

Discovery of *Thecaphora schwarzmaniana* on *Rheum ribes* in Iran and Turkey: implications for the diversity and phylogeny of leaf smuts on rhubarbs

Asieh Vasighzadeh · Doustmorad Zafari · Faruk Selçuk ·
Elsad Hüseyin · Murat Kurşat · Matthias Lutz · Marcin Piątek

Received: 29 October 2013 / Revised: 5 February 2014 / Accepted: 17 February 2014 / Published online: 14 March 2014
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Abstract A leaf smut discovered on wild rhubarb *Rheum ribes* in Iran and Turkey is evaluated to identify the species. Based on morphological analyses using light and scanning electron microscopy and molecular phylogenetic analyses using ITS and LSU ribosomal DNA sequences, it is shown that the smut fungus belongs to *Thecaphora schwarzmaniana*, a rare species previously known on *Rheum cordatum*, *R. lobatum*, *R. macrocarpum*, and *R. turkestanicum* in some central Asian countries. This is the first documented case of *Thecaphora schwarzmaniana* on *Rheum ribes* and significantly expands the range of the species on the Asian continent. The ITS and LSU sequences of the Iranian and Turkish material differed from sequences of *Thecaphora schwarzmaniana* from *Rheum palmatum* from China. The re-study of the morphology of the respective specimen revealed morphological differences allowing the conclusion that *Thecaphora* on *Rheum palmatum*

belongs to a distinct species. The new species is not described here due to the paucity of available material. Molecular phylogenetic analyses, based on ITS and LSU sequences, recovered *Thecaphora* on *Rheum* spp. in a clade including species on host plants from diverse unrelated families, indicating that multiple inter-family host jumps took place in the course of evolution of *Thecaphora* species. Vast similarity of ITS and LSU sequences of *Thecaphora* spp. on *Rheum* spp. point to their close relation suggesting that co-evolution could shape the diversification of *Thecaphora* species on Polygonaceae following an initial host jump from another host plant family.

Keywords Molecular Phylogenetics · Plant Pathogens · *Rheum palmatum* · *R. ribes* · Smut fungi · *Thecaphora*

Introduction

Thecaphora Fingerh. is one of the 104 recognized genera of smut fungi (Vánky 2013). The genus resides in the Glomosporiaceae (Urocystidales) and includes 63 species parasitizing dicotyledonous host plants belonging to 16 families (Vánky and Lutz 2007); (Roets et al. 2008); (Vánky et al. 2008a); (Vánky 2012). All *Thecaphora* species described from monocots, for example *T. herteriana* Cif. on Poaceae (Vánky and Berbee 1988), *T. leptocarpi* Berk. on Restionaceae (Vánky 2000), *T. aterrima* Tul. & C. Tul. on Cyperaceae (Vánky 2000), *T. bulbinellae* P.H.B. Talbot on Xanthorrhoeaceae (Vánky 2008), or *T. anemarrhenae* C.H. Chow & Chi C. Chang on Asparagaceae (Vánky et al. 2008b), were transferred to other genera. *Thecaphora* is characterized by having sori produced in various parts of host plants, composed of loose or permanent spore balls (exceptionally single spores) forming a granular-powdery, yellowish, pale brown or dark reddish-brown mass that is never black or almost black (Vánky et al. 2008a). Sterile cells are usually lacking and have

A. Vasighzadeh · D. Zafari (✉)

Plant Protection Department, Agricultural Faculty, Bu-Ali Sina University, Hamedan, Iran
e-mail: zafari_d@yahoo.com

F. Selçuk · E. Hüseyin

Department of Biology, Arts and Sciences Faculty, Ahi Evran University, 40100 Kırşehir, Turkey

M. Kurşat

Department of Biology, Arts and Sciences Faculty, Bitlis Eren University, Bitlis, Turkey

M. Lutz (✉)

Plant Evolutionary Ecology, Institute of Evolution and Ecology, University of Tübingen, Auf der Morgenstelle 1, D-72076 Tübingen, Germany
e-mail: matthias@chrissilutz.de

M. Piątek (✉)

Department of Mycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland
e-mail: m.piatek@botany.pl

been observed in only one species, namely in *Thecaphora smallanthi* M. Piepenbr., P. Hanson & J. Carranza (Piepenbring 2000).

The rhubarb genus *Rheum* L. belonging to the Polygonaceae includes about 60 species distributed in Asia (one species also in south-east Europe) and having the main centre of diversity in central Asia. *Rheum* was traditionally divided into several sections that, however, were mostly not confirmed by molecular phylogenetic analyses (Lozina-Lozinskaya 1936); (Wang et al. 2005); (Sun et al. 2012); Flora of China and Flora of Pakistan – eFloras, <http://www.efloras.org>). Four species of *Rheum* were reported from Iran (Jafari et al. 2012) and one species from Turkey (Davis 1967). *Rheum ribes* L. is the single species distributed in both countries, and it is additionally known from Afghanistan, Armenia, Iraq, Lebanon, Pakistan, and Palestine (Davis 1967; Flora of Pakistan – eFloras, <http://www.efloras.org>). The vegetative parts of *Rheum ribes* are consumed as a vegetable and are also used as a medicine, for example in treating diabetes, hypertension, obesity and diarrhoea (Krishnaiah et al. 2011).

Three species of smut fungi were reported from different *Rheum* species, all in Asia, namely two seed smuts: *Microbotryum rhei* (Zundel) Vánky, *M. stewartii* (Zundel) Vánky, and one leaf smut: *Thecaphora schwarzmaniana* Byzova (Vánky 2012). None of these species was reported from *Rheum ribes*. Of special interest is *Thecaphora schwarzmaniana* that represents the sole species of *Thecaphora* on Polygonaceae, a host family that otherwise harbours members of *Liriope* Cif., *Melanopsichium* Beck, *Microbotryum* Lév., *Sphacelotheca* de Bary, and *Zundeliomyces* Vánky (Vánky and Oberwinkler 1994); (Vánky 1998); (Piepenbring 2002); (Kemler et al. 2006, 2009); (Piątek et al. 2012a); (Vánky 2012).

In 2010 and 2011 during surveys for phytopathogenic fungi in Iran and Turkey, a smut fungus producing sori in leaves of *Rheum ribes* was collected that superficially resembled *Thecaphora schwarzmaniana*, which, however, had been previously reported from different *Rheum* species. This study aims at resolving the specific status of these two smut collections using morphological and DNA sequence analyses, and to determine their phylogenetic affinities within the genus *Thecaphora*, especially their relation to the only specimen of *T. schwarzmaniana* of which a sequence is available in the NCBI's GenBank nucleotide database.

