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Talaromyces systylus, a new synnematous species from Argentinean semi-arid soil

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With 18 figures and 2 tables

Abstract: The discovery of an interesting new synnematous *Talaromyces* species from Argentinean semi-arid soil has enlarged the number of synnema-producing species. *Talaromyces systylus* sp. nov. is characterized by the production of indeterminate synnemata on Malt Extract Agar and coarsely rough-walled, globose conidia and conidial chains arranged in columns. The optimal growing temperature was 30°C. *Talaromyces systylus* was compared with other related species phylogenetically based on ITS, BenA and CaM markers.

Key words: Ascomycetes, Eurotiales, *Penicillium* subgenus *Biverticillium*, synnemata.

Introduction

In his work of 1979 Pitt accepted the subgenus *Biverticillium* Dierckx *apud* Biourge to accommodate species that present penicilli with metulae of approximately equal length to phialides in symmetrical adpressed or divergent verticils, phialides typically acerose or ampulliform-acerose in few species, and conidia ellipsoidal to fusiform or spheroidal in species with ampulliform-acerose phialides. This author transferred to *Biverticillium* several taxa previously placed by Raper & Thom (1949) in the section *Biverticillata-Symmetrica* Thom. According to him, *Biverticillium* section *Coremigenum* (Biourge) Pitt includes coremial or synnematous species, whereas section *Simplicium* (Biourge)

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Pitt contains species which produces penicilli characteristic of *Biverticillium* but are not known to form coremia or synnemata. He placed in section *Coremigenum* the synnematous species *P. claviforme* Bain., *P. dendriticum* Pitt, *P. duclauxii* Delacr., *P. isariiforme* Stolk & J.Mey. and *P. pseudostromaticum* Hodges, Warner & Rogerson. *Penicillium claviforme* is currently classified in subgenus *Penicillium* (Frisvad & Samson 2004) and *P. isariiforme* has been transferred to the section *Ochrosalmonea* Houbraken & Samson in the subgenus *Aspergilloides* Dierckx (Houbraken & Samson 2011). The following extra synnematous species were also considered as having *Biverticillium*-like penicilli: *P. allahabadense* B.S.Mehrotra & D.Kumar, *P. aureocephalum* Munt.-Cvet., Hoyo & Gómez-Bolea, *P. calidicanium* J.L.Chen, *P. cecidicola* Seifert, Hoekstra & Frisvad, *P. coalescens* Quintan., *P. palmae* Samson, Stolk & Frisvad, *P. panamense* Samson, Stolk & Frisvad, *P. pittii* Quintanilla and *P. ramulosum* C.M.Visagie & K.Jacobs (Quintanilla 1984, Quintanilla 1985, Samson et al. 1989, Van Reenen-Hoekstra et al. 1990, Pitt et al. 2000, Muntañola-Cvetković et al. 2001, Chen et al. 2002, Seifert et al. 2004, Visagie et al. 2009). Samson et al. (2011) transferred all accepted species of *Penicillium* subgenus *Biverticillium* to *Talaromyces* C.R.Benj. and provided new combinations. Visagie & Jacobs (2012) have described a new synnematous penicillate biverticillate species as *Talaromyces chloroloma* Visagie & K.Jacobs. Visagie et al. (2012) found the sexual structures of *P. aureocephalum* on *Quercus suber* L. leaf litter and compared their specimens with the previously described species *Lasioderma flavovirens* Durieu & Mont. (1845). After a careful study, they concluded that *P. aureocephalum* and *L. flavovirens* were the same fungus and, in addition, they decided to place it in the genus *Talaromyces* proposing the new combination *Talaromyces flavovirens* (Durieu & Mont.) Visagie, Llimona & Seifert.

The above mentioned synnematous species bears sub-spheroidal, ellipsoidal or ellipsoidal to fusiform conidia with smooth or finely roughened walls. Regarding the non-synnematous species which produce globose rough-walled conidia, *P. aculeatum* Raper & Fennell was included together with *P. verruculosum* Peyronel in *Biverticillium* section *Simplicium* by Pitt (1979) and placed by Samson et al. (2011) in the genus *Talaromyces*. In addition, Samson et al. (2011) proposed *T. apiculatus* Samson, Yilmaz & Frisvad, and *T. diversus* (Raper & Fennell) Samson, Yilmaz & Frisvad as taxonomic novelties. Visagie & Jacobs (2012) have erected *Talaromyces solicola* Visagie & K.Jacobs. *Talaromyces solicola* presents distinguished spheroidal or subspheroidal rough-walled conidia and non-coremial habit (Raper & Thom 1949, Pitt 1979, Pitt et al. 2000, Visagie & Jacobs 2012). Recently, Yilmaz et al. (2014) in their polyphasic monograph of *Talaromyces* proposed seven sections within the genus based on an ITS, BenA and RPB2 multigene phylogeny, placing the 88 accepted species and providing morphological descriptions for each of these species.

During the course of a thermoresistant soil-borne fungi survey an interesting synnematous isolate with biverticillate adpressed penicilli and spheroidal rough-walled conidia belonging to *Talaromyces* was found. The fungus was isolated into pure culture and considered to be a novelty. The objective of this study was, therefore, to compare it with previously described species using cultural characters, microscopy and phylogenetic analyses. Besides, from a practical point of view, a key to *T. systylus* and related species is presented.

Materials and methods

Isolation: The strain was isolated from a semi-arid soil sample from Catamarca, Argentina. Five g of soil were placed in 100 ml of melted (45–50°C) Malt Extract Agar [(Oxoid CM0059; MEA)] prepared with the addition of 50 ppm of chloramphenicol, and heated at 75°C for 30 min. The mixture was plated and incubated at 30°C up to 30 days (Samson et al. 2000). Cultures and dried specimens were deposited at Facultad de Ciencias Exactas y Naturales of the Universidad de Buenos Aires mycological collection (BAFC). The BAFCcult collection is part of the BAFC, from which specimens can be accessed after signing an agreement (MTA).

