Eur J Plant Pathol (2016) 145:857–869
DOI 10.1007/s10658-016-0874-1

Phylogenetic placement, DNA barcoding, morphology and evidence for the spreading of *Entyloma cosmi*, a species attacking *Cosmos bipinnatus* in temperate climate gardens

Matthias Lutz · Marcin Piątek

Accepted: 26 January 2016 / Published online: 9 February 2016

Abstract The white leaf smut is one of the recently reported and apparently spreading diseases of the garden cosmos (*Cosmos bipinnatus*) causing necrosis and wilting of the leaves and leading to reduction of its ornamental value. The occurrence of this fungal disease was first observed in Japan (Hokkaido Island) in 1996, and later in Germany (Saxony-Anhalt) in 2002, and the causative agent was described as new species, *Entyloma cosmi,* in 2005. This smut was subsequently reported from Canada, Switzerland, and Korea. Here it is reported for the first time from Austria, France, Italy, Poland, and Slovenia. This indicates the rapid spread of *Entyloma cosmi* on *Cosmus bipinnatus* in temperate climate gardens. The phylogenetic position of *Entyloma cosmi* is analyzed using ITS rDNA sequences, the phenotypic characters are critically re-evaluated, and the species is characterized using the Consolidated Species Concept, including morphology, ecology (host plant), and rDNA sequences (ITS and LSU). Selected ITS sequences and one LSU sequence generated in this work are deposited on the BarCode of Life website in GenBank (www.ncbi.nlm.nih.gov/genbank/barcode/) and Fungal Barcoding Database (www.fungalbarcoding.org), and could serve as DNA barcodes to facilitate rapid identification of this economically important species. The ITS sequence from the holotype of *Entyloma cosmi*, sequenced here, is recommended to be deposited in the RefSeq Targeted Loci database.

Keywords *Entyloma* · Garden cosmos · Molecular barcodes · Plant pathogens · Smut fungi · Spreading disease

M. Lutz (✉)
Plant Evolutionary Ecology, Institute of Evolution and Ecology, University of Tübingen, Auf der Morgenstelle 5, D-72076 Tübingen, Germany
e-mail: matthias.lutz@uni-tuebingen.de

M. Piątek
Department of Mycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland

Introduction

The garden cosmos, *Cosmos bipinnatus*, an annual, moderately tall asteracean plant, originates from Mexico and the south-western U.S.A. (Kiger 2006), and is widely planted in the gardens of the temperate climatic zone for its decorative leaves and flowers. Additionally, it escaped cultivation, and naturalized along roadsides and in disturbed places, especially in North America (Kiger 2006). The garden cosmos suffers from a number of fungal diseases, including those caused by *Alternaria cosmosa* (Deng et al. 2015), *Cercospora fagopyri* (Groenewald et al. 2013), *Colletotrichum acutatum* (Kwon et al. 1999), *Diaporthe stewartii* (Udayanga et al. 2012), *Golovinomyces cichoracearum,* and *Podosphaera fusca* s.l. (Jage et al. 2010; Takamatsu et al. 2010), and *Verticillium dahliae* (Carlucci et al. 2009; Blomquist et al. 2011), which affect its health and ornamental value in a number of garden plantations. The USDA Fungal Databases (http://nt.ars-grin.gov/fungaldatabases/) lists more than 60 fungal names reported from seeds or plants

of *Cosmos bipinnatus* though with different levels of identification reliability.

The white leaf smut is one of the recently reported and apparently spreading diseases of *Cosmos bipinnatus* causing necrosis and wilting of the leaves, and leading to reduction of its ornamental value. In 1996, this fungal disease was first observed in Japan on Hokkaido Island, but the causative agent was identified as *Entyloma* sp. (Horita and Haga 1998; Horita 2001). In August 2002, a similar disease of *Cosmos bipinnatus* was observed in Germany (Saxony-Anhalt) and the causative agent was assigned to *Entyloma holwayi*, a species previously known on *Cosmos caudatus* and *C. sulphureus* in Mexico and the U.S.A. (Vánky et al. 2005b). Subsequent detailed comparative morphological study of *Entyloma* specimens on *Cosmos bipinnatus, C. caudatus,* and *C. sulphureus* resulted in the description of the distinct species *Entyloma cosmi* on *C. bipinnatus* differing from *E. holwayi* on *C. caudatus* and *C. sulphureus* mainly by having larger spores, thicker spore walls, and the production of an anamorphic stage (Vánky et al. 2005a). In the original description, *Entyloma cosmi* has been reported from Germany and Japan (Vánky et al. 2005a), and later the species was also found in Korea (Park et al. 2012), Switzerland (Vánky 2012; Bolay 2013), and Canada (Vánky 2012), and again in Germany (Scholz and Scholz 2012; Kruse 2014). *Entyloma cosmi* was one of the most devastating pathogens of *Cosmos bipinnatus* in Japan (Horita 2001) and Korea (Park et al. 2012). The disease may be controlled using systemic ergosterol-biosynthesis-inhibiting (EBI) fungicides like imibenconazole, cyproconazole, and difenoconazole (Horita 2001).

Since 2008, we observed *Entyloma cosmi* in other European countries, in some years causing heavy infection of *Cosmos bipinnatus* in cultivated gardens, but these accumulated data were not published so far. Furthermore, in spite of the progress with sequencing of *Entyloma* species (Begerow et al. 2002; Boekhout et al. 2006; Matheny et al. 2006, 2007; Kemler et al. 2007; Glawe et al. 2010; Koike et al. 2010; Vánky and Lutz 2010; Henricot et al. 2013; Savchenko et al. 2014), *E. cosmi* has not been subjected to molecular analyses as yet, and its phylogenetic relation to other *Entyloma* species and especially to *E. holwayi* (the second *Entyloma* species attacking *Cosmos*) is unknown.

The aim of this study is to resolve the phylogenetic position of *Entyloma cosmi* using ITS rDNA sequences, to provide its detailed description and illustration as well as ITS barcodes for both morphological and molecular identification, and to provide evidence and discuss the spread of *E. cosmi* in particularly European gardens.

Materials and methods

Specimen sampling and documentation

This study is based on phylogenetical and/or morphological analyses of *Entyloma cosmi* specimens originating from seven countries. Nine specimens were selected for molecular phylogenetic analyses, including the holotype and paratype specimen. The voucher specimens are deposited in GZU, KR-M, KRAM F, and BRIP (HUV refers to the personal collection of Kálmán Vánky, "Herbarium *Ustilaginales* Vánky" which was transferred to BRIP) (Table 1, and section Specimens examined). To provide LSU sequences additionally the following specimens were used: *Entyloma bidentis* on *Bidens pilosa*, Costa Rica, Prov. San José, Sabanilla, UCR, 15 Dec. 1993, *leg. M. Piepenbring* (TUB 12576); *Entyloma browalliae* on *Browallia americana*, Costa Rica, Prov. San José, above Santa Ana, El Curio, 5 Nov. 1995, *leg. M. Piepenbring & G. Sancho* (TUB 12577); *Entyloma dahliae* on *Dahlia* x *hortensis*, Switzerland, Grisons, junction of Hinter- and Vorderrhein, 10 Oct. 1993, *leg. M. Piepenbring* (TUB 12574); *Entyloma diastateae* on *Diastatea micrantha*, El Salvador, Dept. La Libertad, Ciudad Merliot, Finca del Espino, 12 Aug. 1995, *leg. M. Piepenbring & O. Molina 1691* (TUB 12579); *Entyloma holwayi* on *Cosmos caudatus*, El Salvador, Dept. Sonsonate, Juayua, La Calera, 27 Aug. 1995, *leg. M. Piepenbring 1769* (USJ).

Morphological examination

Dried fungal spores of the investigated specimens were mounted in lactic acid, heated to boiling point, and then examined under a Nikon Eclipse 80i light microscope at a magnification of × 1000, using Nomarski optics (DIC). 30 spores per investigated specimen were measured using NIS-Elements BR 3.0 imaging software. The extreme measurements were adjusted to the nearest 0.5 μm. The species description includes combined values from all measured specimens. LM micrographs were taken with a Nikon DS-Fi1 camera.

