, Romero IC, Perez Pincheira E, Saxena RK, Saparrat MCN 2022 – Fossil *Tetraploa* redefinition and potential contribution of dark pigments in the preservation of its spores in the fossil record. Mycosphere 13(2), 188–206, Doi 10.5943/mycosphere/si/1f/6

Abstract

The establishment of affinities of fossil fungal spores with extant fungal taxa based mainly on the detailed analysis of morphological traits is difficult. Thus, it is necessary to use mycological terms to properly describe fossil fungi. In this work, we transfer five species of *Frasnacritetrus* Taugourd. (1968) to *Tetraploa* Berk. & Broome (1850) because the spores of the fossil and extant species share the same morphological traits. Here, we also discuss how dark pigments of extant spores of *Tetraploa* are synthesized to assess their potential contribution to the preservation of these diaspores in the fossil record, including their role in the fossilization process. In this work, we conclude that *Frasnacritetrus* should be considered a synonym of *Tetraploa*. Our results also shed light on how melanins have played a role in the preservation of fungal spores and other remains in the fossil record to thus, 1) understand how different burial histories and thermal maturation influenced long-term melanin survival, 2) to study the evolution of fungi lineages such as *Tetraploa*, and 3) to assess how fungal melanins can be used as biomarkers for palaeoecological purposes.

Keywords – Mycopalynology – Melanins – Nearest-living-relative – Non-pollen palynomorphs – Paleomycology – Post-mortem polymerization

Introduction

Naming fossil fungal remains, including spores, could be challenging because most of them belong to Dikarya, a subkingdom of Fungi (Wijayawardene et al. 2020). This taxonomic entity is very diverse in the number of species and in the variation of spore traits and other structural components differentiated within the same taxon (Nuñez Otaño et al. 2021). Recent

Submitted 18 October 2022, Accepted 23 December 2022, Published 29 December 2022
Corresponding Author: Noelia Nuñez Otaño – e-mail – noeliabnunez@gmail.com,
Mario C.N. Saparrat – e-mail – masaparrat@fcnym.unlp.edu.ar

recommendations in the last version of the International Code of Nomenclature (ICN), "Shenzhen code" (Chapter F, May et al. 2019), and several recently published papers (Aime et al. 2021, O'Keefe et al. 2021) proposed to use modern names when is possible to unify taxonomic identifications. This code also recommends to maintain a nomenclatural consistency in mycological practices, such as fungal descriptions, erecting new fungal species names, genus, and transferring species. Thus, in recent years the tendency to assign extant taxa names to fossil fungal spores based on modern fungal analogs has increased to improve the accuracy of paleoecological inferences and paleoenvironmental reconstructions, despite the age of the samples (Nuñez Otaño et al. 2021, Pound et al. 2022).

Several well-known fungi coexist in the literature with "two" putative different names, the fossil and extant names. One example of this dichotomy is the genus *Tetraploa* (Tetraplosphaeriaceae, Pleosporales) (Kumaran et al. 2001, Antoine et al. 2006, Worobiec et al. 2009, Cook et al. 2011, Gelorini et al. 2012). This genus, erected by Berkeley and Broome in 1850, is characterized by easily recognizable stauroconidia commonly associated with *Poaceae* or with submerged wood in humid environments (Tanaka et al. 2009, Afty et al. 2013, Hyde et al. 2013, Dong et al. 2020, Li et al. 2021, Romero et al. 2021, among others). In palynological literature, from the Pliocene to the Quaternary (since 2.58 million years (Ma)), these spores could be found under *Tetraploa*, but in deeper time, from the Miocene to the Late Devonian (– 5.33 to 358.9 Ma), spores with similar morphology are frequently assigned to a fossil genus *Frasnacritetrus* (Taugourdeau 1968, Saxena & Sarkar 1986, Gupta 2002). Several authors have considered the oldest record of *Frasnacritetrus*, (*Frasnacritetrus josettae*, 372.2–382.7 Ma), as a modern form of *Tetraploa* (Head 1993, Kalgutkar & Jansonius 2000, Worobiec et al. 2009).

Conidia of *Tetraploa* are characterized by apical appendages, mostly four, that vary in length, and a body generally short-cylindrical with ornamentations in the highly pigmented wall (Li et al. 2021). Among the phenotypic features of representatives belonging to *Tetraploa* and *Frasnacritetrus*, dark coloration is frequently used for the identification of the spores into the "dematiaceous group" (Hyde et al. 2020, Li et al. 2021, Saxena et al. 2021). However, available data concerning the chemical nature of the pigments synthesized by these fungi and the variation in pigmentation of the conidia are scarce (Zhao et al. 2009, Toledo et al. 2017). It has been hypothesized that the intensity of darkening in spores of *Tetraploa* and other pigmented structures in this genus, as well as in other representatives from the order Pleosporales, could be caused by the chemical structure and types of chromophores derived from the metabolism of this fungal group (Saparrat et al. 2002, Nitiu et al. 2020). Another hypothesis is that post-mortem processes, related to chemo-thermal modifications during the fossilization, might be an additional factor for the coloration grade of the spores from *Frasnacritetrus* and other fossil fungi belonging to Pleosporales, contributing at the same time to their preservation in the fossil record (Nuñez Otaño et al. 2021).

Morphological similarities between *Tetraploa* and *Frasnacritetrus* have been recognized in the literature since 1986 (see Saxena et al. 2021 for a recent mention). However, to avoid this dichotomy no efforts have been made to transfer the fossil species of *Frasnacritetrus* to *Tetraploa*. Following the new nomenclatural rules that encourage taxonomists to avoid using morpho-taxa names for fossil fungi (O'Keefe et al. 2021), we propose disregarding the use of *Frasnacritetrus*, as it was described later, so it should be considered a posterior synonym of *Tetraploa*. The modern genus name has priority in accordance with the San Juan Chapter F, Section 1, Art. F.3, and Chapter II, Section 1, Art. 11 of the ICN Code (May et al. 2019, Turland et al. 2018, Turland 2019). Additionally, we discuss how melanins, which are recalcitrant polymers found in cell walls of pigmented fungal structures, could have had a key role in the preservation of fossil spores of *Tetraploa*. This is based on the different secondary metabolites that *Tetraploa* can synthesize, such as spirodioxynaphthalenes, derivated from the same metabolic pathways that generate the 1.8-dihydroxynaphthalene (DHN)-melanins, found primarily in ascomycetes and related forms (Saparrat et al. 2002, Gómez & Nosanchuk 2003, Chai et al. 2010).

Methods

Selection of species

We carried out a morphological comparison among extant and fossil taxa of accepted species belonging to *Tetraploa* and *Frasnacritetrus* that are registered in Index Fungorum (www.indexfungorum.org) and MycoBank (www.mycobank.org). Species belonging to *Pseudotetraploa* were also included because several species of *Tetraploa* have been recently transferred to this genus, based on DNA analysis (Tanaka et al. 2009).

Data selection and analysis

We did qualitative and quantitative comparisons among extant and fossil specimens using morphological characters commonly selected to describe modern *Tetraploa* conidia (Supplementary Table 1). The qualitative variables, extracted from original descriptions, included conidial shape and ornamentation. Pollen terminology used in fossil species descriptions was changed to mycological terms using the Dictionary of Fungi (Kirk et al. 2008). The quantitative variables considered were length and width of the conidia, number of appendages, length and width of the appendages, numbers of septa per appendage, columns of cells present in the main body of the conidia, number of cells in every column from the base of the conidia to the base of the appendages (Supplementary Table 1). We used a nonmetric multidimensional scaling analysis (NMDS) to compare the species of *Tetraploa, Pseudotetraploa*, and *Frasnacritetrus*. We used a Gower distance to calculate similarity among taxa (Supplementary Table 2) (Gower 1971, Struyf et al. 1997). This analysis was performed in R (R Core Team 2021).

Paleogeographic reconstruction

We compiled geographic points and geological time ranges from published data (Palynodata 2006, Supplementary Table 3). Considering that Palynodata does not include the geographic coordinates of the localities reported, we reviewed the original references to find this information when possible. When exact coordinates could not be found, we estimated a broad geographical location based on the locality name found in the database or in the literature. We used GPlates 2.0.0 (Mathews et al. 2016) to plot occurrence data. GPlates is a paleogeographic mapping software based on the plate tectonic model of Müller et al. (2018).

Changes registered into Faces of Fungi

The transference of *Frasnacritetrus* to *Tetraploa* followed the nomenclature rules in the ICN to publicly validate the changes according to Aime et al. (2021) and O'Keefe et al. (2021).

Results

NMDS results indicate that the fossil spores of *Frasnacritetrus* are more closely related to *Tetraploa* than *Pseudotetraploa* (Fig. 1). The similarity index indicates that the taxonomy of the fossil spores of *Frasnacritetrus* is ~85% similar to the spores of *Tetraploa* (Supplementary Table 2). This indicates that *Frasnacritetrus* is included within *Tetraploa*, and taxonomically *Frasnacritetrus* is a synonym of *Tetraploa*.

Position in classification

Based on Hongsanan et al. (2020) and Wijayawardene et al. (2020)

Kingdom: Fungi
Subkingdom: Dikarya
Phylum: Ascomycota
Subphylum: Pezizomycotina
Class: Dothideomycetes
Order: Pleosporales
Family: Tetraplosphaeriaceae

Genus: *Tetraploa* Berk. & Broome (1850). Index Fungorum Identifier: 10199

Type species: *Tetraploa aristata* Berk. & Broome, Ann. Mag. nat. Hist., Ser. 2 5: 459, 1850. Index Fungorum Identifier: 148113

Tetraplosphaeria Kaz. Tanaka & K. Hiray., in Tanaka, Hirayama, Yonezawa, Hatakeyama, Harada, Sano, Shirouzu & Hosoya, Stud. Mycol. 64: 177 (2009)

Frasnacritetrus Taugourd., Cah. Micropaléontol. 10: 3 (1968)

Original description. "Quadriarticulate oblong spores growing four together and perfectly connate, each crowned with an articulate seta as long as itself".

