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Tranzscheliella helutae sp. nov. on *Agropyron cristatum* from Ukraine

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Abstract

The morphology and phylogeny of *Tranzscheliella* on *Agropyron cristatum* (Triticeae) collected in Ukraine were studied by light microscopy, scanning electron microscopy and a molecular phylogenetic data of two rDNA loci (ITS and 28S). As a result, *T. helutae sp. nov.* is described. The fungus differs from other species from the *T. hypodytes* complex by a combination of morphological and molecular data.

Introduction

Tranzscheliella is a unique group of stem-infecting smut pathogens of grasses. The genus consists of twenty-two species (Vánky 2012, Li *et al.* 2017) occurring on thirty-three genera of grasses, and is found on all continents except Antarctica (Vánky 2012, 2013). The characteristic feature of this genus is the presence of mostly small, smooth or ornamented solitary darkly-colored teliospores, which germinate with *Ustilago*-like basidia. *Tranzscheliella* was proposed as a separate genus by Lavrov (1936) with a type species *T. otophora* on *Stipa pennata* based on the presence of spores with two small bipolar cells. Vánky (2003) demonstrated that the bipolar cells are in fact circular broken parts of the thick spore wall and has broadened the concept of *Tranzscheliella* by including grass-parasitizing species with superficial, blackish-brown sori on culms or floral axis of infected plants, that are either naked or have a peridium of fungal origin, and possess small spores (Vánky 2003, 2003b, 2012). Because of its very simple morphology, *Tranzscheliella* has relatively few characteristics that can be used as criteria for taxonomy. Thus, the delimitation of species relies greatly on the host plant. Furthermore, some species of *Tranzscheliella,* such as *T. hypodytes, T. minima,* and *T. williamsii,* are polyphagous and have been reported on 165, 17, and 38 host species, respectively (Vánky 2012).

The systematics of *Tranzscheliella* is still in its early stage since our knowledge of the phylogenetic relationships in this group is very preliminary as many species have not yet been studied using molecular methods. Prior to the study of Li *et al.* (2017), only three species of *Tranzscheliella, T. hypodytes, T. minima,* and *T. williamsii* had been included in molecular phylogenetic analyses (Begerow *et al.* 2006, Matheny *et al.* 2006, Kellner *et al.* 2011, Piątek *et al.* 2017, McTaggart *et al.* 2020). The results of those studies showed that *Tranzscheliella* is a basal lineage within Ustilaginaceae. The most recent molecular phylogenetic analysis by Li *et al.* (2017) included a much wider variety of species, mostly from China, and confirmed the generic position and monophyly of *Tranzscheliella*.

In the course of an ongoing survey of the biodiversity of stem smuts of grasses, a new species with general characteristics of *Tranzscheliella hypodytes* was collected in Ukraine. In the current paper, we introduce it as a novel species and provide descriptions and illustrations, and phylogenetic evidence to delineate the taxon.

Materials and methods

Specimen sampling and documentation

Specimens were collected in Kherson oblast, Ukraine, and later deposited at the Friesner Herbarium (Butler University, USA). Nomenclatural novelties are registered in MycoBank (Crous *et al.* 2004).

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Morphological examination

Sorus and spore characteristics were studied from dried herbarium material. Specimens were examined by light microscopy (LM) and scanning electron microscopy (SEM). Pictures of sori were taken with a Canon Power Shot G10 camera. For LM, spores were mounted in 90% lactic acid on a microscope slide, covered with a cover glass, gently heated to boiling point and cooled, and then examined under a Carl Zeiss Axiostar microscope at 1000× magnification. LM photographs were taken with a Canon Power Shot G10 camera. At least 30 spores were measured from each collection, and the variation is presented as a range, with extreme values given in parentheses. For SEM studies, spores were attached to metal stubs by double-sided adhesive tape and coated with gold. The surface ornamentation of spores was observed at 15 kV and photographed with a scanning electron microscope JEOL JSM-6700F with a working distance of ca. 12–13 mm. At least eight spores were photographed from each specimen used in SEM analysis to get reliable results on the character of spore ornamentation.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was isolated from spores removed from herbarium specimens using FastPrep 24 (MP Biomedicals, Irvine, California). Tubes were incubated in a water bath for 5 hours or overnight at 55°C, and DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Valencia, California) following the manufacturer's instructions.