Table 1 List of *Entyloma cosmi* specimens from *Cosmos bipinnatus* for which sequences were obtained in the course of this study with GenBank accession numbers, and reference specimens

Species	GenBank acc. no. (ITS/LSU)	Specimen[a]
E. cosmi	KJ728765/-	Austria, Villach, Faak am See, garden, 46°34′16.56″N, 13°54′32.10″E, 6 Aug. 2011, *leg. M. Lutz 2377*, KR-M-29450
E. cosmi	KJ728766/-	Austria, Villach, Oberschütt, garden, 46°33′57.59″N, 13°45′19.50″E, 18 Aug. 2012, *leg. M. Lutz 2431*, KR-M-0042145
E. cosmi	KJ728760/-	Germany, Baden-Württemberg, Tübingen, Hagelloch, Heuberger-Tor-Weg, garden, 48°32′33.05″N, 09°01′11.75″E, 12 Sept. 2010, *leg. M. Lutz 2290*, KR-M-26154
E. cosmi	KJ728761/-	Germany, Baden-Württemberg, Tübingen, Zwehrenbühlstraße, garden, 48°31′40.66″N, 09°02′10.38″E, 8 Sept. 2010, *leg. M. Lutz 2292*, KR-M-26155
E. cosmi	KJ728762*/-	Germany, Saxony-Anhalt, Kreis Wittenberg, Kemberg, Neumühlenweg corner Windmühlenweg, 3 Aug. 2004, *leg. H. Jage*, BRIP: HUV 20948 (paratype)
E. cosmi	KJ728759*/KP668988*	Japan, Hokkaido, Takikawa, Higashi-takikawa, 30 Sept. 2004, *leg. H. Horita*, BRIP: HUV 20935 (holotype)
E. cosmi	KJ728763*/-	Poland, Małopolska Province: Kraków–Nowa Huta, Osiedle Wandy housing estate, garden, 29 Sept. 2010, *leg. M. Piątek*, KRAM F-48753
E. cosmi	KJ728764/-	Poland, Małopolska Province: Nowy Wiśnicz, ca. 40 km SE of Kraków, roadside, 26 Sept. 2009, *leg. M. Piątek*, KRAM F-48760
E. cosmi	KJ728758*/-	Slovenia, Gorenjska, north of Lake Bled, garden, 9 Aug. 2011, *leg. M. Lutz 2363*, KR-M-29459

*Sequences proposed as barcodes. *E.* = *Entyloma*

[a] Acronyms: BRIP – Plant Pathology Herbarium, Queensland Department of Agriculture, Fisheries and Forestry, Brisbane, Australia (HUV refers to the personal collection of Kálmán Vánky, "Herbarium *Ustilaginales* Vánky" which was transferred to BRIP); KR-M – Mycological Herbarium of the Staatliches Museum für Naturkunde, Karlsruhe, Germany; KRAM F – Mycological Herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland

DNA extraction, PCR, and sequencing

Genomic DNA was isolated directly from the herbarium specimens. The methods of isolation and crushing of fungal material, DNA extraction, amplification, purification of PCR products, sequencing, and processing of the raw data followed Lutz et al. (2004). ITS 1 and ITS 2 regions of the rDNA including the 5.8S rDNA (ITS, about 700 bp) were amplified using the primer pair ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990), the 5′-end of the nuclear large subunit ribosomal DNA (LSU, about 650 bp) was amplified using the primer pair NL1 and NL4 (O'Donnell 1993). DNA sequences determined for this study were deposited in GenBank. GenBank accession numbers for *Entyloma cosmi* specimens are given in Figs. 1 and 2 and Table 1. For other *Entyloma* species GenBank accession numbers of LSU sequences are: KP668990 (*E. bidentis*), KP668989 (*E. browalliae*), KP668992 (*E. dahliae*), KP668993 (*E. diastateae*), KP668991 (*E. holwayi*).

Phylogenetic analyses

To elucidate the phylogenetic position of the *Entyloma cosmi* specimens their ITS sequences were analyzed within two datasets. The first dataset covered all ITS sequences available in GenBank that clustered in the clade including species mainly parasitic on *Asteridae* revealed by Begerow et al. (2002). On the basis of the results of the analyses of the first dataset, the second dataset included all sequences that clustered in an unresolved relation to *Entyloma cosmi* and the sequences of *E. diastateae* representing a cluster of sequences that also clustered in an unresolved relation to *E. cosmi*. GenBank accession numbers of the sequences used (Begerow et al. 2002; Boekhout et al. 2006; Glawe et al. 2010; Henricot et al. 2013; Kemler et al. 2007; Matheny et al. 2006, 2007; Savchenko et al. 2014) are given in Figs. 1 and 2. Sequence alignment was obtained using MAFFT 7.215 (Katoh and Standley 2013) using the L-INS-i option. The resulting alignment of the second dataset was used for both the detection of species-specific nucleotide differences and the estimation of genetic distances

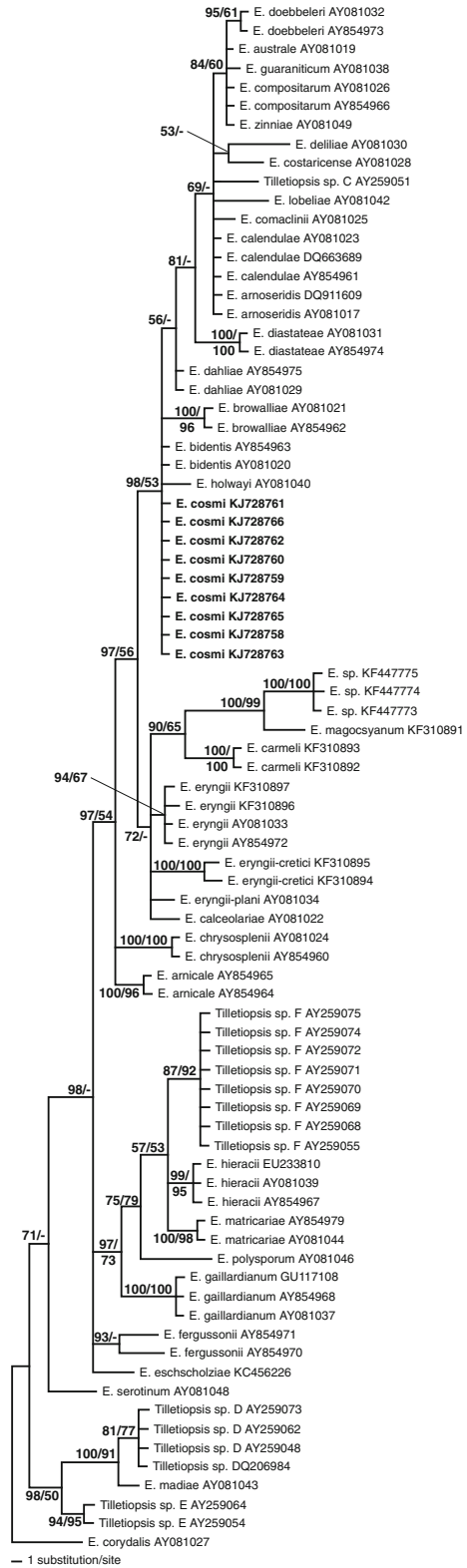


Fig. 1 Bayesian inference of phylogenetic relationships of the sampled *Entyloma* and *Tilletiopsis* species: Markov chain Monte Carlo analysis of an alignment of ITS base sequences using the GTR + I + G model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting trees and default starting parameters of the DNA substitution model. A 50 % majority-rule consensus tree is shown computed from 75 000 trees that were sampled after the process had become stationary. The topology was rooted with *Entyloma corydalis*. Numbers on branches before slashes are estimates for *a posteriori* probabilities; numbers on branches after slashes are ML bootstrap support values. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. E. = *Entyloma*

within species (intraspecific) and between species (interspecific) using the software MEGA (Tamura et al. 2011). We calculated p-distances and report distances as percentage genetic distances. Gaps or different length of sequences were not used for calculations as we chose the pair-wise deletion option in MEGA.