Holotype. Ann. Mag. nat. Hist., Ser. 2 5: 459, 1850. Typification details: Pl. XI, fig. 6 (loc. cit.)

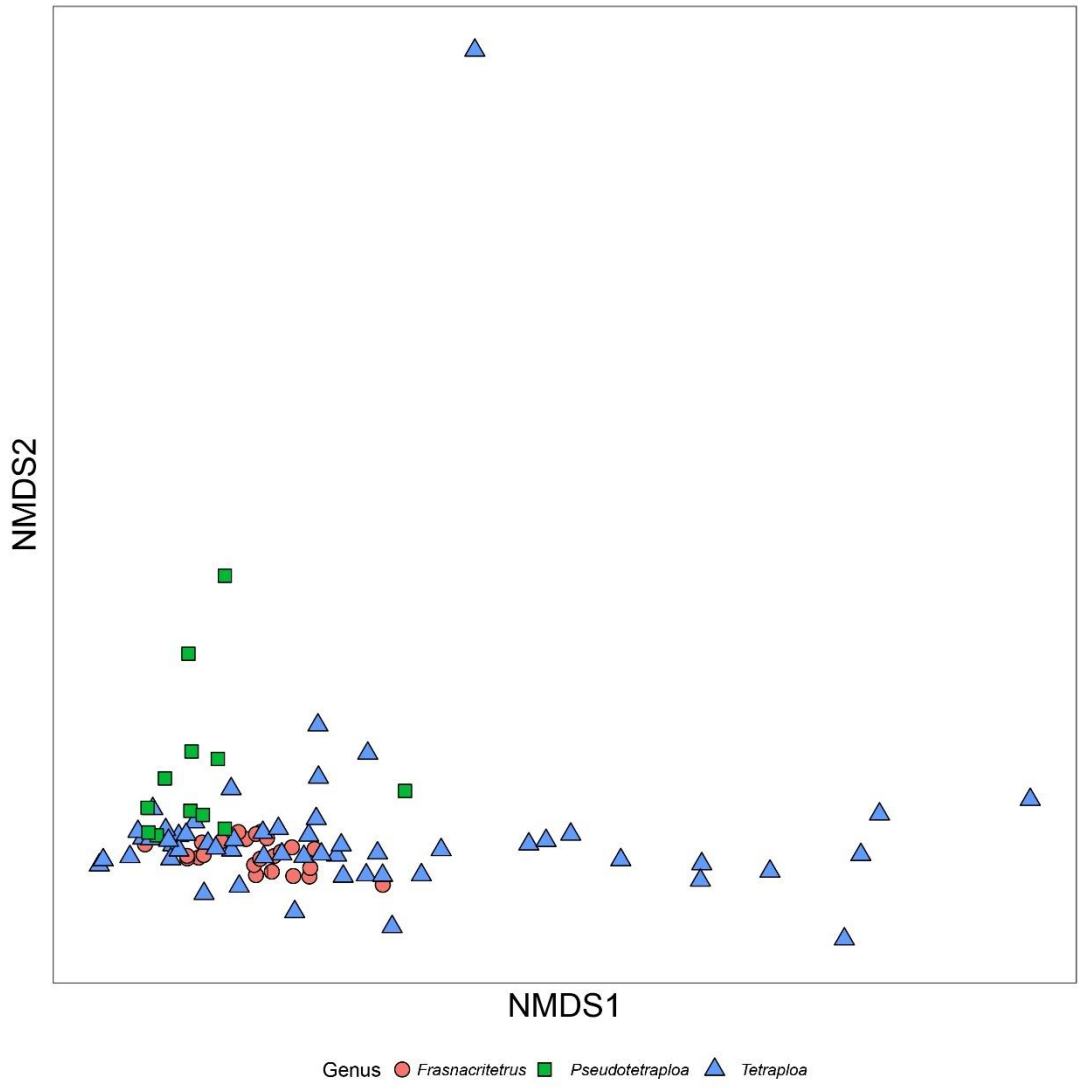


Figure 1 – Non-metric multidimensional scaling ordinations for morphological measurements of modern species of the genera *Tetraploa* and *Pseudotetraploa*, and species of ex. *Frasnacritetrus*. K = 2, non-metric fit: $R^2 = 0.999$, Linear fit $R^2 = 0.998$. Shapes and colors represent different genera. (See Supplementary Table 1 for species information and Supplementary Figs 1-2 for species level NMDS results).

Notes – The spores described in *Frasnacritetrus* Taugourd. are identical to those of *Tetraploa*. According to Li et al. (2020:182) "The asexual morph genus *Tetraploa* s. str. is characterized by micronematous or no conidiophores, monoblastic conidiogenous cells and tetraploid conidia composed of 4-euseptate, short-cylindrical, brown, vertical columns which are verrucose at the base, and with 4 setose, divergent, short or long septate appendages at the apex". Thus, no arguments

sustain the use of the two generic names, then *Frasnacritetrus* is considered a later synonym for *Tetraploa*.

After performing detailed morphological analysis and comparisons, we assigned fossil species to extant ones (Fig. 1, Supplementary Fig. 1). We do not have enough morphological traits of fossil spores to perform detailed comparisons following modern standards or the accepted polyphasic approach for fossil species redefinitions into modern species, but we could establish synonyms within the fossil species after new revisions of some fossil holotypes (Fig. 2). After re-studying *Frasnacritetrus massolensis*, its holotype seems damaged toward the base of the conidium (Fig. 2B), which difficult a correct assessment of the general outline of the spore, despite the similarity values shown in Supplementary Table 2, such as its conidial body size and ornamentation pattern to assess differences between modern *Tetraploa* species. Thus, *F. massolensis* can be defined as *Tetraploa*, but the state of the conidium does not allow an accurate comparison and classification within *Tetraploa* species. This fossil is a doubtful species and therefore excluded. In general, the genus *Frasnacritetrus* can be considered as a synonym of *Tetraploa*, according to the ICN (Turland et al. 2018, Turland 2019), and here, we propose new combinations of several accepted species in *Frasnacritetrus* to *Tetraploa.*

Taxonomy

Tetraploa josettae (Taugourd.) Nuñez Otaño, Bianchin, I.C. Romero, Pérez Pincheira, R.K. Saxena, & Saparrat, comb. nov.

Index Fungorum number: IF900102; Faces of fungi number: FoF13913

Basionym. *Frasnacritetrus josettae* Taugourd., Cah. Micropaléontol. 10: 3 (1968)

Notes – L/W: 2; Outline short cylindrical, surface verrucose, four appendages, conidial size 36-40 um length x 18-20 um width. Locality: France, Late Devonian (Frasnian); Age span: 385.3-374.4 Ma. As mentioned before, the age of this fossil specimen and the lack of some spore traits in the original description difficult its transference to any modern species. Comparing the spore traits available against the rest of the fossil species we recommend keeping this species as *T. josettae*, until more arguments against this could be considered as the oldest species belonging to the modern genus based on their similarity index, in which the similarity was on average 86% (Supplementary Table 2).

Tetraploa taugourdeaui (R.K. Saxena & S. Sarkar**)** Nuñez Otaño, Bianchin, I.C. Romero, Pérez Pincheira, R.K. Saxena, & Saparrat, comb. nov.

Index Fungorum number: IF900103; Faces of fungi number: FoF13912

Basionym. *Frasnacritetrus taugourdeaui* R.K. Saxena & S. Sarkar, Review of Palaeobotany and Palynology (Amsterdam) 46: 213 (1986)

Notes – Ovoid outline, with the base strongly narrower than the medium conidial body (L/W: 1.5: 0.5). Could be comparable with *Tetraploa aristata* (original description). The holotype is not traceable but Saxena & Sarkar (1986) provided good illustrations and descriptions.

Tetraploa conata (R.K. Saxena & S. Sarkar) Nuñez Otaño, Bianchin, I.C. Romero, Pérez Pincheira, R.K. Saxena, & Saparrat, comb. nov. Fig. 2A

Index Fungorum number: IF900104; Faces of fungi number: FoF13915

Basionym. *Frasnacritetrus conatus* R.K. Saxena & S. Sarkar, Review of Palaeobotany and Palynology 46: 215 (1986) [MB#519773]

Taxon synonym. *Frasnacritetrus jamtahensis* A. Gupta, Tertiary Research 21 (1-4): 148 + pl. 5, Fig. 1 (2002)

Notes – L/W: 2.7: 1 to 2.5: 1 (oblong). We consider that the specimen described as *F. jamtahensis* (Fig. 2A) belongs to *T. conata*. We also consider that the original illustration in Gupta (2002:148) does not corresponds to what the author described as *Frasnacritetrus conatus.* Therefore,

our comparisons were based on the description of the illustrated holotype Plate 1-4 in Saxena & Sarkar (1986: 214), as well as on the NMDS results and similarity index (94%, Fig. 1, Supplementary Fig. 1, Supplementary Table 2). Updated color pictures from the holotype are found in Fig. 2A.

Tetraploa indica (R.K. Saxena & S. Khare) Nuñez Otaño, Bianchin, I.C. Romero, Pérez Pincheira, R.K. Saxena, & Saparrat, comb. nov. Fig. 2D

Index Fungorum number: IF900105, Faces of fungi number: FoF13916

Basionym. *Frasnacritetrus indicus* R.K. Saxena & S. Khare, Geophytology 21(1): 42 + pl. 1, fig. 17 (1991)

Notes – L/W: 3:2, short cylindrical outline. According to the original description it has spines on the surface, but this characteristic is not clear after reviewing the holotype which could rather be verruculose (Fig. 2D). In Saxena & Sarkar (1986:214), Plate 1-Fig. 5 resembles *T. Indica* in comparison with the color picture of the holotype. Updated color pictures from the holotype are found in Fig. 2D.

