

Ustilago species causing leaf-stripe smut revisited

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Abstract: Leaf-stripe smuts on grasses are a highly polyphyletic group within *Ustilaginomycotina*, occurring in three genera, *Tilletia*, *Urocystis*, and *Ustilago*. Currently more than 12 *Ustilago* species inciting stripe smuts are recognised. The majority belong to the *Ustilago striiformis*-complex, with about 30 different taxa described from 165 different plant species. This study aims to assess whether host distinct-lineages can be observed amongst the *Ustilago* leaf-stripe smuts using nine different loci on a representative set. Phylogenetic reconstructions supported the monophyly of the *Ustilago striiformis*-complex that causes leaf-stripe and the polyphyly of other leaf-stripe smuts within *Ustilago*. Furthermore, smut specimens from the same host genus generally clustered together in well-supported clades that often had available species names for these lineages. In addition to already-named lineages, three new lineages were observed, and described as new species on the basis of host specificity and molecular differences: namely *Ustilago jagei* sp. nov. on *Agrostis stolonifera*, *U. kummeri* sp. nov. on *Bromus inermis*, and *U. neocopinata* sp. nov. on *Dactylis glomerata*.

Key words:

DNA-based taxonomy
host specificity
molecular species discrimination
multigene phylogeny
new taxa
species complex
Ustilaginaceae

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INTRODUCTION

The term “stripe smut” is commonly used to refer to *Ustilaginomycotina* species that cause dark brown to black linear sori of varying length in the leaves of grasses (*Poaceae*). Black spore masses are released after the spores have matured beneath the epidermis in the mesophyll of the host leaves (Fischer 1953, Vánky 2012). The spore release process of sori is unknown, but may be facilitated either by the withering of dead epidermal cells or by enzymatic action, as in the white blister rusts (Heller & 2009). Of the smut genera that infect grasses, *Ustilago* is the most prevalent (Stoll *et al.* 2003, 2005, Vánky 2012).

The term stripe smut does not reflect phylogenetic relatedness, as at least two other genera, *Tilletia* and *Urocystis*, contain species that manifest similar symptoms. The vast majority of leaf-stripe smuts belong to *Ustilago*, including *U. agropyri*, *U. bahuichivoensis*, *U. bethelii*, *U. calamagrostidis*, *U. calcarea*, *U. davisii*, *U. deyeuxiicola*, *U. echinata*, *U. filiformis*, *U. phlei*, *U. scrobiculata*, *U. serpens* s. lat., *U. sporoboli-indici*, *U. striiformis* s. lat., *U. trebouxii*, *U. trichoneurana*, and *U. ulei* (Vánky 2012). Of these species, *U. striiformis* s. lat., with the type species described on *Holcus lanatus*, is a complex occurring on 164 species of *Poaceae* representing 44 different genera

(*Achnatherum*, *Agropyron*, *Agrostis*, *Alopecurus*, *Ammophila*, *Anthoxanthum*, *Arctagrostis*, *Arrhenatherum*, *Avena*, *Beckmannia*, *Brachypodium*, *Briza*, *Bromus*, *Calamagrostis*, *Cleistogenes*, *Cynosurus*, *Dactylis*, *Danthonia*, *Deschampsia*, *Deyeuxia*, *Elymus*, *Festuca*, *Helictotrichon*, *Hierochloë*, *Holcus*, *Hordeum*, *Hystrix*, *Koeleria*, *Leymus*, *Lolium*, *Melica*, *Milium*, *Pennisetum*, *Phalaris*, *Phleum*, *Piptatherum*, *Poa*, *Polypogon*, *Puccinellia*, *Sesleria*, *Setaria*, *Sitanion*, *Trisetaria*, and *Trisetum*). Based on host specificity and minor differences in spore size and surface ornamentation, approximately 30 different taxa have been described in the *U. striiformis* species complex on various host plants (Vánky 2012, Savchenko *et al.* 2014a). *Ustilago serpens* probably represents an overlooked species complex, occurring on five host genera: *Agropyron*, *Brachypodium*, *Bromus*, *Elymus*, and *Leymus*. Whether other species with large warts on their spores also belong to this complex, such as *U. echinata* and *U. scrobiculata*, is currently unclear.

Ustilago striiformis s. lat. on *Alopecurus pratensis* has often been the sole representative of this group in phylogenetic analyses (Stoll *et al.* 2005, Begerow *et al.* 2006, McTaggart *et al.* 2012a). Stoll *et al.* (2005) supported the recognition of *U. calamagrostidis*, a parasite of several species of *Calamagrostis*, as separate from *U. striiformis*.

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The morphological difference was mainly in spore size and ornamentation. Savchenko *et al.* (2014a) provided a more detailed analysis of the *U. striiformis* species complex using several host-fungus combinations and phylogenetic reconstructions based on the nrITS and partial LSU regions. However, while two additional species were proposed as distinct in the *U. striiformis*-complex, the phylogenetic resolution was too low to draw further conclusions regarding host specificity and potential species boundaries. To resolve undescribed lineages within this species complex, Savchenko *et al.* (2014a) suggested that several additional gene loci and host-fungus combinations should be included. However, in line with Vánky (2012), Savchenko *et al.* (2014a) suggested that it would be difficult to distinguish between these lineages based on morphological characters. DNA-based characteristics, such as diagnostic SNPs, along with host specificity might be a solution towards characterizing and describing previously-named and new species (Denchev *et al.* 2009, Piątek *et al.* 2013). The aim of this study was to use a multigene phylogeny to infer the phylogenetic differentiation in the leaf stripe smuts in the genus *Ustilago*, particularly those in the *U. striiformis* species complex.

MATERIAL AND METHODS

Plant and fungal material

Specimens used in the study are listed in Table 1. The names of the hosts and fungi was derived from the latest version of The International Plant Names Index (www.ipni.org), Index Fungorum (www.indexfungorum.org/) and Vánky (2012), and partly following a broad generic concept for *Ustilago* (Thines 2016). A majority of the samples were collected in Germany (about 76) and most collections were not older than 20 years. Samples are deposited in Herbarium Senckenbergianum Görlitz (GLM). All host identifications were confirmed by ITS sequences.

DNA extraction and PCR

About 2–20 mg of infected plant tissue was taken from fungarium samples, placed in 2 mL plastic reaction tubes and homogenized in a mixer mill (MM2, Retsch) using a combination of three to five 1 mm and two 3 mm metal beads at 25 Hz for 5–10 min. Genomic DNA was extracted using the BioSprint 96 DNA Plant Kit (Qiagen, Hilden) loaded to a KingFisher Flex robot (Thermo Scientific, Dreieich).

The complete nrITS of all DNA extracts were amplified using PCR following the procedure of White *et al.* (1990). The primer pairs M-ITS1 (Stoll *et al.* 2003) / ITS4 (White *et al.* 1990) or M-ITS1 / smITS-R1 (Kruse *et al.* 2017a) were used as the reverse and forward primers, respectively. For DNA samples from historic specimens, including type specimens, the *Ustilaginaceae*-optimised reverse primer ITS-US3R (5'TATCAAACCCGGCAGGGAAG3'), located at the ITS2 region, was used.

The NL1 and NL4 primer pair (O'Donnell 1993) were used to amplify the Large Subunit (LSU) of the nrDNA with an annealing temperature of 53 °C. For other loci, the following regions were amplified with their respective primer pairs and annealing temperatures in brackets: *myosin* R0.5/F3 (55 °C),

map R6/F2 (56 °C), *rpl3* R1/F1 (53 °C), *tif2* R3/F3 (53 °C), *ssc1* R1/F2 (53 °C), *sdh1* R3/F2 (53 °C), *rpl4A* R1/F4 (53 °C), and *atp2* R4/F6 (53 °C) (Kruse *et al.* 2017b).

The plant ITS was amplified using the primer pair ITS1P and ITS4 (Ridgway *et al.* 2003) at 53 °C annealing temperature. The cycling reaction was performed in a thermocycler (Eppendorf Mastercycler 96 vapo protect; Eppendorf, Hamburg) with an initial denaturation at 95 °C for 4 min, 36 PCR cycles of denaturation at 95 °C for 40 s, annealing between 53–56 °C (depending on the specific primer pair) for 40 s and elongation at 72 °C for 60 s, followed by a final elongation at 72 °C for 4 min. For DNA samples older than 50 years, PCR cycles were increased to 46 cycles and a larger amount of DNA (1.5 µL of extracted DNA in a reaction volume of 11 µL) was used. The resulting amplicons were sequenced at the Biodiversity and Climate Research Centre (BiK-F) laboratory using the abovementioned PCR primers. However, amplicons from M-ITS1/smITS-R1 were sequenced using the ITS4 reverse primer. The resulting sequences were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>, Table 1).

Alignments and phylogenetic tree reconstruction

We used 93 samples (including 62 of the *Ustilago striiformis*-complex) for the phylogenetic analysis; 93 had sequences from nrITS, *atp2* (ATP synthase subunit 2) and *ssc1* (member of the heat shock proteins), and 70 had additional sequences from *myosin* (myosin group I), *map* (methionine aminopeptidase), *rpl3* (ribosomal protein L3), *tif2* (initial translation factor of eIF4A), *sdh1* (succinate dehydrogenase ubiquinone flavoprotein), and *rpl4A* (ribosomal protein L4-A) (Table 1). About two thirds of the samples (62) belonged to the *U. striiformis* species complex. *Sporisorium inopinatum* (syn. *Langdonia inopinata*) was chosen as outgroup, according to the findings of McTaggart *et al.* (2012a).

Alignments were made on individual loci using mafft v. 7 (Katoh & Standley 2013) using the G-INS-i algorithm. Both leading and trailing gaps of the alignments were removed manually. Two different sets of concatenated alignments for the phylogenetic constructions were generated. The first multigene-alignment includes three loci (ITS, *atp2*, and *ssc1*) from 93 smut samples. The resulting total alignment was 1502 bp (ITS: 643 bp, *atp2*: 595 bp, *ssc1*: 264 bp). The second multigene-alignment included nine genes with a final alignment of 3156 bp (ITS: 643 bp, *atp2*: 595 bp, *ssc1* 264 bp, *map*: 251 bp, *myosin*: 257 bp, *rpl4A*: 415 bp, *rpl3*: 218 bp, *sdh1*: 269 bp, *tif2*: 244 bp).

The diagnostic bases for the *U. striiformis* species complex for all gene markers were determined using the above mentioned alignments. One further ITS alignment was created (443 bp), with the sequence of the type specimen of *U. bromina* (Table 1), the *U. bromina* sequences from GenBank (KF381006-8) and sequences from the same host-fungus-combination from this study, to check if all specimens were sequence-identical with the type collection of *U. bromina* on *Bromus inermis* (data not shown).

For phylogenetic tree constructions, Minimum Evolution (ME) analysis was done using Mega 6.06 (Tamura *et al.* 2013) with the Tamura-Nei substitution model and assuming

Table 1. Smut specimens used for phylogenetic analysis.

DNA-no.	Species	Host	Collection details				gene loci									
			Location	Date	Collector	Fungarium no.	ITS	atp2	ssc1	map	myosin	rpl4A	rpl3	sdh1	tif2	
2354	<i>Sporisorium</i> aff. <i>inopiatum</i> (<i>Langdonia</i>)	<i>Aristida adscensionis</i>	Zambia	12 Apr. 2001	C., T. & K. Vánky	M-0215944	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929824	KY929964	KY930127
474	<i>Ustilago agrostidis-palustris</i>	<i>Agrostis</i> cf. <i>gigantea</i>	Germany, Bavaria	22 Jun. 2012	J. Kruse	GLM-F105832	KY929551	KY930157	KY929994	KY929709	KY929639	KY929849	KY929779	KY929919	KY930082	
1374		<i>Agrostis gigantea</i>	Switzerland	9 Jul. 2004	V. Kummer	GLM-F107425	KY929582	KY930188	KY930025	KY929729	KY929659	KY929869	KY929799	KY929939	KY930102	
2395		<i>Agrostis</i> sp.	Germany, Lower Saxony	12 Jul. 2014	J. Kruse & H. Jage	GLM-F107439	KY929596	KY930202	KY930039	KY929739	KY929669	KY929879	KY929809	KY929949	KY930112	
2287	<i>Ustilago airae-caespitosae</i>	<i>Deschampsia caespitosa</i>	Polen	13 Jul. 1994	H. Scholz	B 70 0014901	KY929526	KY930132	KY929969	KY929688	KY929618	KY929828	KY929758	KY929898	KY930061	
2401		<i>Deschampsia caespitosa</i>	Austria, Upper Austria	15 Aug. 2014	J. Kruse	GLM-F107444	KY929601	KY930207	KY930044	KY929744	KY929674	KY929884	KY929814	KY929954	KY930117	
2402		<i>Deschampsia caespitosa</i>	Austria, Upper Austria	15 Aug. 2014	J. Kruse	GLM-F107445	KY929602	KY930208	KY930045	KY929745	KY929675	KY929885	KY929815	KY929955	KY930118	
477	<i>Ustilago alopecurivora</i>	<i>Alopecurus pratensis</i>	Germany, Hesse	22 May 2010	J. Kruse	GLM-F105834	KY929553	KY930159	KY929996	KY929711	KY929641	KY929851	KY929781	KY929921	KY930084	
1376		<i>Alopecurus pratensis</i>	Germany, Saxony-Anhalt	20 May 2013	H. Jage	GLM-F107426	KY929583	KY930189	KY930026	–	–	–	–	–	–	–
1822	<i>Ustilago</i> aff. <i>andropogonis</i> (<i>Sporisorium</i>)	<i>Bothriochloa ischaemum</i>	Germany, Saxony-Anhalt	25 Jul. 2004	H. Jage & H. John	GLM-F062665	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929764	KY929904	KY930067
432	<i>Ustilago perennans</i>	<i>Arrhenatherum elatius</i>	Germany, Schleswig-Holstein	21 Jun. 2007	J. Kruse	GLM-F105817	KY929536	KY930142	KY929979	KY929697	KY929627	KY929837	KY929767	KY929907	KY930070	
2398	<i>Ustilago brizae</i>	<i>Briza media</i>	Austria, Tirol	21 Jul. 2014	J. Kruse	GLM-F107442	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929812	KY929952	KY930115
2399		<i>Briza media</i>	Germany, Bavaria	19 Jul. 2014	J. Kruse	GLM-F107443	KY929600	KY930206	KY930043	KY929743	KY929673	KY929883	KY929813	KY929953	KY930116	
498	<i>Ustilago bromina</i>	<i>Bromus inermis</i>	Germany, Saxony-Anhalt	04 Jun. 2011	J. Kruse	GLM-F105843	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929785	KY929925	KY930088
500		<i>Bromus inermis</i>	Germany, Thuringia	15 Jun. 2013	J. Kruse	GLM-F105844	KY929563	KY930169	KY930006	KY929716	KY929646	KY929856	KY929786	KY929926	KY930089	
1180		<i>Bromus inermis</i>	Germany, Berlin	May 1983	H. Scholz	HUV No 498 (TUB)	KY929613	KY930219	KY930056	–	–	–	–	–	–	–
2070		<i>Bromus inermis</i>	Germany, Berlin	Aug. 1892	P. Sydow	B 70 0014775	KY929525	–	–	–	–	–	–	–	–	–
2275		<i>Bromus inermis</i>	Germany, Brandenburg	17 Jul. 2005	H. & I. Scholz	B 70 0014755	KY929524	KY930131	KY929968	–	–	–	–	–	–	–

Table 1. (Continued).

