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Epitypification, morphology, and phylogeny of *Tothia fuscella*

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Abstract — The holotype of *Tothia fuscella* has been re-examined and is re-described and illustrated. An identical fresh specimen from Austria is used to designate an epitype with herbarium material and a living culture. Sequence analyses show *T. fuscella* to be most closely related to *Venturiaceae* and not *Microthyriaceae*, to which it was previously referred.

Key words — *Dothideomycetes*, molecular phylogeny, taxonomy

Introduction

We have been re-describing and illustrating the generic types of *Dothideomycetes* (Zhang et al. 2008, 2009, Wu et al. 2010, 2011, Li et al. 2011) and have tried where possible to obtain fresh specimens for epitypification and use molecular analyses to provide a natural classification.

Our previous studies of genera in the *Microthyriaceae*, a poorly known family within the *Dothideomycetes,* have resulted in several advances (Wu et al. 2010, 2011). The *Microthyriaceae* are characterized by superficial, flattened, dimidiate ascomata with cells of the upper wall that radiate and open by an ostiole. Asci are bitunicate and fissitunicate and ascospores are hyaline to brown (Hawksworth et al. 1995, Kirk et al. 2008).

Tothia is a poorly known monotypic genus forming thyriothecia currently classified in the *Microthyriaceae* (Lumbsch & Huhndorf 2010). To reassess the morphology of *T. fuscella,* we examined its holotype and re-collected the species

from stalks of *Teucrium chamaedrys* in two different regions of Austria. Cultures of these fresh collections were prepared to extract DNA and to sequence LSU and ITS rDNA in order to establish the phylogenetic position of this fungus. In this paper, we provide a detailed description and illustrations of *T. fuscella* and establish its familial placement through nuLSU rDNA sequence analyses.

Materials & methods

Type material of *Tothia fuscella* was borrowed from URM. Ascomata were rehydrated in 3% KOH prior to examination and sectioning. Specimens were examined under a Leica MZ16A stereomicroscope, and fine forceps were used to remove one or two ascomata, which were mounted in water, Melzer's, Congo red, or cotton blue reagents. Observations and photographs were made under a Nikon E800 or Nikon 80i light microscope. For some hyaline structures differential interference contrast microscopy was used. Sections were cut by hand with a sharp razor blade or into 8-µm thick slices using a Leica CM1100 freezing microtome and then were transferred to a drop of water or cotton blue for examination and photography. Measurement ranges cover a minimum of 30 ascospores, 25 asci, or 10 ascomata. Microtome sections were made from one or two ascomata, and ten measurements were made from whole ascoma and peridia.

Fresh specimens were collected in Austria and herbarium material is deposited in IFRD and WU. Single ascospore isolates were obtained following Choi et al. (1999) and grown on 2% malt extract agar (MEA). Cultures are deposited at the CBS.

Total DNA was extracted from liquid cultures according to Voglmayr & Jaklitsch (2011). A ca. 1600 bp fragment containing partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA was amplified with primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990). PCR products were purified by an enzymatic PCR cleanup as described in Voglmayr & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington) with primers V9G, LR3 (Vilgalys & Hester 1990) and LR5, and an automated DNA sequencer (ABI 377 or 3130xl Genetic Analyzer, Applied Biosystems). The GenBank accession numbers of the sequences obtained in the present study are listed in Table 1.

One *Tothia fuscella* sequence was aligned with 12 sequences downloaded from GenBank (Table 1). Preliminary multiple alignments were generated using BioEdit (Hall 1999) and ClustalX v. 1.83 (Thompson et al. 1997) and then checked visually and manually optimized.

The alignment was analyzed using PAUP* 4.0b10 (Swofford 2002) and MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003). *Schismatomma decolorans* (*Arthoniomycetes*), which is basal to other ascomycetes in a recent study (Schoch et al. 2009), was selected as outgroup.

A Maximum Parsimony (MP) tree was inferred with PAUP* using the heuristic search option with 10000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight; gaps were treated as missing data. Maxtrees were unlimited, zero-length branches were collapsed, and all most parsimonious trees were saved. Maximum parsimony bootstrap

Table 1. *Dothideomycetes* and *Arthoniomycetes* specimens analysed (Newly deposited sequences shown in bold).