All amplifications were performed in 20 µl aliquots on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, California). ITS1M primers were used as the forward primer and ITS4 was used as the reverse primer for the ITS region (Stoll *et al.* 2003), NL1 and NL4 were used as the forward primer and the reverse primer, respectively, for the LSU region (O'Donnell 1993). Standard cycling parameters with an annealing temperature of 57°C for the ITS region and 60°C for the LSU region were used for the amplification. PCR products were purified with ExoSAP-IT (USB, Cleveland, Ohio) according to the manufacturer's instructions and amplified with respective forward and reverse PCR primers with the BigDye 3.1 terminator kit (Applied Biosystems, Foster City, California). Amplification products were sequenced on an ABI 3130xl automated DNA sequencer. GenBank accession numbers are included in Table 1.

TABLE 1. Species used in the molecular analyses, including host plants, origin, voucher information, GenBank accession numbers (ITS/28S rDNA), and references

Species	Host plant	Origin	Voucher / Reference	GenBank acc. No. ITS	GenBank acc. No. 28S rDNA	References
T. helutae	*Agropyron cristatum*	Ukraine	BUT-F 304	ON668077	ON953144	This study
T. helutae	*Agropyron cristatum*	Ukraine	BUT-F 305	ON668078	ON953145	This study
T. hypodytes s.l.	*Elymus dahuricus*	China	HMAS 89483	KX832814	KX832847	Li et al. 2017
T. hypodytes s.l.	*Leymus racemosus*	China	HMAS 132683	KX832834	KX832867	Li et al. 2017
T. hypodytes s.l.	*Leymus secalinus*	China	HMAS 88252	KX83281	KX832864	Li et al. 2017
T. lavrovii	*Cleistogenes hackelii*	China	HMAS 807960	KX832843	KX832876	Li et al. 2017
T. linguoae	*Achnatherum inebrians*	China	HMAS 130364	KX832819	KX832852	Li et al. 2017
T. linguoae	*Achnatherum inebrians*	China	HMAS 88253	KX832818	KX832851	Li et al. 2017
T. minima	*Stipa occidentalis*	USA	M 56541	DQ191251	DQ191257	Li et al. 2017
T. reverdattoana	*Achnatherum splendens*	China	HMAS 31398	KX832821	KX832854	Li et al. 2017
T. reverdattoana	*Achnatherum splendens*	China	HMAS 98646	KX832822	KX832855	Li et al. 2017
T. schlechtendalii	*Calamagrostis epigeios*	China	HMAS 73712	KX832846	KX832879	Li et al. 2017
T. schlechtendalii	*Calamagrostis epigeios*	China	HMAS 247038	KX832845	KX832878	Li et al. 2017
Tranzscheliella sp.	*Jarava plumosa*	Argentina	HMAS 84271	KX832816	KX832849	Li et al. 2017
Tranzscheliella sp.	*Jarava plumosa*	Argentina	BRIP 28937	KX832815	KX832848	Li et al. 2017
Tranzscheliella sp.	*Nasella mucronata*	Ecuador	HMAS 68012	KX832817	KX832850	Li et al. 2017
T. williamsii	n.a.	USA	CBS 131475	JN367310	JN367338	Kellner et al. 2011
T. williamsii	*Stipa joannis*	Hungary	BPI 970835	ON668082	ON953146	This study
T. yupeitaniae	*Leymus chinensis*	China	HMAS 84460	KX832840	KX832873	Li et al. 2017
T. yupeitaniae	*Leymus chinensis*	China	HMAS 247040	KX832842	KX832875	Li et al. 2017
Ustanciosporium standleyanum	*Rhynchospora rugosa*	Ecuador	JG 91	DQ846890	DQ846888	Matheny et al. 2006

Phylogenetic analysis

To elucidate the phylogenetic position of the *Tranzscheliella* specimens on *Agropyron cristatum*, their concatenated ITS + 28S rDNA sequences were analyzed within a data set that covered all sequenced *Tranzscheliella* species. *Ustanciosporium standleyanum* was used as an outgroup. GenBank accession numbers of the sequences used (Kellner *et al.* 2011, Li *et al*. 2017) are given in Figure 1 and listed in Table 1.

The DNA sequences included in this study were aligned online with MAFFT v. 7.453 (Katoh & Standley 2013) using the L-INS-i option and concatenated in Geneious Prime. As suggested by Giribet & Wheeler (1999) and Gatesy et al. (1993), to obtain reproducible results, manipulation of the alignment by hand was avoided. Non-homologous parts were curated using Gblocks (Castresana 2000). Maximum Likelihood was implemented as a search criterion in RAxML (Stamatakis, 2014). GTR+I+G was specified as the model of evolution for nucleotide sequence data in MEGA 11 (Tamura et al., 2021). The RAxML analyses were run with a rapid Bootstrap analysis (command –f a) using a random starting tree and 1000 maximum likelihood bootstrap replicates. A Markov Chain Monte Carlo (MCMC) search in a Bayesian analysis was conducted with MrBayes (Ronquist & Huelsenbeck 2003). Four runs were implemented for 5 million generations. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every 500 generations and trees were saved every 1000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (Nylander et al., 2008) and a burn-in of 18000 generations was calculated. The ML and Bayesian analyses were run three times to test accuracy. Host plant determination was verified comparing their ITS sequences to those deposited in GenBank.