Morphological studies: For standard descriptions, spore suspensions in semi-solid agar were inoculated at three points on MEA, Oatmeal Agar (OA), Czapek Yeast Agar (CYA), Czapek Agar (CZ), Blakeslee Malt Extract Agar (BMEA) prepared with malt extract Oxoid (LP0039), 25% Glycerol Nitrate Agar (G25N), Yeast Extract Sucrose Agar (YES) and Creatine Sucrose Agar (CREA) according to formulae provided by Samson et al. (2000). All media were poured into 9 cm plastic Petri dishes. Cultures were three-point inoculated on media with a 5 µl ring loop using a dense conidium suspension. Incubation was carried out in the dark, uncovered, at 25°C during 14 days for all media plates (Frisvad & Samson 2004); cultures on CYA were also incubated at 5 and 37°C. In order to study the radial growth of the fungus against temperature, extra cultures on BMEA were incubated at 5, 10, 20, 37, and 45°C as well. Colonies were examined at 7 and 14 days; their diameters were measured using a ruler. For color standards and color nomenclature Ridgway's table (1912) was utilized to describe colony colors. Stern (2004) was used to prepare the Latin description.

For micro-morphological observations microscopic mounts were made in lactic acid 85% w/w from MEA colonies, a drop of alcohol 70% v/v was added to remove air bubbles and excess of conidia; preparations were observed through a Zeiss Axioskop microscope equipped with a drawing tube. The scanning electron microscope (SEM) micrographs were taken with a Quanta 250, FEI environmental mode.

DNA extraction, PCR amplifications and sequencing: Colonies on PDB (Potato Dextrose Broth) incubated at 25°C in darkness for five d were collected, dried and transferred to 1.5 ml microcentrifuge tubes for DNA extraction. For DNA extraction the DNeasy Qiagen kit was used following the manufacturers' instructions. The DNA obtained was quantified by electrophoresis with 1% agarose gels at 120 mV for 15–20 min. DNA was visualized by fluorescence with ethidium bromide. DNA yield was quantified applying 2 µl of sample with NanoDrop spectrophotometer. The DNA regions studied were internal transcribed spacers rDNA-ITS (ITS) and the genes for calmodulin (CaM) and β-tubulin (BenA). The amplification reactions were performed with the primers pair ITS1 and ITS4 (White et al. 1990); CMD5 and CMD6, Bt2a and Bt2b (Glass & Donaldson 1995).

All the PCR reactions were performed with a Mastercycler gradient thermocycler (Eppendorff, USA). The PCR reactions for ITS were performed as follows: in 50 µl of total volume, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM dNTPs, 0.2 µM primers ITS1 and ITS4, 3 mM $MgCl_2$, 1 U/µl polymerase TAQ (Invitrogen life technologies, Brazil) and 10–100 ng/µl genomic DNA. The amplification program was a first denaturation step at 94°C for 1 min, 30 cycles at 94°C for 15 s, 58°C for 15 s, 72°C for 15 s with a final elongation step at 72°C for 3 min. The PCR reactions for CAL were performed as follows: in 20 µl of total volume of 1x Master Mix New England Biolabs, 1.25 mM $MgCl_2$, 50–100 ng/µl genomic DNA. The amplification program was a first denaturation step at 94°C for 1 min, 30 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min with a final elongation step at 72°C for 3 min. The PCR reaction for BenA were performed as follows: in 50 µl of total volume 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM dNTPs, 0.2 µM primers, 3 mM $MgCl_2$, 1 U Taq polymerase (Invitrogen life technologies, Brasil), 50–100 ng/µl genomic DNA. The amplification program was a first step with 5 cycles: denaturation step at 94°C for 1 min, 68°C for 90 s, 72°C for 2 min; 25 cycles at 94°C for 1 min, 64°C for 90 s, 72°C for 2 min with a final elongation step at 72°C for 10 min. The resulting products were purified with Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing was conducted under Big Dye TM Terminator v 3.1 (Applied Biosystems) based on Sanger's method. The products were purified using ethanol precipitation and run with Genetic Analyzer 3130xl at SIGYSA (Argentina).

Molecular phylogenetic analyses: Alignments were done using Clustal W algorithm analysis in Bioedit v. 7.0.5.3 (Hall 1999). Alignments and phylogenetic analyses for ITS, BenA and CaM are deposited in TreeBASE under the accession numbers S16527.

Heuristic searches were conducted using TNT ver. 1.1 (Goloboff et al. 2008). During the search we used equal weights, uninformative characters were deactivated, and gaps were treated as missing data. The searches were done after a Wagner tree search, which was then submitted to 1000 replicas of TBR swapping, holding a total of 10000 trees. Bootstrap values were calculated from 1000 replicates. All the characters were considered with the same weight. Parsimony-based analyses of sequence data were performed for the three genes separately and combined. Neighbour Joining analysis using MEGA 6.1 (Tamura et al. 2013) was also developed, bootstrap test with 500 replicates. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura & Nei 1993) and are in the units of the number of base substitutions per site.

Results

The formal description is based on MEA and OA because these media supported the most distinguishable morphological features; besides it is provided in Latin. Supplementary cultural and morphological characteristics on MEA, OA, CZ, CYA, G25N, YES and CREA are given as well.