Materials and methods

Specimen sampling and documentation

The specimens of *Rheum ribes* infected with a smut fungus were collected (1) by Mahmood Bahador in the Buqaty mountains, Kabudarahang County, Hamedan Province, Iran, on 15 May 2010 (BASU 4242); and (2) by Elsad Hüseyin around

Meydan road (slopes), Mutki District, Bitlis Province, Turkey, on 17 May 2011 (KRAM F-49788=AHEUM 955). These specimens were used for morphological and molecular phylogenetic analyses. Because of the assumed close relation of the smut collections on *Rheum ribes* to a sequenced specimen of *Thecaphora schwarzmaniana* on *Rheum palmatum* L. (LSU sequence deposited in GenBank: EF647752 – China, Gansu Province, Dangchang, ca. 2800 a.s.l., 6 Aug. 2005, leg. S.R. Wang, H.U.V. 21117=GAU 200502, (Vánky et al. 2008a)), the morphology of the latter smut specimen was examined for comparative purposes and its ITS was sequenced for phylogenetic analyses. The voucher specimens are deposited in AHEUM (Mycological Collection of the Arts and Sciences Faculty of the Ahi Evran University, Kırşehir, Turkey), BASU, KRAM F, and H.U.V. (personal collection of Kálmán Vánky, “Herbarium Ustilaginales Vánky” which is currently transferred to BRIP).

Morphological examination

Sorus, spore ball and spore characteristics were studied using dried herbarium specimens. For light microscopy (LM), spore balls were mounted in lactophenol or lactic acid, heated to the boiling point and cooled, and then examined under a Nikon Eclipse 80i light microscope. Thirty spore balls and 50 spores were measured from each collection, at a magnification of $\times 1000$, using NIS-Elements BR 3.0 imaging software. The measurements are adjusted to the nearest 0.5 μm . The morphological characters of each examined specimen are shown in Table 1. The descriptions include combined values from all measured specimens or respective species. LM micrographs were taken with a Nikon DS-Fi1 camera. Spore germination of *Thecaphora* sp. from *Rheum ribes* (BASU 4242) was obtained by spreading spore balls on 0.2 % malt agar in Petri dishes and incubation at room temperature (ca. 25°C). Observations were made after 36 hours.

The spore balls of *Thecaphora* sp. on *Rheum ribes* (KRAM F-49788=AHEUM 955), and *Thecaphora* sp. on *Rheum palmatum* (H.U.V. 21117) were studied using scanning electron microscopy (SEM). For this purpose, dry spore balls were mounted on carbon tabs and fixed to an aluminium stub with double-sided transparent tape. The tabs were sputter-coated with carbon using a Cressington sputter-coater and viewed with a Hitachi S-4700 scanning electron microscope, with a working distance of ca. 12 mm. SEM micrographs were taken in the Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences, Jagiellonian University, Kraków (Poland).

DNA extraction, PCR, and sequencing

Genomic DNA was isolated directly from the herbarium specimens using a sample of 10 mg of teliospores per specimen.

Table 1 Synopsis of diagnostic characters of *Thecaphora schwarzmaniana* and *Thecaphora* sp. on *Rheum palmatum*

Diagnostic characters	<i>Thecaphora schwarzmaniana</i>	<i>Thecaphora</i> sp.
Sori	<i>Rheum cordatum</i> (Byzova, in Schwarzwamm 1960) as first 2–3 mm in diam., later 6 × 1.5 cm	<i>Rheum palmatum</i> , H.U.V. 21117 (this study) 1 cm
Spore balls	23–62.7 × 19.8–52.8 μm (often 29.7–46.2 × 29.7–42.9 μm, rarely 23–26.4 × 19.8–26.4 μm and 56–62.7 × 51–52.8 μm)	<i>Rheum ribes</i> , Turkey, ¹ KRAM F-49788=AhEUM 955 (this study) 1–6 cm 20–60(–65) × 20–45 μm
Number of spores in spore ball	3–20 (often 6–14)	3–15(–20)
Spores	13.2–19.8 × 9.9–19.8 μm (often 13.2–19.8 × 13.2–18 μm)	(10.0–)11.0–16.0 × (8.5–) 9.5–13.5(–14.0) μm
Spore wall thickness	no data	1.5–2.5(–3.0) μm
Warts	1–1.5 μm	1–1.5 μm

¹ Acronyms: AhEUM – Mycological Collection of the Arts and Sciences Faculty, Ahi Evran University, Kırşehir, Turkey; BASU – Herbarium of the Department of Biology, Bu-Ali Sina University, Hamedan, Iran; H.U.V. – personal collection of Kálmán Vánky, “Herbarium Ustilaginales Vánky” which is currently transferred to BRIP (Plant Pathology Herbarium, Queensland Department of Agriculture, Fisheries and Forestry, Brisbane, Australia); KRAM F – Mycological Herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland