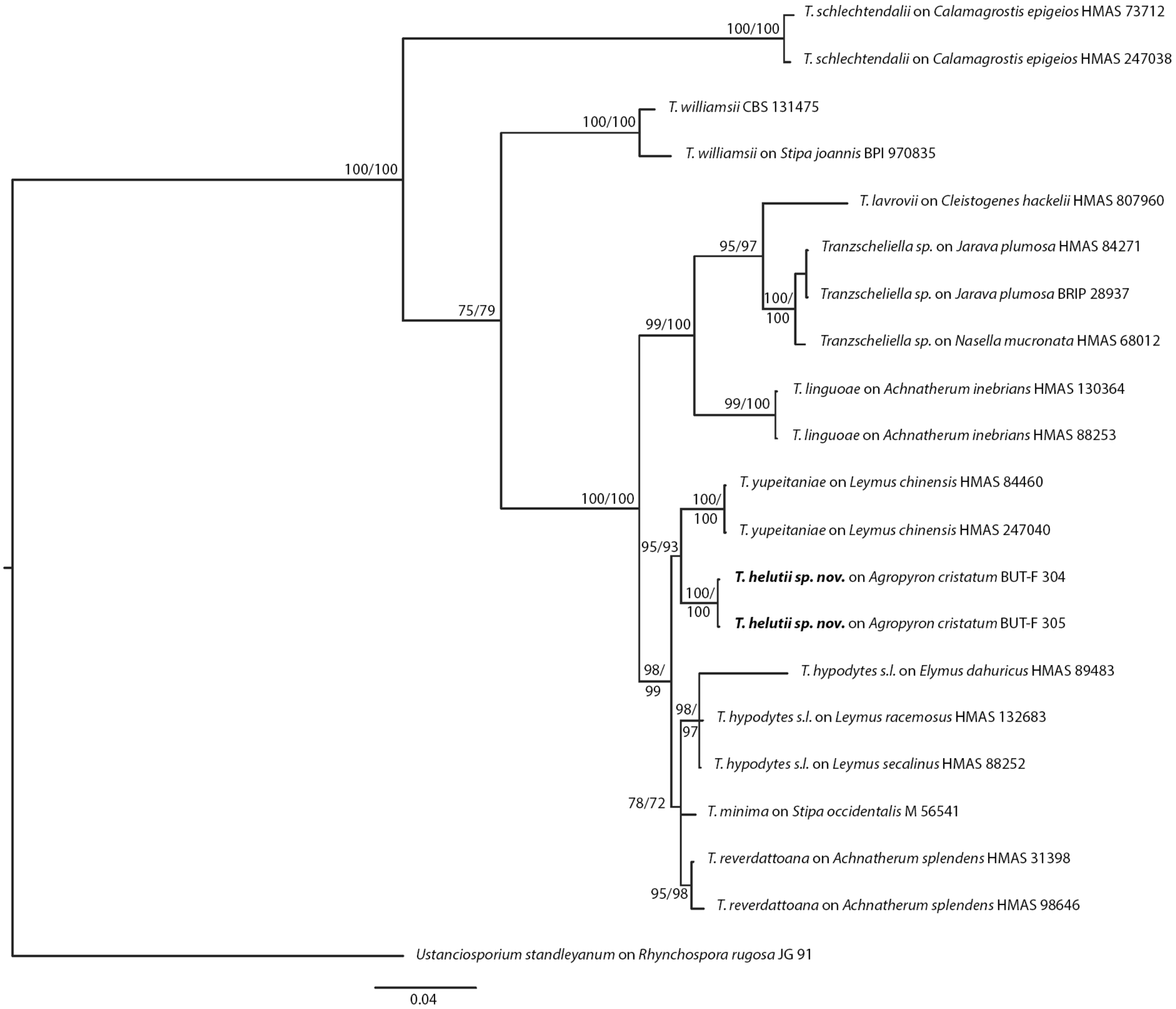

FIGURE 1. Bayesian inference of phylogenetic relationships among the sampled *Tranzscheliella* species: Markov chain Monte Carlo analysis of an alignment of concatenated ITS + 28S rDNA sequences using the GTR+I+G model of DNA substitution with gamma distributed rates and an estimation of invariant sites, random starting trees, and default starting parameters of the DNA substitution model. The topology was rooted with *Ustanciosporium standleyanum.* Numbers on branches are maximum likelihood bootstrap support values/ estimates for a posteriori probabilities. The new species is indicated in bold. *T. = Tranzscheliella.*