For phylogenetic analyses using a Bayesian Approach (BA) and Maximum Likelihood (ML) the resulting alignments of both datasets (number of positions: 871/695, number of variable sites: 144/14) were used throughout their length. Alignments were deposited in TreeBASE (S18071). Methods of BA and ML followed Vasighzadeh et al. (2014). For the second dataset additionally a Neighbour-Joining analysis (NJ) was carried out. For NJ the positions 1–60 and 695–709 of the alignment were excluded for subsequent analyses because for some sequences data were missing for these positions. Then data were analyzed with Modeltest 3.7 (Posada and Crandall 1998) to find the most appropriate model of DNA substitution. The hierarchical likelihood ratio test proposed the Jukes-Cantor DNA substitution model. Bootstrap values were calculated from 1000 replicates. NJ analyses were carried out using PAUP* version 4.0b10 (Swofford 2001).

In line with the results of Begerow et al. (2002) and Boekhout et al. (2006), trees resulting from the analyses of the first dataset were rooted with *Entyloma corydalis*. Trees resulting from the analyses of the second dataset were midpoint rooted.

Results

Phylogenetic analyses

The ITS sequences of all *Entyloma cosmi* specimens analyzed were identical. Intraspecific and interspecific

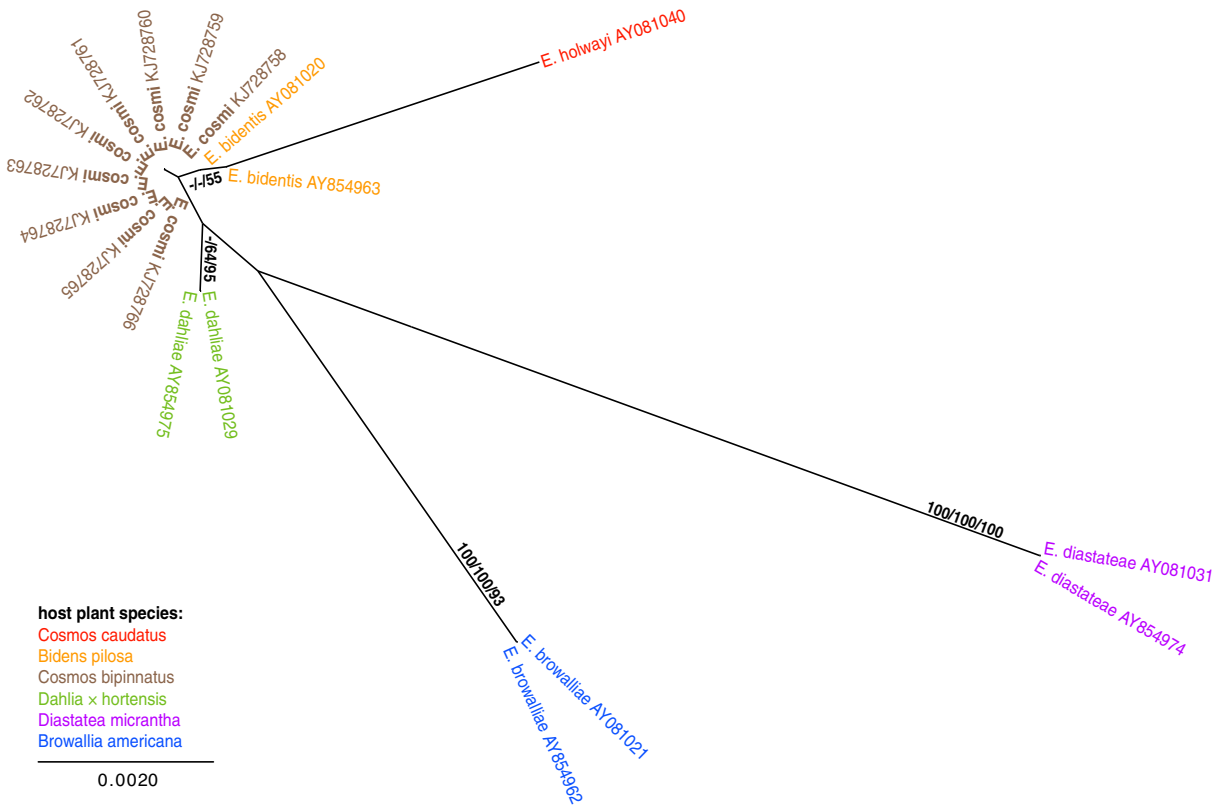


Fig. 2 Hypothesis on phylogenetic relationships within the sampled *Entyloma* species based on neighbour-joining analysis of an alignment of ITS base sequences using the Jukes-Cantor model of DNA substitution. The topology was midpoint rooted. Estimates for *a posteriori* probabilities are indicated before slashes, numbers on branches between slashes are ML bootstrap support values, numbers on branches after slashes are NJ bootstrap values of 1000 replicates. Host plant species are indicated. E. = *Entyloma*

genetic distances of the ITS rDNA sequences included in the second dataset of *Entyloma bidentis*, *E. browalliae*, *E. cosmi*, *E. dahliae*, *E. diastateae*, and *E. holwayi* are given in Table 2. The ITS alignment of the second dataset (Fig. 2) revealed the following numbers of species-specific alignment positions: *E. diastateae* (7 bases in 7 loci), *E. dahliae* (5 in 1), *E. holwayi* (4 in 4), and *E. browalliae* (4 in 4). *Entyloma bidentis* and *E. cosmi* showed no species-specific nucleotides but were distinguished from each other by three specific base deletions in two loci. The LSU of *Entyloma cosmi* revealed the following numbers of nucleotide differences compared to the LSU of the species included in the second dataset: *E. browalliae* (3 in 3), *E. diastateae* (3 in 3), *E. dahliae* (2 in 2), *E. bidentis* (1), and *E. holwayi* (1).

For both datasets, the different runs of BA that were performed and the ML analyses yielded consistent topologies in respect to well supported branchings (ML bootstrap support values greater than 57), which for the second dataset were congruent to the results of the NJ analysis. To illustrate the results, the phylogenetic hypothesis resulting from the BA analysis of the first dataset is presented in Fig. 1; that from the NJ analysis of the second dataset is presented in Fig. 2.

In all analyses the *Entyloma* species included in previous work (Boekhout et al. 2006; Savchenko et al. 2014) were resolved with high support values with the exceptions of *E. arnoseridis*, *E. calendulae*, and *E. compositarum* whose phylogenetic relation was not resolved. The *Entyloma cosmi* specimens clustered together with *E. arnoseridis*, *E. australe*, *E. bidentis*, *E. browalliae*, *E. calendulae*, *E. comaclinii*, *E. compositarum*, *E. costaricense*, *E. dahliae*, *E. deliliae*, *E. diastateae*, *E. doebbeleri*, *E. guaraniticum*, *E. holwayi*, *E. lobeliae*, *E. zinniae*, and *Tilletiopsis* sp. C. However, relations between those species or even the specimens were resolved only poorly, and if resolved relations received weak support. For the second dataset distinct clades for the sampled species

Table 2 Intraspecific and interspecific genetic distances of the ITS rDNA in % between *Entyloma cosmi* and its closest relatives

	E. bidentis	*E. browalliae*	*E. cosmi*	*E. dahliae*	*E. diastateae*	*E. holwayi*
E. bidentis	0	0,64	0	0,16	1,12	0,32
E. browalliae		0	0,64	0,80	1,44	0,96
E. cosmi			0	0,16	1,12	0,32
E. dahliae				0	0,96	0,48
E. diastateae					0	1,44
E. holwayi						-

E. = Entyloma

were revealed by NJ analyses except for *Entyloma bidentis*. However, both support for the clades and for relations between clades were mostly missing or low.

