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ORIGINAL ARTICLE

First record of *Talaromyces udagawae* in soil related to decomposing human remains in Argentina

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KEYWORDS
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Abstract The morphologic features of *Talaromyces udagawae* Stolk and Samson are here described and illustrated. This teleomorphic Ascomycota fungus was isolated from soil obtained in Buenos Aires province (Argentina) from beneath a human cadaver in an advanced state of decomposition. After washing and serial dilution of the soil along with moist-chamber techniques for fungal cultivation, *T. udagawae* formed very restricted colonies of bright yellow color on different growth media with 8-ascospored asci. The ascospores were ellipsoidal and ornamented. The anamorphic state was not observed. Molecular-genetic techniques identified the species. The present record is the first of the species in Argentina, pointing it as a tool to identify soils where cadaver decomposition occurs.

PALABRAS CLAVE
Talaromyces udagawae;
Hongos de suelo;
Descomposición cadavérica;

Primer registro de *Talaromyces udagawae* relacionado con cuerpos humanos en descomposición, en suelo de la Argentina

Resumen Se describen e ilustran las características morfológicas de *Talaromyces udagawae* Stolk y Samson. Se aisló el estado teleomórfico de este hongo Ascomycota de suelo obtenido en la provincia de Buenos Aires (Argentina), por debajo de un cadáver humano en avanzado

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Ascomycota;
Potencial forense

estado de descomposición. Las muestras de suelo fueron analizadas mediante lavado, dilución seriada y cámaras húmedas, técnicas ampliamente usadas para el estudio de hongos de suelo. *T. udagawae* formó colonias muy restringidas de color amarillo brillante en diferentes medios de cultivo, con ascos con 8 ascosporas. Las ascosporas eran elipsoidales y ornamentadas. No fue hallado el estado anamórfico. La especie también fue identificada mediante técnicas moleculares. El presente registro es el primero de la especie en la Argentina y el único que la postula como herramienta para identificar suelos donde ocurre una descomposición cadavérica.

Introduction

The genus *Talaromyces* was established by Chester Ray Benjamin in 1955. The genera mentioned comprise teleomorphic states of *Penicillium*, the species assigned to their corresponding anamorph. *Talaromyces* is characterized by soft, discrete, or confluent globose-to-subglobose surface ascomata of indeterminate growth. Ascomatal coverings vary from scanty to dense and consist of a network of hyphae that may range from very loosely textured to closely knit, but usually surrounded by further generally encrusted growing hyphae that are straight or twisted depending on the species. According to Stolk and Samson[13], ascomatal initials may have various shapes. Asci are short-lived, 4-to-6- or 8-spored, globose to subglobose or slightly ellipsoidal, and borne in chains. Ascospores are likewise globose or ellipsoidal; smooth or showing various ornamentations; and yellow, though rarely becoming reddish[13].

Talaromyces species have been recorded worldwide as being saprophytes utilizing various substrates, and especially organic materials undergoing slow decomposition or rotting wood, in different types of soils. Species such as *Talaromyces stipitatus* (Thom) Bernjamin, *Talaromyces trachyspermus* (Shear) Stolk and Samson, and *Talaromyces flavus* (Klocker) Stolk and Samson are able to degrade cellulose, protein, and keratin; and Eliades *et al.*[8] measured the cellulase, keratinase, and protease activity of *T. stipitatus*, *T. trachyspermus*, and *T. flavus*; *T. stipitatus* also possessed β-glucosidase activity.

The aim of the investigation reported here was to describe and illustrate the presence of *Talaromyces udagawae* in soil taken from beneath a human body in an advanced state of decomposition and to isolate and identify this fungus with a view to its potential forensic use.

This species has been previously described by Stolk and Samson (1972)[13] in relation to decaying wood in soil. The present report, however, constitutes the first documentation of *T. udagawae* in Argentina and the first instance of an association of this species with cadaver decomposition.

Materials and methods

T. udagawae was isolated from a soil sample taken from beneath a human cadaver at the moment it was discovered and removed. The person, whose remains were found by the Buenos Aires province police, had been reported missing 24 days before being discovered. The decaying remains were found in Buenos Aires province (−34°21′41.69″ S; 60°5′22.99″ W), Argentina with the skull already reduced to a skeleton and the arms and legs in a state of advanced decomposition. The site contained temporary puddles from a previous rain. The predominant vegetation was herbaceous, being sorghum the most common plant found in the area. Climatologic data obtained from a government weather station located 60 km away from the site showed that the mean ambient temperature from the time of disappearance to the discovery of the remains had ranged from 15.3 to 24.1 °C, while the average precipitation had ranged between 0 and 41 mm. Soil pH was 5.7 (it corresponds to acid soils of Buenos Aires province)[15].

