

Article Doi 10.5943/mycosphere/8/8/18 Copyright © Guizhou Academy of Agricultural Sciences

Triadelphia moubasherii sp. nov., from the gut of red palm weevils, Rhynchophorus ferrugineus Olivier

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Abdel-Sater MA and Zeinab Soliman 2017 – *Triadelphia moubasherii sp. nov.*, from the gut of red palm weevils, *Rhynchophorus ferrugineus* Olivier. Mycosphere 8(8), 1128–1237, Doi 10.5943/mycosphere/8/8/18

Abstract

During surveys of the mycota inhabiting guts of some insect species in Assiut area, Egypt, an interesting isolate of a dematiaceous hyphomycete was obtained from a gut sample (out of 11 investigated) of the red palm weevil, *Rhynchophorus ferrugineus*. This isolate was morphologically and genotypically identified as a new species in the genus *Triadelphia (Triadelphia moubasherii* Abdel-Sater and Zeinab Soliman, with 94 % ITS similarity with *T. disseminata* Madrid & J. Edathodu, GenBank accession no. KC489510). The type strain of the new species was deposited at Assiut University Mycological Centre Culture Collection and given AUMC number 10746 and the ITS gene sequence was deposited at the National Center for Biotechnological Information (NCBI) and accession numbers is given as KY611849. Description and photos of the new species are presented.

Key words – Assiut – insect – pleomorphic – Triadelphia disseminata – T. pulvinata

Introduction

During search of filamentous and yeast fungi in the guts of two insect species, namely honey bees (*Apis mellifera* L., order: Hymenoptera, family: Apidae), black beetles (*Pterostichus melanarius* Illiger, order: Coleoptera, family: Carabidae), and to our knowledge, for the first time worldwide from the gut of adult red palm weevils, RPW (*Rhynchophorus ferrugineus*, order: Coleoptera, family: Curculionidae), yeasts and filamentous fungi were isolated more frequently from the latter (Moubasher et al. 2017). An interesting filamentous strain was recovered from a gut sample of red palm weevil that could be designated based on macroscopic and microscopic features to the genus *Triadelphia*.

The pleomorphic genus *Triadelphia* Shearer & Crane was erected by Shearer & Crane (1971) to accommodate a sporodochial hyphomycete, *T. heterospora* Shear & Crane, isolated from wood blocks submerged in the Patuxent River, Maryland, USA and characterized by two different forms of conidia. The gregarious to caespitose, globose to subglobose to ampulliform conidiogenous cells borne directly on vegetative hyphae are the hallmark of *Triadelphia*. A similar fungus, *Dicoccum inquinanis* Sacch., found on decaying wood, was transferred into *Triadelphia* by Hughes & Pyrozynski in (1972) as *Triadelphia inquinans* (Sacc.) S. Hughes & Pyroz.. Maggi et al. (1978) described two additional species from the rhizosphere soil of *Loudetia simplex* in the Ivory Coast,

Triadelphia loudetia Maggi, Bartoli & Rambelli, and *T. pulvinata* Maggi, Bartoli & Rambelli, but mentioned only one form of conidia. In 1982, Consantinescu & Samson described a fifth species, *T. romanica* O. Const. & Samson from hen egg shell and proposed a new combination (*Triadelphia alabamensis* (Matsushima) O. Const. & Samson) for *Stemphyliomma alabamense* Matsushima. Reexamination of *Triadelphia heterospora* and *T. inquinanis* dried herbarium specimens, as well as living cultures of *T. loudetia* and *T. pulvinata* showed that these species also possess 3–5 forms of conidia, and the generic description of the genus is emended accordingly and a key for the six species was provided (Constantinescu & Samson 1982).

Later, a new combination, *Triadelphia uniseptata* (Berk. & Br.) P.M. Kirk (1983) was proposed for *Sporidesmium uniseptatum* Berk. & Br. In addition, six species of *Triadelphia* have been described: *Triadelphia stilboidea* Mercado & Oastaneda (1983) from Cuba, *T. hungarica* Révay (1987) on forest litter from Hungary, *T. australiensis* B. C. Sutton (1989) from Queensland, Australia, *T. diversa* Tzean & Chen (1989) on fallen, decayed angiosperm tree stem from Taiwan, and *T. queenslandica* Matsushima (1989); and *T. morgoensis* Revay (1992) on decaying wood from Hungary and a key for 12 species was proposed (Revay 1992). Also, *T. stilboidea* (which is characterized by peculiar synnematous conidiophores, tretic conidiogenous cells and two kinds of conidia) is probably not closely related to *T. heterospora*, the type species of *Triadelphia*, or to other species presently referred to this genus (Revay 1992).