Tetraploa siwalika (R.K. Saxena, H.P. Singh & M.R. Rao) Nuñez Otaño, Bianchin, I.C. Romero, Pérez Pincheira, R.K. Saxena, & Saparrat, comb. nov. Fig. 2C

Index Fungorum number: IF900106; Faces of fungi number: FoF13914

Basionym. *Frasnacritetrus siwalikus* R.K. Saxena, H.P. Singh & M.R. Rao, Geophytology 17 (2): 277 (1987)

Notes – Spore with a triangular outline, obovoid, L/W: 2-2,69. The base of the spore could be deflated probably due to taphonomic processes. If that is the case *T. siwalika* could be comparable with *T. taugourdeaui* but the lack of more spores assignable to *T. siwalika* difficult to synonymize it. Updated color pictures from the holotype are found in Fig. 2C.

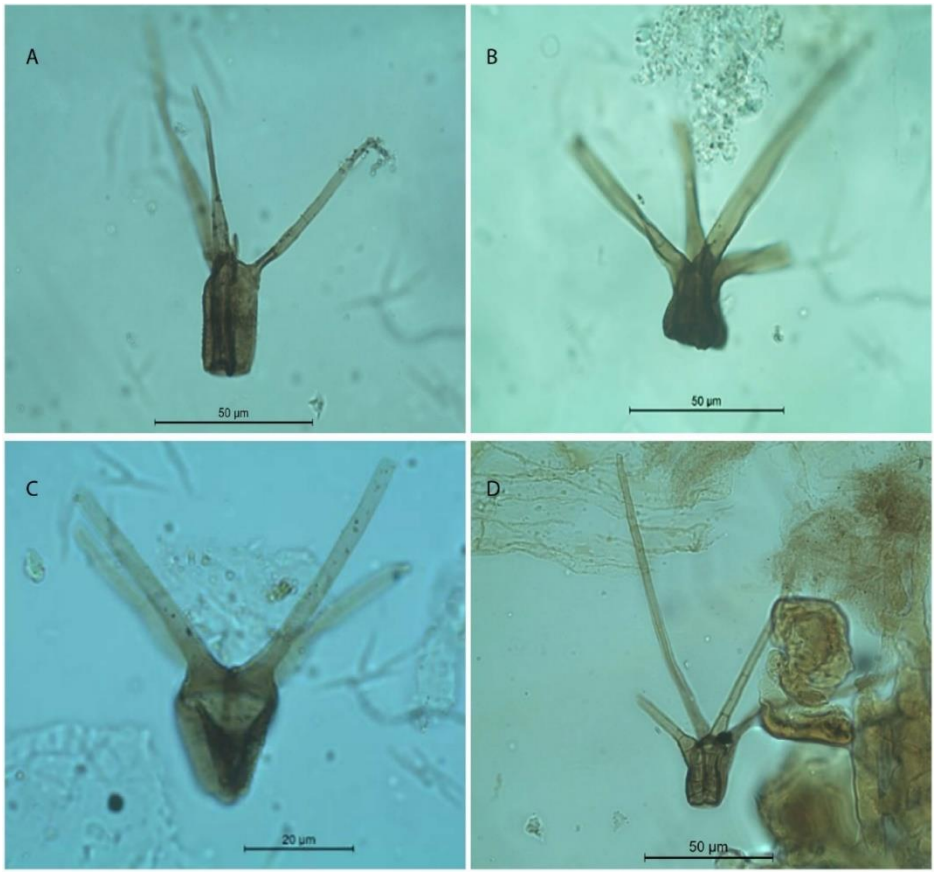


Figure 2 – Color pictures of some fossil *Tetraploa* holotypes. A *Frasnacritetrus jamtahensis* synonym of *T. conata*. B *F. masolensis*. C *Tetraploa siwalika* comb. nov. D *T. indica* comb. nov. Scale bars: A, B, D = 50 um, C = 20 um.

Other tetraploid fossil spores

Several fossil tetraploid spores were designated as '*Frasnacritetrus* sp. 1 to sp. 5' after Saxena & Sarkar (1986) provided detailed descriptions despite lacking an epithet. Following what we propose in this article, we recommend for future research work, where similar spores could be found, to use the modern genus name instead, such as the two fossil tetraploid spores from the Miocene of Colombia shown in this study (Fig. 3A, B). This way the ecology of the NLR *Tetraploa* could be applied for paleoecological and paleoclimatic purposes. In our NMDS analysis, *Tetraploa* sp. 1, sp. 2 and sp. 5 shared similar characteristics with an average similarity of 96% (Supplementary Fig. 1, Supplementary Table 2), while *Tetraploa* sp. 3 and *T*. sp. 4 were grouped together with a similarity of 90% (Supplementary Fig. 1, Supplementary Table 2). Therefore, considering the conservational state of *T*. sp. 1 and *T.* sp. 2, we recommend not taking these spores into consideration for any future comparison. The remaining spores, *T*. sp. 3, *T*. sp. 4, and *T*. sp. 5, cannot be comparable strictly with *Tetraploa* because these only have three appendages, but they can be considered representatives of Tetraplosphaeriaceae, a fungal family with tetraploa-like anamorphic phase.

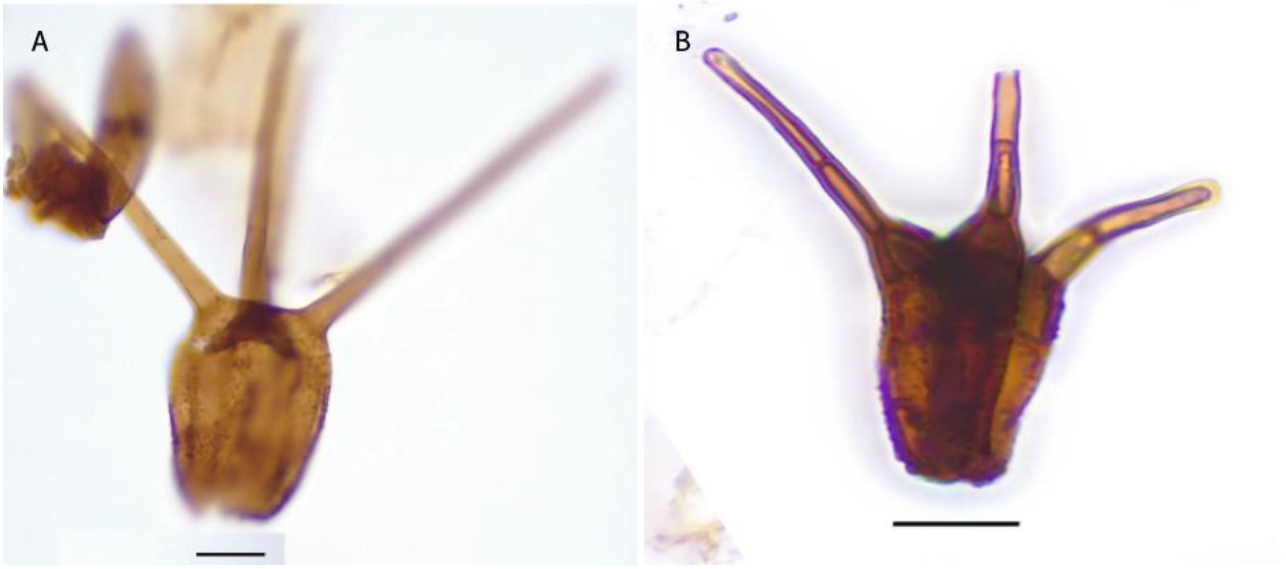


Figure 3 – Fossil spores from the middle Miocene of Colombia. A *Tetraploa* sp. 6. B *Tetraploa* sp. 7. Scale bar: 10 um.

Discussion

Geological Range, Preservation and Ecology

Tetraploid fossil species have been described since the late Devonian, specifically the Frasnian period (374.5 - 385.3 Ma), with most of the erected fossil species found during the Miocene (5.3 - 23 Ma) (Saxena et al. 2021). This coincides with the divergence of the class Dothideomycetes in the Late Devonian, 362 Ma (286 – 476 Ma) (Beimforde et al. 2014). Despite this, the geological range of *Frasnacritetrus* needs to be reviewed because of the large gap between the first record in the Devonian and the next record during the Cretaceous (Jansonius & Hills 1987, Worobiec et al. 2009, Pedrão Ferreira et al. 2005). Additionally, reviewing the phylogeny of Ascomycetes, the group Pleosporales diverged during the late Jurassic around 213 Ma (139-297 Ma), and the divergence time of Tetraplosphaeriaceae was during the early Cretaceous (~110 Ma) which support previous discussions about the validity of the age for the fossil specimen registered in the late Devonian (Liu et al. 2017). A gap of almost 200 Ma in the fossil record seems unlikely (Fig. 4, SupplementaryTable 3) (Worobiec et al. 2009), but our results cannot invalidate the age of the Devonian *Tetraploa* fossil. Further revisions, including other palynomorphs from the same site, need to be performed to determine if this record should be considered a modern contaminant.

Frasnacritetrus has been used as a biostratigraphical marker mostly in India, as paleoecological proxy for environmental inferences around the world, such as an indicator of moist and humid environments (Van Geel & Van der Hammen 1973, Worobiec et al. 2009, Afty et al. 2013), and as co-occurrence with grass pollen, specifically during the Cenozoic (Kumaran et al. 2001, Saxena & Ranhotra 2009, Worobiec et al. 2009, Corona-Esquivel et al. 2010, Saxena & Tripathi 2011, among others). Despite the fossil name assigned to the spores, *Frasnacritetrus* clearly belong to *Tetraploa* (Fig. 1, Supplementary Table 2). The fossil specimens generally have a poor conservational state and commonly are designated as *Frasnacritetrus* sp., while stauroid spores similar to *Tetraploa*, found in quaternary sediments are named *Tetraploa aff. aristata* (Medeanic et al. 2008, Gómez et al. 2007, Musotto et al. 2012, among others). Despite taphonomic processes affecting fossil *Tetraploa* spores from the Frasnian to the Quaternary, the components of the wall of these spores preserve key morphological characteristics, such as shape, appendages, septations, coloration, and ornamentation, that are comparable with modern specimens.