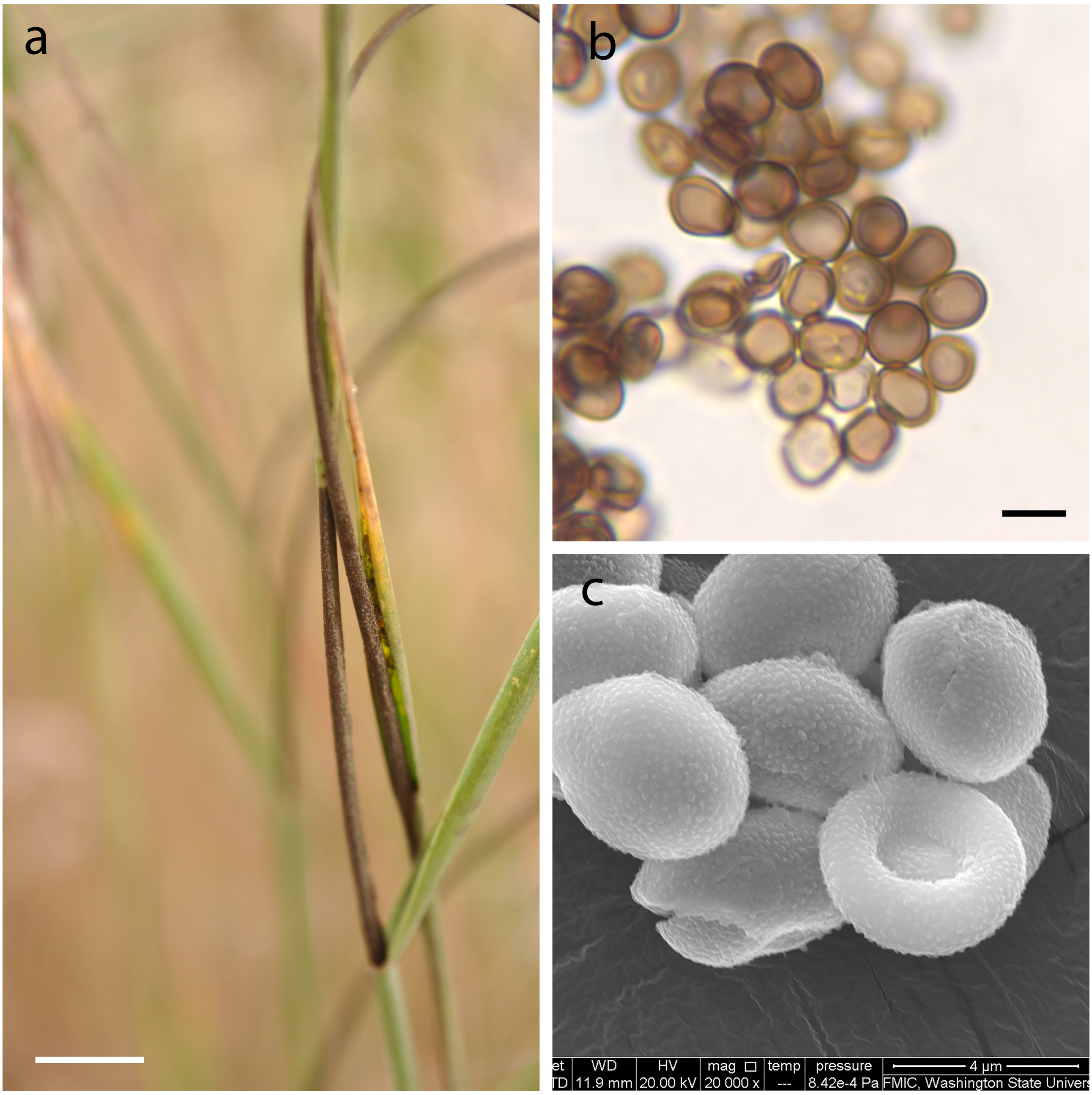

FIGURE 2. *Tranzscheliella helutae* on *Agropyron cristatum.* **A**. Sori of the fungus. **B.** Spores as seen in LM. **C**. Spores as seen in SEM. Bars: a = 5 mm, b = 5 μm, c = 4 μm.