In 1996, *T. centroseptata* was described on dead rachis of *Cocos nucifera* by Venkateshwarlu et al. (1996), however it was not validly published (MycoBank, Index Fungorum, also refer to Patil & Borse 2015).

Triadelphia pulvinata was originally described from the rhizosphere of the grass *Loudetia simplex* in the Ivory Coast as having only one asexual morph (Maggi et al. 1978), but three morphs were reported later by Constantinescu & Samson (1982) and Tzean & Chen (1989). The second isolation of *Triadelphia pulvinata* was also from soil contaminated with bat guano in Saudi Arabia and its pathogenicity to experimentally infected mice has been proven (Al-Hedaithy & Leathers 1987). The third record of this species was from a case of eyelid infection of an apparently immunocompetent patient, represents the first human infection caused by *T. pulvinata* (Al-Hedaithy 2001) and subsequently the fourth isolation from a fatal disseminated infection in a female with leukaemia in Riyadh Province, Saudi Arabia (Edathodu et al. 2013).

In June 2015, the isolate of *Triadelphia pulvinata* that was recovered from the fatal disseminated infection in the immunocompromised human patient in Riyadh Province, Saudi Arabia was reidentified and described as a new species based on ITS gene sequencing as *T. disseminata* Madrid & J. Edathouda (Madrid et al. 2015). Of these 15 species known taxa, only *T. uniseptata*, Kirk 1983, *T. australiensis*, Sutton 1989, and *T. centroseptata*, Venkateshwarlu et al. 1996 show one conidial form, but all other species show at least two (up to five) conidial forms (synasexual morphs) on natural substrates and in culture (Constantinescu & Samson 1982, Tzean & Chen 1989, Révay 1992). Also, no sexual forms have been linked to all *Triadelphia* species except *T. uniseptata* was reported as the asexual morph of *Ascolacicola aquatica*, a member of Sordariales (Ranghoo & Hyde 1998, Ranghoo et al. 1999, Maharachchimbura et al. 2016). Under unitary nomenclature, *T. uniseptata* might need to be considered a synonym of *Ascolacicola aquatica* (Madrid et al. 2015). However, in the analysis of Réblová et al. (2016), *T. uniseptata* nested within the monophyletic *Ascotaiwania* clade as a sister to *A. mitriformis*.

Materials & Methods

Collection and dissection of insects to obtain guts

A total of 11 samples from red palm weevils, RPW (*Rhynchophorus ferrugineus* Olivier, order: Coleoptera, family: Curculionidae) were kindly supplied by the Faculty of Agriculture, Assiut University, in July 2013, February and March 2014. Insects obtained were put in clean glass bottles and kept for 3 days with slightly moistened filter paper so that surface debris is removed. Thereafter, insect samples were put each in a 250 ml conical flask containing 95 % ethyl alcohol

for 1-2 min and washed several times with sterile distilled water for surface sterilization. They were dissected using forceps in a sterile Petri dish, and the gut was removed aseptically from body.

Isolation of gut inhabiting fungi from RPW

The gut is transferred and crushed into a sterile 1.5 ml Eppendorf tube containing 200 μ l of 0.7 % sterile saline solution. All the solution (including gut pieces) was spread over the surface of yeast extract-malt extract-peptone-glucose agar, YM and acidified yeast extract-malt extract-peptone-glucose agar, AYM (Wickerham 1951) plates (at least 3 replicate-plates for each medium type and each gut sample). The inoculated plates were incubated at 25°C for 7 days (Suh & Blackwell 2004). The developing fungal colonies were checked and observed under a microscope. During precise examination, an interesting isolate of a dematiaceous hyphomycete was obtained from a gut sample. This isolate was morphologically identified as a species in the genus *Triadelphia*. However, it was dissimilar in some aspects, when compared, to all known *Triadelphia* species.

Morphology

Culture characteristics and microscopic characters were determined after incubation at 25°C in the dark for 14 days on potato-dextrose agar (PDA), potato-sucrose agar (PSA), potato-carrot agar (PCA), Czapek yeast extract agar (CYA), oatmeal agar (OA) and Sabouraud dextrose agar (SDA) (for media composition refer to Samson et al. 2004). Measurements were assessed from slides mounted in lactophenol cotton blue, using Carl Zeiss, Axiostar Plus microscope, Microimaging GmbH, Göttingen, Germany, magnification up to 1000×.