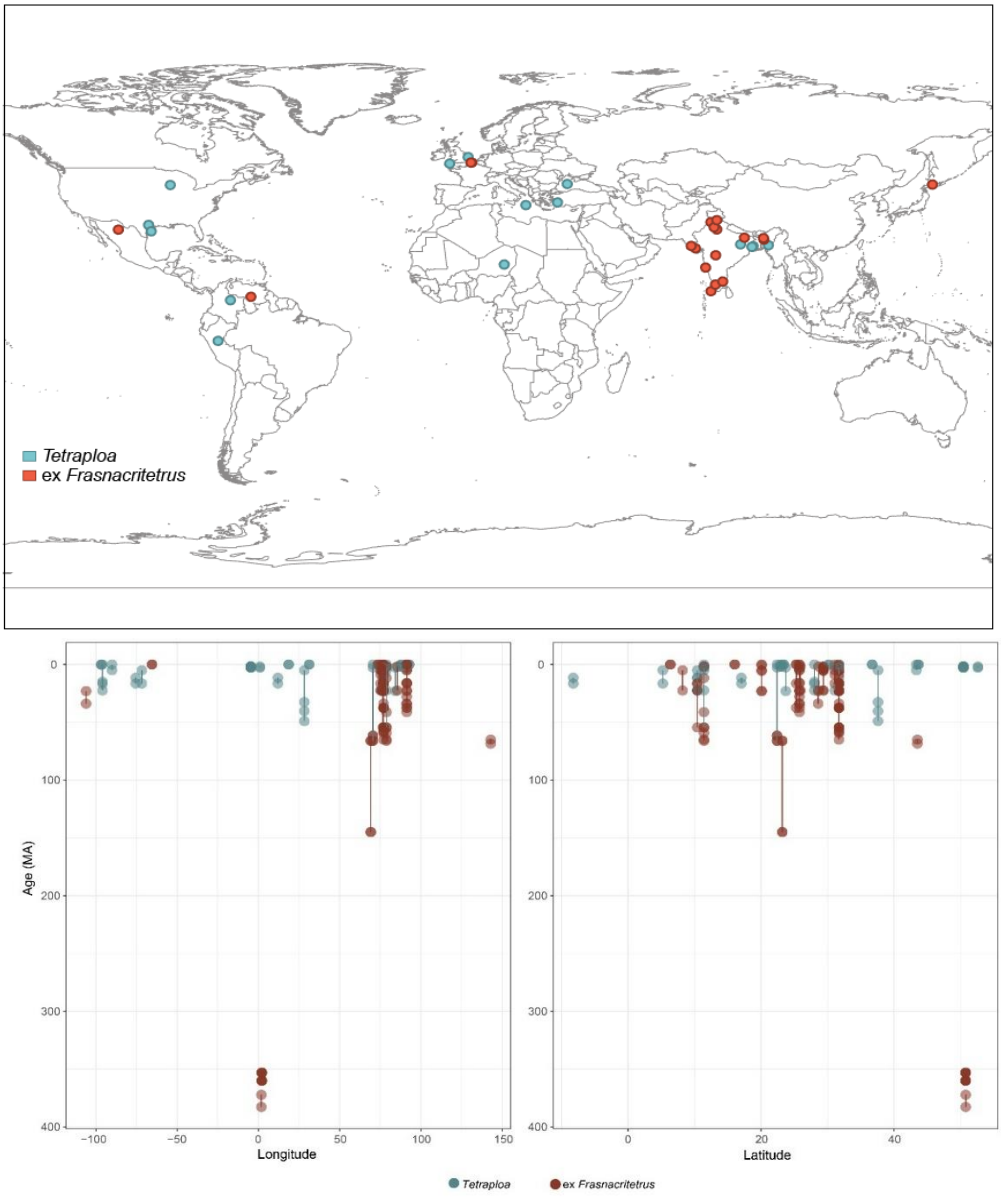


Figure 4 – Top: Paleogeographic distribution of fossil spores of *Tetraploa* designated as *Tetraploa* and/or *ex Frasnacritetrus.* Base: First and last appearance of *Tetraploa* in different localities through time. Data were selected from published records from Palynodata (https://paleobotany.ru/palynodata)

Throughout the earth's history, climatic and environmental changes affected biotic organisms, including fungi, and modified biological communities, causing massive extinctions, as well as dispersal and diversification events (Bender 2013, Kaiho et al. 2016, Smith et al. 2020, Steinthorsdottir et al. 2021, among others). In fungi, these changes are observed in the fossil record when studying the evolution of different lineages (Taylor et al. 2015, Spatafora et al. 2017, Tedersoo et al. 2018, Naranjo-Ortiz & Gabaldón 2019). In fungal spores, such as *Tetraploa*, it appears that the general morphology has been conserved for at least ~110 Ma, facilitating ecological inferences of past depositional environments (Fig. 5) (Taugourdeau 1968, Saxena & Sarkar 1986, Saxena & Trivedi 2009, Saxena & Tripathi 2011, among others). The modern ecology of *Tetraploa* indicates a saprotroph growing on grasses, mostly *Poaceae*, dead stems of herbaceous plants, branches, twigs of Bamboo, *Cocos nucifera*, rotten wood, and on decay submerged wood in freshwater habitats in tropical to subtropical regions (Ellis 1949, Ando 1992, Révay 1993, Watanabe 2002, Pratibha & Bhat 2008, Tanaka et al. 2009, Li et al. 2021, among others). Thus, modern representatives of *Tetraplosphaeriaceae* can be considered modern analogs or NLRs of fossil tetraploid-like spores in future paleoecological research and paleoenvironmental reconstructions. This could also suggest that the initial main host where fossil *Tetraploa* grew and evolved were grasses (Poaceae crown group age ~101Ma (early Cretaceous), Huang et al. 2022), and host substrate preferences evolved from narrow to wider.

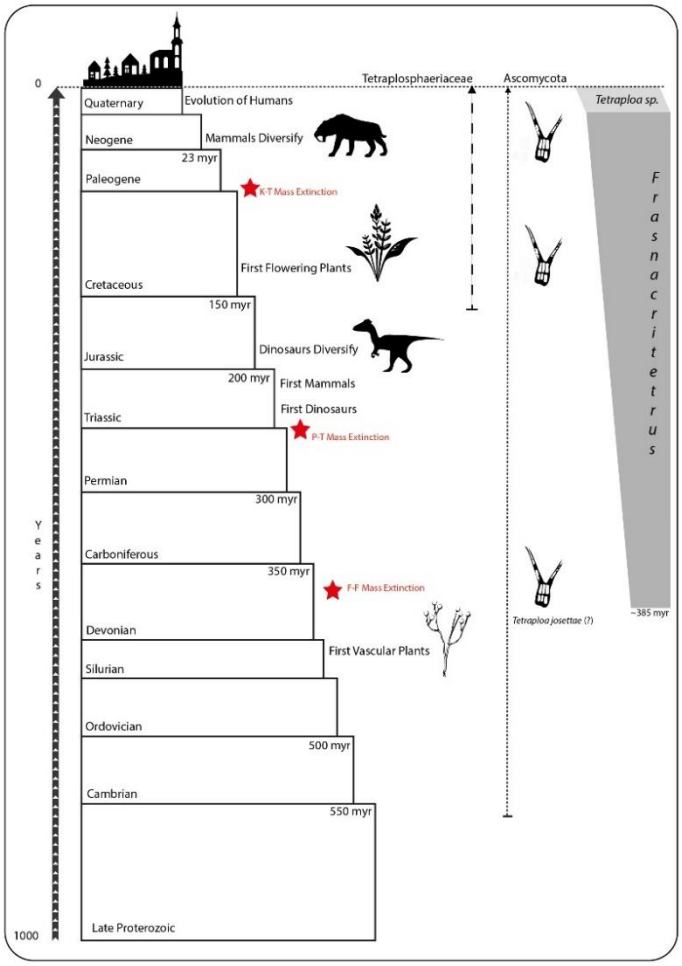


Figure 5 – Geological range of fossil and extant spores of *Tetraploa* during the time gap Quaternary-Late Devonian. The question mark on *T. josettae* indicates doubt about the age of this record. The red stars indicate mass extinction events. The dotted line indicates the estimated time for the origin of the Phylum Ascomycota (Shen et al. 2020). The dashed line indicates the divergence time for Tetraplosphaeriaceae (Liu et al. 2017).

Pigments of *Tetraploa* spores and their long-lasting presence in the fossil record

Fungi can synthesize a broad spectrum of pigments, such as polymeric ones which are the most likely to persist in the fossil record because of their chemical stability and resistance to morphological alterations, among these pigments are sporopollenin and melanins (Brooks & Shaw 1978, Nitiu et al. 2022). Both pigments are structurally complex and made up of a diversity of aromatic and/or aliphatic residues, these are also highly resistant to degradation under different environmental stresses, such as water deficit, salinity (turgor pressure), wounding, and pathogen attack. This is because these pigments work as major extracellular hydrophobic barriers and as structural components that maintain the integrity of the architecture of the cell wall (Feofilova 2010, Glass et al. 2012, Nuñez Otaño et al. 2021). To date, sporopollenin has not been reported for any representatives belonging to Pleosporales, known as the brown-pigmented ones (Martínez-Girón & Martínez-Torre, 2011). However, different classes of melanins have been found in this group and in other orders from the Class Dothideomycetes (Toledo et al. 2017, Nitiu et al. 2022). Sporopollenin has been found only in ascospores of *Neurospora crassa*, *N. tetrasperma* (Sordariales) and in the conidia of *Aspergillus niger* and *Penicillium brevi* (Eurotiales) (Gooday et al. 1974, Brooks & Shaw 1978, Webster & Weber 2007).