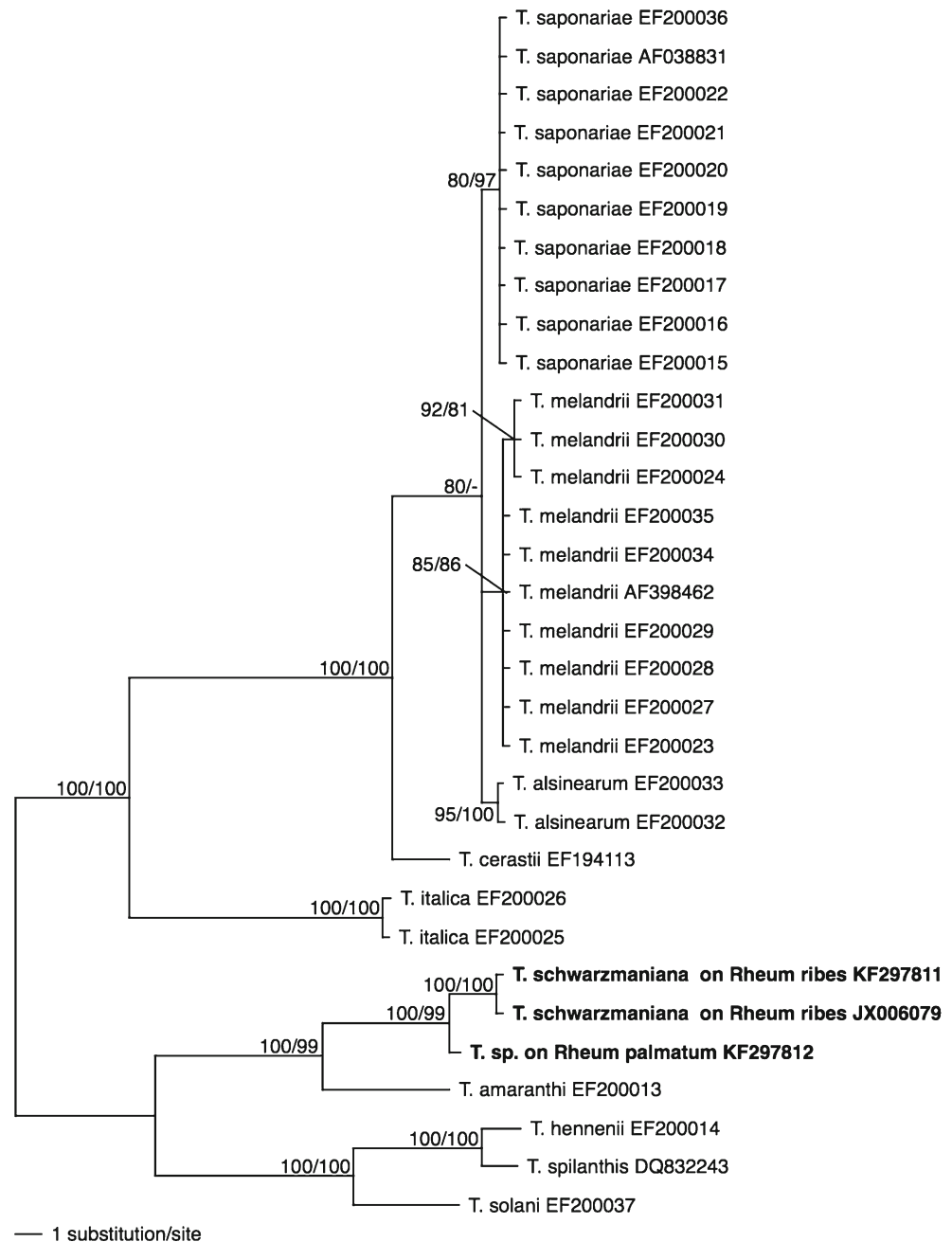
For methods of DNA extraction see (Murray and Thompson 1980). We amplified the ITS 1 and ITS 2 regions of the rDNA, including the 5.8S rDNA (ITS about 710 bp) using the primer pair ITS1-F (5'- CTT GGT CAT TTA GAG GAA GTA A- 3', (Gardes and Bruns 1993)) and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC- 3', (White et al. 1990). The 5'-end of the nuclear large subunit rDNA (LSU, about 640 bp) was amplified using the primer pair NL1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG- 3') and NL4 (5'- GGT CCG TGT TTC AAG ACG G- 3') (O'Donnell 1992, 1993). Primers were used for both PCR and cycle sequencing. Cycling was carried out with an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of 30 s at 94 °C, 1 min at 52 °C, and 50 s at 72 °C. The amplified DNA was visualized by electrophoresis on 1 % agarose gel. The methods of purification of PCR products, sequencing, and processing of the raw data followed (Lutz et al. 2004). DNA sequences prepared in the course of this study were deposited in GenBank [accession numbers (ITS/LSU): BASU 4242 (JX006079/JX006080), H.U.V. 21117 (KF297812/EF647752), KRAM F-49788 (KF297811/ KF297810)].

Phylogenetic analyses

To elucidate the phylogenetic position of the *Thecaphora* specimens on *Rheum ribes*, both their ITS and LSU sequences were analysed within datasets covering all *Thecaphora* ITS or LSU sequences available in GenBank. For the LSU dataset, representatives of all urocystidalean genera of which sequences were available in GenBank were added. If present in GenBank the respective type species were used. GenBank accession numbers of the sequences used (Begerow et al. 1997, 2000, 2006); (Roux et al. 1998); (Andrade et al. 2004); (Matheny et al. 2006); (Bauer et al. 2007, 2008); (Vánky and Lutz 2007); (Roets et al. 2008); (Vánky et al. 2008a, b); (Lutz et al. 2012); (Conforto et al. 2013) are given in Figs. 1 and 2.

Sequence alignment was obtained using MAFFT 6.853 (Kato et al. 2002, 2005); (Kato and Toh 2008) using the L-INS-i option. To obtain reproducible results, manipulation of the alignment by hand as well as manual exclusion of ambiguous sites were avoided as suggested by (Giribet and Wheeler 1999) and (Gatesy et al. 1993), respectively. Instead, highly divergent portions of the alignment were omitted using GBlocks 0.91b (Castresana 2000) with the following options. ITS dataset: ‘Minimum Number of Sequences for a Conserved Position’: 17, ‘Minimum Number of Sequences for a Flank Position’: 17, ‘Maximum Number of Contiguous Non-conserved Positions’: 8, ‘Minimum Length of a Block’: 5, and ‘Allowed Gap Positions’ to ‘With half’. LSU dataset: ‘Minimum Number of Sequences for a Conserved Position’: 34, ‘Minimum Number of Sequences for a Flank Position’: 34, ‘Maximum

Fig. 1 Bayesian inference of phylogenetic relationships between the sampled *Thecaphora* 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 reached stationarity. The topology was rooted with *Thecaphora alsinearum*, *T. haumanii*, *T. hedyssari*, *T. italica*, *T. melandrii*, *T. polymniae*, and *T. saponariae*. 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. T. = *Thecaphora*