Genotypic identification of the Triadelphia isolate

The isolate was characterized by ribosomal DNA sequence analysis. Sequence data were obtained by growing the fungus on MEA for 4 d at 25 °C. DNA was extracted, isolated and the Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified in SolGent Company, Daejeon, South Korea; using the universal primers ITS 1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS 4 (5'- TCC TCC GCT TAT TGA TAT GC -3') (Moubasher & Zeinab Soliman 2011, Abdel-Sater et al. 2016). Contig was created from the sequence data using CLCBio Main Workbench program 8.5.1 and the sequence obtained was further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained together with those retrieved from the GenBank database were subjected to the Clustal W analysis using MegAlign software version 5.05 (DNASTAR Inc., Madison, Wisconsin, USA) for the phylogenetic analysis (Thompson et al. 1994). The type strain and the holotype of the newly descried species were deposited at Assiut University Mycological Centre Culture Collection and Herbarium (AUMC) respectively, and the ITS gene sequence was deposited at the National Center for Biotechnological Information (NCBI).

Results

Morphological and genotypic characters revealed that the current isolate is quite different from all known *Triadelphia* species. Therefore it could be designated to a new species.

Description of the new species

Triadelphia moubasherii Abdel-Sater & Zeinab Soliman, sp nov.

Figs1-2

MycoBank: MB 820030, Facesoffungi number: FoF 03373

Etymology – the specific name refers to the distinguished professor of Mycology Professor AH Moubasher, the founder of Assiut University Mycological Centre, Assiut University, Egypt.

Identification of the current strain was based on phenotypic features and fungal ribosomal DNA sequence analysis.

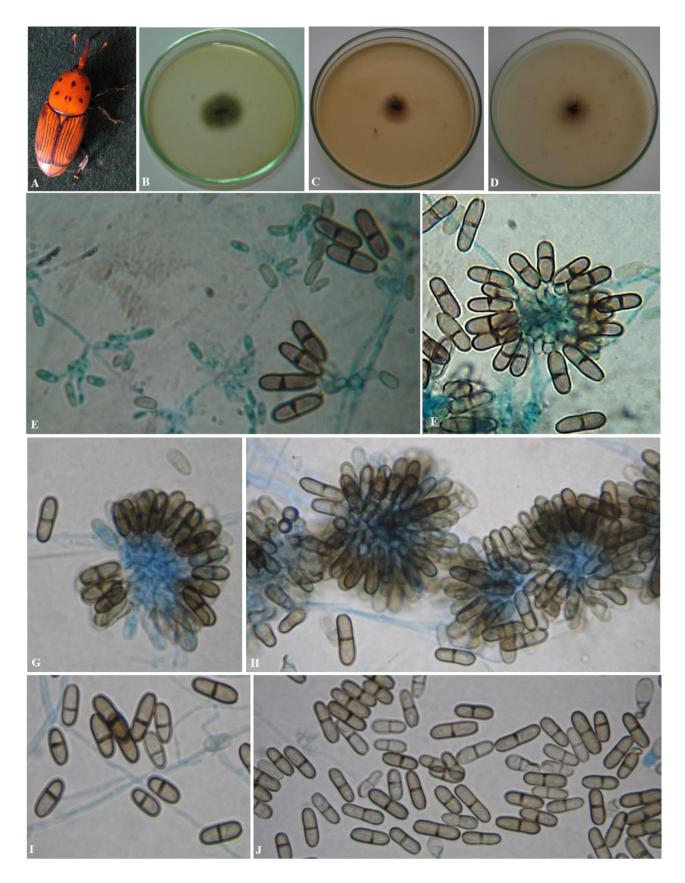


Fig 1 A: The insect *Rhynchophorus ferrugineus* from its gut *Triadelphia moubasherii* AUMC 10746 was isolated, B: on PDA, C: on SDA, D: on OA; E–J: the isolated phialides and sporodochium-like structures bearing one-septate, cylindrical, rounded at both ends, brownish conidia.