Some Pleosporales, synthesize mostly 1,8-dihydroxynaphthalene (DHN) melanins, which accumulate in the cell wall of conidia and hyphae, such as those reported in the genus *Alternaria*. In this genus, specifically, *A. alternata* has also been reported its capacity to synthesize pyomelanin, a different type of melanin, when it is inhibited the synthesis of DHN-melanin, decreasing the cell wall chitin content and the thickness of its structures (Fernandes et al. 2021). Considering that pyomelanin biosynthesis can be activated during the catabolism of aromatic amino-acids, such as L-tyrosine or L-phenylalanine, which involves also auto-oxidation reactions followed by self-polymerization, this opens a question about if there is some possible relationship between proteases-driven autolysis processes in spores exposed to fossilization and the melanization of these diaspores. Fungal autolysis is a natural process of self-digestion of aged structures, that generates micromorphological changes due to hydrolase activity, including modifications in the cell wall (Perez-Leblic et al. 1982, White et al. 2002). The generation of free amino acids in dying fungal structures during diagenesis can lead also to the probable deposition of pyomelanin on the cell wall through the L-tyrosine catabolic pathway. In consequence, early ontogenetic stages of fungal spores with pyomelanin and with a low relative amount of structural DHN-melanin such as reported by Fernandes et al. (2021) in modern analogs of *A. alternata*, could generate morphogenetic alternations during the process of diagenesis, such as damaged and collapsed cell walls, and lacking septa in the remaining structures. It is known that the absence or reduction of DHN-melanin in specific fungal structures is associated with the release of soluble substances, principally glucose and N-acetylglucosamine. These substances increase the synthesis of β-glucans and their accumulation in the cell walls, possibly as a salvage compensatory mechanism, which makes the fungal cell wall more fragile (Perez-Leblic et al. 1982, Fernandes et al. 2021). Consequently, the intraspecific comparisons of fossil and extant taxa based only on spore morphology and pigmentation must be done with caution.

Fungal melanins can be synthesized through different metabolic pathways that involve polyketide synthases and/or oxidative enzymes, such as laccase and/or tyrosinase, that catalyze a specific set of reactions, resulting in polymers that vary in composition, ultrastructure, and color on spores and other propagules (Toledo et al. 2017). In *in-vitro* cultures of *Tetraploa aristata* on three different substrates, lignin (aromatic), glucose (non-aromatic), and sacarose (non-aromatic), has been found that only in lignin the mycelium of *T. aristata* produced coloration (Saparrat et al. 2002). This coloration suggests that the production of heterogeneous melanins on the mycelium is a strategy to detoxify soluble aromatic compounds derived from lignin attack (Saparrat et al. 2002).

There are numerous reports of fossil fungi assigned to *Tetraploa*, which at the same time show their spores with a broad spectrum of brown coloration, ranging from light brown to dark brown, and with differential preservation status (Taugourdeau 1968, Saxena & Sarkar 1986, Gupta 2002, among others). Zhao et al. (2009) also reported more than 10 species of *Tetraploa*, with a range of pigmentation at the conidial system, that comprises from hyaline forms to pale brown ones. Saparrat

et al. (2002) and Li et al. (2021) reported that in Tetraplosphaeriaceae, three strains of this group, including *Tetraploa aristata* LPSC 419, have the ability to synthesize laccases, whose activity is associated with aromatic substrates related to lignin. The polymerization of aromatic compounds associated with the fungal structures of Tetraplosphaeriaceae and its biological function in these, can suggest that the presence of these fungi in the fossil record and its color variation can be used as a paleoenvironmental proxy associated with recalcitrant substrates.

Melanins synthesized by *Tetraploa* during the sporulation process are probably 1,8 DHN-one (Saparrat et al. 2002), while melanin granules in the external layer of the spore wall of *Tetraploa* might correspond to pyomelanin and/or a heterogeneous-type. The variation in the synthesized melanins is the result of a series of autolysis processes and of other chemical transformation reactions, including condensation and polymerization, such as Maillard-type, where amino acids react with reduced sugars to produce molecules with brown colors, according to the formation of humic-type aromatic polymers ("The Browning theory"; Bicchieri et al. 2002). On the other hand, ontogenetic studies on ascospores of *Massarina* species (Massarinaceae, Pleosporales) revealed that initially no melanin-like bodies have been observed in the spore wall (episporium), producing hyaline ascospores (Scheuer, 1991), but the pigmentation was present in mature spores (Tsui et al. 1999). Thus, this polymerization, after spore morphogenesis, could explain the pigmentation variation of fossil spores of *Tetraploa*. The generation of all these pigmented polymers or melanoidins, which can also be stimulated during the postmortem processes, driven mainly by thermal maturity reactions, could contribute to the basis of the broad spectrum in the color variation of fossil spores of *Tetraploa* despite their morphological variability (Glass et al. 2013, Toledo et al. 2017). In addition, spores with pigmented walls have higher density, acting in favor of a faster deposition rate (Rees 1980, Tyson 1995, Quattroccio et al. 2018), fossilization, and differential resistance to reworking (Pross et al. 2007), increasing its recovery in palynological samples.

Additional changes in the coloration of fossil spores of *Tetraploa* can also be explained by three processes: 1) a differential accumulation of dark pigments or biosynthetic melanins in the walls during the ontogenesis of the spore, 2) polymerization of fungal extracellular metabolites available from leachate and derived from the senescence and autolysis of the spores, 3) generation of new chromophores on their native structure of melanin, caused by metal-driven oxidative polymerization due to the higher reactivity of substances from sediments where the spores were buried (Glass et al. 2013, Saitta et al. 2018). The last two processes are triggered by digenetic events, such as low sedimentation rate, exposure to anoxic conditions, and low metamorphism which involve temperature, pressure, burial depth, and time (Molina 1996, Fernández López 2000, Glass et al. 2013, Njoh & Tembi 2017, Jung et al. 2009, Jander-Shagug & Masaphy 2010, Cordero et al. 2020, Nitiu et al. 2020). Thus, biosynthetic melanins and equivalent polymers in pigmented fungal fossils such as those in *Tetraploa*, found in the fossil record, could be one of the main components that contribute to the preservation of spores' structures and color variations within the same genus, despite some degree of morphological differences. It is known that chitin associated with pigmented cell walls could be the main scaffold to cross-link melanins to the cell wall suprastructure, such as demonstrated previously in other fungi (Walker et al. 2010, Camacho et al. 2017). Future investigations must be focused on such specific fungal melanins to analyze if they are severely altered during fossilization, and how is their residual chemistry in fossil spores. This information could help to highlight the diagnostic value of some fungal melanins, either associated with fossil spores or isolated within sedimentary rocks, to be useful as "molecular" fossils such as suggested by Briggs and Summons (2014), or as a biomarker for the study of evolution (Strycker et al. 2021).

Conclusions

There is confusion concerning the dates of the older *Tetraploa,* with *T. josettae* (374.5-385.3 Ma), possibly being a modern contaminant. It is therefore advisable to use the fossils of *T. conata* and *T. indica* from the Cretaceous to early Paleocene as calibration points in evolutionary reconstructions, such as that of Samarakoon et al. (2019). These two-fossil species are among the few fungal fossil taxa from the Mesozoic within the Dothideomycetes lineage.

Taking the *Tetraploa* fossil records into account, along with other palynofossil evidence at the different regions where the species were found, we can speculate that the evolution of *Tetraploa* is in accordance with the geological age already published for Tetraplosphaeriaceae in the late Jurassic to early Cretaceous. Probably this genus evolved from growing on herbaceous plants, mainly *Poaceae*, to other substrate preferences. Taxa may have had a paleogeographic distribution in warm – tropical to subtropical paleoclimates in marshy to shallow pond environments and/or freshwater habitats. The palaeogeographical distribution and climatic preferences coincide with those of extant species of *Tetraploa* but with a less diverse substrate preference for saprotrophic growth.

The synthesis and accumulation of melanins in fossil spore wall during diagenesis, such as morphogenesis and/or postmortem processes of polymerization, could be the main reasons to explain the presence of *Tetraploa* in the fossil record. Since the association between melanin and chitin synthesis is a general principle for dark-pigmented representatives in the fungal kingdom, studies on melanins in fossil fungi are key to understanding the preservation of several extant spore traits since the Cretaceous.

Acknowledgments

We would like to acknowledge the great possibility to perform multidisciplinary work through online meetings during the pandemic times knowing how stressful those times were for all of us. The authors from Argentina would like to thank the Sci-Hub team for the great opportunity to facilitate access of several references needed for this work that could not be possible to reach otherwise. We are grateful to the library loan office at University of Illinois (USA) to allow us to get a copy of the original article from 1968 where *Frasnacritetrus* was defined. We would like to thank the reviewer(s) for their contributions to the original manuscript helping us to improve the final version.

Author Contributions

We use "The first-last-author-emphasis" norm (FLAE) combined with "The percent-contribution-indicated" approach (PCI) to decide the order of the authors between the first and the last one. NNO and MCSN conceived the idea and designed the study. NNO and MVB worked in the classic morphological comparisons. NNO and ICR generated the quantitative data for the study. MCSN provided the background information on *Tetraploa* pigments and lead the writing of that section along with the support of NNO and EPP. NNO and MCNS led the writing with support of MVB, ICR and EPP. ICR performed the statistical analysis, graphs, maps on RStudio, and lead final manuscript editing. RKS provided the color holotype pictures showed in Figure 2. All authors contributed to the article and approved the submitted version. This work contributes to empowerment and representation of women in STEM from Latin America. The lead authors NNO and MCNS maintained the gender balance in leadership and making decisions on this article. All authors tried to keep the gender equality in references as much as possible.

Funding

This research was partially funding by the National Agency for Scientific and Technological Promotion (ANPCyT) of the Ministry of Science, Technology and Productive Innovation through PICT 2019-00207 (to MCNS) project, the National Council of Research (CONICET projects PUE INFIVE and PIP 11220200100527CO (to MCNS), UNLP (Argentina) through the R&D Project A344 (MCNS), PICT 2020-03591 (to NNO), and PIP 3036 (to NNO).