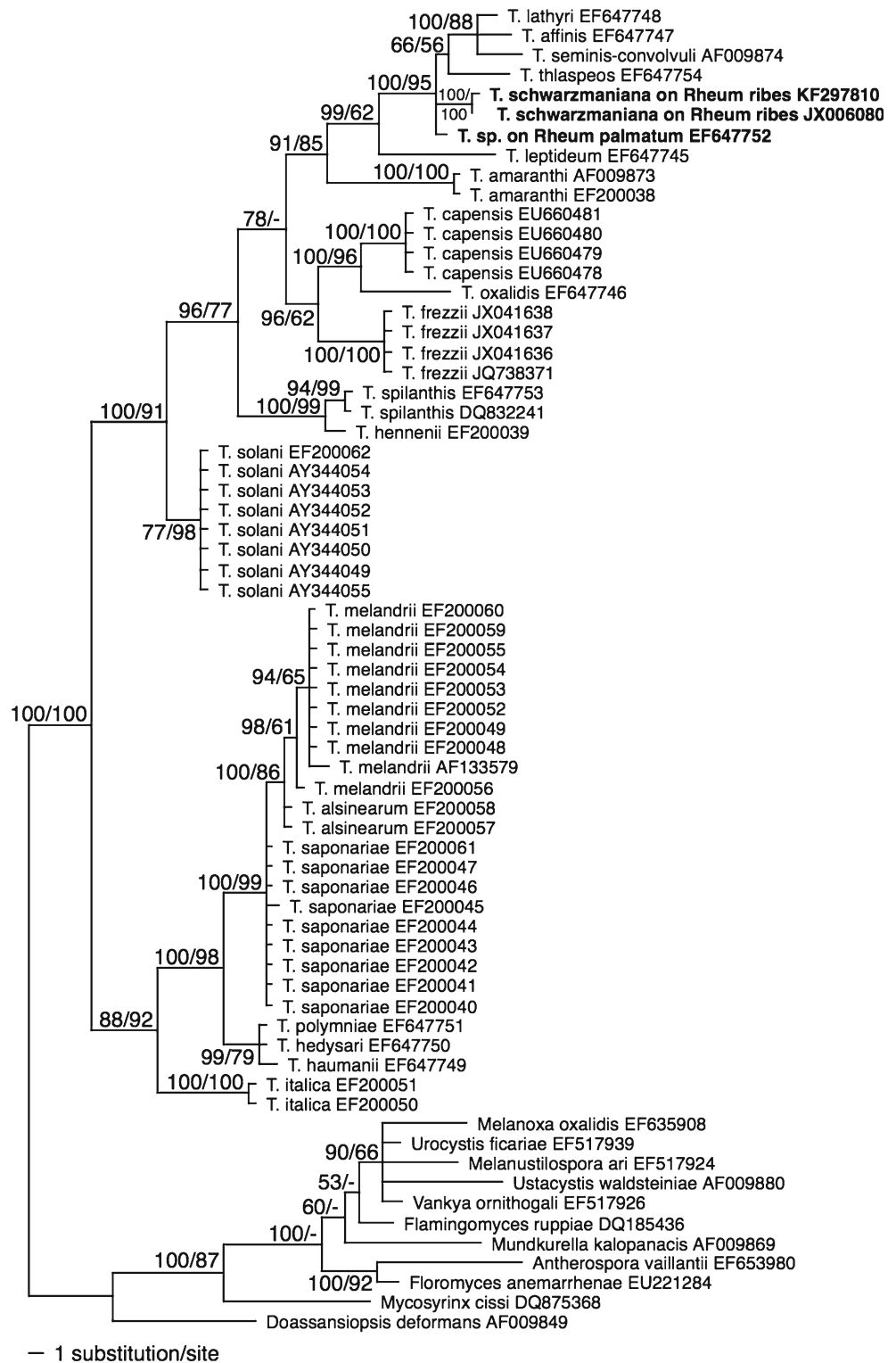
Number of Contiguous Non-conserved Positions': 8, 'Minimum Length of a Block': 5, and 'Allowed Gap Positions' to 'With half'.

The resulting alignments [ITS: new number of positions: 698 (85 % of the original 813 positions), number of variable sites: 177; LSU: new number of positions: 639 (40 % of the original 1576 positions), number of variable sites: 213] were used for phylogenetic analyses using a Maximum Likelihood (ML) and a Bayesian Approach. ML analysis (Felsenstein 1981) was conducted with the RAxML 7.2.8 software (Stamatakis 2006), using raxmlGUI (Silvestro and Michalak

2012), invoking the GTRCAT and the rapid bootstrap option (Stamatakis et al. 2008) with 1,000 replicates.

For BA a Bayesian approach to phylogenetic inference using a Markov chain Monte Carlo technique was used as implemented in the computer program MrBayes 3.2.1 (Ronquist et al. 2012). Two runs over 5,000,000 generations, each consisting of four chains, were implemented using the general time reversible 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 as recommended by (Huelsenbeck and Rannala 2004).

Fig. 2 Bayesian inference of phylogenetic relationships between the sampled Urocystidales: Markov chain Monte Carlo analysis of an alignment of LSU 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 reached stationarity. The topology was rooted with the Urocystidales not belonging to *Thecaphora*. 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 the expected numbers of nucleotide substitutions per site. T. = *Thecaphora*



Trees were sampled every 100th generation, resulting in a sampling of 50,001 trees for each run. From these, the first 25 % of trees for each run were discarded (burnin). The remaining trees were used to compute a 50 % majority rule consensus tree to obtain estimates for the *a posteriori* probabilities of

groups of species. This Bayesian approach to phylogenetic analysis was repeated five times to test the independence of the results from topological priors (Huelsenbeck et al. 2002).

In line with the results of the LSU analyses, ITS trees were rooted with *Thecaphora alsinearum* (Cif.) Vánky & M. Lutz,

T. haumanii Speg., *T. hedsari* Vánky, *T. italica* M. Lutz & Vánky, *T. melandrii* (Syd.) Vánky & M. Lutz, *T. polymniae* Vánky & Pardo-Card., and *T. saponariae* (F. Rudolphi) Vánky; LSU trees were rooted with the Urocystidales not belonging to *Thecaphora*.

Results

Morphological examination

The sori were produced in vegetative tissues of the leaves, namely in the petioles, main midribs or distal veins in *Thecaphora* sp. on *Rheum ribes* and in the distal veins in *Thecaphora* sp. on *R. palmatum*. The spores were firmly united in spore balls that were pale yellow to yellow-brown in colour, and usually globose to subglobose in shape. The ornamentation observed in LM and SEM was similar in specimens on *Rheum ribes* and *R. palmatum*. The specimens on *Rheum ribes* and *R. palmatum* differed in the number of spores per spore ball, the size of spore balls and spores, and the thickness of spore walls (Table 1). The detailed morphological characteristics of the examined *Thecaphora* specimens are included in the species descriptions.

Phylogenetic analyses

The sequences of the specimens of *Thecaphora* sp. on *Rheum ribes* from Iran and Turkey were identical for the ITS and differed in one position (0.15 %) for the LSU. Compared to the sequences of the specimen of *Thecaphora* “*schwarzmaniana*” on *Rheum palmatum* from China, ITS differed in 9 bp (1.22 %), LSU in 6 (0.91 %, specimen from Turkey) or 7 bp (1.06 %, specimen from Iran), respectively.

For both the ITS and LSU dataset, the different runs of the BA that were performed and the ML analyses yielded consistent topologies. To illustrate the results, for both the ITS and LSU analyses, the consensus tree of one run of the BA is presented (Figs. 1, 2).