Taxonomy

Talaromyces systylus S.M.Romero, V.A.Barrera, A.I.Romero & Comerio, **sp. nov.**
Figs 1–13

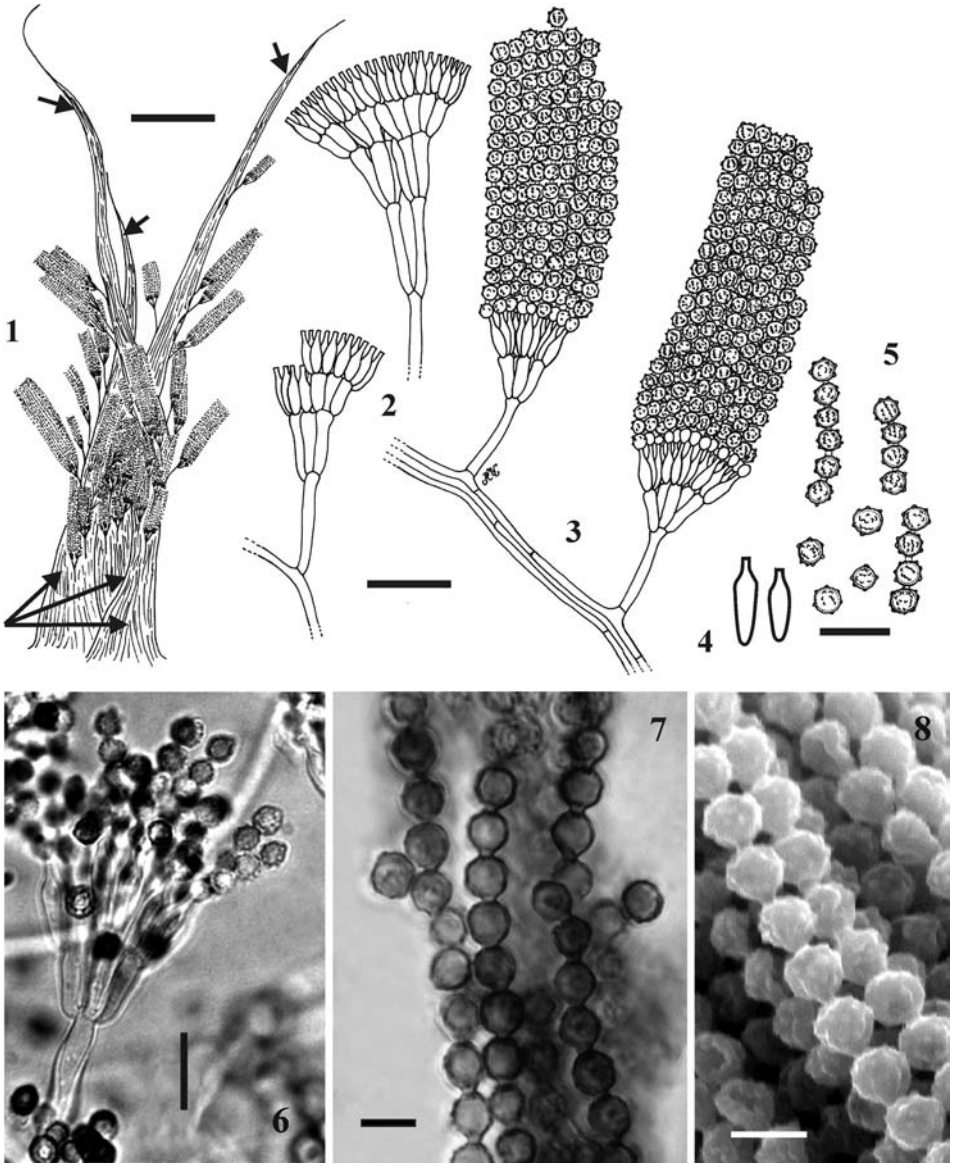
MycoBank MB 812381

Ab affinibus biverticillatis symmetricis penicillatis cum synnematibus aut grossis funiculis speciebus praeclare distinguitur conidiis globosis manifeste rugosis et evidentibus conidiorum columnis.

Etymology: *Syn* (*συν*, prefix), with + *Stylos* (*στυλος*, m), pillar, column = sy + stylus = *systylus*. Referring conidial columns. *Talaromyces systylus* (the basket fungus with the column) in agreement with the white glomeres and dark green conidial columns observed under the stereomicroscope on OA.