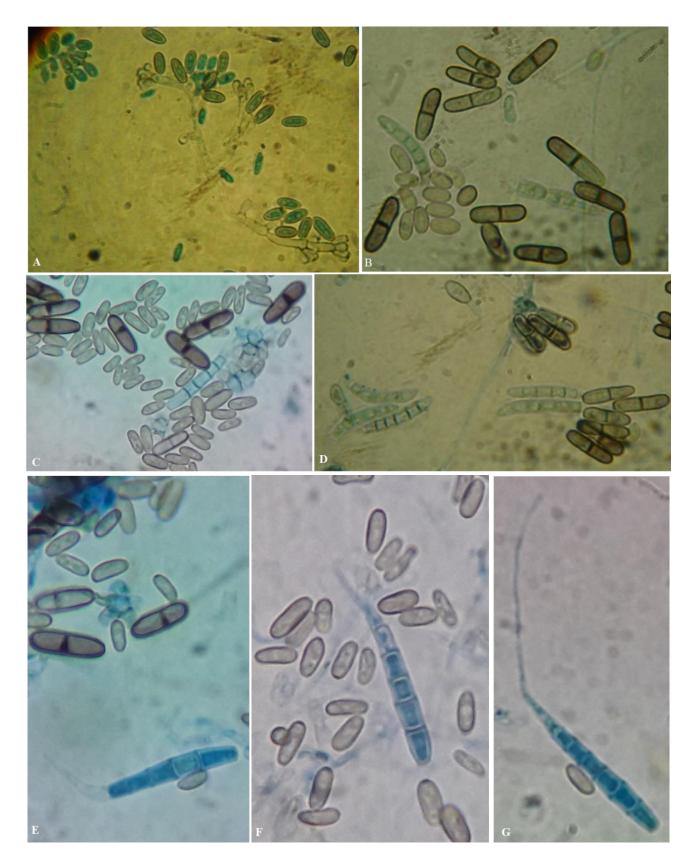


Fig 2 *Triadelphia moubasherii* AUMC 10746, A: phialides bearing one-celled conidia, B–D: the 3 types of conidia (one-celled, two-celled and multiseptate (up to 5-septate), E–G: one-celled and 5–7 multiseptate conidia with long beak).

Examination of microscopic features from potato dextrose agar (PDA) tease mounts in lactophenol cotton blue revealed three distinct types of conidia: 1- cylindrical, rounded at both ends, brown, one-septate, septum thick and situated above the middle, (10-) 14–16 (–19)× 3–4 µm, smooth-walled, conidiogenous cells phialidic, isolated when young but soon aggregated into sporodochium-like structures, hyaline or pale brown; 2- allantoid (curved) to reniform, unicellular, rounded at both ends, hyaline to pale brown, smooth- and thin-walled, 5–9 (–12) × 2–3 (–3.5) µm, conidiogenous cells solitary or aggregated, hyaline, flask-shaped; 3- multiseptate (mostly 5– but up to 7–septate), acicular to obclavate, slightly curved, 20–100 (including the beak) × 3–4 µm, but conidial body only up to (20–) 30–40 (–50)× 3–4 µm, hyaline, smooth and thin-walled, base truncate, conidia gradually tapering into a long 0.5–1 µm wide, hyaline, apical peak; conidiogenous cells solitary (Figs 1, 2).

The three types of conidia are common on potato dextrose agar (PDA), potato sucrose agar (PSA), potato carrot agar (PCA) and Czapek yeast extract agar (CYA) after 14 days of incubation at 25° C, however on oat agar (OA) and Sabouraud dextrose agar (SDA) the most common is the 2nd conidial type, and the 3rd is very rare. Colony surface and reverse brownish on all cultivation media.

The isolate was also characterized by ribosomal DNA sequence analysis (Table 1 & Fig 3). Sequence data obtained was analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website and compared to the ex-type strain of *T. disseminata* CBS 138592=UTHSCSAR-4903, ITS sequence GenBank KC489510. The ITS sequence was 94% identical to the *T. disseminata* ex-type strain. The low percentage identity and the difference in morphology from *T. pulvinata* and *T. disseminata* indicated that it is a different and new species. Thus, the identity of this strain was confirmed to be a new species based on the ITS sequence and consistency with the previously reported morphological features described above.

Material examined – Egypt, Assiut, from the gut of red palm weevils (*Rhynchophorus ferrugineus* Olivier), 12 July 2013, collector and isolator Zeinab Soliman, holotype AUMC 10746, living culture of the new species as well as dried materials are deposited at the culture collection of the Assiut University Mycological Centre, Assiut, Egypt, and ITS sequence GenBank KY611849 was deposited in the National Centre of Biotechnology Information (NCBI) (<u>http:</u>//www.ncbi.nlm.nih.gov.).

Here, the isolation and description of *T. moubasherii* is registered, as the first *Triadelphia* species to be isolated from the gut of red palm weevils, RPW.