Species	Culture	Family (Schoch et al. 2009)	GenBank accession nuLSU rDNA
Apiosporina collinsii (Schwein.) Höhn.	CBS118973	*Venturiaceae*	GU301798
Apiosporina morbosa (Schwein.) Arx.	dimosp	*Venturiaceae*	EF114694
Dothidea insculpta Wallr.	CBS189.58	*Dothideacae*	DQ247802
Dothidea sambuci (Pers.) Fr.	DAOM231303	*Dothideacae*	AY544681
Microthyrium microscopicum Desm.	CBS115976	*Microthyriaceae*	GU301846
Myriangium duriaei Mont. & Berk.	CBS260.36	*Myriangiaceae*	DQ678059
Myriangium hispanicum J.B. Martínez	CBS247.33	*Myriangiaceae*	GU301854
Schismatomma decolorans (Turner & Borrer ex Sm.) Clauzade & Vězda	DUKE0047570	*Roccellaceae*	AY548815
Tothia fuscella	(**TE**)CBS130266	–	JE927786
Tothia fuscella	(**TF1**)CBS130267	-	JE927787
Trichodelitschia bisporula (P. Crouan & H. Crouan) Munk	CBS262.69	*Phaeotrichaceae*	GU348996
Trichodelitschia munkii N. Lundq.	Kruys201	*Phaeotrichaceae*	DQ384096
Venturia inaequalis (Cooke) G. Winter	CBS594.70	*Venturiaceae*	GU301879
Venturia populina (Vuill.) Fabric.	CBS256.38	*Venturiaceae*	GU323212

analyses were done with the same settings, with ten rounds of random sequence addition (Hillis & Bull 1993).

The consistency index (CI; Kluge & Farris 1969), retention index (RI; Farris 1989), and rescaled consistency index (RC; Farris 1989) were obtained from PAUP*.

For Bayesian analyses, evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1 million generations; trees were sampled every 100th generation, resulting in 10 001 saved trees.

The first 2000 trees were discarded and the remaining 8001 trees were used for calculating posterior probabilities in the majority rule consensus tree (Cai et al. 2006, 2008, Liu et al. 2010). The phylogenetic tree was visualized with TREE-VIEW (Page 1996).

Results

Because the SSU-ITS-LSU rDNA sequences obtained for the two *Tothia fuscella* isolates are identical, only one (1535 bp) was included in the analyses. Of the 735 nucleotides analyzed, 478 were constant and 182 variable characters were parsimony informative. Maximum parsimony analysis produced a single