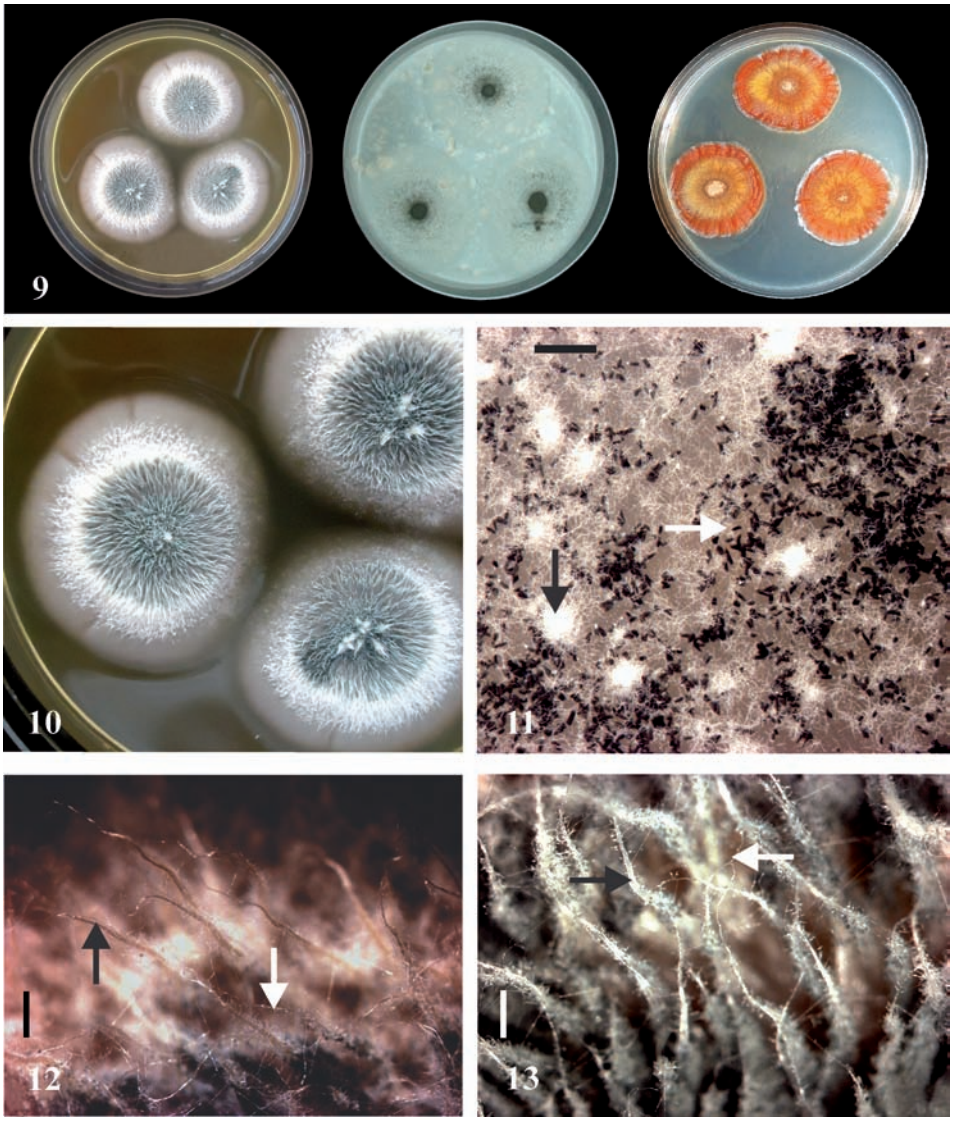
Coloniae in extracto malti agaro (MEA) post 7 dies 25°C 18–21 mm diametro, paulo coriaceae atque humidae, cremeae, cum inmaturis confertisque synnematibus; dorsis luridis vel castaneis. Post duas hebdomades 34–40 mm diametro, viridescentes caesiaeve ob conidia praesentia. Coloniae in agaro avenae farina confecto (OA) post 7 dies 25°C 21–24 mm diametro, planae, conidia paulo genita, atroviridiae in partibus centralibus ob abundantia conidia praesentia; dorsis dilute sulphurinis. Post duas hebdomades 42–48 mm diametro, albi globuli ex laxis hyphis compositi sed neque asci neque ascosporae efficientes 105–350 µm diametro praecipue peripheriam versus adsunt, manifestissimi in vetusta aetate.

Mycelium cremeum in extracto malti agaro, inconspicuum in agaro avenae farina confecto. Synnemata indeterminata parallela, subulata, 1200–3000(–4000) µm alta, conidia in ⅔ basalibus partibus synnematum maxime ostendentia. Conidiophora penicillata in extracto malti agaro e synnematibus oriunda, etsi e pauci funiculi etiam adsunt; in agaro avenae farina confecto ex prostratis funiculis orta, synnemata absentes; plerumque biverticillata ac symmetrica quamquam cum aditiciis ramis aliquando, generatim adpressa in summa. Stipites laeves, 25–60 × 2.5–3.0 µm. Metulae ternae vel quinae, adpressae, laeves, 10–11 × 2.5–3.0 µm. Phialides ternae vel quinae, acerosae vel anguste ampulliformes, 10–12 × 2.5–3.0 µm, cum collis 2–3 µm longis aliquantum visibilibus dimissa magnificatione. Conidia matura globosa, viridia, 3.5–4.0 µm diametro, cum crassis parietibus, sparsis obtusis spinis ornata; spinae saepe combinatae ad cristas formandas; catenae conidiorum consociatae ad columnas 120–180 × 30–50 µm producendas.



Figs 1–8. *Talaromyces systylus* (from holotype). 1. Scheme of three synnemata on MEA (arrows = tips and bases of the three synnemata). 2. Synnematous conidiophores showing additional branches. 3. Funiculose biverticillate conidiophores on OA. 4. Phialides. 5. Conidia. 6. Conidiophore. 7. Chains of conidia (OM). 8. Detail of a conidial column (SEM). Bars = 200 µm (1), 20 µm (2–3), 10 µm (4–6), 5 µm (7–8).

TYPUS: ARGENTINA. CATAMARCA: 27°24'9"S, 66°24'9"W, 3056 m asl., soil, 24 Aug. 2011, leg. S.M.Romero. Holotype BAFC 52367, culture ex type BAFCcult 3419, deposited as dried and living culture at BAFCcult, respectively.



Figs 9–13. *Talaromyces systylus* (from holotype). 9. Colonies from left to right: MEA, OA and CZ, 14 days, 25°C. 10. Detail of the MEA colonies. 11. White globular structures resembling early stages of ascomata (black arrow) and conidium columns (white arrow). 12–13. Synnemata and funicles (black arrow = synnema, white arrow = funicle). Bars = 500 µm (11–13).

Colonies on MEA after 7 days at 25°C with texture leathery and a little bit humid; cream to pale brown colored, reverse pale brown to tan (Clay Color. R. Pl. XXIX); sporulation absent; elongated squared bi-pyramidal crystals abundant in the agar. After

two incubation weeks at 25°C good sporulation, bluish to gray green (Gnaphalium Green or Celandine Green, to Artemisia Green, R. Pl. XLVII). On OA, flat, sporulation sparse but dense at the inoculation area, conidia in masse dark green (Dull Greenish Black, R. Pl. XLVII), reverse light yellow green (Pale Chalcedony Yellow, R. Pl. XVII). After 14 days at 25°C peripheral areas characterized by the presence of white globular structures resembling early stages of ascomata but lacking asci and ascospores; crystals present as in MEA but not so easily seen.