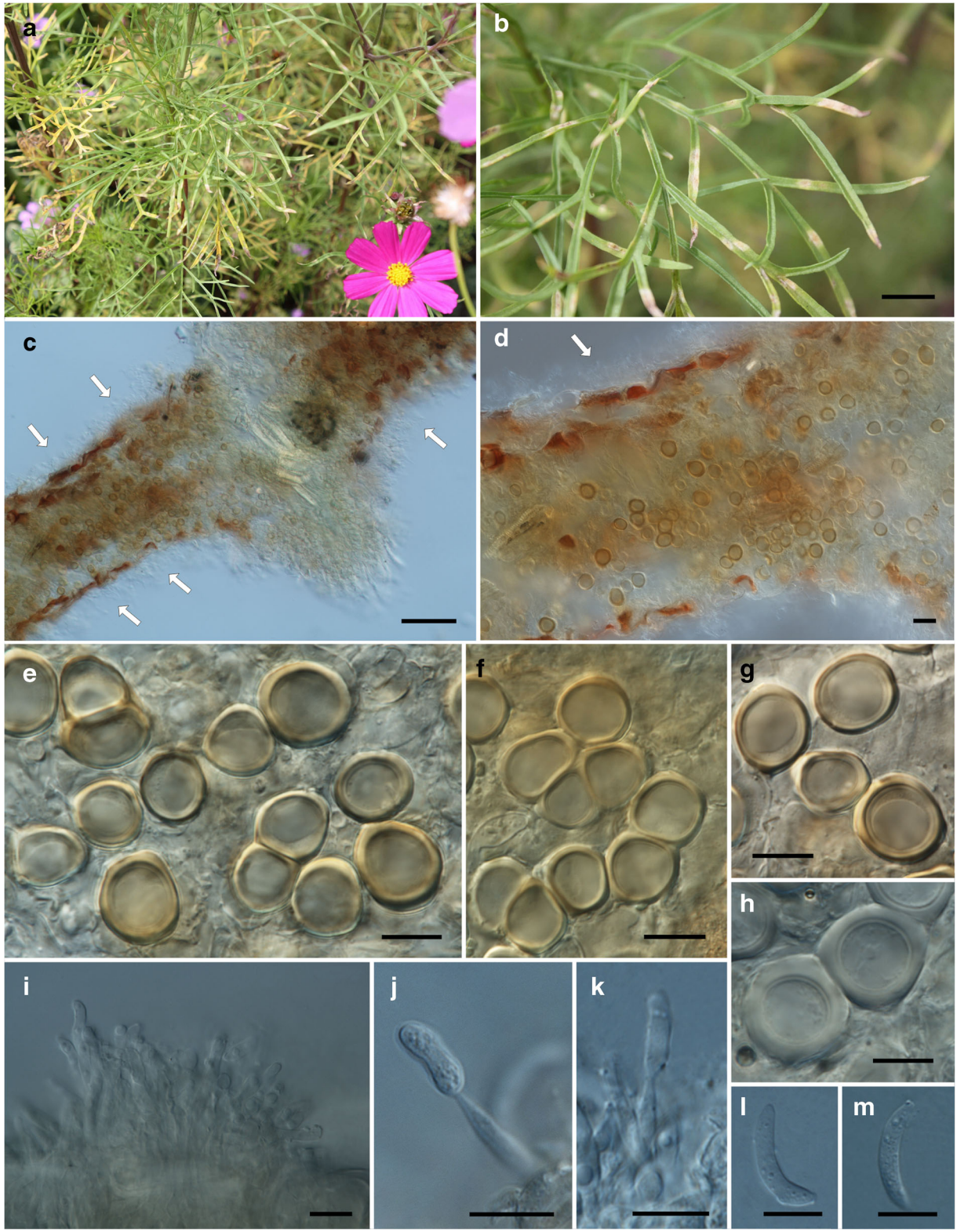
Morphological and molecular characterization

Entyloma cosmi Vánky, Horita & Jage, Mycoscience 46: 365 (2005) Fig. 3

Description: Parasitic on Cosmos bipinnatus Sori in the leaves forming flat spots, 1–5 mm long, 1–1.5 mm wide, at first pale yellowish brown, later brown, in heavy infection leading to necrosis and wilting of the leaves, infected plants become unsightly but do not die, the abaxial side of spots often whitish due to the presence of the conidia and conidiophores of the anamorphic stage. *Spores* embedded in the leaf tissues, single, loosely crowded or scattered in the intercellular spaces of mesophyll cells (the abundance of spores depends on the maturity of sori, i.e., in the young sori only single spores could be detected within the leaf spots); spores subhyaline, pale yellow to yellow-brown, usually regular in shape, rarely slightly angular, globose, subglobose or broadly ellipsoidal, mostly 11.0–15.5 × 10.0–14.5 μm, very rarely smaller/larger reaching extreme sizes with 9.0–20.0 × 8.5–16.5 μm, with smooth context; wall two-layered, mostly 1.5–3.0 μm thick (including inner layer ca. 0.5–0.8 μm thick), very rarely thicker reaching the extremes with 1.5–4.0 μm thickness, sometimes with angles, but usually without angles, layers well visible in LM, inner layer even, outer layer uneven, spore surface smooth. *Anamorph* entylomella-like, usually developed in the initial stage of infection, later disappearing. *Caespituli* both hypophyllous and epiphyllous, conidiophores in dense fascicles, emerging through stomata, hyaline, guttulate, conidiogenous loci (scars) inconspicuous. *Conidia* solitary, hyaline, cylindrical, from almost straight to distinctly curved, 12–18 × 2.5–5.0 μm, non-septate, guttulate, apex rounded, base truncate, hilum inconspicuous, not darkened.

Specimens examined (all on Cosmos bipinnatus) **Austria**, Villach, Faak am See, garden, 46°34′16.56″N, 13°54′32.10″E, 6 Aug. 2011, *leg. M. Lutz 2377* (KR-M-29450, GZU); Villach, Oberschütt, garden, 46°33′57.59″N, 13°45′19.50″E, 18 Aug. 2012, *leg. M. Lutz 2431* (KR-M-0042145); − **France**, Burgundy, Dornecy, 40 km S of Auxerre, flower nursery, 47°26′10″N, 03°34′20″E, 4 Aug. 2014, *leg. M. Lutz & M. Piątek* (KR-M-0042144); − **Germany**, Baden-Württemberg, Tübingen, Hagelloch, Heuberger-Tor-Weg, garden, 48°32′33.05″N, 09°01′11.75″E, 12 Sept. 2010, *leg. M. Lutz 2290* (KR-M-26154); Tübingen, Zwehrenbühlstraße, garden, 48°31′40.66″N, 09°02′10.38″E, 8 Sept. 2010, *leg. M. Lutz 2292* (KR-M-26155); Tübingen, Hasenbühlstraße, garden, 48°31′33.87″N, 09°02′18.76″E, 10 Aug. 2014, *leg. M. Lutz 2494* (KR-M-0042143); Saxony-Anhalt, Kreis Wittenberg, Kemberg, Neumühlenweg corner Windmühlenweg, 3 Aug. 2004, *leg. H. Jage* (paratype BRIP: HUV 20948); − **Italy**, South Tyrol, Vinschgau, NW of Dorf Tirol, Gasthaus Talbauer, garden, 46°42′20.92″N, 11°08′23.79″E, 6 Sept. 2014, *leg. M. Lutz 2486* (KR-M-0042141); South Tyrol, Vinschgau,

Fig. 3 *Entyloma cosmi* on *Cosmos bipinnatus*. **a–b** Macroscopic symptoms of infection. **c–d** Cross section of the leaf with spores (teleomorph) embedded between the intercellular spaces of mesophyll cells and external layer of conidiophores (entylomella-like anamorph, indicated by arrows) (from KRAM F-48754). **e–h** Spores (**e–g** from KRAM F-48753, **h** from KRAM F-48756). **i** Conidiophores emerging through a stoma (from KRAM F-48756). **j–k** Conidiophores with conidia (from KRAM F-48756). **l–m** Conidia (from KRAM F-48756). Scale bars: b = 5 mm, c = 100 μm, d = 20 μm, e–m = 10 μm