A sample (composed of random subsamples) of soil in contact with the remains and another sample taken 15 m away (control sample) were collected with a sterile spoon at the time of the discovery and transported to the laboratory in hermetic plastic bags. In the laboratory, the soil specimens were processed in accordance with the following steps as detailed in Tranchida et al.[15] Soil washing was done as described by Parkinson and Williams[10], and serial dilutions were made following the Warcup's method[16]. The moist-chamber incubation was carried out as proposed by Elíades et al.[7]

The fungal morphologic structures were observed by optical microscopy, after lactophenol cotton-blue staining[14]. The ultrastructure of the fungal isolate was examined under a Jeol JSM-6390 scanning electron microscope (JSM-6390LV, Jeol, Akishima, Tokyo, Japan) in the Electronic Microscope Service at the Museum of Natural Sciences of La Plata. The morphology of the colonies was evaluated on malta-extract agar (MEA) (Difco™, USA), potato-dextrose agar (PDA) (Britania S.A., Argentina), and corn-meal–yeast agar (CMYA) (BBL™, USA).

For molecular-genetic identification, the fungal DNA was extracted according to Stenglein and Balatti[12]. The ribosomal DNA internal transcribed spacer (rDNA-ITS) region was amplified by the polymerase-chain reaction (PCR) in an XP thermal cycler (Bioer Technology Co, Hangzhou, China) with primer pairs ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′)[18]. The success of the amplification was confirmed by gel electrophoresis using 1.5% (w/v) agarose gels containing GelRed TM (Biotium Inc., CA, USA) at 90 V in 1× Trisborate-EDTA

buffer at room temperature. The PCR product was purified with the aid of the PureLink™ PCR-purification kit (Invitrogen, Löhne, Germany) and then sequenced from both the sense and antisense ends by using the Big Dye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA, USA) in an Applied Biosystems Sequencer (ABI/Hitachi Genetic Analyzer 3130). Similarities of the fragment sequence to those previously published were investigated by using the BLASTn software[1] in the National Center for Biotechnology Information (NCBI) web page. The sequence was registered in GenBank (accession number, KF723838).

Results

T. udagawae was isolated from soil obtained from beneath a human cadaver by using the three techniques mentioned above. It is related to species such as *Dichotomomyces cejpii* (Milko) Scott (Ascomycota, Eurotiomycetes), *T. trachyspermus*, *T. flavus* and anamorphic stage of Ascomycota such as *Penicillium* and *Aspergillus* as mentioned by Tranchida *et al.* (2014)[15].

No colony of *T. udagawae* was found in the control sample. Species found were identified as *Mucor hiemalis* (Hagem) Schipper and *Mortierella* sp. (Zygomycota), and anamorphic stages of Ascomycota as *Penicillium frequentans* (Wehmer) Westling, *Aspergillus* sp., and *Penicillium* sp., as mentioned by Tranchida *et al.*[15].

The *T. udagawae* species was identified on the basis of macro- and micromorphological characteristics, the fungus development in different culture media, and the DNA sequence. These characteristics were compared to the description published by Stolk and Samson[13]. Before the present report, *T. udagawae* had never been observed anywhere in the world in association with decomposing human remains. The fungal isolate identified as *T. udagawae* was deposited in the collection of Botanical Institute C. Spegazzini, National University of La Plata under accession number LPSC 1163.

T. udagawae grew very restrictedly on MEA, attaining a diameter of 0.8–1.1 cm within 2 weeks at 25 °C. The resulting colonies – consisting of an interwoven felt that presented numerous ascomata near the agar surface – were bright yellow in color ranging from lemon yellow at the margins to empire yellow in the central areas[11] surrounded by a thin, regular submerged margin. The underside was orange in color, near orange yellow, becoming an ocherous orange in the center[11]. An exudate was present in the form of colorless to orange droplets. The fungus odor was not pronounced.

Likewise, colonies on PDA grew very restrictedly, reaching a diameter of 0.3–0.8 cm in 2 weeks at 25 °C. The ascomata and color of the colony were similar to their appearance on MEA.

Colonies on CMYA – attained 0.3–0.7 cm in 20 days at 25 °C – resembled those on PDA and MEA; but ascomata were less abundantly overgrown by an aerial mycelium, and the submerged colony margin was much broader, exhibiting pale-yellow shades. The underside appeared the same as on malt agar.

The asci were 8-spored (Fig. 1), ellipsoidal to subglobose, and 7.5–8.7 by 6–8 μm in dimensions. The ascospores

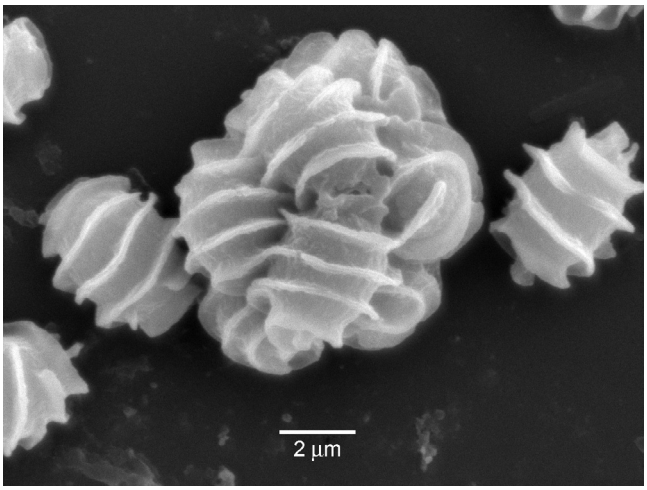

Figure 1 Ascus of *T. udagawae* with eight ascospores observed by scanning electron microscopy.