Results

The combined curated data matrix contained 21 specimens and a total of 1197 characters, including 572 in the ITS and 625 in the LSU loci. The different runs of BA and the ML analyses yielded consistent topologies in respect to well-supported branches (*a posteriori* probability greater than 90 in most cases). The consensus tree of one run of Bayesian phylogenetic analyses is presented in Fig. 1. In all analyses specimens of *Tranzscheliella* on *Agropyron cristatum* were sister to *T. yupeitaniae* in a clade with *Tranzscheliella hypodytes* s.l., *T. minima,* and *T. reverdattoana.*

Taxonomy

Tranzscheliella helutae K.G. Savchenko *sp. nov.*
MycoBank: 846947

Etymology: After a Ukrainian mycologist Dr. Vasyl P. Heluta.

Sori in the culms and surrounding the upper internodes and axes of abortive inflorescences, initially covered by the leaf sheath, later exposed. Peridium absent. Spore mass semi-agglutinated to powdery, dark brown. Spores globose to subglobose, 4.5–5.5 × 5–6.5 µm, dark olive-brown. Spores rarely with polar cups, medium brown, without appendages; spore wall c. 0.5–1 µm, smooth. In SEM unevenly, moderately verruculose.

Typification: UKRAINE. Kherson Oblast: Hola Prystan District, Chernomorsky Biosphere Reserve, 46°45′15″ N, 32°12′74″ E, 7 m. asl, On *Agropyron cristatum,* 07 June 2009*,* K.G. Savchenko, BUT-F 304 (holotype).

Other specimens examined: UKRAINE. Kherson Oblast: Hola Prystan District, Chernomorsky Biosphere Reserve, 46°31′35″ N, 31°94′22″ E, 1 m. asl, on *Agropyron cristatum,* 09 June 2009*,* K.G. Savchenko, BUT-F 305 (paratype).

Note – *Tranzscheliella helutae* is a part of *T. hypodytes* species complex. *Tranzscheliella helutae* is the only species from this genus occurring on *Agropyron* (subfamily Pooideae, tribe Triticeae). *Agropyron* contains about seven species found in temperate, mostly arid regions of Eurasia (Dewey 1986). Several species of *Agropyron* were listed as hosts of *T. hypodytes* s.l. by Vánky (2012). *Tranzscheliella helutae* has slightly larger and darker spores than *T. hypodytes* s.str. (4 – 4.5 × 4.5 – 5.5 µm). The spores of *T. helutae* are unevenly, and moderately verruculose in SEM, which differ from the densely, minutely, uniformly verruculose spores of *T. hypodytes* s.str. In the phylogenetic analysis, specimens of *T. helutae* were resolved in a well-supported monophyletic clade (Fig. 1). In a concatenated alignment there are 25 base pair differences between *T. helutae* and the most closely related species, *T. yupeitaniae.*

Discussion

The current study identified a novel species of smut fungi (*Tranzscheliella helutae*) parasitizing *Agropyron* in Ukraine. The species is introduced based on evidence from morphology, host data, and combined ITS and 28S phylogenetic analyses. Although, several *Tranzscheliella* species have been recently described based on molecular data from China (Li *et al.* 2017) the evolutionary relationships of *Tranzscheliella* from various grass hosts have been poorly studied.

Phylogenetically, *T. helutae* is grouped together with *T. hypodytes* s.l., *T. minima, T. reverdattoana* and *T. yupeitaniae. Tranzscheliella helutae* differs from *T. minima* in the absence of peridium, from *T. hypodytes* s.str. and *T. reverdattoana* in having slightly larger spores ((3.5) 4–5 × 4.5–5.5 (6.5) µm) and the absence of the punctuation between warts as seen in SEM, and from *T. yupeitaniae* in having slightly darker spores, with thicker spore walls and moderately verruculose vs. densely verruculose spore surface as seen in SEM.

Species identification criteria in *Tranzscheliella* were previously based on macro- and micromorphological characters and host associations (Vánky 2012). A study of Li *et al.* (2017) confirmed that *T. hypodytes* is a species complex, as was postulated by earlier mycologists (Fischer & Hirschhorn 1945, Vánky & McKenzie 2002, Vánky 2012). It seems that many species of *Tranzscheliella* are host-specific to a high degree, therefore phylogenetic relationships combined with morphological and host data are needed to accurately distinguish *Tranzscheliella* species. It seems that the spore wall ornamentation pattern as seen in SEM can be a useful character in separating various taxa. Clearly, the taxonomy of *Tranzscheliella* species still requires extensive sampling from a wide distribution and host range, as numerous undescribed species associated with important hosts remain undiscovered worldwide.

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