DNA-no.	Species	Host	Collection details					gene loci								
			Location	Date	Collector	Fungarium no.	ITS	atp2	ssc1	map	myosin	rpl4A	rpl3	sdh1	tif2	
2276		<i>Bromus inermis</i>	Germany, Thuringia	10 Sep. 1999	I. Scholz	B 70 0021843	KY929527	KY930133	KY929970	–	–	–	–	–	–	
1591	<i>Ustilago</i> aff. <i>bromivora</i>	<i>Bromus rigidus</i>	Greece	23 Apr. 2013	C. & F. Klenke	GLM-F107429	KY929586	KY930192	KY930029	KY929731	KY929661	KY929871	KY929801	KY929941	KY930104	
3370		<i>Bromus sterilis</i>	Spain, Andalusia	2 May 2015	J. Kruse	GLM-F107449	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929818	KY929958	KY930121	
442	<i>Ustilago calamagrostidis</i>	<i>Calamagrostis epigejos</i>	Germany, Lower Saxony	03 Aug. 2011	J. Kruse	GLM-F105818	KY929537	KY930143	KY929980	–	–	–	–	–	–	
445		<i>Calamagrostis epigejos</i>	Germany, Baden-Württemberg	20 Jul. 2013	J. Kruse	GLM-F105819	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929768	KY929908	KY930071	
1383		<i>Calamagrostis epigejos</i>	Germany, Saxony-Anhalt	29 Jun. 2013	H. Zimmermann, U. Richter	GLM-F107427	KY929584	KY930190	KY930027	KY929730	KY929660	KY929870	KY929800	KY929940	KY930103	
1912		<i>Calamagrostis epigejos</i>	Germany, Saxony-Anhalt	09 Aug. 1996	H. Jage	GLM-F048100	KY929530	KY930136	KY929973	KY929691	KY929621	KY929831	KY929761	KY929901	KY930064	
1182	<i>Ustilago corcontica</i>	<i>Calamagrostis villosa</i>	Germany, Saxony	22 Aug. 1987	W. Dietrich	HUV No 794 (TUB)	KY929615	KY930221	KY930058	–	–	–	–	–	–	
1611		<i>Calamagrostis villosa</i>	Germany, Saxony-Anhalt	26 Jul. 2003	H. & U. Richter	GLM-F107434	KY929591	KY930197	KY930034	–	–	–	–	–	–	
1825	<i>Ustilago cruenta</i> (<i>Sporisorium</i>)	<i>Sorghum bicolor</i>	Greece	11 May 2006	H-W, Otto	GLM-F078871	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929766	KY929906	KY930069	
3375	<i>Ustilago cynodontis</i>	<i>Cynodon dactylon</i>	Spain, Andalusia	3 May 2015	J. Kruse	GLM-F107450	KY929607	KY930213	KY930050	KY929749	KY929679	KY929889	KY929819	KY929959	KY930122	
3376		<i>Cynodon dactylon</i>	Spain, Andalusia	3 May 2015	J. Kruse	GLM-F107451	KY929608	KY930214	KY930051	KY929750	KY929680	KY929890	KY929820	KY929960	KY930123	
1596	<i>Ustilago</i> aff. <i>dactyloctenii</i> (<i>Sporisorium</i>)	<i>Dactyloctenium australe</i>	South-Africa	22 Feb. 2000	V. Kummer	GLM-F107430	KY929587	KY930193	KY930030	KY929732	KY929662	KY929872	KY929802	KY929942	KY930105	
478	<i>Ustilago denotarisi</i>	<i>Arrhenatherum elatius</i>	Germany, Schleswig-Holstein	13 May 2007	J. Kruse	GLM-F105835	KY929554	KY930160	KY929997	–	–	–	–	–	–	
481		<i>Arrhenatherum elatius</i>	Germany, Rhineland-Palatinate	23 May 2010	J. Kruse	GLM-F105836	KY929555	KY930161	KY929998	–	–	–	–	–	–	
483		<i>Arrhenatherum elatius</i>	Germany, Lower Saxony	31 Jul. 2011	J. Kruse	GLM-F105837	KY929556	KY930162	KY929999	–	–	–	–	–	–	
486		<i>Arrhenatherum elatius</i>	Germany, Thuringia	04 Jun. 2012	J. Kruse	GLM-F105838	KY929557	KY930163	KY930000	–	–	–	–	–	–	
488		<i>Arrhenatherum elatius</i>	Germany, Bavaria	16 May 2013	J. Kruse	GLM-F105839	KY929558	KY930164	KY930001	–	–	–	–	–	–	

Table 1. (Continued).

DNA-no.	Species	Host	Collection details				gene loci									
			Location	Date	Collector	Fungarium no.	ITS	atp2	ssc1	map	myosin	rpl4A	rpl3	sdh1	tif2	
447	<i>Ustilago echinata</i>	<i>Phalaris arundinacea</i>	Germany, Lower Saxony	01 Jul. 2010	J. Kruse	GLM-F105820	KY929539	KY930145	KY929982	KY929699	KY929629	KY929839	KY929769	KY929909	KY930072	
449		<i>Phalaris arundinacea</i>	Germany, Lower Saxony	29 Aug. 2011	J. Kruse	GLM-F105821	KY929540	KY930146	KY929983	KY929700	KY929630	KY929840	KY929770	KY929910	KY930073	
1914		<i>Phalaris arundinacea</i>	Switzerland, St. Gallen	26 Jul. 2000	H. Jage	GLM-F048338	KY929531	KY930137	KY929974	KY929692	KY929622	KY929832	KY929762	KY929902	KY930065	
451	<i>Ustilago aff. filiformis</i>	<i>Glyceria fluitans</i>	Germany, Lower Saxony	17 May 2007	J. Kruse	GLM-F105822	KY929541	KY930147	KY929984	KY929701	KY929631	KY929841	KY929771	KY929911	KY930074	
454		<i>Glyceria fluitans</i>	Germany, Bavaria	24 Jun. 2012	J. Kruse	GLM-F105823	KY929542	KY930148	KY929985	KY929702	KY929632	KY929842	KY929772	KY929912	KY930075	
455		<i>Glyceria fluitans</i>	Germany, Bavaria	10 May 2013	J. Kruse	GLM-F105824	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929773	KY929913	KY930076	
456	<i>Ustilago filiformis</i>	<i>Glyceria maxima</i>	Germany, Lower Saxony	01 Jul. 2010	J. Kruse	GLM-F105825	KY929544	KY930150	KY929987	KY929704	KY929634	KY929844	KY929774	KY929914	KY930077	
472	<i>Ustilago jagei</i> sp. nov.	<i>Agrostis rupestris</i>	Switzerland, Grisons	02 Aug. 2009	J. Kruse	GLM-F105830	KY929549	KY930155	KY929992	–	–	–	–	–	–	
473		<i>Agrostis stolonifera</i>	Germany, Bavaria	20 May 2012	J. Kruse	GLM-F105831	KY929550	KY930156	KY929993	–	–	–	–	–	–	
476		<i>Agrostis stolonifera</i>	Germany, Hesse	22 May 2010	J. Kruse	GLM-F105833	KY929552	KY930158	KY929995	KY929710	KY929640	KY929850	KY929780	KY929920	KY930083	
551		<i>Agrostis</i> sp.	Germany, Lower Saxony	11 Jun. 2010	J. Kruse	GLM-F107423	KY929580	KY930186	KY930023	KY929727	KY929657	KY929867	KY929797	KY929937	KY930100	
2396		<i>Agrostis stolonifera</i>	Germany, Bavaria	20 Jul. 2014	J. Kruse	GLM-F107440	KY929597	KY930203	KY930040	KY929740	KY929670	KY929880	KY929810	KY929950	KY930113	
2397		<i>Agrostis stolonifera</i>	Germany, Hesse	27 Jun. 2014	J. Kruse	GLM-F107441	KY929598	KY930204	KY930041	KY929741	KY929671	KY929881	KY929811	KY929951	KY930114	
494		<i>Agrostis</i> sp.	Germany, Bavaria	04 Jul. 2013	J. Kruse	GLM-F105841	KY929560	KY930166	KY930003	KY929713	KY929643	KY929853	KY929783	KY929923	KY930086	
1375		<i>Agrostis stolonifera</i>	Germany, Saxony-Anhalt	16 Sep. 2001	H. Jage	GLM-F047379	KY929528	KY930134	KY929971	KY929689	KY929619	KY929829	KY929759	KY929899	KY930062	
1612	<i>Ustilago kummeri</i> sp. nov.	<i>Bromus inermis</i>	Germany, Brandenburg	19 Jun. 2010	V. Kummer	GLM-F107435	KY929592	KY930198	KY930035	KY929736	KY929666	KY929876	KY929806	KY929946	KY930109	
1948		<i>Bromus inermis</i>	Germany, Saxony-Anhalt	17 Jul. 2001	H. Jage, W. Lehman	GLM-F047380	KY929529	KY930135	KY929972	KY929690	KY929620	KY929830	KY929760	KY929900	KY930063	
501	<i>Ustilago lollicola</i>	<i>Lolium perenne</i>	Germany, Bavaria	14 May 2013	J. Kruse	GLM-F105845	KY929564	KY930170	KY930007	–	–	–	–	–	–	
2288A		<i>Festuca pratensis</i>	Germany, Hesse	25 May 2014	J. Kruse	GLM-F107437	KY929594	KY930200	KY930037	–	–	–	–	–	–	

Table 1. (Continued).

DNA-no.	Species	Host	Collection details				gene loci									
			Location	Date	Collector	Fungarium no.	ITS	atp2	ssc1	map	myosin	rpl4A	rpl3	sdh1	tif2	
3386		<i>Festuca arundinacea</i>	Germany, Hesse	02 Nov. 2014	J. Kruse	GLM-F107454	KY929611	KY930217	KY930054	KY929753	KY929683	KY929893	KY929823	KY929963	KY930126	
2815A	<i>Ustilago maydis</i>	<i>Zea mays</i>	Germany, Saxony-Anhalt	10 Jul. 2007	H. Jage	GLM-F107446	KY929603	KY930209	KY930046	KY929746	KY929676	KY929886	KY929816	KY929956	KY930119	
1404	<i>Ustilago milii</i>	<i>Milium effusum</i>	Germany, Saxony-Anhalt	02 Jun. 2002	H. Jage	GLM-F107428	KY929585	KY930191	KY930028	–	–	–	–	–	–	
2303		<i>Milium effusum</i>	Germany, Saxony	03 Jun. 2012	W. Dietrich	GLM-F107438	KY929595	KY930201	KY930038	KY929738	KY929668	KY929878	KY929808	KY929948	KY930111	
3385		<i>Milium effusum</i>	Germany, Hesse	11 Jun. 2015	J. Kruse	GLM-F107453	KY929610	KY930216	KY930053	KY929752	KY929682	KY929892	KY929822	KY929962	KY930125	
503	<i>Ustilago neocopinata</i> sp. nov.	<i>Dactylis glomerata</i>	Germany, Lower Saxony	01 Jul. 2010	J. Kruse	GLM-F105846	KY929565	KY930171	KY930008	–	–	–	–	–	–	
505		<i>Dactylis glomerata</i>	Germany, Bavaria	20 Jun. 2010	J. Kruse	GLM-F105847	KY929566	KY930172	KY930009	–	–	–	–	–	–	
506		<i>Dactylis glomerata</i>	Germany, Lower Saxony	19 May 2011	J. Kruse	GLM-F105848	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929787	KY929927	KY930090	
508		<i>Dactylis glomerata</i>	Germany, Bavaria	19 Jul. 2011	J. Kruse	GLM-F105849	KY929568	KY930174	KY930011	KY929718	KY929648	KY929858	KY929788	KY929928	KY930091	
510		<i>Dactylis glomerata</i>	Germany, Bavaria	24 May 2012	J. Kruse	GLM-F105850	KY929569	KY930175	KY930012	KY929719	KY929649	KY929859	KY929789	KY929929	KY930092	
512		<i>Dactylis glomerata</i>	Germany, Bavaria	15 Jun. 2012	J. Kruse	GLM-F107413	KY929570	KY930176	KY930013	–	–	–	–	–	–	
521		<i>Dactylis glomerata</i>	Germany, Thuringia	15 Jun. 2013	J. Kruse	GLM-F107414	KY929571	KY930177	KY930014	–	–	–	–	–	–	
463	<i>Ustilago nuda</i>	<i>Hordeum vulgare</i>	Germany, Bavaria	12 May 2012	J. Kruse	GLM-F105826	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929775	KY929915	KY930078	
884	<i>Sporisorium</i> aff. <i>occidentale</i>	<i>Andropogon gerardii</i>	USA	30 Jul. 1989	not known	HUV No 758 (TUB)	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929825	KY929965	KY930128	
471	<i>Ustilago salweyi</i>	<i>Holcus mollis</i>	Germany, Bavaria	11 Jun. 2012	J. Kruse	GLM-F105829	KY929548	KY930154	KY929991	KY929708	KY929638	KY929848	KY929778	KY929918	KY930081	
489		<i>Holcus mollis</i>	Germany, Bavaria	16 May 2013	J. Kruse	GLM-F105840	KY929559	KY930165	KY930002	KY929712	KY929642	KY929852	KY929782	KY929922	KY930085	
523		<i>Holcus lanatus</i>	Germany, Lower Saxony	24 May 2009	J. Kruse	GLM-F107415	KY929572	KY930178	KY930015	KY929720	KY929650	KY929860	KY929790	KY929930	KY930093	
524		<i>Holcus lanatus</i>	Germany, Lower Saxony	22 May 2010	J. Kruse	GLM-F107416	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929791	KY929931	KY930094	
525		<i>Holcus lanatus</i>	Germany, Lower Saxony	27 May 2010	J. Kruse	GLM-F107417	KY929574	KY930180	KY930017	KY929722	KY929652	KY929862	KY929792	KY929932	KY930095	
531		<i>Holcus lanatus</i>	Germany, Bavaria	17 May 2012	J. Kruse	GLM-F107418	KY929575	KY930181	KY930018	–	–	–	–	–	–	

Table 1. (Continued).