All *Thecaphora* species included in previous analyses were inferred with high support values except for *Thecaphora alsinearum* and *T. saponariae* in the LSU analyses. In all analyses the specimens of *Thecaphora* sp. on *Rheum ribes* from Iran and Turkey clustered together with perfect support forming the sister lineage of *Thecaphora* “*schwarzmaniana*” on *R. palmatum* (ITS analyses) or clustering in an unresolved relation together with *T. “schwarzmaniana”* on *R. palmatum* and a clade including *T. affinis* A. Schneid., *T. lathyri* J.G. Kühn, *T. seminis-convolvuli* (Desm.) Ito, and *T. thlaspeos* (Beck) Vánky (LSU analyses), respectively.

Taxonomy

Thecaphora schwarzmaniana Byzova, in Schwarzman, Flora Sporovykh Rastenii Kazakhstana 2: 221 (1960) Figs. 3, 4, 5

Sori in the leaf tissues, forming hypophyllous, fusiform or pulvinate swellings of the petioles, main midribs or distal veins, about 1–10 cm long (or longer by fusion), at first covered by host epidermis that during maturity ruptures longitudinally to expose the granular-powdery and dark reddish-brown mass of spore balls embedded between numerous sinuous or straight, stout or filiform remnants of vascular bundles. Spore balls permanent, pale yellow to yellow-brown, globose, subglobose, ovoid, sometimes somewhat irregular, 20–60(–65)×20–45 μm, composed of 2–20 (usually 4–15) firmly united spores. Spores yellow-brown, globose, subglobose, broadly ellipsoidal to usually subcuneiform, 11.0–24.0×8.0–17.0(–18.5) μm; wall on the contact sides ca. 0.5 μm thick, smooth, wall on the free surface convex, 2.0–5.0 μm (usually 3.0–5.0 μm) thick, coarsely and densely verruculose under LM, irregularly verrucose under SEM, with warts usually confluent in short, irregular rows, warts up to 1.5 μm high (measured from SEM micrographs). Spore germination results in germ tubes, initially aseptate, later septate, with or without ramifications, growing out as filaments producing apically or subapically one to several long ellipsoidal sporidia.

Specimens examined: Iran, Hamedan Province, Kabudarahang County, Buqaty mountains, ca. 2850 m a.s.l., on *Rheum ribes*, 15 May 2010, leg. M. Bahador (BASU 4242). – Turkey, Bitlis Province, Mutki District, around Meydan road (slopes), on *Rheum ribes*, 17 May 2011, leg. E. Hüseyin (KRAM F-49788=AhEUM 955).

Comments: The specimens on *Rheum ribes* from Iran and Turkey agree morphologically with the description of *Thecaphora schwarzmaniana* on *R. cordatum* Losinsk. (type host) included in the protologue (Byzova, in (Schwarzman 1960). This is the most detailed description of this species while other descriptions are only slight modifications of the original version (Uljanishchev 1968); (Karatygin and Azbukina 1989); (Vánky 1994, 2012); (Vánky and Oberwinkler 1994). The latter three publications include, however, the original illustration of the macroscopic symptoms of the infection as well as LM and SEM micrographs of spore balls. Furthermore, (Vánky 2012) added information about thickness of spore walls on the free surface of spores that is a valuable character for species delimitation in *Thecaphora*. This feature was not included in any other description of *Thecaphora schwarzmaniana*.

***Thecaphora* sp.** Fig. 6

Sori in the leaf tissues, forming hypophyllous, fusiform swellings of the distal veins, up to 1 cm long, or connected together in the form of a reticulate network about 1.5 cm in

Fig. 3 Macroscopic symptoms caused by *Thecaphora schwarzmaniana* infecting *Rheum ribes*: **a** Iranian specimen, **b** Turkish specimen. – Bars= 10 mm



diameter, covered by host epidermis, were composed of granular-powdery and dark reddish-brown mass of spore balls. Spore balls permanent, pale yellow to yellow-brown, globose, subglobose, broadly ellipsoidal, sometimes somewhat irregular, 15–40(–45)×15–35 μm, were composed of 3–15(–20) firmly united spores. Spores were yellow-brown, globose, subglobose, broadly ellipsoidal to usually subcuneiform, (10.0–)11.0–16.0×(8.5–)9.5–13.5(–14.0) μm;

wall on the contact sides ca. 0.5 μm thick, smooth, wall on the free surface convex, 1.5–2.5(–3.0) μm thick, coarsely and densely verruculose under LM, irregularly verruculose under SEM, with warts usually confluent in short, irregular rows, warts up to 1.5 μm high (measured from SEM micrographs).

Specimen examined: China, Gansu Province, Dangchang, ca. 2800 a.s.l., on *Rheum palmatum*, 6 Aug. 2005, leg. S.R. Wang (H.U.V. 21117).

Comments: The leaf smut on *Rheum palmatum* from China was assigned to *Thecaphora schwarzmaniana* by (Bai and Wang 1991, 1993), (Wang et al. 2009), and (Vánky et al. 2008a). The sequenced specimen in the latter study (LSU sequence deposited in GenBank: EF647752) does not belong to *Thecaphora schwarzmaniana* but most probably represents a distinct species. It differs morphologically from *Thecaphora schwarzmaniana* in having smaller spore balls, smaller spores and thinner spore walls. However, the available material was poor and could be not fully mature (the sori are not open). Thus, collection and examination of fresh material is desirable before describing a new species.