a
b
c
d
e
f
g
h
i
j
k
l
m

Latsch, garden, 46°37′5.26″N, 10°50′58.76″E, 7 Sept. 2014, *leg. M. Lutz 2484* (KR-M-0042142); − **Japan**, Hokkaido, Takikawa, Higashi-takikawa, 30 Sept. 2004, *leg. H. Horita* (holotype BRIP: HUV 20935); − **Poland**, Małopolska Province, Kraków–Nowa Huta, Osiedle Na Skarpie housing estate, garden, 30 Aug. 2008, *leg. M. Piątek*, (KRAM F-48755); Kraków–Nowa Huta, Osiedle Młodości housing estate, garden, 26 July 2009, *leg. M. Piątek* (KRAM F-48758); Kraków–Nowa Huta, at Żaglowa street, garden, 29 Aug. 2009, *leg. M. Piątek* (KRAM F-48756); Kraków–Nowa Huta, at Żaglowa street, garden, 27 Sept. 2009, *leg. M. Piątek* (KRAM F-48757); Kraków–Nowa Huta, Nad Dłubnią gardens at Bulwarowa street, garden, 14 Sept. 2010, *leg. M. Piątek* (KRAM F-48759); Kraków–Nowa Huta, Osiedle Na Skarpie housing estate, garden, 22 Sept. 2010, *leg. M. Piątek* (KRAM F-48754); Kraków–Nowa Huta, Osiedle Na Sportowe housing estate, garden, 22 Sept. 2010, *leg. M. Piątek* (KRAM F-48752); Kraków–Nowa Huta, Osiedle Wandy housing estate, garden, 29 Sept. 2010, *leg. M. Piątek* (KRAM F-48753); Nowy Wiśnicz, ca. 40 km SE of Kraków, roadside, 26 Sept. 2009, *leg. M. Piątek* (KRAM F-48760); Wygiełzów, ca. 36 km W of Kraków, open ethnographic museum, 10 Sept. 2014, *leg. M. Piątek* (KRAM F-57735); − **Slovenia**, Gorenjska, north of Lake Bled, garden, 46°22′ 23.88″N, 14°05′52.60″E, 9 Aug. 2011, *leg. M. Lutz 2363* (KR-M-29459, GZU).

Comments In the original description, Vánky et al. (2005a) reported larger spore sizes of *Entyloma cosmi* with thicker walls [spores 10.5–21 × 9.5–16 μm, wall 1.5–4(−5) μm thick] than typical spore sizes obtained in the present study. In the specimens examined here the typical spores were 11.0–15.5 × 10.0–14.5 μm, and the wall thickness was 1.5–3.0 μm. In the re-examined holotype specimen the spore size length was 11.0–14.5(−15.0) μm, the spore size width was 10.0–12.5(−15.0) μm, and the spore wall thickness was 1.5–3.0 μm thick according to our measurements. On the other hand, Vánky (2012) depicted the spores of *Entyloma cosmi* that, according to the attached scale bar, were about 20 μm, while Park et al. (2012) reported the spore size range for Korean material as 12.5–20.0 × 10–15 μm and spore wall thickness as 2–4 μm (though they depicted only spores with a length not exceeding 15 μm). Likewise, in one specimen examined here (KRAM F-48756) the spore size range and wall thickness were indeed similar to those reported in the original description: (11.5–)14.0–20.0 × (10.5–)12.0–16.5 μm, wall 1.5–4.0 μm thick. This could indicate that spores with a length of about 20 μm are sometimes present in *Entyloma cosmi* but constitute only a very small fraction amongst typical sizes. This fact must be taken into account when *Entyloma cosmi* is identified morphologically. Conidia of the anamorph were originally reported to measure 10–13 × 2–2.5 μm (Vánky et al. 2005a), while Park et al. (2012) reported the conidial size range for Korean material as 17–24 × 3.5–5 μm, and concluded that the measurements in the original description are the result of a mistake. In the material examined here, the conidia measured 12–18 × 2.5–5.0 μm. The sizes of spores and conidia given by Bolay (2013) and the sizes of conidia given by Klenke and Scholler (2015) are the same as given by Vánky (2012), so they are probably not original counts.

Molecular characterization Considering *Entyloma cosmi* and its closest relatives, interspecific genetic distances of the ITS rDNA are very low and range between 0 and 1.44 % (Table 2). However, for each of the species ITS sequences of all sampled specimens are identical, and for the ITS each species can be characterized unambiguously. In addition, the LSU of *Entyloma cosmi* reveals one species specific nucleotide position. Therefore we deposited selected ITS sequences and one LSU sequence (see Table 1) that may serve as DNA barcodes for *Entyloma cosmi* on the BarCode of Life website in GenBank (www.ncbi.nlm.nih.gov/genbank/barcode/) and Fungal Barcoding Database (www.fungalbarcoding.org). The ITS sequence from the holotype of *Entyloma cosmi*, sequenced here, is recommended to be deposited in the RefSeq Targeted Loci database (Schoch et al. 2014).

Evidence for spreading The first world outbreak of *Entyloma cosmi*, in Japan, took place in 1996 (Horita and Haga 1998; Horita 2001). In Asia this species was also reported from Korea where it was first observed in 2011 (Park et al. 2012). The occurrence of *Entyloma cosmi* in Europe is dating back to its first detection in Germany, Saxony-Anhalt in 2002 (Vánky et al. 2005a, b). The species is now common all over Germany, including Brandenburg (first finding in 2004), Mecklenburg-West Pomerania (2005), Baden-Württemberg (2006), Lower Saxony (2006), Saxony (2006), Thuringia (2007), Hesse (2008), Schleswig-

Holstein (2010), and Bavaria (2012) (Scholz and Scholz 2012; Kruse 2014). In Switzerland, the species has been for the first time found in 2008 (Vánky 2012; Bolay 2013). In Poland, *Entyloma cosmi* was first observed in 2008, in Austria and Slovenia in 2011, and in France and Italy in 2014. *Entyloma cosmi* is reported here for the first time from Austria, Poland, France, Italy, and Slovenia. This species seems to be widespread in Europe, with most reported localities from Germany, which may be a sampling bias resulting from the field activity of local mycologists. During the special surveys in Kraków in southern Poland, in the years 2008–2010, *Entyloma cosmi* was commonly observed in many city gardens, especially in late summer and early autumn months. In North America, the species has been for the first time reported from Canada in 2012, but the exact date of the first collection was not reported (Vánky 2012).

Discussion

The molecular phylogenetic analyses (Fig. 1) revealed *Entyloma cosmi* within a clade including *E. arnoseridis*, *E. australe*, *E. bidentis*, *E. browalliae*, *E. calendulae*, *E. comaclinii*, *E. compositarum*, *E. costaricense*, *E. dahliae*, *E. deliliae*, *E. diastateae*, *E. doebbeleri*, *E. guaraniticum*, *E. holwayi*, *E. lobeliae*, *E. zinniae*, and *Tilletiopsis* sp. C. Within that clade relations are resolved only poorly and if resolved support is weak. Nevertheless *Entyloma bidentis*, *E. browalliae*, *E. dahliae*, and *E. holwayi* may be closest relatives of *E. cosmi.* Interestingly, except *Entyloma browalliae* on *Browallia americana* (*Solanaceae*), the host genera of these species, *Bidens*, *Dahlia,* and *Cosmos*, are phylogenetically closely related and are included in the asteracean tribe *Coreopsideae* (Mort et al. 2008). Therefore, the host relation could support the close relation between these four *Entyloma* species on *Coreopsideae*. Three other *Entyloma* species on hosts from the tribe *Coreopsideae*, namely *E. coreopsis* on *Coreopsis borianiana*, *E. spegazzinii* on *Bidens* spp. and *E. frondosa* on *Bidens frondosa* (Vánky 2012), might belong to this group, but their sequences are not available in public sequence databases. On the other hand, two other species on *Coreopsideae*, *Entyloma doebbeleri* on *Dahlia imperialis* and *E. guaraniticum* on *Bidens pilosa*, which were already sequenced in previous studies (Begerow et al. 2002; Boekhout et al. 2006), are more distantly related to *E. bidentis*, *E. dahliae*, *E. cosmi*, and *E. holwayi*. This indicates that host plants in the tribe *Coreopsideae* were colonized more than once from different ancestors in the course of *Entyloma* evolution. This confirms the similar conclusion of Begerow et al. (2002) who pointed out that two morphological species evolved independently on the same host plant (*Entyloma bidentis* and *E. guaraniticum* on *Bidens pilosa*) or the same host genus (*E. dahliae* and *E. doebbeleri* on *Dahlia*). Other examples of host species-sharing *Entyloma* species pairs, based on DNA-sequence and morphological evidence, are *E. ficariae* and *E. majewskii* on *Ficaria verna,* phylogenetically closely related species (Vánky and Lutz 2010), and *E. microsporum* and *E. ranunculirepentis* on *Ranunculus repens*, phylogenetically distantly related species (Begerow et al. 2002). Furthermore, *Entyloma* species parasitic on different *Eryngium* species represent a suite of phylogenetically related parasites, all on the same host genus but each restricted to one host species (Savchenko et al. 2014). Further host-sharing *Entyloma* species pairs on the same host species (e.g., *E. corydalis* and *E. urocystoides* on *Corydalis bulbosa*) or host genus (e.g., *Entyloma* species on *Ambrosia*, *Aster*, *Asteriscus*, *Crepis*, *Hydrocotyle*, *Rudbeckia*, *Tragopogon*) are described on the basis of morphological characters (Vánky 2012). These *Entyloma* species are not sequenced yet and it is therefore still unclear how they are related to each other and if they evolved from a common ancestral species. Current evidence suggests however that in the evolution of *Entyloma* both host switches and radiation on closely related hosts took place.