DNA-no.	Species	Host	Collection details				gene loci									
			Location	Date	Collector	Fungarium no.	ITS	atp2	ssc1	map	myosin	rpl4A	rpl3	sdh1	tif2	
541		<i>Holcus mollis</i>	Germany, Saxony	03 Jun. 2011	J. Kruse	GLM-F107419	KY929576	KY930182	KY930019	KY929723	KY929653	KY929863	KY929793	KY929933	KY930096	
543		<i>Holcus mollis</i>	Germany, Saxony-Anhalt	05 Jun. 2011	J. Kruse	GLM-F107420	KY929577	KY930183	KY930020	KY929724	KY929654	KY929864	KY929794	KY929934	KY930097	
544		<i>Holcus mollis</i>	Germany, Saxony-Anhalt	05 Jun. 2011	J. Kruse	GLM-F107421	KY929578	KY930184	KY930021	KY929725	KY929655	KY929865	KY929795	KY929935	KY930098	
545		<i>Holcus mollis</i>	Germany, Lower Saxony	17 Aug. 2011	J. Kruse	GLM-F107422	KY929579	KY930185	KY930022	KY929726	KY929656	KY929866	KY929796	KY929936	KY930099	
497	<i>Ustilago scaura</i>	<i>Helictotrichon pubescens</i>	Germany, Rhineland-Palatinate	23 May 2010	J. Kruse	GLM-F105842	KY929561	KY930167	KY930004	KY929714	KY929644	KY929854	KY929784	KY929924	KY930087	
3384		<i>Helictotrichon pubescens</i>	Germany, Hesse	10 Jun. 2015	J. Kruse	GLM-F107452	KY929609	KY930215	KY930052	KY929751	KY929681	KY929891	KY929821	KY929961	KY930124	
1359	<i>Ustilago</i> aff. <i>schroeteriana</i> (<i>Sporisorium</i>)	<i>Paspalum virgatum</i>	Costa Rica	15 Mar. 1991	T. & K. Vánky	HUV No 888 (TUB)	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929826	KY929966	KY930129	
1608	<i>Ustilago scrobiculata</i>	<i>Calamagrostis epigejos</i>	Germany, Brandenburg	17 Aug. 2011	V. Kummer & C. Buhr	GLM-F107431	KY929588	KY930194	KY930031	KY929733	KY929663	KY929873	KY929803	KY929943	KY930106	
1609		<i>Calamagrostis epigejos</i>	Germany, Thuringia	27 May 2010	V. Kummer	GLM-F107432	KY929589	KY930195	KY930032	KY929734	KY929664	KY929874	KY929804	KY929944	KY930107	
1610		<i>Calamagrostis epigejos</i>	Germany, Brandenburg	24 Jun. 2007	V. Kummer	GLM-F107433	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929805	KY929945	KY930108	
467	<i>Ustilago serpens</i>	<i>Elymus repens</i>	Germany, Schleswig-Holstein	31 Jul. 2012	J. Kruse	GLM-F105827	KY929546	KY930152	KY929989	KY929706	KY929636	KY929846	KY929776	KY929916	KY930079	
469		<i>Elymus repens</i>	Germany, Thuringia	15 Jun. 2013	J. Kruse	GLM-F105828	KY929547	KY930153	KY929990	KY929707	KY929637	KY929847	KY929777	KY929917	KY930080	
3110		<i>Elymus repens</i>	Germany, Brandenburg	29 Jun. 2014	V. Kummer	GLM-F107447	KY929604	KY930210	KY930047	–	–	–	–	–	–	
1305	<i>Ustilago</i> aff. <i>sorghii</i> (<i>Sporisorium</i>)	<i>Sorghum plumosum</i>	Australia	20 Feb. 1996	A. A. Mitchell, C. & K. Vánky	HUV No 970 (TUB)	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929827	KY929967	KY930130	
1951	<i>Ustilago</i> aff. <i>syntherismae</i>	<i>Digitaria sanguinalis</i>	Germany, Saxony-Anhalt	01 Oct. 2004	H. Jage	GLM-F064759	KY929534	KY930140	KY929977	KY929695	KY929625	KY929835	KY929765	KY929905	KY930068	
1617		<i>Digitaria sanguinalis</i>	Germany, Brandenburg	11 Aug. 2001	V. Kummer	GLM-F107436	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929807	KY929947	KY930110	
553	<i>Ustilago trichophora</i>	<i>Echinochloa crus-galli</i>	Germany, North Rhine-Westphalia	04 Oct. 2010	J. Kruse	GLM-F107424	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	Kruse <i>et al.</i> 2017b	KY929798	KY929938	KY930101	

Table 1. (Continued).

DNA-no.	Species	Host	Location	Date	Collection details		gene loci								
					Collector	Fungarium no.	ITS	atp2	ssc1	map	myosin	rpl4A	rpl3	sdh1	tif2
1957	<i>Echinochloa crus-galli</i>	Germany, Saxony-Anhalt	01 Oct. 2003	H. Jäge	GLM-F062638	KY929532	KY930138	KY929975	KY929693	KY929623	KY929833	KY929763	KY929903	KY930066	
3347	<i>Ustilago</i> aff. <i>vanderystii</i> (<i>Sporisorium</i>)	Spain, Andalusia	22 Apr. 2015	J. Kruse	GLM-F107448	KY929605	KY930211	KY929747	KY929677	KY929887	KY929817	KY929957	KY930120		

Type specimens are printed in bold face.

complete deletion at 80 % cut-off with 1000 bootstrap replicates. All other parameters were set to default values. Maximum Likelihood (ML) analysis was done using RAxML (Stamatakis 2014) with parameters set to default values and Bayesian analysis was done using MrBayes 3.2 (Ronquist & Huelsenbeck 2003) running five times with model 6 (GTR) using four incrementally heated chains for 10 million generations, sampling every 1000th tree discarding the first 30 % of the obtained trees, all other parameters were set to default on the TrEase webserver (<http://www.thines-lab.senckenberg.de/trease>).

To account for potentially deviating evolutionary properties, the analysis in ME was done also on a partitioned concatenated dataset. As no supported differences within the topology of the trees were observed in comparison with the un-partitioned dataset, the other analyses were carried out without partitioning.

Morphological examination

For light microscopy, fungarium specimens (GLM-F107417, GLM-F105836, GLM-F107435, GLM-F107413, GLM-F047379, GLM-F105827) were transferred to 60 % lactic acid on a slide. Morphological examination was carried out using a Zeiss Imager M2 AX10 microscope (Carl Zeiss, Göttingen). Measurements of the spores were performed at x400. The measurements are reported as maxima and minima in parentheses, and the mean plus and minus the standard deviation of a number of measurements is given in parenthesis. The means are placed in italics.

RESULTS

Phylogenetic inference

The LSU sequence data were excluded from further analysis since sequences were identical for all members of the *Ustilago striiformis* species complex (data not shown). All other loci showed SNPs within the *U. striiformis* cluster. The diagnostic bases (SNPs) with their specific positions are given in Fig. 6.

There were no supported conflicts in the topology of the trees of the single loci and the concatenated trees. Thus, the datasets were combined and used as concatenated for further analysis. The multigene tree based on nine different loci (Fig. 1) showed strong to maximum support for a monophyly of the *U. striiformis* species complex. If multiple specimens from one host species were included, these grouped together with strong to maximum support, except for the clades corresponding to *U. scaura s. lat.* (ME 64, ML 63, BA 0.99), *U. brizae* (ME 63, ML 68, BA 0.99), and *U. agrostidis-palustris* (ME 71, ML 68, BA 0.99), which received weak to strong support (Fig. 1).

A phylogenetic reconstruction (Fig. 2) with an additional 21 specimens but based on only half of the characters per specimen (ITS, *atp2*, and *ssc1*) revealed the same groups as the double-sized alignment, but expectedly with weaker statistical support. For example, the three weak to strongly supported lineages shown in Fig. 1 still grouped together, but with no or weak support (*U. brizae* – ME 64, ML -, BA 0.79; *U. scaura s. lat.* – ME -, ML -, BA 0.79; *U. agrostidis-palustris* – no support), highlighting the importance of gene selection.

In the phylogenetic reconstruction based on fewer genes (Fig. 2) additional specimens were included, which further supported the high degree of genetic differentiation in conjunction with the host species infected. Specimens from *Festuca* and *Lolium* grouped together with strong support, while the monophyly of the clade containing samples from *Alopecurus* species was unsupported to weakly supported (ME 64, ML -, BA -). Two monophyletic groups were absent from the tree with more loci (Fig. 1): one on *Calamagrostis villosa* and another on *Arrhenatherum elatius*. Both of these groups were highly supported (*Calamagrostis*: ME 76, ML 94, BA 0.99; *Arrhenatherum*: ME 92, ML 99, BA 1) in the tree based on fewer loci (Fig. 2).

In both phylogenetic trees (Figs 1–2), *U. cynodontis* was inferred as the sister species to the whole *U. striiformis* species complex. To illustrate the relationships within this species complex further, two additional phylogenetic

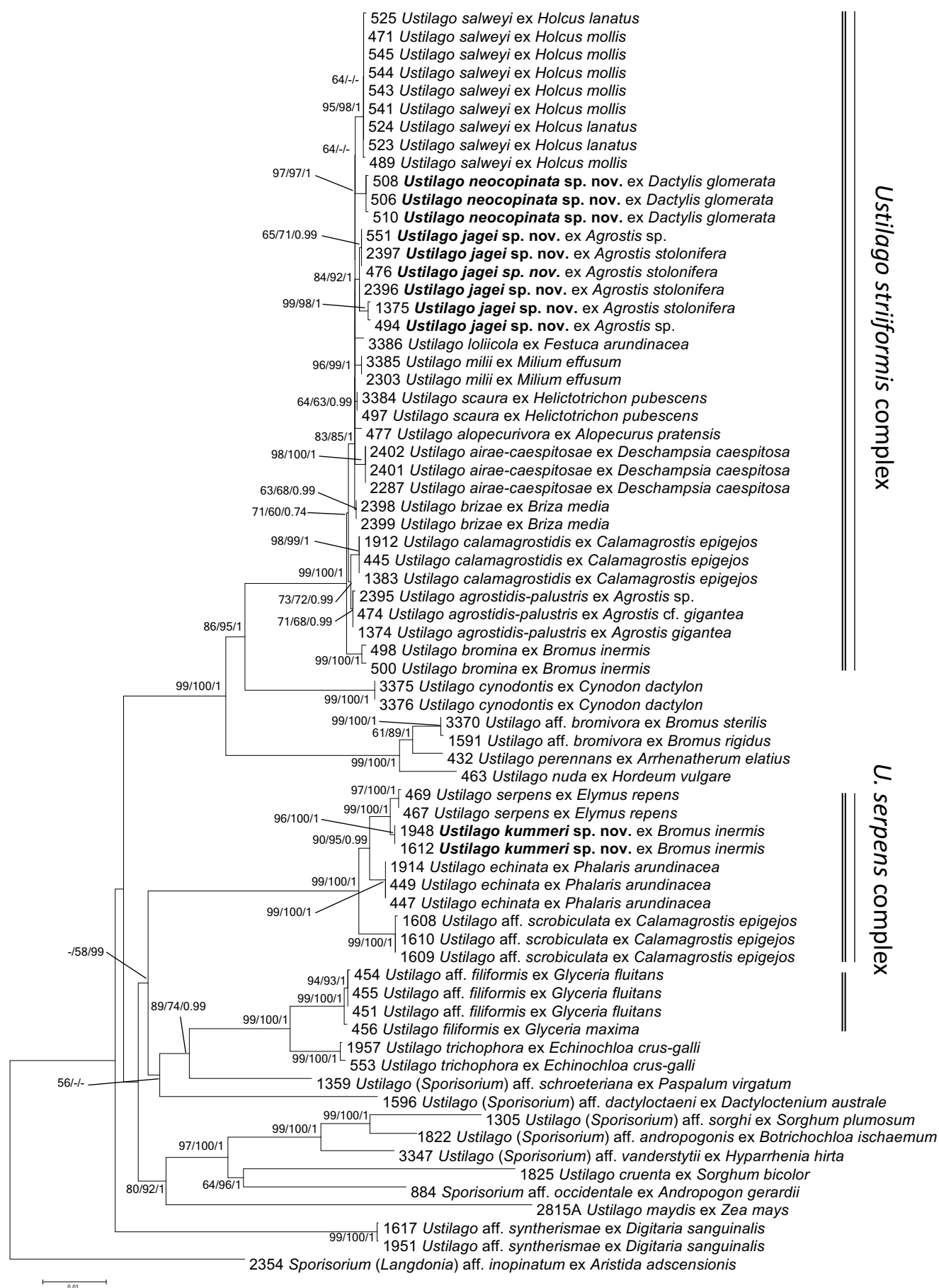


Fig. 1. Phylogenetic tree based on Minimum Evolution analysis of nine loci (ITS, *myosin*, *map*, *rpl3*, *tif2*, *ssc1*, *sdh1*, *rpl4A*, *atp2*). Numbers on branches denote support in Minimum Evolution, Maximum Likelihood and Bayesian Analyses, in the respective order. Values below 55 % are denoted by '-'. The bar indicates the number of substitutions per site.