Colonies on CYA 14–18 mm diam. after 7 days at 25°C, leathery, white to pale pink (Cartridge Buff, R. Pl. XXX), bundles of mycelium resembling synnemata at the center and unripe synnemata at the periphery, sporulation very poor or absent, exudate lacking; reverse pale brown (Cream-Buff, R. PL. XXX); soluble pigment absent. After 7 days at 5 °C no sporulation. After one week at 37°C 16–19 mm diam., convex, compounded unripen synnemata in central areas and white mycelial tufts resembling synnemata at periphery, sporulation absent, exudate lacking; reverse pale brown. On CZ 21-23 mm diam. after 7 days at 25°C, white, bundles of mycelium resembling synnemata at the center, no synnematous structures at the periphery, sporulation absent, exudate lacking; reverse pale yellow brown (Chamois to Honey Yellow, R. Pl. XXX). After 14 days of incubation 31–34 mm diam., with little sporulation, obverse pale grey at the inoculation point (Pearl Gray, R. Pl. LII) surrounded by three peripheral mycelium rings in orange tones: orange brown (Antique Brown to Amber Brown, R. Pl. III), yellow orange (Pale Orange-Yellow to Orange-Buff, R. PL. III) and brick color (Mars Orange to Burnt Sienna, R. Pl. II); a subtle transparent halo surrounds the whole colonies; reverse of the same color. Sectors of inconspicuous mycelium often present. On BMEA 21–25 mm diam. after 7 days at 25°C, low, plane, bundles of mycelium resembling synnemata at the center, no synnematous structures at the periphery, white, pale bluish green at the center (Pale Blue Green to Pale Turquoise Green, R. Pl. VII) due to sparse sporulation, exudate and soluble pigment lacking; reverse pale to yellowish (Ivory Yellow to Colonial Buff, R. Pl. XXX). On G25N conidial germination but no linear growth. Germination observed mainly in those conidia aggregated in clumps and almost not present in isolated conidia. Colonies on YES 9–12 mm diam. convex, pale yellow (Cream-Buff, R. Pl. XXX), compounded unripen synnemata in central areas and mycelial tufts resembling synnemata at periphery; reverse pale orange (Chamois, R. Pl. XXX). On CREA growth absent or weak and attaining 11 mm diam, no sporulation; good acid and no base production.

The first week yeast like odor in MEA, OA, CYA, CZ, BMEA and YES.

Radial Growth: Colony diameters were measured at different temperatures on BMEA cultures incubated for a one week period. A curve representing linear growth against temperature is presented in Fig. 14. No fungal development was observed at 5, 10 and 45°C; nevertheless, further incubation of these cultures at 25°C has shown good growth from inoculation points. The maximum linear growth was obtained at 30°C.

Molecular phylogenetic analyses: The isolate representing new species was found to be different from all allied species of *Talaromyces* based on either ITS, BenA, CaM sequences. The trees from the maximum parsimony (MP) and Neighbor-Joining (NJ) analyses showed no difference in the position of the *Talaromyces* clades.

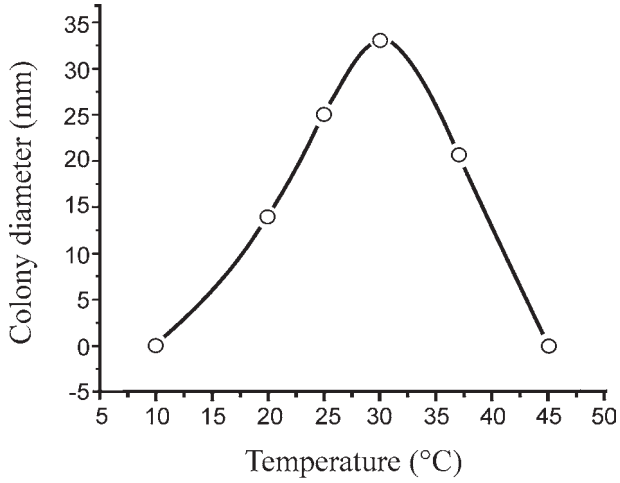


Fig. 14. Radial growth (mm) on BMEA against different incubation temperatures (°C).