The species identification in *Entyloma* is complicated because of its simple morphology with weak or no differentiating characters (Vánky 2012) and small genetic differences between species, at least using the ITS region (Begerow et al. 2002). However combination of morphology, host plant (ecology) and genetic information seems sufficient for identification of particular species. This approach was already used in studies on different fungi, including smuts and false smuts (Bauer et al. 2008; Lutz et al. 2008; Piątek et al. 2012a, b, 2013a, b, 2015; Li et al. 2014; Savchenko et al. 2013, 2014) and was recently formally defined and named the Consolidated Species Concept (Quaedvlieg et al. 2014).

Based on the original description and measurements of spores from the original description (Vánky et al. 2005a), *Entyloma cosmi* could easily be differentiated from the

putative neighbours detected in phylogenetic analyses (*Entyloma browalliae*, *E. bidentis, E. dahliae,* and *E. holwayi*) in having larger spores (Table 3). This is also true for one specimen (KRAM F-48756) observed in the current study that had similar spore sizes compared to those reported in the original description of *Entyloma cosmi*. This is however less obvious when the spore sizes and wall thickness detected in most specimens of *Entyloma cosmi* in the current study are used for morphological differentiation (see species description). Then, the spore sizes are similar in *Entyloma cosmi* and its phylogenetic neighbours (*E. browalliae*, *E. bidentis, E. dahliae, E. holwayi*), and the spore wall thickness is similar in all of them except *E. bidentis* (Table 3). *Entyloma holwayi* is reported to be devoid of the anamorph by Vánky et al. (2005a) and Vánky (2012), contrasting with *E. cosmi* in which the anamorph is usually developed. However, Piepenbring (2003) recorded an anamorphic stage in *Entyloma holwayi*, which suggests that an anamorph may be developed at least in some specimens of *Entyloma holwayi* as well, and therefore indicating that this differentiating morphological character is probably dubious. In contrast *Entyloma bidentis* is morphologically clearly divergent from *E. cosmi* in having thinner spore walls and lacking the anamorphic stage (Table 3), despite that both species show no interspecific genetic distances of the ITS rDNA and were distinguished from each other only by three specific base deletions in two loci. The important point is that *Entyloma browalliae*, *E. bidentis*, *E. cosmi*, *E. dahliae*, and *E. holwayi* infect different host genera or species, and based on current DNA sequence-evidence none of the reported host species could be infected by two different *Entyloma* species from that lineage. Thus, the differentiation between these species is possible by combination of morphology, host plant (ecology) and molecules, i.e., the Consolidated Species Concept, with special focus on differences in host specificity and genetic characters. To facilitate molecular identification of *Entyloma cosmi* for rapid identification procedures, ITS (barcode marker for *Fungi*, Schoch et al. 2012) and LSU barcodes are deposited on the BarCode of Life website in GenBank (www.ncbi.nlm.nih.gov/genbank/barcode/), and in the Fungal Barcoding Database (www.fungalbarcoding.org/).

Global trade, plantings of exotic plants and global environmental changes over the past years were major driving forces of invasions of phytopathogenic fungi to new environments, continents, and countries (Kreisel and Scholler 1994; Desprez-Loustau et al. 2010; Mułenko et al. 2010; Wingfield et al. 2010), including such remarkable fungal invasions of recent years as *Hymenoscyphus fraxineus* (Timmermann et al. 2011), *Puccinia lagenophorae* (Scholler at al. 2011), *Puccinia psidii* (Machado et al. 2015), *Peronospora belbahrii* (Thines et al. 2009; Voglmayr and Piątek 2009; Wyenandt et al. 2015), or powdery mildews (Ale-Agha et al. 2004; Bolay et al. 2005; Kiss et al. 2005). Considering smut fungi, several important introductions and subsequent invasions were already noticed in Europe in the past such as those caused by *Entyloma calendulae* since the 19th century, *Entyloma gaillardianum*, and *Thecaphora oxalidis* in the 20th century, or the model species *Ustilago maydis* dating

Table 3 Morphological characters of *Entyloma cosmi* and its closest relatives detected in phylogenetic analyses

Species	Host plants	Spore sizes (μm)	Spore wall thickness (μm)	Anamorph	References
E. bidentis	*Bidens* spp.	10–16 × 9–14	1–1.5(−2.5)	absent	Vánky 2012
E. browalliae	*Browallia americana*	9.5–15 × 9–12.5	1–3	present	Vánky 2012
E. cosmi	*Cosmos bipinnatus*	10.5–21 × 9.5–16	1.5–4(−5)	present	Vánky 2012
E. cosmi	*Cosmos bipinnatus*[a]	11.0–15.5 × 10.0–14.5 (very rarely smaller/larger reaching the extreme sizes as 9.0–20.0 × 8.5–16.5)	1.5–3.0(−4.0)	present	this study
E. dahliae	*Dahlia* spp.	9.5–17(−20) × 8–16	1–4	present	Vánky 2012
E. holwayi	*Cosmos caudatus, C. sulphureus*[b]	9–15 × 8–13	(1−)1.5–3(−3.5)	absent	Vánky 2012

E. = *Entyloma*

[a] Klenke and Scholler (2015) reported also *Cosmos sulphureus*, but the occurrence of *E. cosmi* on this host plant is dubious; the report should be reconsidered

[b] Vánky (2012) erroneously reported also *Cosmos bipinnatus*

back to at least 18th century (Kreisel and Scholler 1994). In this study we provided evidence for spreading of another smut fungus: *Entyloma cosmi* in Europe. This species is now widespread in that area, with most reported localities from Germany, which may be a sampling bias resulting from the field activity of local mycologists. *Entyloma cosmi* is reported here for the first time from Austria, Poland, France, Italy, and Slovenia, but likely the species is also present in other European countries where *Cosmos bipinnatus* is cultivated, and its occurrence and incidence should be monitored. An unresolved problem is the origin of *Entyloma cosmi*. The first outbreaks of this pathogen in Germany and Japan resulted most probably from introductions of *Entyloma cosmi*, likely with infected seeds, from its native area of occurrence. It could be speculated that this smut pathogen originated from Mexico or south-western U.S.A., like the host plant, but remained unnoticed there until now. Interestingly, there are still no published records of *Entyloma cosmi* from the U.S.A. though report of the white smut of *Cosmos* on the web page of the New England Greenhouse Update assigned to *Entyloma calendulae* (http://negreenhouseupdate.info/photos/cosmos-%E2%80%93-entyloma-white-smut) may in fact refer to *Entyloma cosmi*. The species was however already reported from Canada (Vánky 2012). Therefore, in addition to Europe and Asia, the occurrence and incidence of this important and emerging smut disease should be monitored in North America and in other parts of the world where *Cosmos bipinnatus* is cultivated.

Acknowledgments The work of Marcin Piątek was supported by the statutory funds of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland.

Compliance with Ethical Standards The authors declare that this work complies to the Ethical Standards of the journal, and that they have no conflict of interest.

References

Ale-Agha, N., Bolay, A., Braun, U., Feige, B., Jage, H., Kummer, V., Lebeda, A., Piątek, M., Shin, H. D., & Zimmermannová-Pastirčaková, K. (2004). *Erysiphe catalpae* and *E. elevata* in Europe. *Mycological Progress, 3*, 291–296.

Bauer, R., Lutz, M., Begerow, D., Piątek, M., Vánky, K., Bacigálová, K., & Oberwinkler, F. (2008). Anther smut fungi on monocots. *Mycological Research, 112*, 1297–1306.