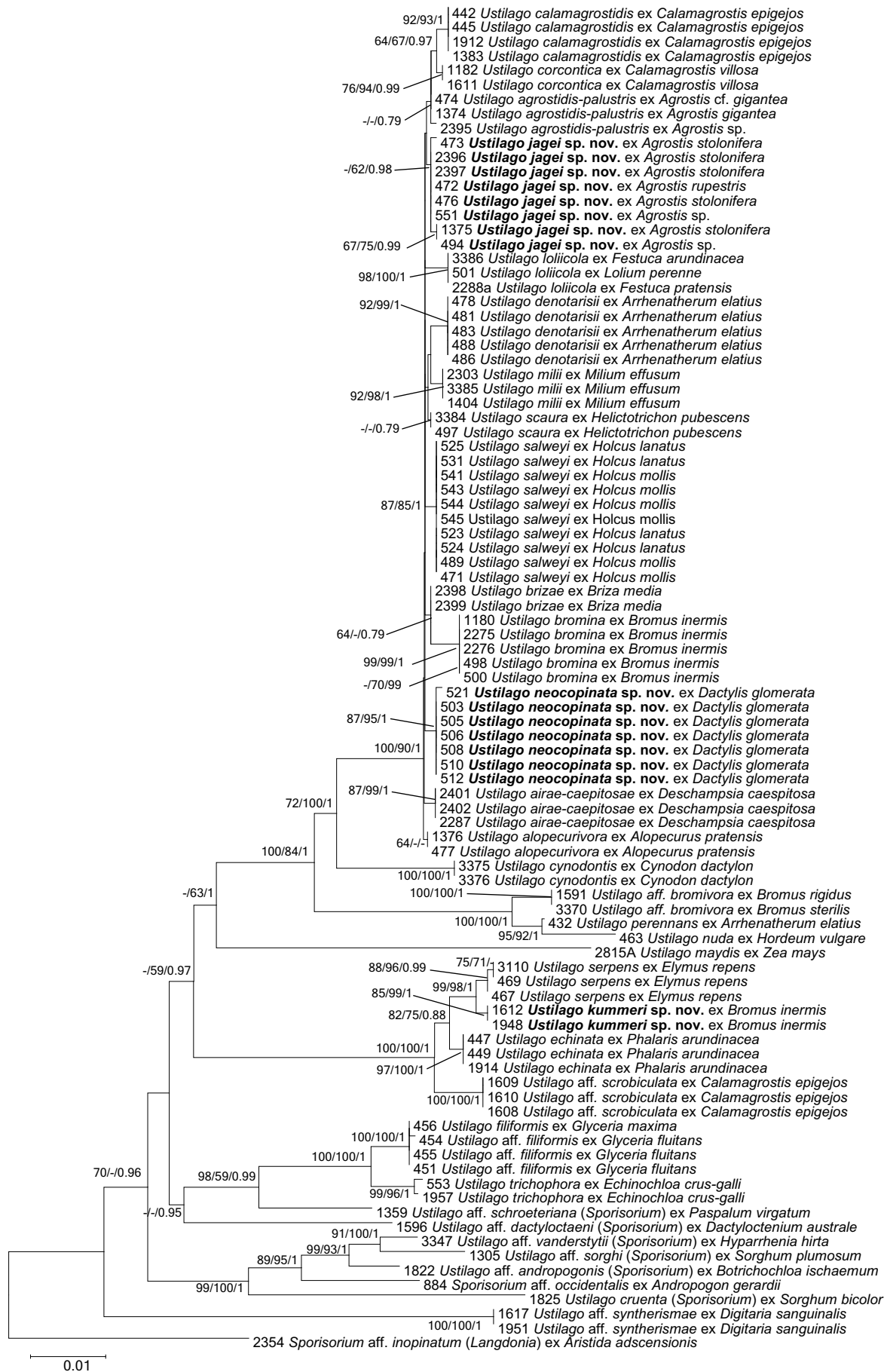


Fig. 2. Phylogenetic tree based on Minimum Evolution analysis of three loci (ITS, *ssc1*, *atp2*). Numbers on branches denote support in Minimum Evolution, Maximum Likelihood and Bayesian Analyses, in the respective order. Values below 55% are denoted by '-'. The bar indicates the number of substitutions per site.

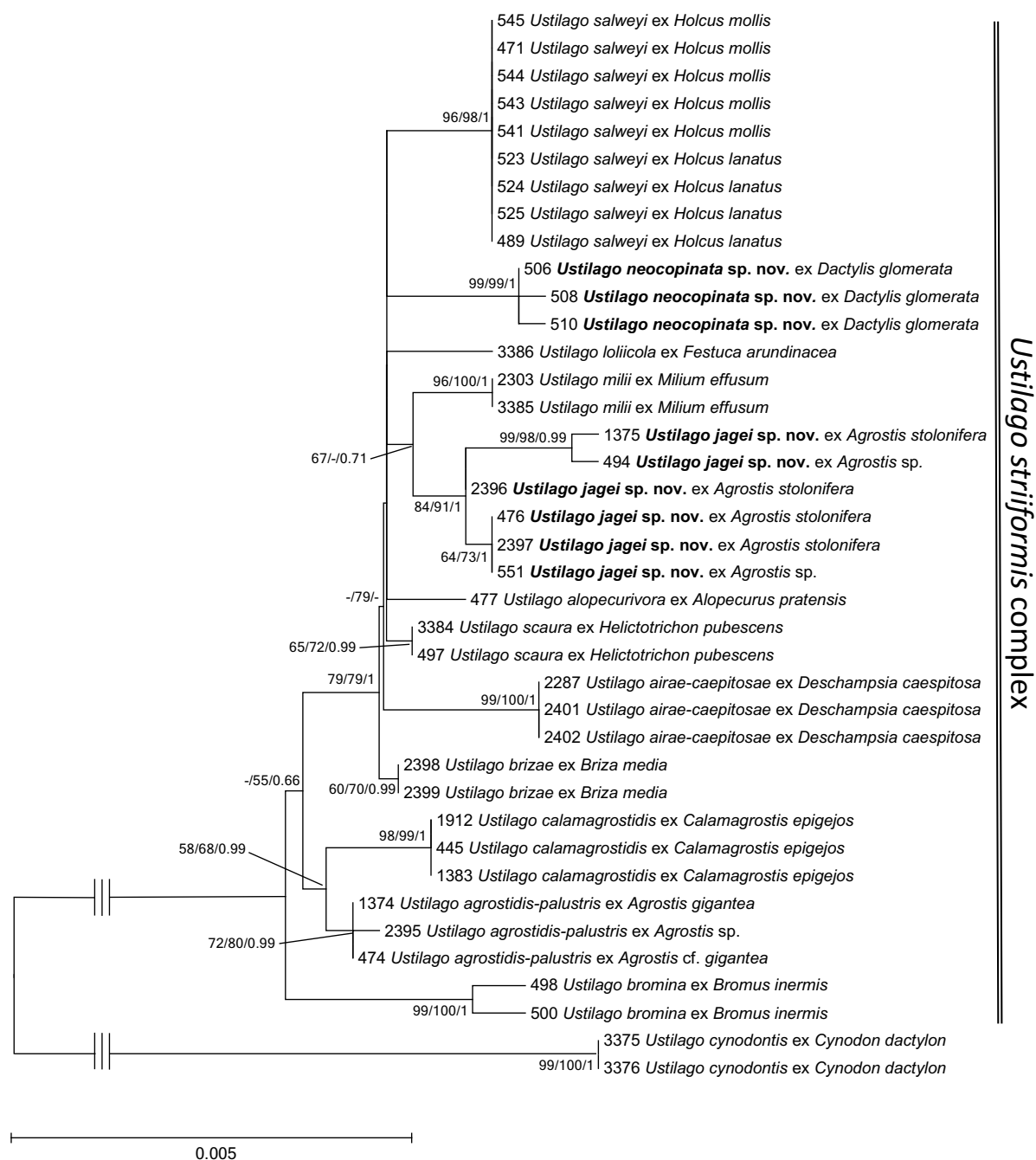


Fig. 3. Phylogenetic tree based on Minimum Evolution analysis of nine loci (ITS, *myosin*, *map*, *rpl3*, *tif2*, *ssc1*, *sdh1*, *rpl4A*, *atp2*) detailed showing the *Ustilago striiformis*-complex with the outgroup *U. cynodontis*. Numbers on branches denote support in Minimum Evolution, Maximum Likelihood and Bayesian Analyses, in the respective order. Values below 55 % are denoted by '-'. The bar indicates the number of substitutions per site.

trees with a reduced sampling and *U. cynodontis* as outgroup are shown in Figs 3 (9 loci) and 4 (3 loci). The support values and the topology were comparable to the phylogenetic reconstructions in Figs 1–2. In both phylogenetic trees, *U. serpens* on *Elymus repens* and on *Bromus inermis* grouped together with high to maximum support. This group clustered with two further lineages with larger echinulate spores compared to the *U. striiformis* species complex, which is considered a synapomorphy of this lineage.

The resolution on the backbone was rather low, as highlighted also by the ambiguous placement of *U. maydis*, which was resolved as a sister group to the pathogens on the

majority of panicoid hosts in the tree based on 9 loci (Fig. 1) with moderate to maximum support, while being inferred as a sister to the clade containing the *U.* species complex as well as the *U. nuda* species group with lacking to maximum support in the tree based on three loci (Fig. 2).

Morphology

The degree of overlap in morphological characteristics was too high in both species complexes to provide easily accessible characteristics for species delimitation (Fig. 5). The individual measurements are included in the species descriptions below and summarized in Table 3.

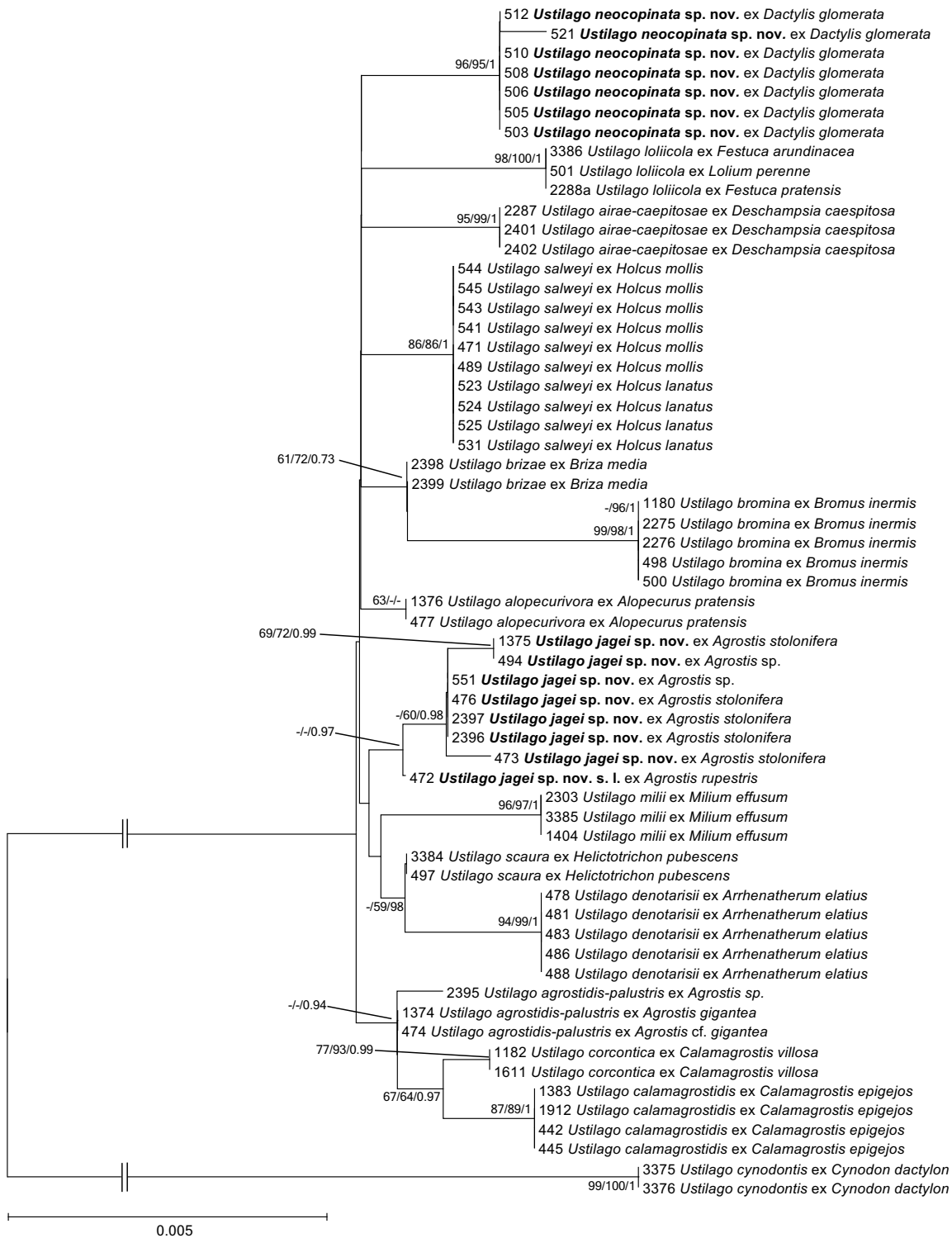


Fig. 4. Phylogenetic tree based on Minimum Evolution analysis of three loci (ITS, *ssc1*, *atp2*) detailed showing the *Ustilago striiformis*-complex with the outgroup *U. cynodontis*. Numbers on branches denote support in Minimum Evolution, Maximum Likelihood and Bayesian Analyses, in the respective order. Values below 55 % are denoted by ‘-’. The bar indicates the number of substitutions per site.

TAXONOMY

Based on our phylogenetic analyses, the following nomenclature and taxonomic changes are proposed for leaf stripe smuts caused by species of *Ustilago*. The positions given for the diagnostic bases refer to specific positions in the alignments as highlighted in the alignment consensus sequences in Fig. 4. Only selected synonyms are given

here. For a complete synonymy reference should be made to Vánky (2012) and references therein.

***Ustilago agrostidis-palustris* W. H. Davis ex Ciferri, *Ann. Mycol.* 29: 54 (1931).**

Type: USA: Wisconsin: Madison, on cultivated ‘redtop’ (i.e. *Agrostis* “*palustris* Huds.”, now *Agrostis gigantea*),

8 July 1921, W. H. & J. J. Davis (BPI 166994 – **lectotype designated here**, MBT 380628).

Confirmed host: *Agrostis gigantea*.

Confirmed distribution: Germany and USA.

Notes: *Ustilago agrostidis-palustris* can be distinguished from other leaf stripe smuts of the *U. striiformis* species complex based on its host specific occurrence on *Agrostis gigantea* s. lat. Furthermore, it differs in one diagnostic base from all other species of the *U. striiformis*-complex included in this study – in the *sdh1* gene there is a C instead of a T at position 138 (Table 2, Fig. 6).

Ustilago airae-caespitosae (Lindr.) Liro, *Ann. Acad. Sci. Fenn.*, ser. A **17** (1): 71 (1924).

Basionym: *Tilletia airae-caespitosae* Lindr., *Acta Soc. Fauna Flora Fenn.* **26**:15 (1904).

Type: Finland: Nyland: Helsingfors, Hagasund, on Aira caespitosa (i.e. *Deschampsia caespitosa*), 10 Aug. 1902, J. I. Lindroth [Vestergren, *Micr. Rar. Sel.* no. 806; Sydow, *Ustil.* no. 316] (M-0236198 – **lectotype designated here**, MBT 380628; from one of the several duplicate collections treated as “lectotype” by Lindeberg, *Symb. Bot. Upsal.* **16** (2): 135, 1959).

Confirmed host: *Deschampsia caespitosa*.

Confirmed distribution: Austria and Finland.

Notes: Within the *Ustilago striiformis* species complex, *U. airae-caespitosae* can be distinguished from other species based on the host-specific occurrence on *Deschampsia caespitosa*. Furthermore, it differs in six diagnostic bases from all other species within the *U. striiformis* species complex included in this study – in the *atp2* gene it has an A instead of a G at position 22 and 94, in the *map* gene there is a T instead of a C at position 227, in the *myosin* gene there is an A instead of a G at position 133, in the *rp3* gene a T instead of a C at position 199, and an A instead of a G at position 576 in the ITS region (Table 2, Fig. 6).