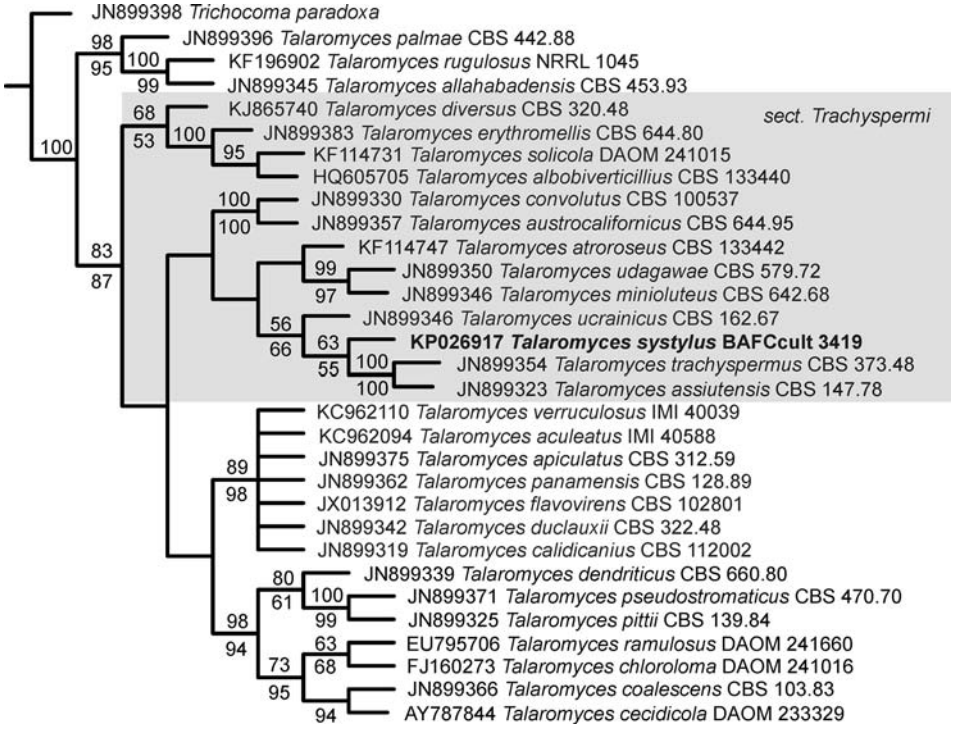


Fig. 15. Strict consensus phylogenetic cladogram constructed with maximum parsimony analysis with ITS sequences. MP and NJ bootstrap values >50% are shown above and below branches, respectively. Terminal nodes given as GenBank accession number, species name, and collection number.

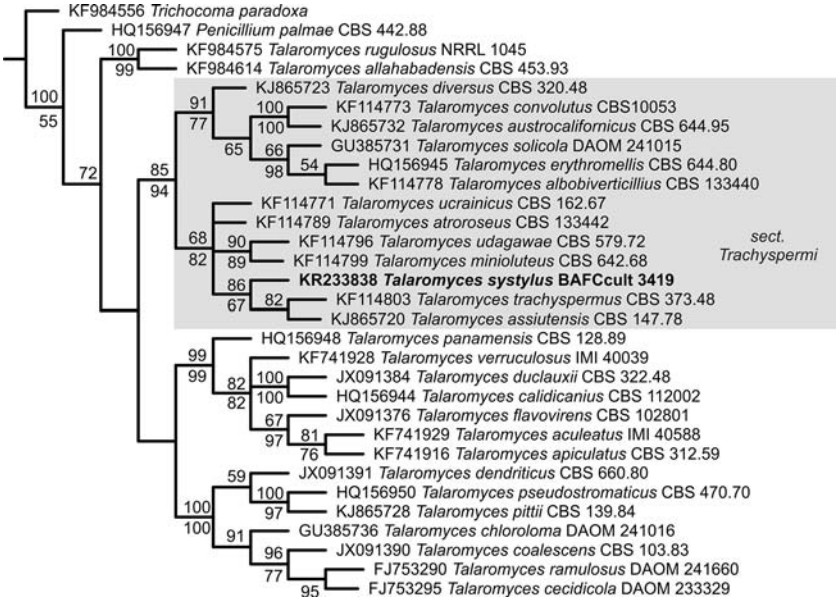


Fig. 16. Strict consensus phylogenetic cladogram constructed with maximum parsimony analysis with BenA sequences. MP and NJ bootstrap values >50% are shown above and below branches, respectively. Terminal nodes given as GenBank accession number, species name, and collection number.

The ITS data set included 31 taxa and 574 characters; 31 and 503 for BenA and for CaM 29 and 537, respectively. The ITS MP analysis yielded 17 optimal trees of 917 steps with a consistency index CI = 29 and a retention index RI = 23 from 155 informative characters. The BenA MP analysis yielded 6 optimal trees of 1618 steps with a CI = 30, RI = 30 from 238 informative characters. The CaM MP analysis yielded 4 optimal trees of 2394 steps with CI = 27, RI = 29 from 297 informative characters. The combined data set (ITS + BenA + CaM), using 1614 characters with 689 informative characters from the three markers produced four most parsimonious trees (4934 steps; CI = 28; RI = 28).

The MP strict consensus trees based on ITS, BenA and CaM, separated and combined are shown in Figs 15–18 with the accession numbers of the query sequences and the 29 reference sequences, obtained from GenBank and *Trichocoma paradoxa*, as outgroup (all them were selected following Yilmaz et al. 2014). Also the NJ bootstrap values are shown on the same cladogram.

The position of the BAFCcult 3419 sequence was in the same clade for both, MP and NJ, analyses for the three genetic markers (ITS, BenA, CaM) when were performed separately or altogether. It was always grouped within section *Trachyspermi* according to Yilmaz et al. (2014). In the ITS MP tree, *T. systylus* (GenBank KP026917) was grouped with the clade (MP = 63 bv; NJ = 55 bv) formed by *T. assiutensis* JN899323

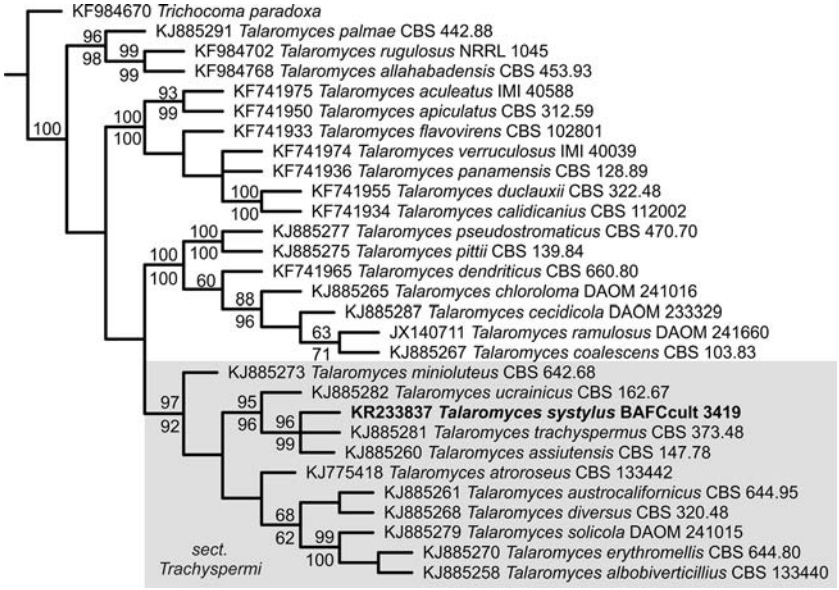


Fig. 17. Strict consensus phylogenetic cladogram constructed with maximum parsimony analysis with CaM sequences. MP and NJ bootstrap values >50% are shown above and below branches, respectively. Terminal nodes given as GenBank accession number, species name, and collection number.