Discussion

In this paper *Triadelphia moubasherii* is introduced as a new species that is phylogenetically distinct from other Triadelphia species with DNA sequence data in GenBank. Unfortunately no DNA sequence data are available for most Triadelphia species, therefore all the known species need to be sequenced to establish a complete phylogeny of the genus. The new species is also phenotypically different from all other species in the genus. It produces three forms of conidia in culture [one-septate of cylindrical shape (a-type), multiseptate acicular to obclavate (c-type), and non-septate allantoid to reniform conidia (e-type)]. It shared this feature with other 4 Triadelphia species, its cylindrical conidia (together with T. pulvinata and T. disseminata) but differs in having only one septum from those of T. loudetiae and T. morgoensis whose cylindrical conidia having two septa. Also, it could be distinguished from the other two closely related species (T. pulvinata and T. disseminata) by their conidial sizes (Table 2). Species of Triadelphia were reported from decaying wood (Shearer & Crane 1971, Hughes & Pyrozynski 1972, Revay 1992), decayed angiosperm tree stem (Tzean & Chen 1989), forest litter (Revay 1987), dead rachis of Cocos nucifera (Venkateshwarlu et al. 1996), rhizosphere soil (Maggi et al. 1978), soil contaminated with bat guano (Al-Hedaithy & Leathers 1987), hen egg shell (Constantinescu & Samson 1982), a case of evelid infection of an apparently immunocompetent patient (Al-Hedaithy 2001) and from a fatal disseminated infection in a female with leukaemia (Edathodu et al. 2013, Madrid et al. 2015). From ecological point of view, the new species broaden, and for the first time, the distribution of the

genus *Triadelphia* to include other habitats (guts of insects, anaerobic environments), that probably give the opportunity to these fungi to play, in part, a role in their life cycles on their hosts.

Key of *Triadelphia* species (modified from those of Constantinescu & Samson 1982, Revay 1992)

1. Only one conidial form is present
2. More than one conidial form are present
3. Conidia clavate, $8.5-10 \ge 4.5-6 \ \mu m$
3. Conidia clavate, 12.5–16 x 6.5–10.5 µm <i>T. uniseptata</i>
3. Conidia clavate, 12.5–21 (17.5) x 4.0–8.5 (5.5) µm in size <i>T. centroseptata</i>
4. At least one conidial form is broadly obclavate, fusiform ellipsoidal, with black band at the septa
4. Not as above, acicular to obclavate conidia present
5. Conidia fusiform-ellipsoidal, without acerose end cells <i>T. alabamensis</i>
5. Conidia broadly obclavate or fusiform-ellipsoidal with acerose end cells
6. Cylindrical conidia absent
6. Cylindrical conidia present
7. Fusiform-ellipsoidal conidia 32–36 x 8–10 μm <i>T. hungarica</i>
7. Fusiform conidia 30–42 x 12–18 μm
8. Allantoid or reniform conidia absent
8. Allantoid or reniform conidia present
9. Clavate conidia absent
9. Clavate conidia present
10. Cylindrical conidia 1-septate
10. Cylindrical conidia 2-septate 13
11. Clavate conidia present, cylindrical conidia $6.5-14 \ge 2.5-4 \ \mu m$ <i>T. romanica</i>
11. Clavate conidia absent
12. Cylindrical conidia 5–11 x 2.5–4 μ m, acicular to obclavate conidia 3–4 septate, 43–227 μ m
long
12. Cylindrical conidia 9–12 x 3–3.5 μ m, acicular to obclavate conidia 5–7 septate, 40–160 μ m
long
12. Cylindrical conidia 10–19 x 3–4 μ m, acicular to obclavate conidia up to 4–7 septate,20–100 μ m
long
13. Cylindrical conidia brownish, 9–19 x 3–5 μm <i>T. loudetiae</i>
13. Cells of the cylindrical conidia differently pigmented, 16–25.6 x 3.2–4.8 µm <i>T. morgóensis</i>
15. Cons of the cymuncul contrational inference, 10–25.0 x 5.2–4.0 µm1. morgoensis

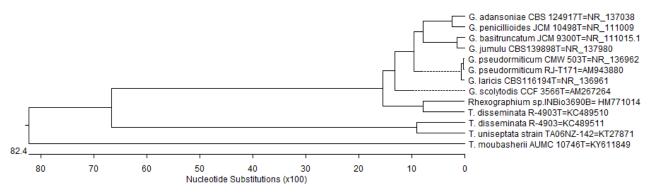


Fig 3 The phylogenetic tree of *Triadelphia moubasherii* based on partial nucleotide sequences (bp) of the ribosomal DNA internal transcribed spacer regions. The scale indicates the number of nucleotide substitutions per site.