Ustilago alopecurivora (Ule) Liro, *Ann. Acad. Sci. fenn.*, ser. A **17** (1): 72 (1924).

Basionym: *Tilletia alopecurivora* Ule, *Hedwigia* **25**: 113 (1886).

Synonyms: *Uredo longissima* var. *megalospora* Riess, in Rabenhorst, *Herb. Viv. Myc.* no. 1897 (1854).

Ustilago megalospora (Riess) Cif., *Nuovo Giorn. Bot. Ital.* **40**: 261 (1933).

Type: Germany: Bavaria: Coburg, Hofgarten, on *Alopecurus pratensis*, June 1879, E. Ule (B – holotype lost); *Berlin:* Charlottenburg-Nord, Kolonie Königsdamm, slope of ditch, 9 Aug. 1988, H. Scholz (B 70 0014985 – **neotype designated here**, MBT 380629).

Confirmed host: *Alopecurus pratensis*.

Confirmed distribution: Germany.

Notes: Within the *U. striiformis* species complex, *U. alopecurivora* can be distinguished from other species based on the host-specific occurrence on *Alopecurus pratensis*. Furthermore, *U. alopecurivora* differs in three diagnostic bases from all other species within the *U. striiformis* species complex included in this study – in the *atp2* gene, there is an A instead of a G at position 358, in the *map* gene there is a G instead of a T at position 192, and in the *myosin* gene there is a T instead of a C at position 83 (Table 2, Fig. 6).

Ustilago brizae (Ule) Liro, *Ann. Acad. Sci. Fenn.*, Ser. A **17** (1): 74 (1924).

Basionym: *Tilletia brizae* Ule, *Verh. Bot. Ver. Prov. Brandenb.* **25**: 214 (1884).

Type: Germany: Bavaria: Coburg, Rögner Berg, on *Briza media*, July 1879, E. Ule [Rabenhorst, *Fungi Eur.* no. 3604] (M-0147750 – **lectotype designated here**, MBT 380630; from one of the several duplicate collections treated as “lectotype” by Lindeberg, *Symb. Bot. Upsal.* **16**(2): 135, 1959).

Confirmed hosts: *Briza media*.

Confirmed distribution: Austria and Germany.

Notes: Within the *U. striiformis* species complex, *U. brizae* can be distinguished from other species based on the host-specific occurrence on *Briza media*. Furthermore, *U. brizae* differs in one diagnostic base from all other species within the *U. striiformis* species complex included in this study, except *U. bromina* on *Bromus inermis*, in having a C instead of a T at position 621 in the ITS region, and differs from *U. bromina* by having an A instead of a G at position 223 in the ITS region (Table 2, Fig. 6).

Ustilago corcontica (Bubák) Liro, *Ann. Acad. Sci. Fenn.*, Ser. A **17** (1): 383 (1924).

Basionym: *Tilletia corcontica* Bubák, *Houby Ceské, Hemibasidii* **2**: 47 (1912).

Type: Czech Republic: on the crest of Riesengebirge Mts, on *Calamagrostis halleriana* (i.e. *C. villosa*), 20 July 1872, J. Gerhardt (BPI 172761 – **lectotype designated here**, MBT 380631; one of the “isolectotypes” of Lindeberg, *Symb. Bot. Upsal.* **16**(2): 114, 1959).

Confirmed host: *Calamagrostis villosa*.

Confirmed distribution: Czech Republic and Germany.

Notes: Within the *U. striiformis* species complex, *U. corcontica* can be distinguished from other species based on the host-specific occurrence on *Calamagrostis villosa*. Furthermore, *U. corcontica* differs in one diagnostic bases from all other species within the *U. striiformis* species complex included in this study – in the *atp2* gene there is an T instead of a C at position 535 (Table 2, Fig. 6).



Ustilago denotarisii A. A. Fischer v. Waldheim, *Aperçu Syst. Ustil.*: 22 (1877); as “de Notarisii”.

Type: Italy: on *Arrhenatherum* spp. (not located but could also not be confirmed as lost; a neotype may need to be designated for this species in the future).

Confirmed hosts: *Arrhenatherum* species.

Confirmed distribution: Germany and Italy.

Notes: Spores globose to ovoid, standard range (9.0–)10.5–(av. 11.2)–12.0 (–13.5) × (8.0–)9.0–(av. 9.7)–10.5(–12.0) μm, length/breadth ratio of 1.10–(av. 1.20)–1.38, olive-brown, and finely echinulate. Within the *U. striiformis* species complex, *U. denotarisii* can be distinguished from other species based on the host-specific occurrence on *Arrhenatherum* species. Furthermore, *U. denotarisii* differs in two diagnostic bases from all other species within the *U. striiformis* species complex included in this study – in the *atp2* gene there is an A instead of a G at position 346, and in the gene *ssc1* there is an A instead of a C at position 182 (Table 2, Fig. 6).

Ustilago echinata J. Schröt., *Abh. Schles. Ges. Vaterl. Kult., Abth. Naturwiss.*: 48: 4 (1870 [“1869”]).

Type: Poland: Silesia: ‘Schwarzwasserbruch’, near Legnica, on *Phalaris arundinacea*, June 1869, W. G. Schneider [Rabenhorst, *Fungi Eur.* no. 1497] (FR – **lectotype designated here**, MBT 380632; one of the several duplicate collections previously treated as “lectotype” in Rabenhorst, *Fungi Eur.* No. 1497).

Reported hosts: *Glyceria grandis*, *Phalaris arundinacea*, and *Scolochloa festucacea*.

Confirmed host: *Phalaris arundinacea*.

Known distribution: Asia, North America, and Europe.

Notes: This species shares one sequence motif (AACCCAAC) at positions 20–27 in the ITS region with other coarsely ornamented stripe smuts (*U. serpens* clade in Fig. 1), and many SNPs which distinguish *U. echinata* from species of the *U. striiformis*-complex. Within the *U. serpens*-complex, *U. echinata* can be distinguished from other species based on its host-specific occurrence on *Phalaris arundinacea* (type host). Whether the other hosts of a similar ecotype are infected by the same species could not be clarified in the current study, but the high degree of host specificity observed in *Ustilago* renders it possible that specimens from other host genera will have to be described as new species. Furthermore, *U. echinata* differs

in eight diagnostic bases from all other species within the *U. serpens* species complex included in this study – in the *atp2* gene there is a G instead of an A at position 85, in the *map* gene there is an A instead of a G at position 208, in the *myosin* gene there is a C instead of an A at position 141 and a T instead of a C at position 156, in the *rp13* gene there is a T instead of a C at position 91 and an A instead of a G at position 146, in the *sdh1* gene there is an A instead of a G and at positions 58 and 256, and in the ITS locus there is a C instead of an A at position 19, a C instead of a T at position 38, an A instead of a gap at position 186 and 596 and a G instead of an A at positions 188 and 604 (Tab. 2, Fig. 6).

Due to the generally narrow host specificity of smut fungi, it is conceivable that *U. echinata* will be revealed to be a species group.

Ustilago jagei J. Kruse & Thines, **sp. nov.**

Mycobank MB819627

(Fig. 5A–B)

Etymology: Named after mycologist Horst Jage from Kemberg (Germany), who has made significant contributions to the knowledge of phytopathogenic fungi and has enabled well-sampled phylogenetic investigations in various plant pathogens by his outstanding collections.

Diagnosis: Within the *U. striiformis* species complex, *U. jagei* can be distinguished from other species based on its host-specific occurrence on *Agrostis stolonifera* s. lat. Furthermore, *U. jagei* differs in two diagnostic bases from all other species within the *U. striiformis* species complex included in this study – in the *atp2* gene there is an A instead of a G at position 466 and in the gene *rp13* there is an A instead of a G at position 92 (Table 2, Fig. 6).

Type: Germany: Saxony-Anhalt: Dessau, Kühnauer See, southern shore east-southeast of Großkuhnau, wayside, on *Agrostis stolonifera*, 16 Sept. 2001, H. Jage (GLM-F047379 – holotype).

Description: Sori as long narrow streaks parallel to vascular bundles, mostly in the leaves, rarely ascending into the inflorescence, initially covered by the epidermis of the plants, which soon frays. Spore mass dark brown to almost black, powdery. Infection systemic, infected plants usually sterile. Spores globose to ovoid, (9.5–) 10.0–(av. 10.9) –11.5(–13.5) × (7.5–) 8.5–(av. 9.3)–10.0(–11.5) μm, length/breadth ratio 1.04–(av. 1.24)–1.5, olive-brown, finely echinulate (Table 3, Figs 3–4).

Confirmed hosts: *Agrostis rupestris* and *A. stolonifera*.

Fig. 5. Sori and spores of *Ustilago jagei* (A–B), *U. denotarisii* (C–D), *U. neocopinata* (E–F), *U. salweyi* (G–H), *U. kummeri* (I–J), and *U. serpens* s. str. (K–L). **A.** Sori of *U. jagei* on *Agrostis stolonifera* (GLM-F047379); **B.** Teliospores seen by LM; **C.** Sori of *U. denotarisii* on *Arrhenatherum elatius* (GLM-F105836); **D.** Teliospores seen by LM; **E.** Sori of *U. neocopinata* on *Dactylis glomerata* (GLM-F107413); **F.** Teliospores seen by LM; **G.** Sori of *U. salweyi* on *Holcus lanatus* (GLM-F107417); **H.** Teliospores seen by LM; **I.** Sori of *U. kummeri* on *Bromus inermis* (GLM-F107435); **J.** Teliospores seen by LM; **K.** Sori of *U. serpens* s. str. on *Elymus repens* (GLM-F105827); and **L.** Teliospores seen by LM.

Table 2. Diagnostic bases within the *Ustilago striiformis* and the *Ustilago serpens* complexes.

	Gen Loci																		
	<i>atp2</i>		<i>map</i>		<i>ssc1</i>		<i>myosin</i>		<i>rpl4A</i>		<i>rpl3</i>		<i>sdh1</i>		<i>tif2</i>		ITS		
	Pos.	Base	Pos.	Base	Pos.	Base	Pos.	Base	Pos.	Base	Pos.	Base	Pos.	Base	Pos.	Base	Pos.	Base	
<i>U. striiformis</i>-complex																			
on <i>Agrostis gigantea</i>	x	x	x	x	x	x	x	x	x	x	x	x	138	C / T	x	x	x	x	
on <i>Agrostis stolonifera</i> and <i>A. rupestris</i>	466	A / G	x	x	x	x	x	x	x	x	92	A / G	x	x	x	x	x	x	
on <i>Alopecurus pratensis</i>	358	A / G	192	G / T	x	x	83	T / C	x	x	x	x	x	x	x	x	x	x	
on <i>Arrhenatherum elatius</i>	346	A / G	x	x	182	A / C	x	x	x	x	x	x	x	x	x	x	x	x	
on <i>Bromus inermis</i>	191, 244	G / A	x	x	232	C / T	x	x	228, 311 292	A / G C / T	x	x	x	x	23	A / G	621	C / T	
on <i>Calamagrostis epigejos</i>	91	A / G	x	x	x	x	x	x	x	x	x	x	x	x	65	T / C	102	T / C	
on <i>Calamagrostis villosa</i>	535	T / C	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
on <i>Dactylis glomerata</i>	x	x	x	x	69, 198	A / G	x	x	120	T / C	40	A / G	x	x	x	x	617	A / G	
on <i>Deschampsia caespitosa</i>	22, 94	A / G	227	T / C	x	x	133	A / G	x	x	199	T / C	x	x	x	x	576	A / G	
on <i>Festuca</i> spp. and <i>Lolium</i> spp.	x	x	x	x	210, 214, 231 243	A / G T / C	x	x	x	x	x	x	x	x	x	x	x	x	
on <i>Holcus</i> spp.	x	x	x	x	x	x	x	x	85	T / C	133	T / C	x	x	x	x	103	A / G	
on <i>Milium effusum</i>	301	A / G	x	x	x	x	x	x	x	x	x	x	x	x	x	x	206	T / C	
<i>U. serpens</i>-complex																			
on <i>Bromus inermis</i>	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x	x	260 629	C / T G / A	
on <i>Calamagrostis epigejos</i>	175 181, 429, 496 352	G / A T / C A / G	92 203	A / C C / T	88, 99 93 255	C / G G / A G / T	70 225	A / G T / C	7 91 187	C / G T / C G / T		28	G / T	13 25 100	G / A G / T A / G	x x	x x	215 522 597 606	C / T T / - A / T A / G
on <i>Elymus repens</i>	x	x	x	x	x	x	93	A / G	232	T / C	x	x	x	x	x	x	157 170	A / G C / T	
on <i>Phalaris arundinacea</i>	85	G / A	208	A / G	x	x	141 156	C / A T / C	x	x	91 146	T / C A / G	58, 256	A / G	x	x	38 186, 596 188, 604	C / A C / T A / - G / A	

Slash (/) = instead of, x = no diagnostic bases.

atp2 - 595bp

CTTVGAGTCVAGRGGGTCTBA **C**R**G**CRGGGTARATACCRAGC TCGGCRATACCACGVGAHAR BACRGTGGTGGCGTCCAAGT GKG**C****R**AAGGT**R**G**T**R**G**CGGGR GCAGGRTCMGTC AAGTCATC GCGGGGCACRTARACGGCCT GBACSGARGTRATGGARCCC
 TTCTTGGTGGTRGT**R**ATKCG **Y**TCCTGCATG**R**SACCCATGT CRGTRGCDAGGGTRGGCTGR TAWCKACRGCBCGARGGRAT **A**CG**R**CCRAGMARRGCSGAGG TCTCGGAACCRGCYTGGGTG AARCGGAAAATRTTGTCTRAT **R**AARAGAAGCACRTCTGTDC
 CCTCYTCGTACGGAAGTAC TCRG**C****R**ATGGT**R**AKACC**V**GT VAGRGCRACRGGGCRGGG CWCCRGGRGCTCGTTCATC TGACCGAAVACRAGVGCCKAC CTTGGAKT**Y**RCCBTYVAGGT TGATRACACCRGTCTCTRATC ATY**T****C****R**TGGTARAGATCRTT
 RCCCTCACGRGTDC**Y**TCAC CGACACCRGTGAARACVAG TAACCACCGTRRG**C****Y**TTRGC RACGTTGTTRATSAGYTCYT GRATRAGYACVGTCTTDCCG ACMCCRCRCCACCR