References

Afty HE, Brocke R, Uhl D. 2013 – A fungal proliferation near the probable Oligocene/Miocene boundary, Nukhul Formation, Gulf of Suez, Egypt. Journal of Micropaleontology 32, 183–195. Doi 10.1144/jmpaleo2013-004

Aime MC, Miller AN, Aoki T, Bensh L et al. 2021 – How to publish a new fungal species, or name, version 3.0. IMA Fungus 12, 11. Doi 10.1186/s43008-021-00063-1

Ando K. 1992 – A study of terrestrial aquatic hyphomycetes (in Japanese). Transactions of the Mycological Society of Japan 33, 415–425.
Antoine PO, De Franceschi D, Flynn JJ, Nel A et al. 2006 – Amber from western Amazonia reveals Neotropical diversity during the middle Miocene. Proceedings of the National Academy of Sciences of the United States of America 103, 13595–13600.
Beimforde C, Feldberg K, Nylinder S, Rikkinen J et al. 2014 – Estimating the Phanerozoic history of the Ascomycota lineages: combining fossil and molecular data. Molecular Phylogenetics and Evolution 78, 386–398. Doi 10.1016/j.ympev.2014.04.024
Bender ML. 2013 – Paleoclimate. Princeton University Press, Princeton, NJ.
Berkeley MJ, Broome CE. 1850 – Notices of British fungi. Annals and Magazine of Natural History Serie 2, 5, 455–466. Nature Reviews Microbiology 18, 717–730.
Bicchieri M, Ronconi S, Romano FP, Pappalardo L et al. 2002 – Study of foxing stains on paper by chemical methods, infrared spectroscopy, micro-X-ray fluorescence spectrometry and laser induced breakdown spectroscopy. Spectrochimica Acta B 57, 7, 1235–1249.
Briggs DEG, Summons RE. 2014 – Ancient biomolecules: Their origins, fossilization, and role in revealing the history of life. BioEssays 36, 5, 482–490.
Brooks J, Shaw G. 1978 – Sporopollenin: a review of its chemistry, paleochemistry and geochemistry. Grana 17, 91-97.
Chai LY, Netea MG, Sugui J, Vonk AG, van de Sande WW, Warris A, Kwon-Chung KJ, Kullberg BJ. 2010 – *Aspergillus fumigatus* conidial melanin modulates host cytokine response. Immunobiology 215, 915–920.
Cook EJ, van Geel B, van der Kaars S, van Arkel J. 2011 – A review of the use of non-pollen palynomorphs in palaeoecology with examples from Australia. Palynology 35, 2, 155–178. Doi 10.1080/01916122.2010.545515
Corona-Esquivel R, Martínez-Hernández E, Henríquez F, Nyström JO, Tritlla J. 2010 – Palynologic evidence for iron-oxide ash fall at La Perla, an Oligocene Kiruna-type iron ore deposit in northern Mexico. GFF 132, 3–4, 173–181.
Dong W, Wang B, Hyde KD, McKenzie ERH et al. 2020 – Freshwater Dothideomycetes. Fungal Diversity 105, 319–575. Doi 10.1007/s13225-020-00463-5
Ellis MB. 1949 – *Tetraploa*. Transactions of the British Mycological Society 32, 246–251.
Feofilova EP. 2010 – The fungal cell wall: Modern concepts of its composition and biological function. Microbiology 79, 711–720. https://doi.org/10.1134/S0026261710060019
Fernandes C, Mota M, Barros L, Días MI et al. 2021 – Pyomelanin synthesis in *Alternaria alternata* inhibits DHN-Melanin synthesis and decreases cell wall chitin content and thickness. Frontiers in Microbiology: Fungi and their interactions 12: 691433. Doi 10.3389/fmicb.2021.691433
Fernández López SR. 2000 – Temas de tafononía. Departamento de Paleontología, Universidad Complutense de Madrid.
Gelorini V, Verbeken A, Lens L, Eggermont H et al. 2012 – Effects of land use on the fungal spore richness in small crater-lake basins of western Uganda. Fungal Diversity 55, 125–142. Doi 10.1007/s13225-012-0155-z
Glass K, Ito S, Wilby PR, Sota T et al. 2012 – Direct chemical evidence for eumelanin pigment from the Jurassic period. Proceedings of the National Academy of Science 109, 10218–10223.
Glass K, Ito S, Wilby PR, Sota T et al. 2013 – Impact of diagenesis and maturation on the survival of eumelanin in the fossil record. Organic Geochemistry, 64, 29–37. Doi 10.1016/j.orggeochem.2013.09.002
Gómez BL, Nosanchuk JD. 2003 – Melanin and fungi. Current Opinion in Infectious Diseases, 16, 2, 91–96. Doi 10.1097/00001432-200304000-00005
Gooday GW, Green D, Fawcett P, Shaw G. 1974 – Sporopollenin formation in the ascospore wall of *Neurospora crassa*, Archives of Microbiology 101, 145–151.
Gower JC. 1971 – A general coefficient of similarity and some of its properties. Biometrics 27, 4, 857–871.

Gupta A. 2002 – Algal/fungal spores from Early Tertiary sediments of Sirmaur District, Himachal Pradesh, India. Tertiary Research 21, 14, 123–153.
Head MJ.1993 – Dinoflagellates, sporomorphs, and other palynomorphs from the Upper Pliocene St. Erth Beds of Cornwall, Southwestern England. Journal of Paleontology 67, Suppl. 3, 3), 1–62.
Hongsanan S, Hyde KD, Phookamsak R, Wanasinghe DN et al. 2020 – Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. Mycosphere 11(1): 1553–2107.
Huang W, Zhang L, Columbus JT, Hu Y et al. 2022 – A well-supported nuclear phylogeny of *Poaceae* and implications for the evolution of C4 photosynthesis. Molecular Plant 15, 755–777.
Hyde KD, Jones EBG, Liu JK, Ariyawansa H et al. 2013 – Families of Dothideomycetes. Fungal Diversity 63, 1–313. Doi 10.1007/s13225-013-0263-4
Hyde KD, Dong Y, Phookamsak R, Jeewon R et al. 2020 – Fungal diversity notes 1151–1276: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 100, 1, 5–277. Doi 10.1007/s13225-020-00439-5
Index Fungorum. 2021 – Available from: www.indexfungorum.org/ Names/Names.asp (Accessed on November 28, 2022).
Jander-Shagug G, Masaphy S. 2010 – Free radical scavenging activity of culinary-medicinal morel mushrooms, *Morchella* Dill. ex Pers. (Ascomycetes): Relation to color and phenol contents. International journal of medicinal mushrooms 12, 5, 299–307.
Jansonius J, Hills LV. 1987 – Genera file of fossil spores and pollen. Department of Geology, University of Calgary, Alberta, Canada. Supplement 9, cards 4361–4575. Special publication.
Jarzen DM, Corbett SL, Manchester R. 2010 – Palynology and paleoecology of the middle Miocene Alum Bluff flora, Liberty County, Florida, USA. Palynology, 34, 2, 261–286.
Jung JH, Lee JE, Lee CH, Kim SS, Lee BU. 2009 – Treatment of fungal bioaerosols by a high-temperature, short-time process in a continuous-flow system. Applied and environmental microbiology 75, 9, 2742-9. Doi 10.1128/AEM.01790-08
Kaiho K, Oshima N, Adachi K, Mizukami T et al. 2016 – Global climate change driven by soot at the K-Pg boundary as the cause of the mass extinction. Scientific Reports 6, 28427. Doi 10.1038/srep28427
Kalgutkar RM, Jansonius J. 2000 – Synopsis of fossil fungal spores, mycelia and fructifications. Am. Assoc. Strat. Palynol. Contrib. Ser. 39, 1–423.
Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008 – Dictionary of the Fungi. 10th Edition, Wallingford, CABI, 22.
Kumaran KPN, Limaye RB, Rajshekhar C, Rajagopalan G. 2001 – Palynoflora and radiocarbon dates of Holocene deposits of Dhamapur, Sindhudurg district, Maharashtra. Current Science 80, 10, 331–1336.
Li WL, Bao DF, Bhat DJ, Su HY. 2021 – *Tetraploa aquatica* (Tetraplosphaeriaceae), a new freshwater fungal species from Yunnan Province, China. Phytotaxa, 459, 2, 181–189. Doi 10.11646/phytotaxa.459.2.8
Liu JK, Hyde KD, Jeewon R, Phillips AJL, Maharachchikumbura SSN, Ryberg M, Liu ZY, Zhao Q. 2017 – Ranking higher taxa using divergence times: a case study in Dothideomycetes. Fungal Diversity 84, 75–99. Doi 10.1007/s13225-017-0385-1
Martínez-Girón R, Martínez-Torre S. 2011 – *Tetraploa aristata* conidium in sputum cytology. Cytopathology 24, 2, 132–133. Doi 10.1111/j.1365-2303.2011.00936.x
Matthews KJ, Maloney KT, Zahirovic S, Williams SE et al. 2016 – Global plate boundary evolution and kinematics since the late Paleozoic. Global and Planetary Change 146, 226–250. Doi 10.1016/j.gloplacha.2016.10.002
May TW, Redhead SA, Bensch K, Hawksworth DL et al. 2019 – Chapter F of the International Code of Nomenclature for algae, fungi, and plants as approved by the 11th International Mycological Congress, San Juan, Puerto Rico, July 2018. IMA Fungus 10, 21, 10–24. Doi 10.1186/s43008-019-0019-1

Medeanic S, Zamora N, Corrêa ICS. 2008 – Non-pollen palynomorphs as environmental indicator in the surface samples from mangrove in Costa Rica. Revista geológica de America Central, 39, 27–52. ISSN: 02567024

Molina E. 1996 – El análisis tafonómico en Micropaleontología: particularidades tafonómicas de los microfósiles. Comunicación de la II Reunión de Tafonomía y fosilización 241–246.