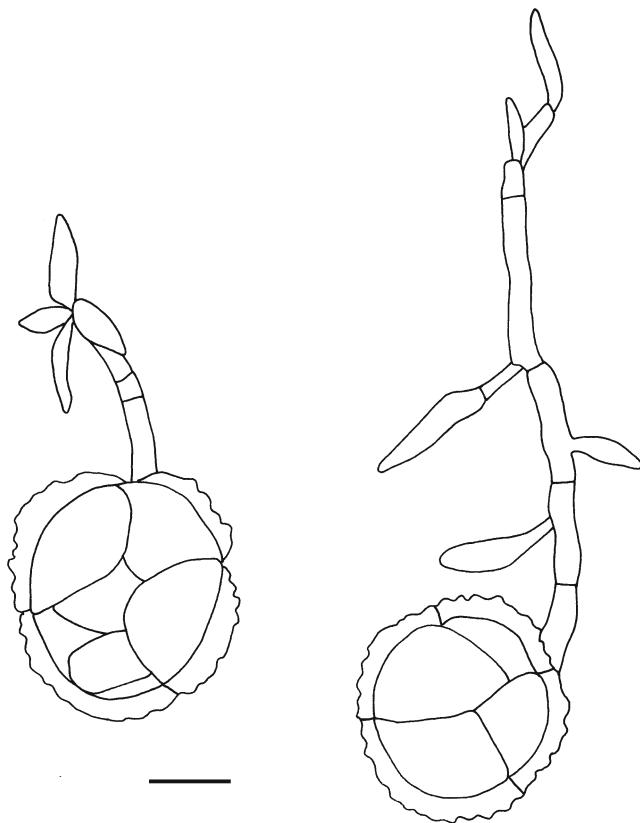
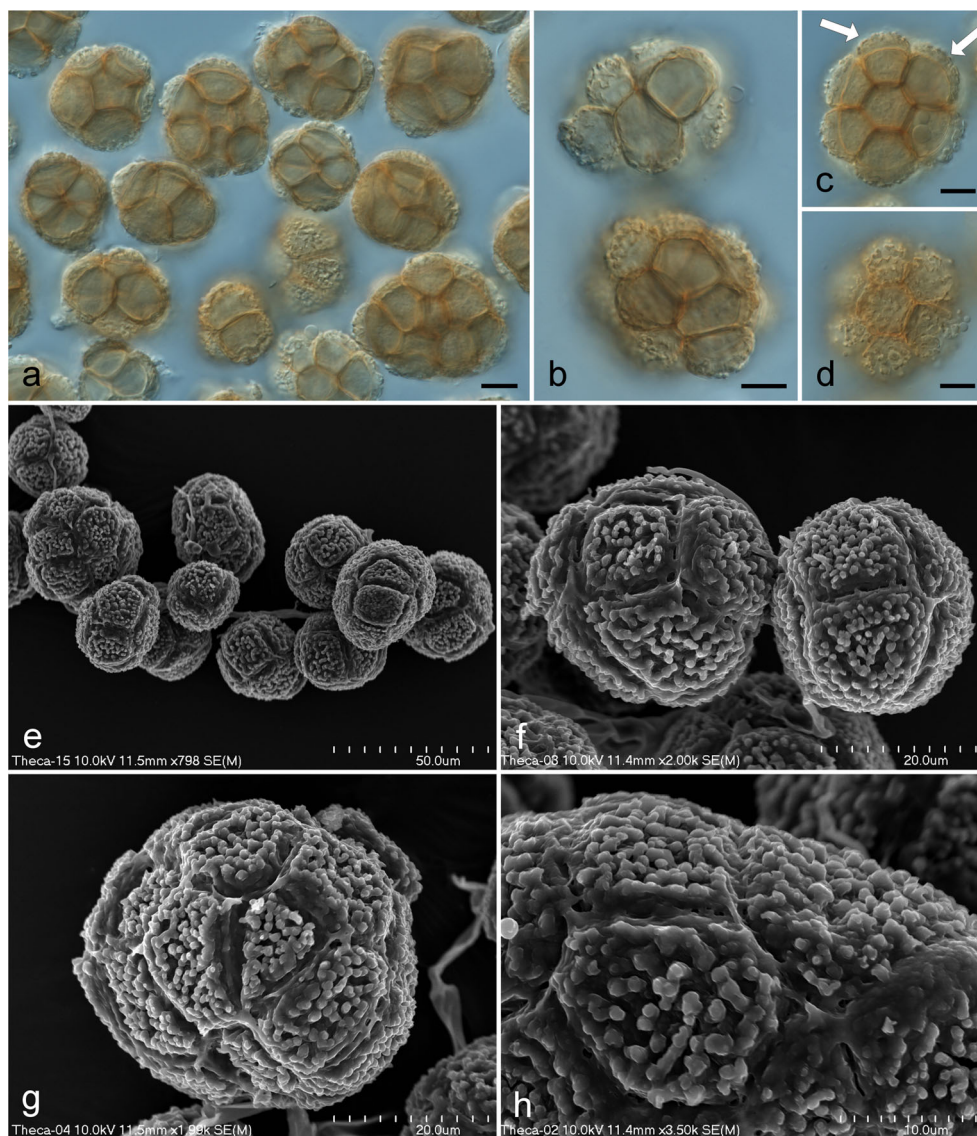


Fig. 4 Spore germination of *Thecaphora schwarzmaniana* from *Rheum ribes* (BASU 4242). Spore balls are drawn schematically. – Bar=10 μm

Discussion

This study reports the first smut species discovered on *Rheum ribes*. The soral and spore ball characters indicate that it belongs to the genus *Thecaphora* and in crucial diagnostic characters it is morphologically indistinguishable from *Thecaphora schwarzmaniana* as described by Byzova (in Schwarzman 1960). In the protologue the sori were described as occurring in leaves, under the epidermis, in parenchyma and veins, first in the form of small scattered pustules that are later connected together to cover larger areas. This description was simplified by (Uljanishchev 1968) and (Karatygin and Azbukina 1989) who reported that the sori occur on leaves, often in veins. (Vánky 1994) and (Vánky and Oberwinkler 1994) described

Fig. 5 Spore balls of *Thecaphora schwarzmaniana* from *Rheum ribes* (KRAM F-49788): **a–d** observed in LM, note the wall thickness on the free surface of spores indicated by arrows on figure c, **e–g** observed in SEM, **h** spore walls observed in SEM. – Bars **a–d, h**=10 μ m, **e**=50 μ m, **f–g**=20 μ m