ITS 643bp

HCGTYGCGTCSRGCYHNN**V** MYNNNYBHYNHBNY**C**YCC GAAGYGGYAYANNKTATCA AAACCCGGCAGGNWVGMNNN NNNNVGRVNRNRANNNN **W**B**R**AGCTTTCGTCGGTCTNTY KCNNCYDTHVAANNNNNNR CTMATSYWTTTCRAGR**R**AGC
 CAYGRYDMAYGGC**N**NN**N**NNN NNNNNNVVMYCCTCAVTAC CRAK**C****Y**RYCVDCC**Y****Y**YHNNN NNNNNNNNNNNNNNNNNN NRARRRRNNNNBBK**C**GY**Y** CGAAACDATTGCGGGCCCTC AAACMGGCATGCTCCCAGA TTARATCTGCMGGGAGCGCA
 AGGTGCGTTCAAGATTCGA TGATTCTCTTCTGCAATTCA CATTACTTATCGCAATTGCG TGGCTTCTTATCGAKGGGA RAACCAAGAGATCCGTTGCC AAAAGTTVNTTTKNNNNNW TTAGACGACCCYATTACCAG YCGNNNNNNNNNNNNNAW
 RAYNNNNNNNNNNNTYHHA ATCCTANNTSWTCNAAAARK **K****N**KTNDDDDNNNNNNN BRYMNRBYNNBYBBHNNN NNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNN NNN**N**NNNNNNNNNN**N**CD**B** **B**DNYBN**Y****R**NRNCYRMCCSR
 TGC

sscl 264bp

CCRTCVCGRGCRGTVGARAA SACYTGCCTTCTGGTDG GGATVGTGGTRTRCGGTTR ATVAGRC**G****V**TGAAVACACC RCCVAG**N**GTCT**G**RATACC**S**A GVGARAGVGGVGTTRACR**T**CG AGSARVARAA**Y**RTCGGTRAC CTGRCCGGAVAGVACACCAC
 CCTGRATSGARGCRCCRATR **G****M**VACRGCCTCR**T**CKGG**R**TT RACRCCCT**T****R**CT**R**GG**T**CR**C** GCTTGAARAT**R****Y**CTTGACV **G****T****Y**TCRAGVACCT**K**GGCAT DCGG

map 251bp

TYCGHKCCGARATCAARGCN CACMTCAARAATGTBCAGCA RGAYACBGGAARCTYGCCA ACTCGGTGMMMAAGGAGCGY GARATGTBCAC**M**AARWYGAT YACCGARCTYGCYGHGTCCA TCACYTTSCTCAARAAYACB CCYATGAGYGTACBGCBCG
 HGARGAYCCYTACGCGYCCA AYCAREBMSG**C****K**WYCGYCAR **C****T****Y**CAGC**R**CCAGGTCAAYGA GGARAA**Y**GCYCTBCARAART CSATYATCATC

myosin 257bp

CTCTTRTTCTGRTTDDGGCTT GATKGTDCRATRTAVGAHG GCTGNGCNYKCATVARVTTY TCNACHAR**V**CR**T**TKGCRCT NG**H**YTTKATAC**G**R**T**CRCCVG CRGTRGGVGGVCKCTTYTTR YTRTYRGGRT**Y****N**R**G**DCGDTC **N**GGRAARAGYTTY**G****V**ARRT
 ASRAGTTDKBRCTY**G**ARTCR ACVARRTCVARRATRTCYTT RAGVAGYGMRTCYTTRTTBT TRTY**B**GTCATDCCYTSRACR TTGTACATGACR**T**CRCC

rp14A 415bp

SCTNKM**N**GARRTHCCBYTSG TYRTBKCBGAYSMBGCGYAG RRYYSACSAARACCAAGGA GGCYR**T**YRCBCTBC**T**CAAGK CBVT**Y**AAYGC**Y**TWCRMSGAY GTYRYAAGGTSTCSA**A**CT**Y** GCGCAAGRTYGCYGCNGGYG TKGYYAAGMTSCGCAACCGY
 CGCCAYACSCAGCGHCGYGG TCCYCT**K**G**T**BATY**T**ACAACM AGGAYCGCGYATYGTAAAG GCBTT**C****R****Y**AA**H**G**T**BCCYGG YG**T**KGAGCTSTGCTCNGYYG ASCGYCTSAACMTSCTCCAG CTYGCYCCYGG**H**GGACAYMT BGGYCGHTTY**R**TCATYTTCA
 CBSAGTCYGCYTTYGGCCCGC CTCGACRAGGTSTTYGCYGC CAAGWMBRGYTT**C**AYBCTNC CYAAGGCAAGATYGCY**A**AC ACBAYGTBACSCGC

rp13 218bp

CANYGGYGCYTSSATCT**S**BC GHGAGCT**B**GAGCGCAT**A**A**R** AAGTACTGCACYGTBGTBCG TSTBCTYGCYCACACYCAGR TBCGCAAGAC**B**R**G**YCTSAAG CAGAAGAAGKCRCA**Y**CTBAT GGAGRTBCARAT**Y**AACGGWG GYTC**B****R**TYGYGAVAARGTC
 GACTTYGCSAAGGAGCACTT YGAGAAGACBT**T**Y**G**MBGT**Y**A AGTCVRTBT**T**Y**G**AGSASA

sdh1 269bp

RTCYTGAVAGRG**R**AGVCCRG CWCG**B**GARACCATRGCCATR CCRTCACCVGTGCAVGT**R**TG VGCVGARGTGGCVGAGAAGT ARGCACGDCCRTADCC**R**CC**D** GTGGCVAGVACRG**T**CT**T****R**TG RGCRCGGAADCGGTGRAYKG TRCCTCTCCATRTTTRAGD
 GCRGYVACACCGACRCAYTC GCCRTCYTCCATGATVARGT CRAGVGCRAARTACTCGATR AARAAGT**K**G**T**TRTGKCG RAGCGACTGCCRTAVAGSG TGTGMAGCA

tif2 244bp

TGTCGTARACACGRCCRGV **G****T****R**CCRACRACGACCTGRGC RCCBCTRTTGAGCTTRGCCA TG**T****C****Y**TCRCGRACG**T**TRGTR CCRCRATGCARGCGTGGCA GTYRAYCTTATGTAGTCRC CRAGNGCRATRACGACCTTY TGRATCTGCTGRGCVAGCTC
 RCGRGTGGVGC**R**AGGATVA GVGCTYGRACVGC**T**KGATS YYRGGTCRATDCGCTGVAR RATGGCRATSAGAA**G**TRG CBGT

Fig. 6. Alignment consensus sequences for the alignments used in this study with positions of diagnostic bases highlighted in bold face.

Confirmed distribution: Germany and Switzerland.

Notes: It seems possible that *U. jagei* on *Agrostis stolonifera* s. lat. represents a species complex, and further investigations with more specimens and additional gene loci are needed to clarify this situation.

Ustilago kummeri J. Kruse & Thines, **sp. nov.**

Mycobank MB819628

(Fig. 5I–J)

Etymology: Named after the mycologist Volker Kummer from Potsdam (Germany), who has made significant contributions to the knowledge of phytopathogenic fungi and has enabled well-sampled phylogenetic investigations in various plant pathogens by his outstanding ability to recognise easily overlooked plant pathogens.

Diagnosis: Differs from species of the *U. striiformis* species complex in the larger spores and taller warts. Furthermore, *U. kummeri* shares one sequence motif at positions 20–27 (AACCCAAC) with other coarsely ornamented stripe smuts, and many SNPs distinguishing it from species of the *U. striiformis* species complex. Within the *U. serpens*-complex, *U. kummeri* can be distinguished from other species based on the host-specific occurrence on *Bromus inermis*. Furthermore, *U. kummeri* differs in two diagnostic bases from *U. serpens* on *Elymus repens* – in the ITS region there is an C instead of a G at position 260 and G instead of an A at position 629 (Table 2, Fig. 6).

Type: Germany: Brandenburg: Middlemark, Uetz: Hinterer Werder, southwest corner between Sacrow-Paretzer-Channel und Havel-Channel, on *Bromus inermis*, 19 June 2010, V. Kummer (GLM-F107435 – holotype; VK 2577/17 – isotype).

Description: Sori as long, narrow streaks parallel to vascular bundles, mostly in the leaves, rarely ascending to the inflorescence, initially covered by the epidermis of the plants, which soon frays. Spore mass dark brown, powdery. Infection systemic, infected plants mostly sterile. Spores ovoid to globose, (11.0–) 12.0– (av. 13.0) –14.0 (–15.5) × (9.0–) 10.5– (av. 11.5) –12.0 (–13.5), length/breadth ratio 1.04– (av. 1.15) –1.41, olive-brown, coarsely verrucose to echinulate (Table 3, Figs 5–6).

Confirmed host: *Bromus inermis*.

Confirmed distribution: Germany.

Notes: It seems likely that additional species will be discovered in the *U. serpens* clade once more stripe-smuts with coarse spore ornamentation will be scrutinised.

Ustilago loliicola Ciferri, *Fl. Ital. Crypt., Par. I. Fungi, Fasc. 17:* 345 (1938).

Type: Germany: Berlin: Berlin-Weissensee, on *Lolium perenne*, Sept. 1877, E. Ule [Rabenhorst, Fungi Eur. no.

2491] (FR – **lectotype designated here**, MBT 380633; from one of the several duplicate collections treated as “lectotype” by Lindeberg, *Symb. Bot. Upsal.* **16** (2): 136, 1959).

Confirmed hosts: *Festuca arundinacea* s. lat. and *Lolium perenne*.

Confirmed distribution: Germany.

Notes: Within the *U. striiformis* species complex, *U. loliicola* can be distinguished from other species based on the specific occurrence on the closely related hosts *Festuca arundinacea* s. lat. and *Lolium perenne*. Furthermore, *U. loliicola* differs in four diagnostic bases from all other species within the *U. striiformis* species complex included in this study – in the *ssc1* locus there is an A instead of a G at positions 210, 214 and 231, and a T instead of a C at position 243 (Table 2, Fig. 6).

Ustilago milii (Fuckel) Liro, *Ann. Acad. Sci. Fenn., ser. A 17* (1): 78 (1924).

Basionym: *Tilletia milii* Fuckel, *Jb. nassau. Ver. Naturk.* **23–24:** 40 (1870).

Type: Germany: Hesse: Rabenkopf Mt., near Oestrich, on *Milium effusum*, L. Fuckel [Fungi Rhenani no. 2410] (FR – **lectotype designated here**, MBT 380634, from one of the several duplicate collections treated as “lectotype” in Fuckel, Fungi Rhenani no. 2410).

Confirmed host: *Milium effusum*.

Confirmed distribution: Germany.

Notes: Within the *U. striiformis* species complex, *U. milii* can be distinguished from other species based on the host-specific occurrence on *Milium effusum*. Furthermore, *U. milii* differs in two diagnostic bases from all other species within the *U. striiformis* species complex included in this study – in the *atp2* gene there is an A instead of a G at position 301, and in the ITS there is a T instead of a C at position 206 (Table 2, Fig. 6).

Ustilago neocopinata J. Kruse & Thines, **sp. nov.**

Mycobank MB819630

(Fig. 5E–F)

Etymology: Highlights the unexpected finding that there are several distinct and host-specific species within the *U. striiformis* species complex.

Diagnosis: Within the *U. striiformis* species complex, *U. neocopinata* can be distinguished from other species based on the host-specific occurrence on *Dactylis glomerata*. Furthermore, *U. neocopinata* differs in five diagnostic bases from all other species within the *U. striiformis* species complex included in this study – in the *ssc1* gene there is an A instead of a G at positions 69 and 198, in the *rpl4A* gene there is a T instead of a C at position 120, in the *rpl3* gene there is an A instead of a G at position 40, and in the ITS region there is an A instead of a G at position 617 (Table 2, Figs 5–6).

Table 3. Measurements from 100 teliospores for four different species of the *Ustilago striiformis*-complex on *Agrostis stolonifera*, *Dactylis glomerata*, *Arrhenatherum elatius*, and *Holcus lanatus*, as well as two species of the *Ustilago serpens*-complex on *Elymus repens* and *Bromus inermis*.