and *T. trachyspermus* JN899354. In the BenA MP tree, *T. trachyspermus* KF114803 was grouped with the clade (MP = 86 bv; NJ = 67) formed by *T. systylus* KR233838 and *T. assiutensis* KJ865720. In CaM phylogenetic analysis, the three species were grouped together in a polytomy (MP = 96 bv; NJ = 99). In the combined MP tree with ITS, BenA and CaM sequences, the clades are identical to the ITS tree with higher bv = 100 in the clade corresponding to *T. systylus*, *T. trachyspermus* and *T. assiutensis*. However, the length of the branches is different, being the branch length of 38 for *T. systylus* vs 21 for the pair composed by *T. assiutensis* and *T. trachyspermus.*

Discussion

Talaromyces systylus constitutes a special case within the genus because it combines the production of indeterminate synnemata and coarsely rough-walled, globose conidia. Taking into account Samson et al. (2011), Visagie et al. (2009), Visagie & Jacobs (2012) and Yilmaz et al. (2014) the species described in the present work is, on the bases of its penicilli and conidial shape, similar to *T. aculeatus*, *T. apiculatus*, *T. diversus*, *T. solicola* and *T. verruculosus*. Despite these features, the concomitant production of synnemata with an elongated fertile zone suggests a connection with *T. allahabadensis*, previously studied in connection with the *P. funiculosum* complex by Van Reenen-Hoekstra et al. (1990), and *T. duclauxii*. In addition, the presence of indeterminate

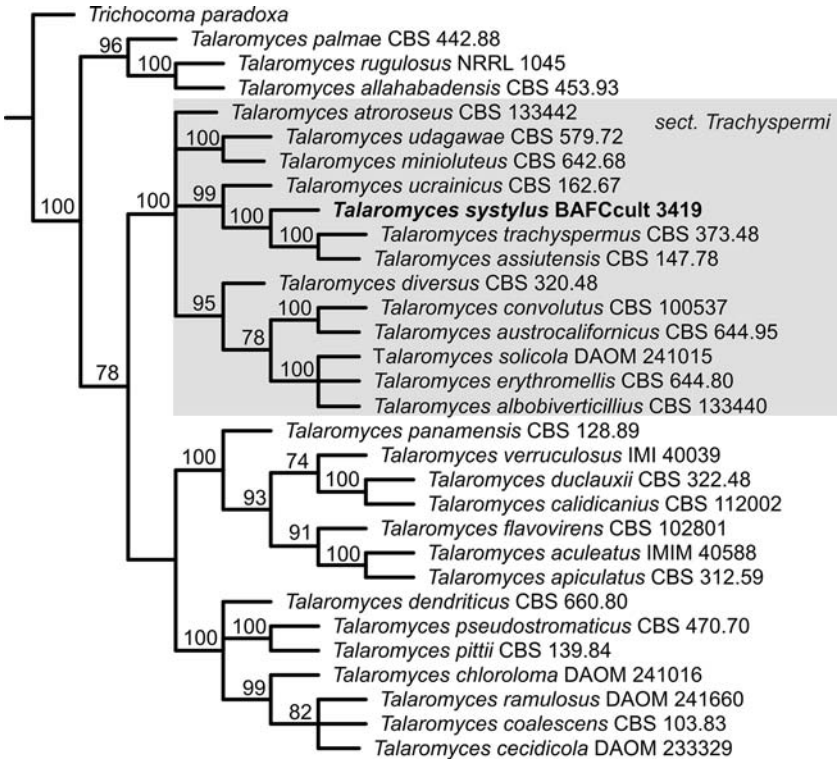


Fig. 18. Strict consensus phylogenetic cladogram, constructed with maximum parsimony analysis, from ITS, BenA and CaM combined sequences. MP bootstrap values >50% are shown above branches. Terminal nodes given as GenBank accession number, species name, and collection number.

synnemata and conidial columns might indicate a relation with *T. palmae* as well. Furthermore, the MEA colony texture of *T. systylus* resembles that of *T. funiculosus* (Thom) Samson et al. exposed in the work of Yilmaz et al. (2014) and represented by their Fig. 39 B. These authors states as a distinguishing character that *T. funiculosus* produces colonies that are strongly funiculose. Concerning *T. systylus* colonies on MEA, after two incubation weeks and despite the presence of few synnemata at the center, the colony texture was dominated by compacted groups of conidiophores placed in an oblique position regarding the substrate. These structures consisted in coriaceous axes supporting penicilli, with a free apex, that can be individually and entirely excised from the basal mycelium felt with a needle. In addition, the analysis of colony texture under the stereomicroscope revealed delicate funicles connecting synnemata one to each other (Fig. 12). The particular oblique structures mentioned, neither perpendicular nor parallel to the substrate, were interpreted as synnemata in the present work; nevertheless, such structures could probably also suggest cord-like funicles.

Table 1. Morphological features of synnematous species of *Talaromyces*.