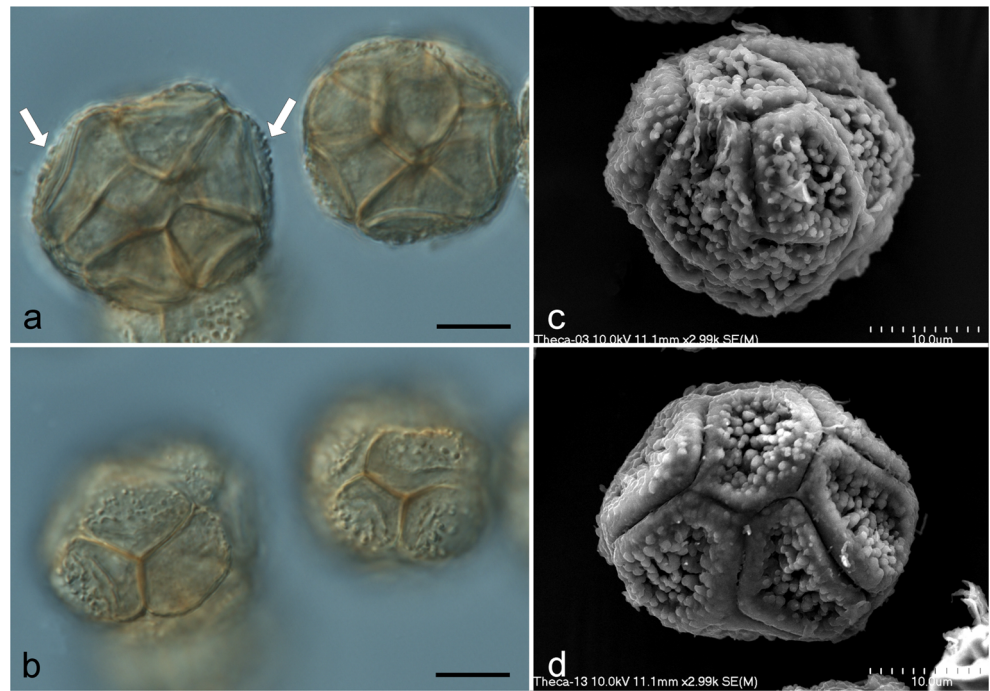


the sori as occurring in leaves, on small distal veins or on main midrib-branches, while (Vánky 2012) described them as forming “small, epiphyllous pustules on leaves, or (...) conspicuous, hypophyllous, fusiform (...) swellings of the mid-ribs”. In the materials from Iran and Turkey the sori were restricted to the vein system of the leaves. This observation allows a more accurate description of the sori in *Thecaphora schwarzmaniana*. It seems that the mycelium of this smut spreads through the vein system where it produces the sori filled with spore balls. This detail is a quite important aspect of the morphology of *Thecaphora schwarzmaniana*, because smut fungi are organ-specific and characters of sori are valuable for the characterization of species and genera in this group of parasitic basidiomycetes (e.g., (McTaggart et al. 2012).

Thecaphora schwarzmaniana was described as a leaf smut infecting *Rheum cordatum* in Kazakhstan (Byzova, in (Schwarzman 1960). This species was later found on

R. cordatum, *R. lobatum* Litv. ex Losinsk., and *R. macrocarpum* Losinsk. in Uzbekistan (Karatygin and Azbukina 1989); (Vánky 1994); (Vánky and Oberwinkler 1994), on *R. turkestanicum* Janischew. in Tajikistan (Korbonskaia 1990), and on *Rheum* sp. in Afghanistan (Vánky 1994); (Vánky and Oberwinkler 1994). The discovery of *Thecaphora schwarzmaniana* in Iran and Turkey significantly expands the geographical range of this species to the west of its main area of occurrence in Central Asia (Fig. 7). A leaf smut on *Rheum palmatum* from China assigned to *Thecaphora schwarzmaniana* does not belong to this species (see results and discussion below). The host species are classified within two morphological *Rheum* sections, that is sect. *Deserticola* (*R. turkestanicum*), and sect. *Ribesiformia* (*R. cordatum*, *R. lobatum*, *R. macrocarpum*, *R. ribes*) (Lozina-Lozinskaya 1936). However, because none of them has so far been included in molecular phylogenetic analyses

Fig. 6 Spore balls of *Thecaphora* sp. on *Rheum palmatum* (H.U.V. 21117): **a–b** observed in LM, note the wall thickness on the free surface of spores indicated by arrows, **c–d** observed in SEM. – Bars=10 μm



(Wang et al. 2005); (Sun et al. 2012), their sectional placement should still be treated with caution.

The application of molecular phylogenetic analyses to the systematics of smut fungi revealed that many morphological species reported from multiple host plants are complexes of closely related cryptic or pseudo-cryptic species. In some cases in-depth assessment of morphological characters

revealed some phenotypic differentiation, and the recognized species often appeared to be highly specialized to the host plant species (Lutz et al. 2005, 2008); (Carris et al. 2007); (Bauer et al. 2008); (Kemler et al. 2009); (Prillinger et al. 2009); (Piątek et al. 2011, 2012b, 2013a, b); (Savchenko et al. 2013)). The level of host specialization in *Thecaphora* differs between species: except for strictly host species specific taxa

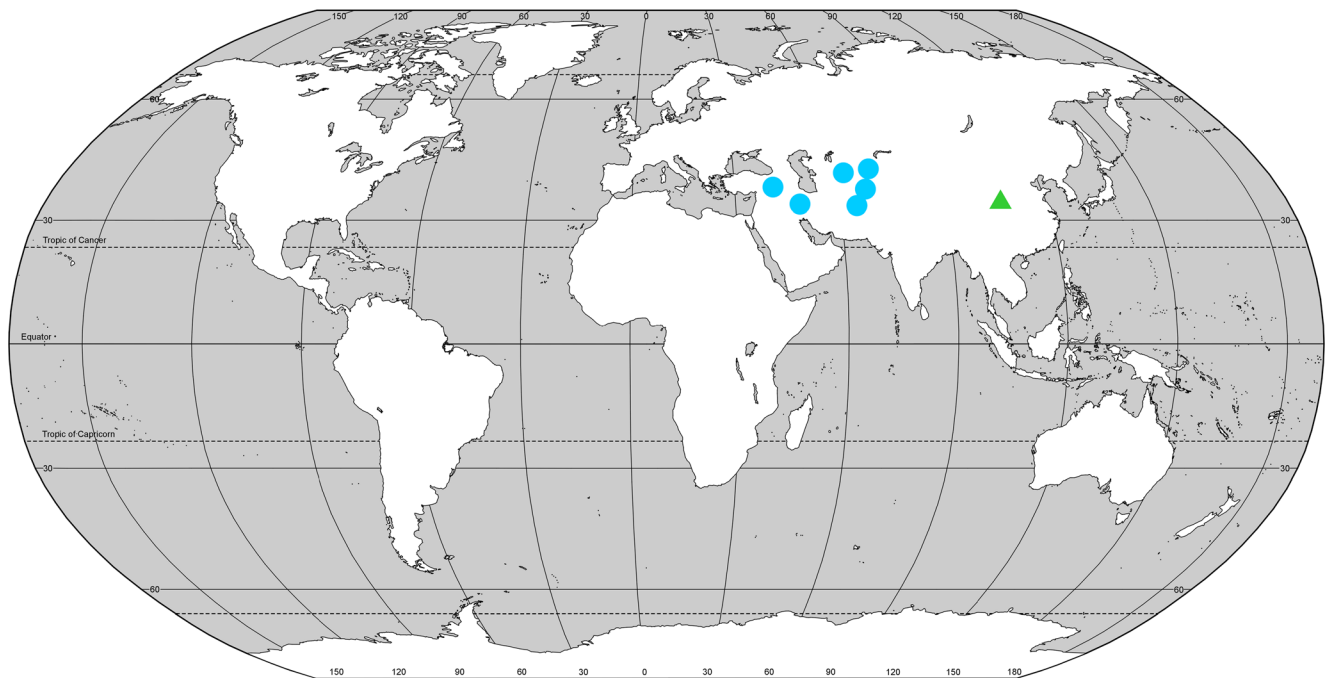


Fig. 7 Global distribution of *Thecaphora* spp. on *Rheum*: spots = *Thecaphora schwarzmaniana*, triangle = *Thecaphora* sp. on *Rheum palmatum*. One spot refers to a country record (only in China and Uzbekistan the *Thecaphora* spp. were reported for more than one locality)