No.	<i>Ustilago striiformis</i> -complex									<i>Ustilago serpens</i> -complex										
	<i>U. jagei</i> sp. nov. on <i>Agrostis stolonifera</i>			<i>U. denotarisii</i> on <i>Arrhenatherum elatius</i>			<i>U. neocopinata</i> sp. nov. on <i>Dactylis glomerata</i>			<i>U. salveii</i> on <i>Holcus lanatus</i>			<i>U. serpens</i> on <i>Elymus repens</i>			<i>U. kummeri</i> sp. nov. on <i>Bromus inermis</i>				
	spores			spores			spores			spores			spores			spores				
length	width	l/b	length	width	l/b	length	width	l/b	length	width	l/b	length	width	l/b	length	width	l/b	length	width	l/b
1	10.5	9.5	1.11	10	9	1.11	11	10.5	1.05	11.5	10.5	1.1	10.5	1.05	14	13	1.08			
2	10	7.5	1.33	11.5	9	1.28	9.5	9.5	1	11.5	10	1.15	12	10	1.2	13.5	11	1.23		
3	10.5	8.5	1.24	9	8	1.13	11	10	1.1	10	9.5	1.05	12.5	10	1.25	14.5	12	1.21		
4	13.5	9.5	1.42	10	8	1.25	10.5	9.5	1.11	11	9.5	1.16	13	12	1.08	14	12.5	1.12		
5	11	9	1.22	10.5	8.5	1.24	10.5	10	1.05	12	9.5	1.26	12.5	10.5	1.19	14	12	1.17		
6	11	10	1.1	11.5	9	1.28	11	9.5	1.16	12	9	1.33	13	12.5	1.04	11.5	11.5	1		
7	9.5	8	1.19	10.5	9.5	1.11	10	8.5	1.18	11	9	1.22	12.5	11.5	1.09	14	12	1.17		
8	11	8	1.38	10.5	9.5	1.11	10.5	10	1.05	10.5	9	1.17	12.5	9.5	1.32	14	13.5	1.04		
9	10.5	10	1.05	11.5	10	1.15	10.5	10	1.05	10	10	1	13.5	11	1.23	13	12.5	1.04		
10	11.5	9	1.28	11.5	8.5	1.35	10.5	10.5	1	10.5	9.5	1.11	13	11	1.18	13.5	13.5	1		
11	11.5	10	1.15	11	8	1.38	11	10	1.1	10.5	9.5	1.11	14.5	13.5	1.07	13.5	11.5	1.17		
12	11.5	8	1.44	11	10	1.1	11	11	1	10.5	10	1.05	14.5	12	1.21	12.5	11	1.14		
13	12	8	1.5	10.5	9	1.17	12	10.5	1.14	12.5	9	1.39	15.5	11	1.41	13.5	12.5	1.08		
14	12	10.5	1.14	12	9	1.33	10	10	1	10	8	1.25	13	12.5	1.04	12	12	1		
15	10	8.5	1.18	10.5	9	1.17	10.5	10.5	1	11	10	1.1	12.5	12	1.04	13.5	12.5	1.08		
16	12	11.5	1.04	10.5	9.5	1.11	10.5	9.5	1.11	10.5	9.5	1.11	13	12.5	1.04	12	11.5	1.04		
17	11	8	1.38	12	9	1.33	10	9	1.11	10	9	1.11	12	11.5	1.04	13.5	13	1.04		
18	11	9.5	1.16	12.5	10.5	1.19	10	9.5	1.05	11.5	9.5	1.21	13	10.5	1.24	14.5	13	1.12		
19	11	9	1.22	10	9	1.11	10.5	9.5	1.11	10	9	1.11	13	11.5	1.13	13	11.5	1.13		
20	12	9.5	1.26	12.5	11	1.14	11	10.5	1.05	10.5	9.5	1.11	13	12	1.08	13.5	13	1.04		
21	11	9.5	1.16	12.5	11.5	1.09	10.5	10	1.05	10.5	9	1.17	12.5	11	1.14	14.5	12	1.21		
22	13	9.5	1.37	13.5	12	1.13	11.5	10.5	1.1	10	8.5	1.18	12	11.5	1.04	13.5	12.5	1.08		
23	12.5	10	1.25	13.5	10	1.35	11	11	1	11.5	9	1.28	13	11.5	1.13	13	12.5	1.04		
24	11.5	10	1.15	11.5	10.5	1.1	10	9.5	1.05	10	9.5	1.05	13.5	12	1.13	12.5	12	1.04		
25	10.5	8.5	1.24	11.5	9.5	1.21	11	10.5	1.05	11	9.5	1.16	13	10.5	1.24	15	13.5	1.11		
26	10.5	10	1.05	12.5	11	1.14	11	10.5	1.05	9.5	9	1.06	12	10.5	1.14	13	11.5	1.13		
27	11	9	1.22	12.5	11.5	1.09	11	10	1.1	10.5	9	1.17	12.5	12	1.04	13.5	13.5	1		
28	10.5	10.5	1	11	10.5	1.05	10.5	9	1.17	10	9.5	1.05	13	12	1.08	13.5	11.5	1.17		
29	11	9.5	1.16	11	11	1	11	11	1	10	9	1.11	14	12.5	1.12	13.5	11.5	1.17		
30	10.5	7.5	1.4	11	9.5	1.16	10	10	1	10	9	1.11	12	11	1.09	13	12.5	1.04		
31	10.5	9	1.17	11	9	1.22	10	9.5	1.05	10	9	1.11	12.5	11.5	1.09	13	11	1.18		
32	10	8.5	1.18	11.5	10.5	1.1	10	9.5	1.05	11	10	1.1	14.5	12.5	1.16	14	13	1.08		
33	10.5	9.5	1.11	11	8.5	1.29	10.5	10.5	1	10	9	1.11	13	11.5	1.13	14	13	1.08		
34	10.5	9.5	1.11	11.5	9	1.28	10.5	10.5	1	11	8.5	1.29	14	12.5	1.12	14	13	1.08		
35	11.5	10	1.15	12.5	9.5	1.32	11	10	1.1	11	10	1.1	12	11.5	1.04	12.5	12	1.04		
36	12	9	1.33	10.5	8.5	1.24	10.5	9.5	1.11	10	9	1.11	14.5	11.5	1.26	13	11	1.18		
37	11	9.5	1.16	12.5	10.5	1.19	11	10.5	1.05	10	8.5	1.18	12.5	11.5	1.09	15	13.5	1.11		
38	10.5	9	1.17	11	9.5	1.16	10.5	10	1.05	10.5	9.5	1.11	14	12	1.17	14	13	1.08		
39	9.5	9	1.06	10.5	10	1.05	11	10	1.1	10.5	10.5	1	13	10	1.3	14	13.5	1.04		
40	10	8.5	1.18	12.5	10.5	1.19	10.5	9.5	1.11	10	9	1.11	11.5	11	1.05	13	13	1		
41	10.5	9.5	1.11	11	9.5	1.16	10	9.5	1.05	10	9	1.11	13.5	10.5	1.29	14.5	12.5	1.16		
42	11.5	10.5	1.1	11.5	11	1.05	10	10	1	10.5	10	1.05	12.5	9.5	1.32	13	12	1.08		
43	11	10.5	1.05	10	10	1	10.5	9.5	1.11	10.5	9.5	1.11	13.5	11	1.23	13.5	11.5	1.17		
44	10	9	1.11	11	9.5	1.16	10.5	10	1.05	10	10	1	14	12	1.17	14	11.5	1.22		
45	10.5	8.5	1.24	11.5	10	1.15	10.5	9.5	1.11	12	10	1.2	13.5	10.5	1.29	13	11.5	1.13		
46	10.5	8	1.31	11.5	11.5	1	10.5	9.5	1.11	10.5	10	1.05	14	12	1.17	13.5	12	1.13		
47	12.5	10.5	1.19	11	10.5	1.05	9.5	8.5	1.12	9.5	9.5	1	12	11.5	1.04	12.5	11.5	1.09		
48	11	9.5	1.16	10.5	10	1.05	10	10	1	11.5	10	1.15	13.5	12	1.13	13.5	11.5	1.17		
49	11	9.5	1.16	11.5	9.5	1.21	10.5	9.5	1.11	10.5	10	1.05	13	11.5	1.13	13	12	1.08		
50	10	9.5	1.05	11.5	9.5	1.21	11	10.5	1.05	10.5	8	1.31	13.5	12	1.13	13	12.5	1.04		
51	10	9.5	1.05	10	8.5	1.18	10	9	1.11	11	9	1.22	14	11.5	1.22	12.5	10.5	1.19		
52	10.5	8.5	1.24	10.5	8	1.31	10	11	0.91	10.5	10.5	1	13.5	10.5	1.29	14.5	12	1.21		

Table 3. (Continued).

<i>Ustilago striiformis</i> -complex										<i>Ustilago serpens</i> -complex								
<i>U. jagei</i> sp. nov.			<i>U. denotarisii</i>			<i>U. neocopinata</i> sp. nov.			<i>U. salweyi</i>			<i>U. serpens</i>			<i>U. kummeri</i> sp. nov.			
on <i>Agrostis stolonifera</i>			on <i>Arrhenatherum elatius</i>			on <i>Dactylis glomerata</i>			on <i>Holcus lanatus</i>			on <i>Elymus repens</i>			on <i>Bromus inermis</i>			
No.	spores			spores			spores			spores			spores			spores		
	length	width	l/b	length	width	l/b	length	width	l/b	length	width	l/b	length	width	l/b	length	width	l/b
53	12	9.5	1.26	10	8.5	1.18	9	9	1	11.5	10	1.15	14	11.5	1.22	14	12	1.17
54	10.5	10.5	1	11	8.5	1.29	10	9.5	1.05	10.5	9	1.17	13	12	1.08	13.5	12	1.13
55	10.5	10.5	1	12	9.5	1.26	10	9.5	1.05	10	10	1	12	11	1.09	14	12	1.17
56	11	10	1.1	9.5	9	1.06	9.5	9.5	1	10.5	10.5	1	12	12	1	13	13	1
57	10.5	9	1.17	10	8.5	1.18	11.5	10.5	1.1	11	9.5	1.16	13	10.5	1.24	14	13	1.08
58	10	10	1	11.5	9.5	1.21	10	9	1.11	10.5	10	1.05	14.5	10.5	1.38	13	12.5	1.04
59	11	10	1.1	11	10	1.1	10	7.5	1.33	10.5	9.5	1.11	13	11.5	1.13	13.5	12	1.13
60	10.5	10.5	1	12	9.5	1.26	10	10	1	10	9.5	1.05	13	12	1.08	14.5	12	1.21
61	10.5	8.5	1.24	11	10	1.1	10.5	10	1.05	10.5	9.5	1.11	13.5	10	1.35	14.5	13	1.12
62	11.5	9	1.28	10.5	10	1.05	11	9	1.22	10.5	10	1.05	13	11.5	1.13	14.5	13	1.12
63	10.5	8.5	1.24	10.5	9.5	1.11	10.5	9.5	1.11	9.5	9.5	1	12.5	12	1.04	13.5	12	1.13
64	10.5	9.5	1.11	10.5	10	1.05	11	10.5	1.05	10	9.5	1.05	12.5	12	1.04	14	12.5	1.12
65	10	10	1	10	8.5	1.18	10.5	10.5	1	11	9	1.22	14.5	10.5	1.38	13	12.5	1.04
66	10.5	8.5	1.24	11.5	11	1.05	11	11	1	10.5	8.5	1.24	11.5	10.5	1.1	13	12.5	1.04
67	11	10.5	1.05	11	9.5	1.16	11.5	10	1.15	11	9.5	1.16	15	12	1.25	13.5	12.5	1.08
68	10.5	8.5	1.24	11	9.5	1.16	11	11	1	10.5	9.5	1.11	12.5	11	1.14	14	13	1.08
69	10	10	1	11.5	10.5	1.1	11.5	11	1.05	10	9	1.11	14	11	1.27	14.5	14	1.04
70	10	10	1	11.5	11	1.05	10	9.5	1.05	11	10	1.1	12	11	1.09	13.5	12.5	1.08
71	11	9	1.22	11	10	1.1	9	9	1	10.5	9	1.17	13	10.5	1.24	13	12.5	1.04
72	10	10	1	10.5	10	1.05	10	9.5	1.05	11	10.5	1.05	13	12	1.08	13.5	12.5	1.08
73	10	10	1	13	10	1.3	11	10	1.1	9.5	9	1.06	11.5	11.5	1	13.5	13	1.04
74	10.5	8	1.31	11	9	1.22	10.5	9.5	1.11	11	9.5	1.16	14	11	1.27	13.5	13	1.04
75	10	9.5	1.05	11.5	10	1.15	10.5	9.5	1.11	10.5	10.5	1	12	10.5	1.14	15.5	13.5	1.15
76	11.5	9	1.28	12	10.5	1.14	11	9.5	1.16	10.5	10	1.05	11.5	9	1.28	13	12.5	1.04
77	11	10	1.1	10.5	10	1.05	9	9	1	11.5	9.5	1.21	12.5	11	1.14	14	12.5	1.12
78	11.5	9.5	1.21	10.5	10	1.05	9.5	9.5	1	9.5	8.5	1.12	11	10.5	1.05	14.5	12	1.21
79	11	9	1.22	11.5	8.5	1.35	11	10	1.1	10	9	1.11	13	11	1.18	13.5	12	1.13
80	11.5	9.5	1.21	10.5	9.5	1.11	10	8.5	1.18	11	8.5	1.29	11.5	11	1.05	13.5	12.5	1.08
81	9.5	9.5	1	11	9.5	1.16	10.5	10	1.05	11.5	9	1.28	11.5	11	1.05	13.5	10.5	1.29
82	11	9.5	1.16	10.5	10	1.05	11	9.5	1.16	10.5	10	1.05	12.5	11	1.14	13	13	1
83	10.5	10.5	1	11.5	9	1.28	11.5	9.5	1.21	11	9.5	1.16	12.5	12	1.04	14.5	13	1.12
84	11.5	10	1.15	11	9	1.22	10	9.5	1.05	10	9.5	1.05	12	9.5	1.26	13.5	12.5	1.08
85	11	10	1.1	10	10	1	11	9.5	1.16	10	8	1.25	13.5	11	1.23	14	13	1.08
86	11	9	1.22	10.5	9.5	1.11	11.5	11	1.05	9.5	7.5	1.27	14	9.5	1.47	14	12	1.17
87	11	10.5	1.05	10.5	9	1.17	11.5	10.5	1.1	11	8.5	1.29	12.5	12	1.04	15	13.5	1.11
88	11	9	1.22	11	9.5	1.16	10	9	1.11	10	9	1.11	12	12	1	12.5	12.5	1
89	10	7.5	1.33	11.5	8.5	1.35	9.5	9	1.06	10	9.5	1.05	15	12.5	1.2	13.5	11.5	1.17
90	11	9.5	1.16	10	9.5	1.05	11	9.5	1.16	11	10	1.1	14.5	12	1.21	12	12	1
91	10.5	9	1.17	13.5	11	1.23	11	9.5	1.16	11	10.5	1.05	12	11.5	1.04	13.5	13	1.04
92	10.5	9	1.17	13.5	10.5	1.29	9.5	9.5	1	11.5	10	1.15	14	11	1.27	13	12	1.08
93	10	8.5	1.18	13	10.5	1.24	9.5	7.5	1.27	11.5	9.5	1.21	13.5	11	1.23	14	12.5	1.12
94	11	9.5	1.16	11.5	10.5	1.1	11	11	1	10.5	9.5	1.11	13	11	1.18	13.5	11.5	1.17
95	10.5	9	1.17	11	11	1	11	10	1.1	10.5	10	1.05	14.5	12	1.21	14.5	12	1.21
96	10.5	7.5	1.4	12	10.5	1.14	10.5	8.5	1.24	10.5	9.5	1.11	12.5	10.5	1.19	14.5	13	1.12
97	11.5	9.5	1.21	11	11	1	10.5	10	1.05	10.5	9.5	1.11	12.5	11.5	1.09	13	11	1.18
98	11.5	9.5	1.21	11	9	1.22	13	11	1.18	11	10	1.1	11.5	11	1.05	13.5	11.5	1.17
99	13.5	11.5	1.17	10.5	10	1.05	10	9	1.11	10.5	9.5	1.11	12.5	11.5	1.09	14.5	13.5	1.07
100	10.5	9.5	1.11	11	10	1.1	10	10	1	11	9.5	1.16	13.5	12.5	1.08	13.5	11.5	1.17

Type: **Germany:** *Bavaria:* Upper Franconia, Kronach county, Wallenfels, in the direction of the sewage treatment plant downstream of Stumpfenschneidmühle, on *Dactylis glomerata*, 15 July 2012, J. Kruse (GLM-F107413 – holotype).

Description: *Sori* as long small streaks parallel to vascular bundles, mostly in the leaves, very rarely ascending to the inflorescence, initially covered by the epidermis of the plants, which soon frays. *Spore mass* dark brown to almost black, powdery. Infection systemic, infected plants mostly sterile. *Spores* mostly globose, rarely ovoid, (9.0–) 10.0– (av. 10.5)–11.0 (–13.0) × (7.5–) 9.0– (av. 9.8) –10.5 (–11) µm, length/breadth ratio 1.00– (av. 1.07) –1.18, olive-brown, finely echinulate (Table 3, Figs 5–6).

Notes: As the host is widespread throughout the Holarctic region, it is conceivable that the species will prove to have a much wider distribution range than currently known.