Müller RD, Cannon J, Qin X, Watson RJ et al. 2018 – GPlates: Building a Virtual Earth Through Deep Time. Geochemistry, Geophysics, Geosystems 19, 2243–2261. Doi 10.1029/2018GC007584

Naranjo-Ortiz MA, Gabaldón T. 2019 – Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. Biological Reviews 94, 6, 2101–2137. Doi 10.1111/brv.12550

Nitiu DS, Mallo AC, Saparrat MCN. 2020 – Fungal melanins that deteriorate paper cultural heritage: an overview, Mycologia, 112, 5, 859–870. Doi 10.1080/00275514.2020.1788846

Nitiu D S, Mallo C, Saparrat MCN. 2022 – Pigmentos sintetizados por hongos negros y su impacto en el deterioro del patrimonio documental en papel. Boletín de la Sociedad Argentina de Botánica 57, 169–184.

Njoh O, Tembi A. 2017 – Spores and pollen colour changes and thermal maturation of mid-cretaceous lacustrine organic-rich sediments in the Mamfe Basin, S.W. Cameroon. Journal of Geography and Geology 9, 4, 22–32. ISSN 1916-9779 E-ISSN 1916-9787. Published by Canadian Center of Science and Education.

Nuñez Otaño NB, Bianchinotti MV, Saparrat MCN. 2021 – Palaeomycology: a modern mycological view of fungal palynomorphs. In: Marret, F., O'Keefe, J., Osterloff, P., Pound, M., Shumilovskikh (eds). Applications of non-pollen palynomorphs: from Palaeoenvironmental Reconstructions to Biostratigraphy. Geological Society, London, Special Publications, 511. Doi 10.1144/SP511-2020-47

O'Keefe JMK, Nuñez Otaño NB, Bianchinotti MV. 2021 – Nomenclature: how do we designate NPP taxa? in: Marret, F., O'Keefe, J., Osterloff, P., Pound, M. and Shumilovskikh, L. (eds). Applications of Non-Pollen Palynomorphs: from Palaeoenvironmental Reconstructions to Biostratigraphy. Geological Society, London, Special Publications, 511. Doi 10.1144/SP511-2020-119

Pedrão Ferreira E, De Araujo Carvalho M, Viviers MC, 2005 – Palinologia (fungos) da formação Calumbi, Paleoceno da Bacia de Sergipe, Brasil. Arquivos do Museu Nacional 63, 3, 395–410.

Perez-Leblic MI, Reyes F, Martinez MJ, Lahoz R. 1982 – Cell wall degradation in the autolysis of filamentous fungi. Mycopathologia 80, 3, 147–55. Doi 10.1007/BF00437577.

Pound MJ, Nuñez Otaño NB, Romero IC, Lim M et al. 2022 – The fungal ecology of the Brassington Formation (Middle Miocene) of Derbyshire, United Kingdom, and a new method for palaeoclimate reconstruction. Frontiers in Ecology and Evolution, Paleoecology 10: 947623. Doi 10.3389/fevo.2022.947623

Pratibha J, Bhat DJ. 2008 – New and unusual hyphomycetes from Mahabaleshwar, India. Mycotaxon 105, 423–431.

Pross J, Pletsch T, Shillington DJ, Ligouis B et al. 2007 – Thermal alteration of terrestrial palynomorphs in mid-Cretaceous organic-rich mudstones intruded by an igneous sill (Newfoundland Margin, ODP Hole 1276A). International Journal of Coal Geology 70, 277–291.

Quattroccio ME, Olivera DE, Martínez MA, Ponce JJ, Carmona NB. 2018 – Palynofacies associated to hyperpycnite deposits of the Miocene, Cabo Viamonte Beds, Austral Basin, Argentina. Facies, 64, 22. Doi 10.1007/s10347-018-0535-2

R Core Team. 2021 – R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rees G. 1980 – Factors affecting the sedimentation rate of marine fungal spores. Botanica Marina, 23, 375–85.

Révay A. 1993 – Some new or interesting hyphomycetes from Hungary. Nova Hedwigia, 56, 473–482.

Romero IC, Kong S, Fowlkes CC, Jaramillo C et al. 2020 – Improving the taxonomy of fossil pollen using convolutional neural networks and superresolution microscopy. Proceedings of the National Academy of Sciences of the United States of America 117, 45, 28496–28505. Doi 10.1073/pnas.2007324117

Romero IC, Nuñez Otaño NB, Gibson ME, Spears TM et al. 2021 – First record of fungal diversity in the tropical and warm-temperate Middle Miocene Climate Optimum forests of Eurasia. Frontiers in Forest and Global Change 4: 76840. Doi 10.3389/ffgc.2021.768405

Saitta ET, Kaye EG, Vinther J. 2018 – Sediment-encased maturation: a novel method for simulating diagenesis in organic fossil preservation. Paleontology 62, 1, 135–150. Doi 10.5061/dryad.0t67n

Samarakoon MC, Hyde KD, Hongsanan S, McKenzie EHC. 2019 – Divergence time calibrations for ancient lineages of Ascomycota classification based on a modern review of estimations. Fungal Diversity 96, 285–346.

Saparrat MCN, Cabello M, Arambarri AA. 2002 – Extracellular laccase activity in *Tetraploa aristata*. Biotechnology Letters 24, 1375–1377.

Saxena RK, Sarkar S. 1986 – Morphological study of *Frasnacritetrus taugourdeaui* emend. from tertiary sediments of Himachal Pradesh, India. Review of Palaeobotany and Palynology 46, 209-225.

Saxena RK, Khare S. 1992 – Fungal remains from the Neyveli Formation of Tiruchirapalli District, Tamil Nadu, India. Geophytology 21, 3743.

Saxena RK, Ranhotra PS. 2009 – Palynofloral study of the Intertrappean Bed Exposed at a New Locality in Kutch District, Gujarat and its implications on palaeoenvironment and age. Journal Geological Society of India 74, 690–696.

Saxena RK, Singh HP, Rao MR. 1987 – Palynology of the Tatrot - Pinjor sequence exposed between Masol and Kiratpur in Ambala District, Haryana. Geophytology 17, 270–284.

Saxena RK, Trivedi GK. 2009 – Palynological investigationof the Kopili formation (late Eocene) in North Cachar Hills, Assam, India. Acta Palaeobotanica 49, 2, 253-277.

Saxena RK, Tripathi SKM. 2011 – Indian Fossil Fungi. The Palaeobotanist 60, 1, 1–208.

Saxena RK, Wijayawardene NN, Dai DQ, Hyde KD, Kirk PM. 2021 – Diversity in fossil fungal spores. Mycosphere 12, 1, 670–874.

Scheuer CH. 1991 – *Massarina tetraploa* sp. nov., the teleomorph of *Tetraploa aristata*. Mycological Research, 95, 126–128.

Shen XX, Steenwik JL, LaBella AL, Opulente DA et al. 2020 – Genome-scale phylogeny and contrasting modes of genome evolution in the fungal phylum Ascomycota. Sciences Advances 6, eabd0079.

Smith V, Warny S, Grice K, Schaefer B et al. 2020 – Life and death in the Chicxulub impact crater: A record of the Paleocene-Eocene Thermal Maximum. Climate of the Past 16, 5, 1889–1899. Doi 10.5194/cp-16-1889-2020

Spatafora JW, Aime MC, Grigoriev IV, Martin F et al. 2017 – The Fungal Tree of Life: from Molecular Systematics to Genome-Scale Phylogenies. Microbiology Spectrum 5, 5, FUNK-0053–2016. Doi 10.1128/microbiolspec.FUNK-0053-2016

Steinthorsdottir M, Coxall HK, de Boer AM. Huber M et al. 2021 – The Miocene: The future of the past. Paleoceanography and Paleoclimatology 36, e2020PA004037. Doi 10.1029/2020PA004037

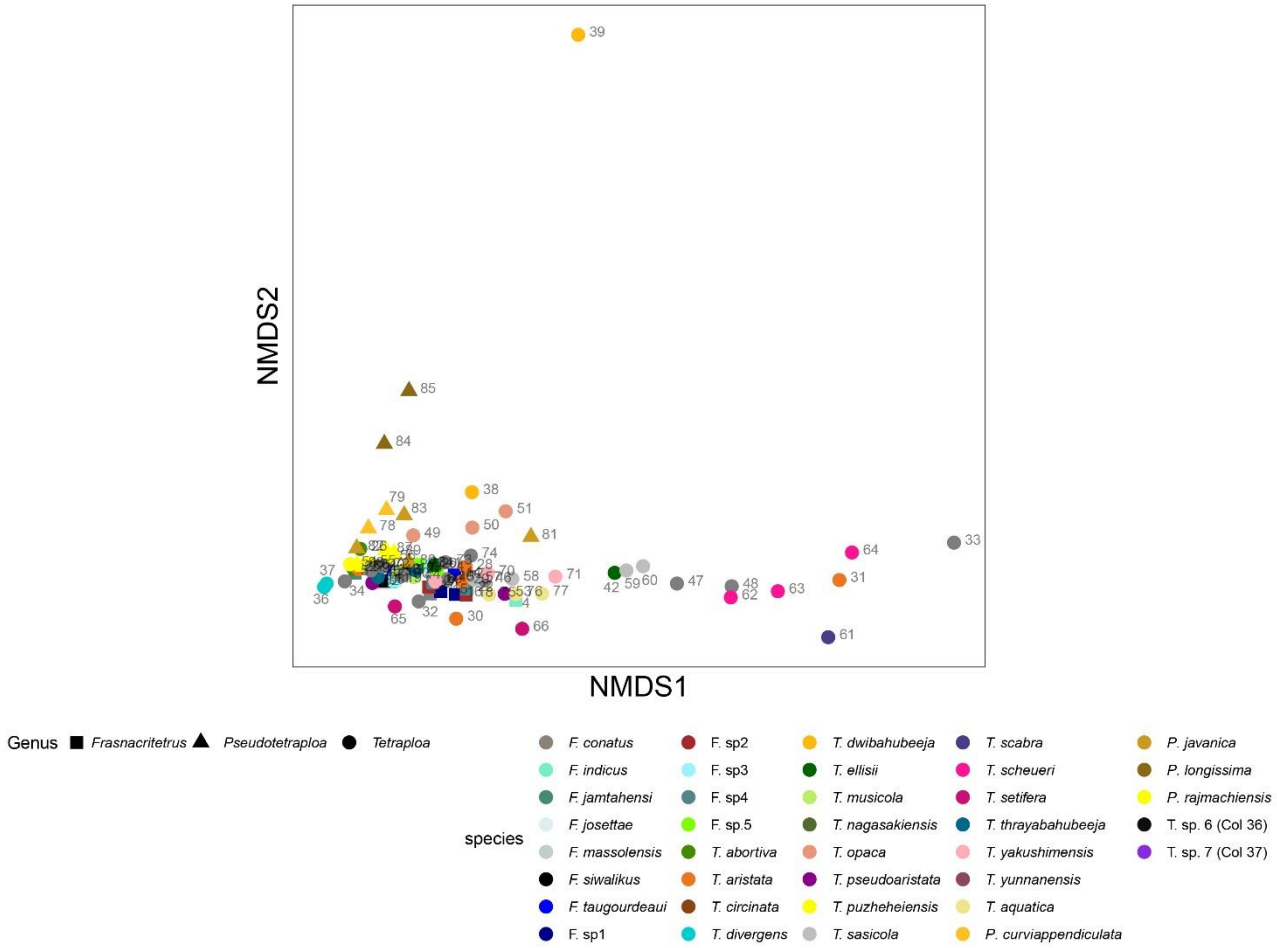
Struyf A, Hubert M, Rousseeuw PJ. 1997 – Integrating robust clustering techniques in S-PLUS, Computational Statistics and Data Analysis 26, 17–37.