there are species with a broader host range (based on DNA-sequence evidence), for example *T. capensis* Roets & L.L. Dreyer is known from 12 *Oxalis* L. species (Roets et al. 2008, 2012); (Curran et al. 2009), *T. melandrii* from four species in two genera (*Silene* L., *Stellaria* L.), *T. saponariae* from six species in three genera (*Dianthus* L., *Petrorhagia* (Ser.) Link, *Saponaria* L.) (Vánky and Lutz 2007) and *T. solani* Barrus from two *Solanum* L. species (Vánky et al. 2008a).

This study is the first explicit evidence that more than one species could be hidden within collections assigned to *Thecaphora schwarzmaniana*. Molecular phylogenetic analyses revealed two lineages of *Thecaphora* on *Rheum* spp. with significant genetic divergence between them. Despite the absence of sequences of *Thecaphora schwarzmaniana* on its type host *Rheum cordatum*, the lineage including the specimens from Iran and Turkey on *Rheum ribes* that could be assigned to this species on the basis of morphological similarity and the placement of the host plant in the same morphological section – *Rheum* sect. *Ribesiformia* (Lozina-Lozinskaya 1936), though there still is no molecular confirmation of host relations). Obviously, it is possible that, once sequenced, *Thecaphora schwarzmaniana* on *Rheum cordatum* will be revealed being genetically distinct from the specimens on *Rheum ribes* indicating that the latter could be a cryptic species, but for now there is no evidence for that hypothesis. The re-examination of the *Thecaphora* “*schwarzmaniana*” specimen on *Rheum palmatum* from China indicates that it cannot be assigned to this species as it differs in several morphological characters. This is also congruent with host plant taxonomy. *Rheum palmatum* belongs to a distinct section – *Rheum* sect. *Palmata* (Lozina-Lozinskaya 1936), section confirmed in molecular studies, see (Wang et al. 2005); (Sun et al. 2012), unlike all other host species reported for *Thecaphora schwarzmaniana*. The description of the new species for this smut is, however, not possible in the context of this study because of the paucity of the available material.

The host plants in Polygonaceae harbour diverse smut fungi, including 49 recognized species (Vánky 2012). The vast majority of them belong to false smuts.¹ in the pucciniomycotinous genera *Liroa*, *Microbotryum*, *Sphacelotheca*, and *Zundeliomyces* (Microbotryaceae), with only three recognized species belonging to smuts in the ustilaginomycotinous genera *Melanopsichium* (Ustilaginaceae), and *Thecaphora* (Glomosporiaceae). These three groups have different evolutionary histories. The microbotryacean species have their origin on Polygonaceae

(Kemler et al. 2006); (Oberwinkler 2012), later resulting in a major radiation on polygonaceous and other hosts. Conversely, *Melanopsichium* species could have evolved in the consequence of a host jump from Poaceae to Polygonaceae (Weiss et al. 2004). The interpretation of *Thecaphora schwarzmaniana* evolution is more difficult as *Thecaphora* species infect many unrelated host families, a fact quite uncommon in smut genera (with other exceptions in *Doassansia* Cornu, *Doassansiopsis* (Setch.) Dietel, *Entyloma* de Bary, *Heterodoassansia* Vánky, *Melanotaenium* de Bary, *Microbotryum*, *Yelsemia* J. Walker, and *Urocystis* Rabenh. ex Fuckel, see (Vánky 2002, 2013). Molecular phylogenetic analyses, based on ITS and LSU sequences, recovered two sister lineages in *Thecaphora*: one including the parasites on Caryophyllaceae (and *T. polymniae* on Asteraceae, *T. hedydari* on Fabaceae, *T. haumanii* on Amaranthaceae), the other containing the remaining analysed species. *Thecaphora schwarzmaniana* clusters in this latter lineage that is composed of *Thecaphora* species on Amaranthaceae, Asteraceae, Brassicaceae, Convolvulaceae, Fabaceae, Oxalidaceae, and Solanaceae, of which only *Thecaphora* sp. on Oxalidaceae form a host family-monophyletic subgroup. Therefore, it could be assumed that multiple inter-family host jumps took place in the course of evolution of *Thecaphora* species, although on the basis of available molecular data it is not possible to indicate the likely ancestral host family for this genus. Vast similarity of ITS and LSU sequences of *Thecaphora* spp. on *Rheum* spp. point to their close relation suggesting that, following an initial host jump from another host plant family, co-evolution could shape further diversification of *Thecaphora* species on Polygonaceae.

Although, this study reports that at least two *Thecaphora* species occur on Polygonaceae, more intensive sampling is necessary to understand host specialization and evolution of these parasites. Especially, it will be crucial to incorporate specimens from the remaining hosts reported for *Thecaphora schwarzmaniana*, including the materials on the type host *Rheum cordatum*, to reveal whether more than two species evolved on *Rheum*. The collaborative effort, as conducted during the present study, could be the most effective way to push the molecular systematics of smut fungi forward by incorporating smut specimens from hardly accessible parts of the world into a molecular phylogenetic context.

Acknowledgements We thank Mahmood Bahador (Hamedan, Iran) and Kálmán Vánky (Tübingen, Germany) for providing specimens, Michael Weiß, Sigisfredo Garnica and Robert Bauer (Tübingen, Germany) for providing facilities for molecular analyses, Jolanta Piątek (Kraków, Poland) for preparing the world map and the drawing of germinating spores, and Anna Łatkiewicz (Kraków, Poland) for her help with the SEM pictures. The work of Marcin Piątek was partly financed through the statutory fund of the W. Szafer Institute of Botany of the Polish Academy of Sciences, Kraków.

¹ (Oberwinkler 2012) introduced the term “false smuts” to species having a similar life strategy like smut fungi but classified in the pucciniomycotinous order Microbotryales. It should be noted that the term “false smuts” is also commonly used for species of the ascomycetous genus *Ustilagoidea* Bref. that have similar appearance like smut fungi.

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