Begerow, D., Lutz, M., & Oberwinkler, F. (2002). Implications of molecular characters for the phylogeny of the genus *Entyloma*. *Mycological Research, 106*, 1392–1399.

Blomquist, C. L., Rooney-Latham, S., Haynes, J. L., & Scheck, H. J. (2011). First report of *Verticillium* wilt on field-grown cosmos caused by *Verticillium dahliae* in California. *Plant Disease, 95*, 361.

Boekhout, T., Gildemacher, P., Theelen, B., Müller, W. H., Heijne, B., & Lutz, M. (2006). Extensive colonization of apples by smut anamorphs causes a new postharvest disorder. *FEMS Yeast Research, 6*, 63–76.

Bolay, A. (2013). Les champignons parasites des plantes vasculaires des Conservatoire et Jardin botaniques de la Ville de Genève. *Boissiera, 66*, 1–147.

Bolay, A., Braun, U., Delhey, R., Kummer, V., Piątek, M., & Wołczańska, A. (2005). *Erysiphe deutziae* – a new epidemic spread in Europe. *Cryptogamie Mycologie, 26*, 293–298.

Carlucci, A., Lops, F., & Frisullo, S. (2009). First report of wilt caused by *Verticillium dahliae* on cosmos (*Cosmos bipinnatus*) in Italy. *Plant Disease, 93*, 846.

Deng, J. X., Lee, J. H., Paul, N. C., Cho, H. S., Lee, H. B., & Yu, S. H. (2015). Occurrence of leaf blight on *Cosmos* caused by *Alternaria cosmosa* in Korea. *Plant Pathology Journal, 31*, 78–82.

Desprez-Loustau, M. L., Courtecuisse, R., Robin, C., Husson, C., Moreau, P. A., Blancard, D., Selosse, M. A., Lung-Escarmant, B., Piou, D., & Sache, I. (2010). Species diversity and drivers of spread of alien fungi (sensu lato) in Europe with a particular focus on France. *Biological Invasions, 12*, 157–172.

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology, 2*, 113–118.

Glawe, D. A., Barlow, T., & Koike, S. T. (2010). First report of leaf smut of *Gaillardia* × *grandiflora* caused by *Entyloma gaillardianum* in North America. Online. *Plant Health Progress*. doi:10.1094/PHP-2010-0428-01-BR.

Groenewald, J. Z., Nakashima, C., Nishikawa, J., Shin, H. D., Park, J. H., Jama, A. N., Groenewald, M., Braun, U., & Crous, P. W. (2013). Species concepts in *Cercospora*: spotting the weeds among the roses. *Studies in Mycology, 75*, 115–170.

Henricot, B., Scrace, J., & Spooner, B. (2013). First record of smut caused by *Entyloma eschscholziae* on California poppy in the United Kingdom. *New Disease Reports, 28*, 10.

Horita, H. (2001). Disease development and effects of various fungicides for the control of white smut of *Cosmos* caused by *Entyloma* sp. *Annual Report of the Society of Plant Protection of North Japan, 52*, 79–81 (in Japanese).

Horita, H., & Haga, Y. (1998). White smut of *Cosmos* caused by *Entyloma* sp. *Annals of the Phytopathological Society of Japan, 64*, 582 (abstract, in Japanese).

Jage, H., Klenke, F., & Kummer, V. (2010). Neufunde und bemerkenswerte Bestätigungen von phytoparasitischen Kleinpilzen in Deutschland – *Erysiphales* (Echte Mehltaupilze). *Schlechtendalia, 21*, 1–140.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution, 30*, 772–780.

Kemler, M., Begerow, D., & Lutz, M. (2007). Biodiversität von Brand- und Rostpilzen auf der Alp Flix. *Jahresbericht der Naturforschenden Gesellschaft Graubünden, 114*, 65–72.

Kiger, R. W. (2006). *Cosmos* Cavanilles. In: Flora of North America Editorial Committee (Eds.), *Flora of North America North of Mexico. Volume 21: Magnoliophyta: Asteridae, part 8: Asteraceae, part 3* (pp. 203–205). New York-Oxford: Oxford University Press.

Kiss, L., Takamatsu, S., & Cunnington, J. H. (2005). Molecular identification of *Oidium neolycopersici* as the causal agent of the recent tomato powdery mildew epidemics in North America. *Plant Disease, 89*, 491–496.

Klenke, F., & Scholler M. (2015). *Pflanzenparasitische Kleinpilze. Bestimmungsbuch für Brand-, Rost-, Mehltau-, Flagellatenpilze und Wucherlingsverwandte in Deutschland, Österreich, der Schweiz und Südtirol*. Berlin-Heidelberg: Springer Verlag.

Koike, S. T., Glawe, D. A., & Barlow, T. (2010). First report of leaf smut of tomatillo caused by *Entyloma australe.* Online. *Plant Health Progress*. doi:10.1094/PHP-2010-0216-01-BR.

Kreisel, H., & Scholler, M. (1994). Chronology of phytoparasitic fungi introduced to Germany and adjacent countries. *Botanica Acta, 107*, 387–392.

Kruse, J. (2014). Diversität der pflanzenpathogenen Kleinpilze im Ökologisch-Botanischen Garten der Universität Bayreuth. *Zeitschrift für Mykologie, 80*, 169–226.

Kwon, J. H., Kang, S. W., Son, K. E., & Park, C. S. (1999). Anthracnose of *Cosmos* caused by *Colletotrichum acutatum* in Korea. *Plant Pathology Journal, 15*, 172–174.

Li, Y. M., Shivas, R. G., & Cai, L. (2014). Three new species of *Tilletia* on *Eriachne* from north-western Australia. *Mycoscience, 55*, 361–366.

Lutz, M., Bauer, R., Begerow, D., Oberwinkler, F., & Triebel, D. (2004). *Tuberculina*, rust relatives attack rusts. *Mycologia, 96*, 614–626.

Lutz, M., Piątek, M., Kemler, M., Chlebicki, A., & Oberwinkler, F. (2008). Anther smuts of *Caryophyllaceae*: molecular analyses reveal further new species. *Mycological Research, 112*, 1280–1296.

Machado, P. S., Glen, M., Pereira, O. L., Silva, A. A., & Alfenas, A. C. (2015). Epitypification of *Puccinia psidii*, causal agent of guava rust. *Tropical Plant Pathology, 40*, 5–12.

Matheny, P. B., Gossmann, J. A., Zalar, P., Arun Kumar, T. K., & Hibbett, D. S. (2006). Resolving the phylogenetic position of the *Wallemiomycetes*: an enigmatic major lineage of *Basidiomycota. Canadian Journal of Botany, 84*, 1794–1805.

Matheny, P. B., Wang, Z., Binder, M., Curtis, J. M., Lim, Y. W., Nilsson, R. H., Hughes, K. W., Hofstetter, V., Ammirati, J. F., Schoch, C. L., Langer, E., Langer, G., McLaughlin, D. J., Wilson, A. W., Froslev, T., Ge, Z. W., Kerrigan, R. W., Slot, J. C., Yang, Z. L., Baroni, T. J., Fischer, M., Hosaka, K., Matsuura, K., Seidl, M. T., Vauras, J., & Hibbett, D. S. (2007). Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (*Basidiomycota*, *Fungi*). *Molecular Phylogenetics and Evolution, 43*, 430–451.

Mort, M. E., Randle, C. P., Kimball, R. T., Tadesse, M., & Crawford, D. J. (2008). Phylogeny of *Coreopsidae* (*Asteraceae*) inferred from nuclear and plastid DNA sequences. *Taxon, 57*, 1–12.

Mułenko, W., Piątek, M., Wołczańska, A., Kozłowska, M., & Ruszkiewicz-Michalska, M. (2010). Plant parasitic fungi introduced to Poland in modern times. Alien and invasive species. *Biological Invasions in Poland, 1*, 49–71.

O'Donnell, K. L. (1993). *Fusarium* and its near relatives. In D. R. Reynolds & J. W. Taylor (Eds.), *The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics* (pp. 225–233). Wallingford: CAB International.

Park, J. H., Cho, S. E., Park, M. J., Hong, S. H., & Shin, H. D. (2012). Occurrence of white smut caused by *Entyloma cosmi* on *Cosmos bipinnatus* in Korea. *Research in Plant Disease, 18*, 250–254.