Species	Stipe wall	Phialide shape	Conidial shape	Conidial wall	Conidial chains pattern
T. allahabadensis[a]	smooth	acerose[c]	ellipsoidal to somewhat fusiform	smooth, occasionally finely roughened	loose columns disordered[c]
T. calidicanius	finely roughened[d]	ampulliform to acerose	ellipsoidal, short fusiform, ovoid or subglobose	spiral-striated transversely	long disordered
T. cecidicola	smooth	acerose or narrowly ampulliform	ellipsoidal	smooth or slightly roughened	disordered[b]
T. chloroloma	smooth	acerose	ellipsoidal	smooth	disordered
T. coalescens	smooth, seldom rough[d]	acerose	commonly ellipsoidal but also subspheroidal	smooth	aggregated in compact columns sometimes twisted
T. dendriticus	smooth	acerose	ellipsoidal	smooth	aggregated in long columns
T. duclauxii	smooth to finely roughened[d]	acerose	ellipsoidal to apiculate	smooth to finely roughened	disordered
T. flavovirens	smooth	cylindroid	ovoid-ellipsoidal	slightly verrucose	-
T. palmae	smooth	acerose	ellipsoidal	smooth	long becoming columnar
T. panamensis	smooth	acerose	ellipsoidal	smooth	-
T. pittii	smooth	acerose	subspherical to ellipsoidal	smooth	entangled chains
T. pseudostromaticus	smooth	acerose	ellipsoidal	smooth	disordered
T. ramulosus	smooth	acerose	subspheroidal to ellipsoidal	smooth	closely packed
T. systylus	smooth	acerose or narrowly ampulliform	globose	rough, spines combined forming ridges	ordered in columns (rows)

[a] described as strongly funiculose and becoming synnematous after a prolonged incubation period (Van Reenen-Hoekstra et al.1990).

[b]Not expressly described but conjecturable from the protologue.

[c]According the illustration from Van Reenen-Hoekstra et al. (1990).

[d]Smooth according to Yilmaz et al. (2014).

(-): information not found.

Taking into account the morphological information included within the literature cited, two tables are offered for rapid comparison purposes. The Table 1 presents some features displayed by synnematous *Talaromyces* species; the Table 2 describes some features of non synnematous rough-conidia-bearing *Talaromyces* spp. The presence of white globular structures resembling early stages of ascomata might reinforce the connection with the sexual state. However, *Talaromyces systylus* is not only supported by the present phylogenetic analysis as new, it seems to be close to the members of the *Trachyspermi* Section (as defined by Yilmaz et al. 2014). *T. systylus* agrees with the main features of this Section as the slow growth on CYA and YES and faster on MEA, poor growth on CREA, and production of strong orange pigment. Additionally, within *Trachyspermi* Section the new species is segregated from the other members by its globose coarsely roughened conidia arranged in columns. In the whole phylogenetic analyses carried out in the present study, *T. systylus* always appears associated in the same clade with *T. trachyspermus* and *T. assiutensis* but analyzing the length of the branches in the tree based on the combined matrix with the three markers, 38 changes can be appreciated which separate *T. systylus* from the other two species. From the morphological point of view, *T. trachyspermus* and *T. assiutensis* have acerose phialides, ovoid to ellipsoidal and smooth conidia in disordered chain; these features are different from those of *T. systylus* (see Table 1). Besides, *T. systylus* produces acid in CREA and *T. trachyspermus* and *T. assiutensis* do not. These two species develop ascomata, but a teleomorph of *T. systylus* is not known.

From a practical point of view it would be worth to point out that only the MEA Oxoid favored good synnemata development in 7 days and their sporulation in two weeks. This fact suggests MEA useful to check synnemata production in *Talaromyces* species. In addition, CZ was the most useful media for mycelial pigments expression.

Key to related indeterminate synnematous or strongly funiculose species of *Talaromyces*

As an aid for determining species, a key based on the cited literature is provided. Any isolate should be inoculated on one MEA and two CYA plates respectively. Incubation of MEA cultures: 25°C, 7–21 days. Incubation of CYA cultures: 25 and 37°C for 7 days.

1 Growth on CYA 37°C, 7 days, present 2
1' Growth on CYA 37°C, 7 days, absent ..... *T. palmae*

2 Colonies on CYA 37°C, 7 days, more than 20 mm diam 3
2' Colonies on CYA 37°C, 7 days, less than 20 mm diam 4

3 Colonies on CYA 25°C less than 25 mm diam.; velvety on MEA; conidia ellipsoidal to fusiform ..... *T. allahabadensis*
3' Colonies on CYA 25°C more than 25 mm diam.; strongly funiculose on MEA; conidia ellipsoidal ..... *T. funiculosus*

4 Conidia ellipsoidal, walls smooth to finely roughened ..... *T. duclauxii*
4' Conidia globose, walls coarsely roughened ..... *T. systylus*

Table 2. Morphological features of non-synnematous *Talaromyces* species close to *T. systylus.*

Species	Stipe wall	Phialide shape	Conidial shape	Conidial wall	Conidial chains pattern
T. aculeatus	smooth	ampulliform-acerose	mostly spheroidal	heavy verrucose to spinose	disordered
T. apiculatus	smooth	flask-shaped	globose with a minor proportion apiculate	echinulate	-
T. diversus	smooth or nearly so	acerose	ellipsoidal to subglobose	smooth to delicately roughened	loosely parallel chains
T. solicola	smooth	acerose	subspheroidal	verrucose, rough-walled	disordered
T. verruculosus	smooth	ampulliform-acerose	spheroidal, subespheroidal	thick verrucose	disordered

(-): information not found.

At the present, the holotype of *T. systylus* is the only known strain of the species. The isolate was obtained in the course of a four-year screening work on thermoresistant ascospores from native semiarid soils. A single strain is not enough to completely draw the natural history of *T. systylus*; nevertheless, it was considered that the particular morphological, physiological and molecular features of the strain suffices to set it as a different biological entity in the context of species with biverticillate penicilli. Further ecological studies could throw light on the biology of *T. systylus* and variations among strains.

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