Strycker BD, Han Z, Bahari A, Pham T et al. 2021 – Raman characterization of fungal DHN and DOPA Melanin Biosynthesis Pathways. Journal of Fungi 7, 10, 841. Doi 10.3390/jof7100841

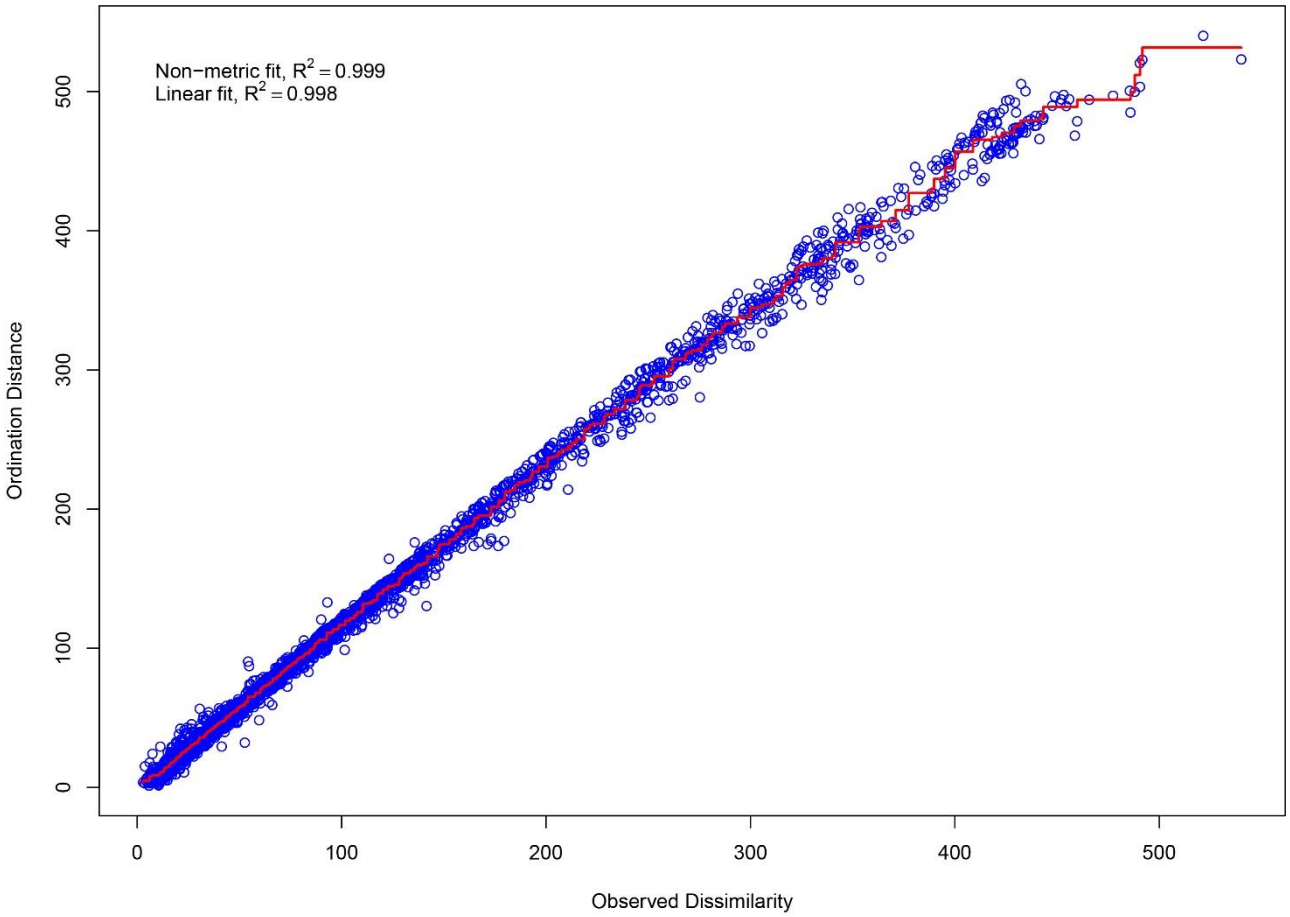
Tanaka K, Hirayama K, Yonezawa H, Hatakeyama S et al. 2009 – Molecular taxonomy of bambusicolous fungi: Tetraplosphaeriaceae, a new pleosporalean family with Tetraploa-like anamorphs. Studies in Mycology 64, 175-209. Doi 10.3114/sim.2009.64.10

Taugourdeau P. 1968 – Sur un curiex microfossile incertae sedis du Frasnien du Boulonnais *Fransnacritetrus* nov. gen. (Acritarche). Cahiers de Micropaleontologie Série 1, 10, 1–4.
Taylor TN, Krings M, Taylor EL. 2015 – Fungal Spores. Doi 10.1016/B978-0-12-387731-4.00011-6
Tedersoo L, Sánchez-Ramírez S, Kõljalg U, Bharam M et al. 2018 – High-level classification of the Fungi and a tool for evolutionary ecological analyses. Fungal Diversity 90, 135–159. Doi 10.1007/s13225-018-0401-0
Toledo AV, Franco MEE, Yanil Lopez SM, Troncozo MI et al. 2017 – Melanins in fungi: types, localization and putative biological roles. Physiological and Molecular Plant Pathology 99, 2–6. Doi 10.1016/j.pmpp.2017.04.004
Tsui KM, Hyde KD, Hodgkiss IJ. 1999 – *Massarina proprietunicata* sp. nov., from submerged wood in streams in Hong Kong. Mycological Research 103, 1575–1578.
Turland NJ, Wiersema JH, Barrie FR, Greuter W et al. 2018 – (eds.) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. Doi 10.12705/Code.2018
Turland N. 2019 – The Code Decoded: A user's guide to the International Code of Nomenclature for algae, fungi, and plants, Second Edition. Pensoft Publishers, Sofia, Bulgaria.
Tyson RV. 1995 – Sedimentary organic matter. Organic facies and palynofacies. Ed. Chapman and Hali. ISBN 978-94-01 0-4318-2
Van Geel B, Van der Hammen T. 1973 – Upper quaternary vegetational and climatic sequence of the fuquene area (Eastern Cordillera, Colombia). Palaeogeography, Palaeoclimatology, Palaeoecology 14, 1, 9-92.
Watanabe T. 2002 – Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species, 2nd Ed, CRC Press, Boca Raton. Doi 10.1201/EBK1439804193
Webster J, Weber RWS. 2007 – Introduction to fungi 3rd Edition. Cambridge University Press, UK.
Walker CA, Gómez BL, Mora-Montes HM, Mackenzie KS, Munro CA, Brown AJ, Gow NA, Kibbler CC, Odds FC. 2010 – Melanin externalization in *Candida albicans* depends on cell wall chitin structures. Eukaryotic Cell 9, 1329-1342. Doi 10.1128/EC.00051-10
White S, McIntyre M, Berry DR, McNeil B. 2002 – The autolysis of industrial filamentous fungi. Critical Reviews in Biotechnology, 22, 1, 1–14. Doi 10.1080/07388550290789432
Wijayawardene NN, Hyde KD, Al-Ani LKT, Tedersoo L et al. 2020 – Outline of Fungi and fungus-like taxa. Mycosphere 11, 1060–1456.
Worobiec E, Worobiec G, Gedl P. 2009 – Occurrence of fossil bamboo pollen and a fungal conidium of *Tetraploa cf. aristata* in Upper Miocene deposits of Józefina (Poland). Review of Paleobotany and Palynology 157, 3, 211–217. Doi 10.1016/j.revpalbo.2009.05.002
Zhao G, Liu X, Xie X, Cao A. 2009 – Saprobic dematiaceous hyphomycetes from Shennongjia region, China. Nova Hedwigia 88, 1–2, 217–227. Doi 10.1127/0029-5035/2009/0088-0217

Supplementary materials



Supplementary Figure 1 – Species level NMDS results. Symbols indicate different genera: *Frasnacritetrus* (Square), *Pseudotetraploa* (Triangle), and *Tetraploa* (circle). Species numbers in Supplementary Table 1.



Supplementary Figure 2 – Stress values from NMDS analysis in Supplementary Figure 1.

Below is the link to the electronic supplementary materials.

Supplementary Table 1 List of species, quantitative, and qualitative variables considered for the morphological analysis.

Supplementary Table 1

Supplementary Table 2 Gower distance showing similarity values among taxa.

Supplementary Table 2

Supplementary Table 3 Fossil *Tetraploa* species records with age, locality, and reference information.

Supplementary Table 3