Table 1 Assiut University Mycological Center (AUMC no.) of the *Triadelphia moubasherii* strain isolated from red palm weevil together with GenBank accession numbers given together with the closest match in the GenBank database and sequence similarity in percent to the match as inferred from Blastn searches of ITS sequences.

AUMC no.	GenBank accession	Length	Closest Genbank match # ITS		Sequence	Identification	Source
	no.	(pb)	Accession no.	Culture collection code	similarity (%)		
			KC489510	R-4903 ^T	567/601(94%)	Triadelphia disseminata	A patient with acute myeloid leukemia
10746	KY611849	600	HM771014	INBio3690B	443/516(86%)	Rhexographium sp.	Guts of beetle larvae
			AM943880	RJ-T171	360/413(87%)	Graphium pseudormiticum	Tetropiuim sp. colonizing Piceaabies
	as Triadelphia		NR_137980	CBS 139898 ^T	358/413(87%)	Graphium jumulu	Adansonia gregorii (Malvaceae)
	moubasherii sp.		NR_136962	CMW 503 ^T	290/319(91%)	Graphium pseudormiticum	Pine bark beetle
	nov.		NR_136961	CBS 116194 ^T	290/319(91%)	Graphium laricis	A larvae of Ips cembrae on Larixdecidua
			NR_137038	CBS 124917 ^T	290/320(91%)	Graphium adansoniae	African baobab tree (Adansonia digitata)
			NR_111015	JCM 9300 ^T	290/322(90%)	Graphium basitruncatum	Forest soil
			AM267264	CCF 3566 ^T	287/322(89%)	Graphium scolytodis	Ambrosia beetle Scolytodes unipunctatus
			NR_111009	JCM 10498 ^T	289/325(89%)	Graphium penicillioides	Wood core of Populus nigra

Table 2 Synoptic table of different conidial forms (a-f) of different *Triadelphia* species recorded up-to-date.

Species	Conidial forms	Type*	Reference
T. uniseptata, T. australiensis&T. centroseptata	1	a (1-septate)	Kirk 1983, Sutton 1989, Venkateshwarlu et. al. 1996
T. alabamensis	2	d (multiseptate) & e (1-3 septate)	Constantinescu & Samson 1982
T. pulvinata, T. disseminata & T. moubasherii	3	a (1-septate), c (multiseptate) &	Maggi et al. 1978, Constantinescu & Samson 1982,
		e (0-septate)	Madrid et al. 2015, the present work
T. morgoensis	3	a (2–septate), c (multiseptate) &	Revay 1992
		e (0-septate)	
T. loudetiae	3	a (2–septate), c (multiseptate) &	Maggi et al. 1978, Constantinescu & Samson 1982
		e (1–septate)	
T. heterospora	4	a (2-septate), d (multiseptate),	Shearer & Crane 1971, Constantinescu & Samson 1982
		e (1–septate) & f (0–septate)	
T. romanica	4	a (1–septate), b (1–septate),	Constantinescu & Samson 1982
		c (multiseptate) & e (0–septate)	
T diversa	5	a (1–2 septate), b (1–septate),	Tzean & Chen 1989
		d (multiseptate), e (1-septate) &	
		f (0–septate)	
T inquinans	5	a (2-septate), b (1-septate),	Hughes & Pirozynski 1972, Constantinescu & Samson 1982
		c (multiseptate), e (1–septate) &	
		f (0-septate)	
T. hungarica	at least 2	a (not recorded), d (multiseptate), no more available data	Revay 1987 (not available)
T. queenslandica	at least 2	a (not recorded), d (multiseptate), no more available data	Matsushima 1989 (not available)

*Six forms of conidia recognized in different species of the pleomorphic genus *Triadelphia* a: cylindrical, brownish, 1-2-septate; b: clavate, dark brown, uniseptate; c & d obclavate to acicular with a narrow long tip, hyaline or yellowish brown, multiseptate; e allantoid (curved) to reniform, hyaline or pale brown, 0—3 septate; and/or f obclavate to broadly ellipsoidal, pale brown, 0-septate (After Constantinescu & Samson 1982).

Acknowledgements

The authors are deeply indebted for Assiut University Mycological Centre for the financial support provided.

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