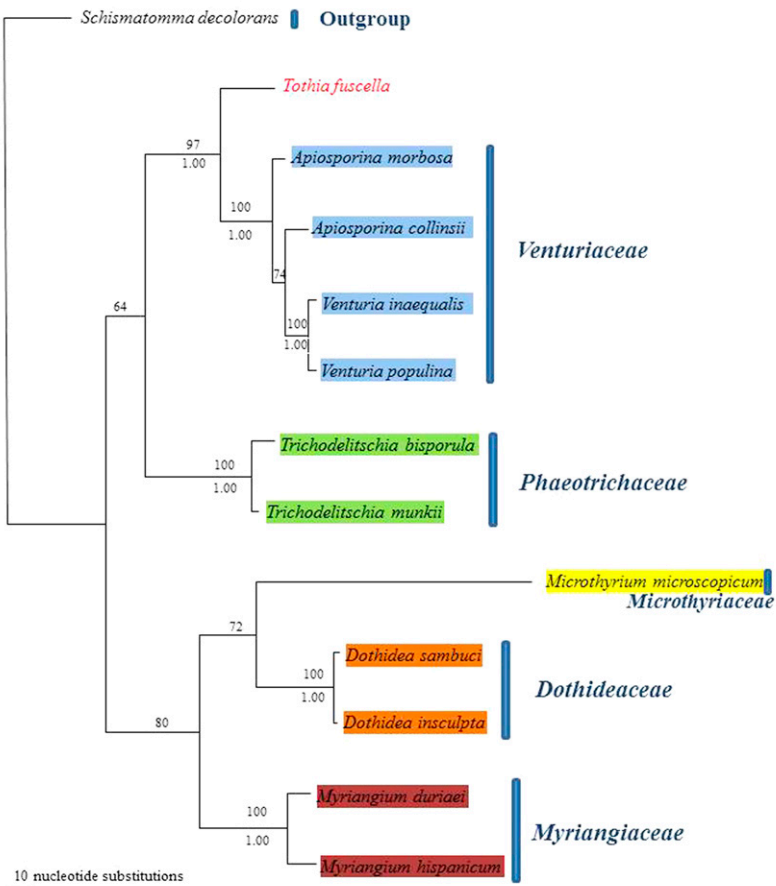

Fig. 1. Phylogram showing the single MP tree of 487 steps, inferred from a heuristic parsimony analysis of the nuLSU rDNA using PAUP*. MP bootstrap support values ≥ 50 % and Bayesian values ≥ 90 % are shown above and below branches, respectively.

MP tree of 487 steps (Fig. 1), with CI = 0.729, RI = 0.735, RC = 0.536 and HI = 0.271. The overall topology of trees sampled in the Bayesian analysis was compatible with the MP tree. MP bootstrap support values and posterior probabilities are shown in Fig. 1.

In the phylogenetic analyses (Fig. 1), *Tothia fuscella* was remote from the *Microthyriaceae* clade but clustered near the *Venturiaceae* clade with 97% bootstrap support and 1.0 posterior probability.

Taxonomy

Tothia Bat., in Toth, Annls hist.-nat. Mus. natn. hung. 52: 105 (1960).

Saprobic on stems. Ascomata, superficial, thyriothecial; in section conical, relatively small, opening with a minute flat or slightly papillate ostiole. Asci 8-spored, bitunicate, fissitunicate, cylindrical or obclavate. Ascospores, 1-septate, light brown.

Anamorphs: None reported for the genus (Hyde et al. 2011).

Tothia fuscella (Sacc.) Bat., in Toth, Annls hist.-nat. Mus. natn. hung. 52: 106 (1960). Fig. 2

≡ *Microthyrium fuscellum* Sacc., Michelia 2(6): 57 (1880).

Saprobic on stems of *Teucrium chamaedrys*. Superficial mycelium absent. Ascomata, solitary or gregarious, superficial, appearing as small black dots on the host surface, thyriothecial; in section 130–270 µm diam, 60–122 µm high, dome-shaped or flat-conical, dark brown, membranaceous, opening by a short papillate ostiole. Peridium 7–15 µm wide above, dark brown, comprising a single upper layer of cells of textura angularis; ascoma base, comprising hyaline ellipsoidal cells. Hamathecium of pseudoparaphyses 1.5–3 µm wide, longer than asci. Asci 57–71 × 9–13 µm (mean = 64 × 10.5 µm, n = 25), 8-spored, bitunicate, fissitunicate, obclavate, with a knob-like short pedicel about 4–6 × 5–7 µm, lacking an ocular chamber. Ascospores 24–28 × 4–6 µm (mean = 25.7 × 4.7 µm, n = 30), 2–3 seriate, fusiform or oblong-ellipsoid, light brown, one-septate, slightly constricted at the septum, upper cell slightly wider than the lower, with four guttules, smooth-walled.

After critical morphological comparisons showed that the fresh collections were identical with the holotype, we designated one specimen as the epitype for *T. fuscella*.

Materials examined: **AUSTRIA**, **Kärnten**, St. Margareten im Rosental, Aussicht, grid square 9452/3, on stalks of *Teucrium chamaedrys* (*Lamiaceae*), soc. *Ophiobolus erythrosporus*, 3 July 2010, W. Jaklitsch (**epitype designated here,** WU31396; ex-epitype culture TF1; LSU-ITS sequence JF927787; iso-epitype IFRD8982); 12 Sep 2010, W. Jaklitsch & O. Sükösd (WU 31397). **Niederösterreich**, Krems, Egelsee, on stems of *Teucrium chamaedrys*, soc. *Ophiobolus* sp., 15 Sep 2010, H. Voglmayr (WU 31398; culture TE; ITS-LSU sequence JF927786). **HUNGARY**, on stems of *Teucrium chamaedrys*, further data not given (holotype, URM 8210).