Piątek, M., Lutz, M., & Welton, P. (2012a). *Exobasidium darwinii*, a new Hawaiian species infecting endemic *Vaccinium reticulatum* in Haleakala National Park. *Mycological Progress, 11*, 361–371.

Piątek, M., Lutz, M., Ronikier, A., Kemler, M., & Świderska-Burek, U. (2012b). *Microbotryum heliospermae,* a new anther smut fungus parasitic on *Heliosperma pusillum* in the mountains of the European Alpine System. *Fungal Biology, 116*, 185–195.

Piątek, M., Lutz, M., & Chater, A. O. (2013a). Cryptic diversity in the *Antherospora vaillantii* complex on *Muscari* species. *IMA Fungus, 4*, 5–19.

Piątek, M., Lutz, M., & Kemler, M. (2013b). *Microbotryum silenes-saxifragae* sp. nov. sporulating in the anthers of *Silene saxifraga* in southern European mountains. *IMA Fungus, 4*, 29–40.

Piątek, M., Lutz, M., Jacobs, A., Villablanca, F., & Wood, A. R. (2015). Epitypification of *Tilletia ehrhartae*, a smut fungus with potential for nature conservation, biosecurity and biocontrol. *European Journal of Plant Pathology, 143*, 151–158.

Piepenbring, M. (2003). Smut fungi (*Ustilaginomycetes* p.p. and *Microbotryales*, *Basidiomycota*). *Flora Neotropica*, *86:(iv)*, 1–291.

Posada, D., & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics, 14*, 817–818.

Quaedvlieg, W., Binder, M., Groenewald, J. Z., Summerell, B. A., Carnegie, A. J., Burgess, T. I., & Crous, P. W. (2014). Introducing the Consolidated Species Concept to resolve species in the *Teratosphaeriaceae. Persoonia, 33*, 1–40.

Savchenko, K. G., Lutz, M., Piątek, M., Heluta, V. P., & Nevo, E. (2013). *Anthracoidea caricis-meadii* is a new North American smut fungus on *Carex* sect. *Paniceae*. *Mycologia, 105*, 181–193.

Savchenko, K. G., Carris, L. M., Castlebury, L. A., Heluta, V. P., Wasser, S. P., & Nevo, E. (2014). Revision of *Entyloma* (*Entylomatales*, *Exobasidiomycetes*) on *Eryngium*. *Mycologia, 106*, 797–810.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., & Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi. Proceedings of the National Academy of Sciences, USA, 109*, 6241–6246.

Schoch, C. L., Robbertse, B., Robert, V., Vu, D., Cardinali, G., Irinyi, L., Meyer, W., Nilsson, R. H., Hughes, K., Miller, A. N., Kirk, P. M., Abarenkov, K., Aime, M. C., Ariyawansa, H. A., Bidartondo, M., Boekhout, T., Buyck, B., Cai, Q., Chen, J., Crespo, A., Crous, P. W., Damm, U., De Beer, Z. W., Dentinger, B. T. M., Divakar, P. K., Dueñas, M., Feau, N., Fliegerova, K., García, M. A., Ge, Z. W., Griffith, G. W., Groenewald, J. Z., Groenewald, M., Grube, M., Gryzenhout, M., Gueidan, C., Guo, L., Hambleton, S., Hamelin, R.,

Hansen, K., Hofstetter, V., Hong, S. B., Houbraken, J., Hyde, K. D., Inderbitzin, P., Johnston, P. R., Karunarathna, S. C., Kõljalg, U., Kovács, G. M., Kraichak, E., Krizsan, K., Kurtzman, C. P., Larsson, K. H., Leavitt, S., Letcher, P. M., Liimatainen, K., Liu, J. K., Lodge, D. J., Luangsaard, J. J., Lumbsch, H. T., Maharachchikumbura, S. S. N., Manamgoda, D., Martín, M. P., Minnis, A. M., Moncalvo, J. M., Mulè, G., Nakasone, K. K., Niskanen, T., Olariaga, I., Papp, T., Petkovits, T., Pino-Bodas, R., Powell, M. J., Raja, H. A., Redecker, D., Sarmiento-Ramirez, J. M., Seifert, K. A., Shrestha, B., Stenroos, S., Stielow, B., Suh, S. O., Tanaka, K., Tedersoo, L., Telleria, M. T., Udayanga, D., Untereiner, W. A., Uribeondo, J. D., Subbarao, K. V., Vágvölgyi, C., Visagie, C., Voigt, K., Walker, D. M., Weir, B. S., Weiß, M., Wijayawardene, N. N., Wingfield, M. J., Xu, J. P., Yang, Z. L., Zhang, N., Zhuang, W. Y., & Federhen, S. (2014). Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. *Database*, *2014*, bau061.

Scholler, M., Lutz, M., Wood, A. R., Hagedorn, G., & Mennicken, M. (2011). Taxonomy and phylogeny of *Puccinia lagenophorae*: a study using rDNA sequence data, morphological and host range features. *Mycological Progress, 10*, 175–187.

Scholz, H., & Scholz, I. (2012). Die Brandpilze Deutschlands, 3. Nachtrag. *Verhandlungen des Botanischen Vereins von Berlin und Brandenburg, 145*, 161–217.

Swofford, D. L. (2001). *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Massachusetts: Sinauer Associates, Sunderland.

Takamatsu, S., Niinomi, S., Harada, M., & Havrylenko, M. (2010). Molecular phylogenetic analyses reveal a close evolutionary relationship between *Podosphaera* (*Erysiphales*: *Erysiphaceae*) and its rosaceous hosts. *Persoonia, 24*, 38–48.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*, 2731–2739.

Thines, M., Telle, S., Ploch, S., & Runge, F. (2009). Identity of the downy mildew pathogens of basil, coleus and sage with implications for quarantine measures. *Mycological Research, 113*, 532–540.

Timmermann, V., Børja, I., Hietala, A. M., Kirisits, T., & Solheim, H. (2011). Ash dieback: pathogen spread and diurnal patterns of ascospore dispersal, with special emphasis on Norway. *EPPO Bulletin, 41*, 14–20.

Udayanga, D., Liu, X. Z., Crous, P. W., McKenzie, E. H. C., Chukeatirote, E., & Hyde, K. D. (2012). A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). *Fungal Diversity, 56*, 157–171.

Vánky, K. (2012). *Smut fungi of the world*. St. Paul: APS Press.

Vánky, K., & Lutz, M. (2010). *Entyloma majewskii* sp. nov. (*Entylomataceae*) on *Ranunculus ficaria* from Iran. *Polish Botanical Journal, 55*, 271–279.

Vánky, K., Horita, H., & Jage, H. (2005a). *Entyloma cosmi* sp. nov. on *Cosmos bipinnatus* (*Compositae*). *Mycoscience, 46*, 364–366.

Vánky, K., Jage, H., & Scholz, H. (2005b). Three smut fungi new for Europe. *Nova Hedwigia, 80*, 387–395.

Vasighzadeh, A., Zafari, D., Selçuk, F., Hüseyin, E., Kurşat, M., Lutz, M., & Piątek, M. (2014). Discovery of *Thecaphora schwarzmaniana* on *Rheum ribes* in Iran and Turkey: implications for the diversity and phylogeny of leaf smuts on rhubarbs. *Mycological Progress, 13*, 881–892.

Voglmayr, H., & Piątek, M. (2009). *Peronospora* causing downy mildew disease of sweet basil newly reported in Cameroon. *Plant Pathology, 58*, 805–805.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols, a guide to methods and applications* (pp. 315–322). San Diego: Academic.

Wingfield, M. J., Slippers, B., Roux, J., & Wingfield, B. D. (2010). Fifty years of tree pest and pathogen invasions, increasingly threatening world forests. In D. M. Richardson (Ed.), *Fifty years of invasion ecology: The legacy of Charles Elton* (pp. 89–100). Oxford: Wiley-Blackwell.

Wyenandt, A., Simon, J. E., Pyne, R. M., Homa, K., McGrath, M., Zhang, S., Raid, R. N., Ma, L. J., Wick, R., Guo, L., & Madeiras, A. (2015). Basil downy mildew (*Peronospora belbahrii*): discoveries and challenges relative to its control. *Phytopathology, 105*, 885–894.