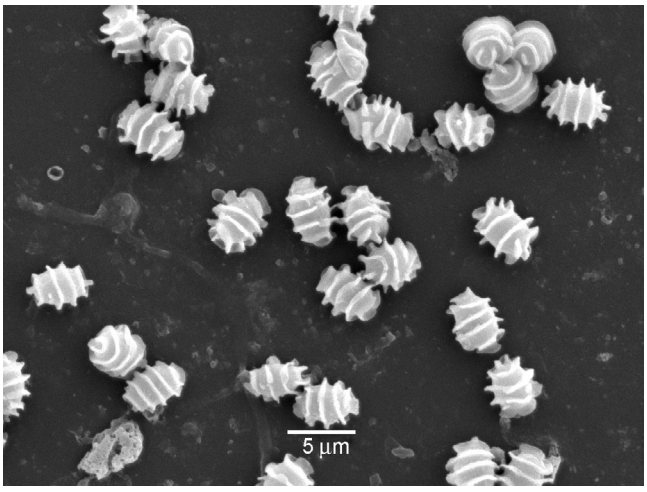

Figure 2 Ascopores of *T. udagawae* under scanning electron microscopy.

were ellipsoidal, 3.5–5 by 2.5–3.3 μm in dimensions and were ornamented with 3–5 regularly transverse, nearly parallel ridges, approximately 600–700 nm wide and often spirally arranged (Fig. 2).

The anamorph was not observed in the cultures maintained in this way under laboratory conditions.

The rDNA ITS sequence of the isolated fungus was 730 bp in length, and BLAST searches showed 98–100% similarity with the *T. udagawae* specimens of accession numbers AB176634 and JN899350.

Discussion

The results obtained in this work represent the first record of the presence of *T. udagawae* in Argentina. This fungal species was identified by molecular genetics, growth, and micromorphologic characteristics. The anamorph of *T. udagawae* was not observed under laboratory conditions.

The genus *Talaromyces* is frequent in Argentine soils, as Cabello *et al.* demonstrated in 2003[3] and Elíades et al. in 2007[7] in several studies carried out in a native dry forest

of Buenos Aires province. The present finding is relevant to both general mycology and forensic science because the species was found in a soil sample beneath a decaying human corpse. The characteristics of the soil had been likely modified through the contribution of compounds released by bodily decomposition[15]. Thus, Tranchida et al.[15] found that soil fungal communities had been modified in the place where a human cadaver had decomposed. Furthermore, we observed differences in the composition of the fungal communities between the soil sample taken from beneath the body and the control sample. In the latter, many teleomorph species of Ascomycota, such as *T. trachyspermus*, *D. cejpii*, and *T. flavus* were identified.

The burial of human cadavers in natural and seminatural ecosystems occasionally takes place in an attempt to conceal the evidence of a crime. The ability to locate these clandestine graves can be an integral part of the investigative process, while the finding and identification of the cadaver allows the victim's relatives to bring closure. The burial of a body and its subsequent decay influences the chemical and physical properties of the soil, and the biological processes taking place therein, thus generating a complex and dynamic system within that immediate environment. Nevertheless, although taphonomy continues to be a valuable forensic tool, until now most of the attention has been focused on the cadaver rather than on the environment immediately surrounding the interment[4,5].

In addition, certain species of *Talaromyces* have been registered as human pathogens. For example, Weisenborn et al.[17] reported the presence of a *Talaromyces* species during a survey of human-skin and -nail lesions in Panama; and in that study *Talaromyces indigoticus* Takada and Udagawa was isolated from a male affected with onychomycosis, being the first record of *Talaromyces* in the teleomorphic state as a human pathogen. In contrast, members of the *Penicillium* genus have been shown to be involved in different opportunistic fungal infections in humans – such as infections of the respiratory and gastrointestinal tract and in both human-cutaneous and animal mycoses[2,6,9].

These studies represent the beginning of a new line of mycological investigation for Argentina. The present finding establishes a new horizon for further research aimed at gaining deeper understanding of the complexity of fungal communities beneath buried and/or decomposing cadavers. Nevertheless, the fungal communities found in soil under decaying corpses need additional studies in order to be used as effective forensic tools. For example, after sufficient studies, the discovery and identification of *T. udagawae* might be able to provide valuable clues for estimating the time and detecting the site of death, though much more research will be necessary to develop this new discipline in mycology.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

We have no conflict of interest to declare regarding the publication of the information contained in the submitted manuscript.

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