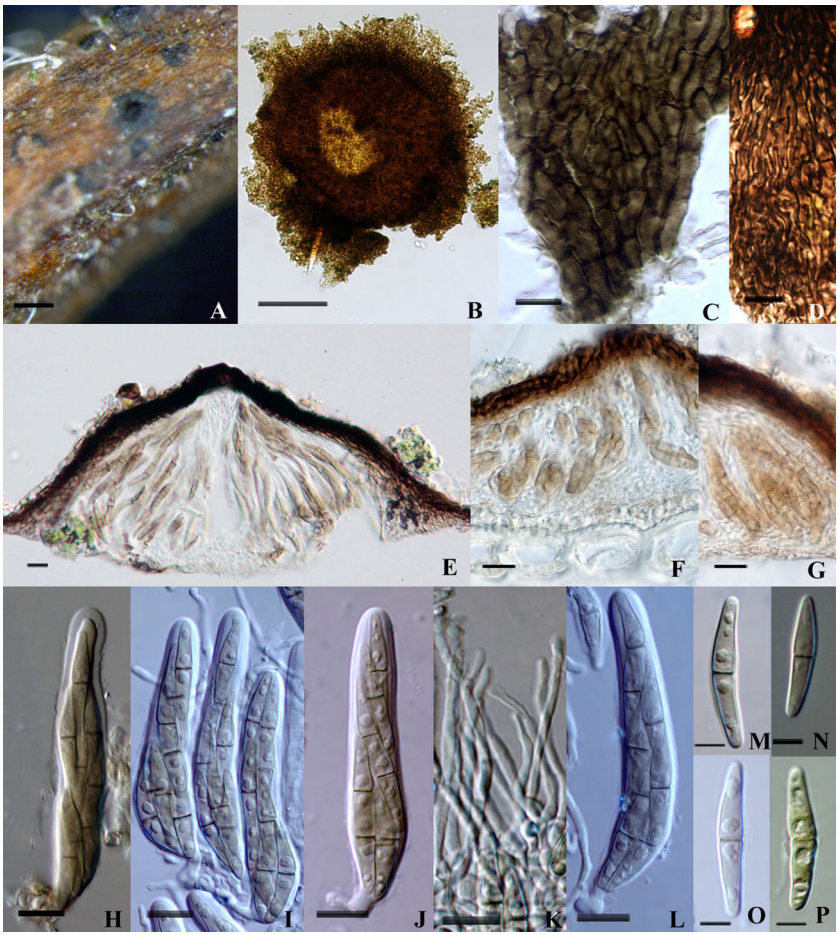

Fig. 2. *Tothia fuscella* (A, D–E, H, N from epitype; B–C, F–G, I–M, O–P, from holotype). A. Appearance of ascomata on the host surface. B, C, D. Squash mount of ascoma. E–G. Ascomata in Section. H–J, L. Asci. K. Hamathecium. M–P. Ascospores. Scale bars: A = 500μm, B = 40 μm, C–L = 10 μm. M–P = 5 μm.

Comments—In this paper we redescribe the type of *Tothia fuscella* and designate an epitype with dried material and an associated living ex-type culture. Ascomata of *T. fuscella* are morphologically indistinguishable from typical representatives of *Microthyriaceae*. Its ascospores resemble *Microthyrium* in being one-septate, guttulate, and slightly asymmetrical but differ in their dilute brown color. Phylogenetic analyses indicate that *T. fuscella* is most closely related to the *Venturiaceae*, to which it should be assigned.

Thyriothecial ascomata are unusual for the *Venturiaceae*, which illustrates that this ascoma type has evolved independently several times within *Dothideomycetes*. Genera in *Venturiaceae* usually have yellowish, greenish brown to brown, one-septate ascospores and obclavate asci (Barr 1968, 1989), which agrees well with *Tothia*.

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