Ustilago salweyi Berk. & Broome, *Ann. Mag. Nat. Hist.* **5**: 463 (1850).
(Fig. 5G–H)

Type: **UK:** *Channel Islands:* Guernsey, St Martin's, on *Holcus lanatus* [originally misidentified as *Dactylis glomerata* fide Hubbard, in Stevenson, *Plant Dis. Rep.* **30**: 57, 1946], 1847, *T. Salwey* (K-M – holotype; K-M00022071 – isotype).

Synonyms: *Uredo striiformis* Westend., *Bull. Acad. R. Sci. Belg., cl. sci.* **18**: 406 (1852); as “*striaeformis*”.

Uredo salveii (Berk. & Broome) Oudem., *Prodromus Florae Bataviae*, 2nd edn, **4**: 180 (1866).

Tilletia debaryana A.A. Fisch. Waldh., in Rabenhorst, *Fungi eur.* no. 1097 (1867).

Tilletia striiformis (Westend.) Magnus, *Malpighia* **1**: 8 (1875).

Ustilago striiformis (Westend.) Niessl, *Hedwigia* **15**: 1 (1876).

Tilletia salveii (Berk. & Broome) P. Karst., *Bidrag. Kännedom. Finlands Naurt. Folk.* **6**: 102 (1884).

Confirmed hosts: *Holcus lanatus* and *H. mollis*.

Confirmed distribution: Belgium, Germany, and UK.

Notes: Spores globose to ovoid, standard range (9.5–)10.0– (av. 10.6)–11.0 (–12.5) × (7.5–)9.0– (av. 9.4)–10.0 (–10.5) µm, finely echinulate, length/breadth ratio 1.00– (av. 1.15)–1.39. Within the *U. striiformis* species complex, *U. salweyi* can be distinguished from other species based on the host-specific occurrence on *Holcus lanatus* and *H. mollis*. Furthermore, *U. salweyi* differs in three diagnostic bases from all other species within the *striiformis* species complex included in this study – in the *rp14A* gene there is a T instead of a C at position 85, in *rp13* there is a T instead of a C at position 133, and in the ITS region there is an A instead of a G at positions 103 (Table 2, Fig. 6).

The original host was misidentified as *Dactylis glomerata*, but this was found to be incorrect and actually *Holcus lanatus* by the leading grass specialist C.E. Hubbard (in Stevenson 1946). David Hawksworth also studied the type materials in K-M and concurs. Hosts in their vegetative stage can be

misidentified, as some characteristics, such as leaf shape, ligula, and general habit can be modified as a consequence of infection.

Ustilago scaura Liro s. lat., *Ann. Acad. Sci. Fenn., ser. A*, **17**(1): 73 (1924).

Replaced name: *Tilletia avenae* Ule, *Verh. Bot. Vereins Prov. Brandenburg* **25**: 214 (1884).

Type: **Germany:** *Bavaria:* Coburg, Fortress, on *Avena pratensis* (i.e. *Helictotrichon pratense*), June 1879, E. Ule (s. n. – lost); *Hesse:* county Tann/Rhön, at Galgenmount, on *Avena pubescens* [now, *Helictotrichon pubescens*], 16 Sept. 1990, H. Scholz (B 70 0014830 – **neotype designated here**, MBT 380637).

Non *Ustilago avenae* (Pers.) Rostrup, *Overs. K. danske Vidensk. Selsk. Forh. Medlemmers Arbeider.* **13** (1890).

Confirmed host: *Helictotrichon pubescens*, *H. pratense*?

Confirmed distribution: Germany.

Notes: Within the *U. striiformis* species complex, *U. scaura* s. lat. can be distinguished from other species based on the host-specific occurrence on *Helictotrichon pratense* and *H. pubescens*. Furthermore, *U. scaura* s. lat. differs in one diagnostic base from all other species within the *U. salweyi* species complex included in this study, except *U. denotarisii* on *Arrhenatherum* spp., in having a T instead of a C at position 628 in the ITS region, and from *U. denotarisii* on *Arrhenatherum elatius* in having a 13 nucleotide deletion at positions 222–241 in the ITS alignment (Table 2, Fig. 6).

Since the type has been lost, we designate a neotype for *Ustilago scaura* with material on the closely related *H. pubescens*.

Ustilago scrobiculata Liro, *Ann. Acad. Sci. Fenn., ser. A* **17**(1): 68 (1924).

Type: **Finland:** *Nyland:* Pornainen, Kirveskoski, on *Calamagrostis arundinacea*, 9 Aug. 1916, T. Putkonen & J. I. Liro (H – lectotype, designated by Lindeberg, *Symb. Bot. Upsal.* **16** (2): 130 (1959).

Synonym: ? *Ustilago deyeuxiae* L. Guo, *Mycosystema* **6**: 51 (1993).

Reported hosts: *Calamagrostis* spp. (see Vánky 2012: 1265).

Reported distribution: Asia and Europe.

Notes: This species shares one sequence motif with other coarsely ornamented stripe smuts (AACCCAAC at positions 20–27), which distinguishes it from species of the *Ustilago striiformis* species complex, and many additional single SNPs. Within the *U. serpens* species complex, *U. scrobiculata* differs in 21 diagnostic bases from other species (Table 2, Fig. 6). It seems possible that *U. deyeuxiae* has not been sampled

on *Calamagrostis arundinacea*, as the host of *U. deyeuxiae* is given as “*Deyeuxia arundinacea*” by Guo (1993), which is often seen as a synonym of *D. pyramidalis* in Asian literature (e.g. Shenglian *et al.* 2006). Thus, it seems possible that the species needs to be reconsidered as independent from *U. scrobiculata* once sequence data from the type specimen become available.

Ustilago serpens (P. Karst.) B. Lindeb., *Symb. Bot. Upsal.* **16**(2): 133 (1959).

Basionym: *Tilletia serpens* P. Karst., *Fungi Fenn. Exs.*, fasc. **6**: no. 599 (1866).

Type: **Finland:** Merimasku, on “*Dactylis glomerata*” [re-determined as *Elymus repens* by Lindeberg, *Symb. Bot. Upsal.* **16**(2): 133, 1959], July 1862, P. Karsten [Fungi Fenn. Exs no. 599] (HUV 10432 – **lectotype designated here**; MBT 380638 from one of the several duplicate collections treated as “lectotype” by Lindeberg, *Symb. Bot. Upsal.* **16**(2): 133, 1959).

Confirmed host: *Elymus repens*.

Confirmed distribution: Finland and Germany.

Notes: The spores are small to medium sized, (11.5–) 13.0–(av. 13.5)–14.5 (–15.5) × (10.5–) 11.5 (av. 12.5)–13.0 (–14.0) µm, with a length/breadth ratio of 1.00–(av. 1.09)–1.23 and with coarsely verrucose ornamentation. This species shares one sequence motif with other coarsely ornamented stripe smuts (AACCCAAC at position 20–27), which distinguishes it from species of the *U. striiformis* species complex and many additional SNPs. Within the *U. serpens*-complex, *U. serpens* can be distinguished from other species based on four diagnostic bases: in the *myosin* gene there is an A instead of G at position 93, in *rpl4A* gene there is a T instead of a C at position 232 and in the ITS locus there is a C instead of a T at position 260, and a G instead of an A at position 629 (Table 2, Fig. 6).

Vánky (2012) lists several additional hosts for *U. serpens*. Due to the narrow specialization of stripe-smut revealed in this study, however, it seems likely that these harbour several distinct species. Until sequence data become available for these host-pathogen combinations, *Ustilago* on these other hosts is probably best referred to as *U. serpens* s. lat.

DISCUSSION

In this study, the closely related species of the *Ustilago striiformis*-complex and some other leaf stripe *Ustilago* smuts were investigated using multigene phylogenetic reconstructions to clarify their relationships. In total, 62 specimens of the *U. striiformis* species complex (incl. *U. calamagrostidis*) and four other leaf stripe smuts (*U. echinata*, *U. filiformis*, *U. scrobiculata*, and *U. serpens* s. lat.) were studied.

Phylogenetic analyses provided strong support for the polyphyly of the leaf-stripe smuts within *Ustilago*. However, the multilocus-based phylogenetic trees support the monophyly of the *U. striiformis* species complex, in contrast to the analysis

by Savchenko *et al.* (2014a), where it was concluded that the *U. striiformis* group was polyphyletic and the segregation of two species was necessary to render it monophyletic. That interpretation was mainly based on a combined LSU-ITS tree of *U. striiformis* species, where *U. bromina* and *U. nunavutica* were located outside the *U. striiformis* s. lat. clade. Because of this conflicting result, the ITS region of the type specimen of *U. bromina* was sequenced (Table 1) and compared with the deposited GenBank sequences of Savchenko *et al.* (2014a). The type specimen of *U. bromina* on *Bromus inermis* had an ITS sequence nearly identical (except for a base exchange in a poly A/T region) with the other specimens identified as this species in the current study. It differed in nine bases compared to the three sequences labelled as *U. bromina* in Savchenko *et al.* (2014a). It is conceivable that these specimens belong to another undescribed smut species (the three sequences were obtained from material from Israel and USA, while the type collection was from Germany), or the quality of the sequences was not optimal; almost all differences in the sequences from Savchenko *et al.* (2014a) in comparison to the sequences from this study were located behind a poly A/T site, which necessitated re-sequencing for several of the specimens used in this study. Furthermore, misidentification of the host plant seems also possible, as no records were found for the occurrence of *Bromus inermis* in the floras of Israel (<http://flora.org.il/en/plants/>) or Palestine (Feinbrun-Dothan 1986).

Ustilago nunavutica was the second species that led Savchenko *et al.* (2014a) to assume that the *U. striiformis* species complex was polyphyletic. Comparing the ITS and LSU sequences of *U. nunavutica* with sequences from the current study, the LSU sequence used by Savchenko *et al.* (2014a) showed several SNPs (data not shown), while all other *U. striiformis* samples investigated in this study were identical in the LSU region. In contrast, the ITS sequence of *U. nunavutica* has only few SNPs in comparison to other members of the *U. striiformis* species complex and is identical with *U. neocopinata*. It seems possible that the LSU sequence of *U. nunavutica* either was of bad quality or shows the amplification of a contaminant smut fungus. However, as the genera *Puccinellia* and *Dactylis* are not closely related (Schneider *et al.* 2009) and very high host specificity has been revealed for the closely related species of the *U. striiformis* species complex in this study, it is unlikely that *U. neocopinata* and *U. nunavutica* are conspecific.

In agreement with Stoll *et al.* (2005) and Spooner & Legon (2006), we found that *U. calamagrostidis* and *U. corcontica* belonged to the *U. striiformis* species complex. However, further resolution within the *U. striiformis* species complex was only achieved when the protein-coding loci introduced by Kruse *et al.* (2017b) were employed. The trees revealed a host genus or host species specific occurrence for almost all lineages within the *U. striiformis* species complex, thus they should be treated as distinct species, supported by the observations of Liro (1924). All specimens from a single host species formed a clade according to the host species (or the host genus, in case of *Holcus*), with the exception of the rather closely related species *Lolium perenne* and *Festuca arundinacea* (Malik & Thomas 1966, Catalán *et al.* 2004, Hand *et al.* 2010). As most of these clades received high to

maximum support, they should be considered to represent distinct species, which can be distinguished based on the host and diagnostic SNPs (Fig. 6). For most of the 14 lineages of the *U. striiformis* species complex validly published names are available, necessitating the description of only two new species in this complex, *U. neocopinata* on *Dactylis glomerata* and *U. jagei* on *Agrostis stolonifera* s. lat. Vánky (2012) and Savchenko *et al.* (2014a) mentioned that different species on different hosts within this complex vary remarkably in spore shape, size, and ornamentation. However, morphological variation was observed to be high even within the same host species in the current study and also by Vánky (2012). Thus it is difficult to distinguish these closely related species based on morphology, necessitating the consideration of hosts and SNPs for diagnosis. The host range of at least two species of *Ustilago* parasitic to *Agrostis* could not be inferred with certainty, as both ITS and chloroplast loci did not resolve closely related species in the *A. stolonifera* and *A. gigantea* clusters (Amundsen & Warnke 2012).

While investigating synonymies of the *U. striiformis* species complex, it was found that the name *U. salweyi* is the correct name for the stripe smut on *Holcus lanatus*. Stevenson (1946) flagged *U. salweyi* as a “*nomen ambiguum*”, although no action was taken to formally reject the name. Following the ICN (McNeill *et al.* 2012), the name *U. salweyi* has priority over *Uredo striiformis* as it was published two years earlier (Berkeley & Broome 1850: 463). Although the group generally referred to as the *U. striiformis*-group does not contain a species with that as the correct name, as it is still included as a synonym we feel that it is best to continue to use “*U. striiformis*-group” or “species complex” for these fungi as it is so well established and recalls the symptoms all species of the complex exhibit, although this feature is shared by some leaf-stripe smuts not belonging to this complex.

The species within the *U. striiformis* species complex have sometimes been recognised as special forms based on infection trials (Liro 1924, Davis 1930, 1935, Fischer 1940). However, it has been shown for various biotrophic pathogens that the special form concept, in which there is a population continuum with somewhat specialised forms, cannot be upheld (Göker *et al.* 2004, Lutz *et al.* 2005, Kemler *et al.* 2009, Thines *et al.* 2009, Ploch *et al.* 2011, Savchenko *et al.* 2014b, Choi & Thines 2015).

Similar to the situation in the *U. striiformis* species complex, *Ustilago serpens* s. lat. on different hosts clustered in phylogenetically distinct subgroups. As the type host for *U. serpens* is *Elymus repens*, the collections from *Bromus inermis* warrants recognition as a new species. *Ustilago serpens* is another example illustrating the narrow host specialization among smut fungi. As for both the coarsely ornamented stripe-smuts (*U. serpens* clade) and the finely ornamented stripe smuts (*U. striiformis* clade) only a subset of the known hosts could be included in the current study. It is therefore conceivable that some older names published for specific host-pathogen combinations in these groups warrant recognition and several new species await discovery.

With respect to the global phylogeny of *Ustilago* it is noteworthy that even based on nine loci the backbone of the phylogenetic tree was only poorly resolved. Conflicting supported topologies were inferred with respect to the

phylogenetic position of *U. maydis* in the reconstructions based on three (sister to a clade comprising, among others, the *U. nuda* and the *U. salweyi* clade) and nine loci (sister to a clade comprising the majority of smuts on panicoid grasses).

This highlights the high degree of uncertainty that there still is with respect to the global phylogeny of *Ustilago* s. lat. (Thines 2016). Considering the diversity of anatomical characteristics and disease syndromes caused, many of which have arisen several times independently (such as the stripe-smut habit; McTaggart *et al.* 2012a, b, c), any splitting of *Ustilago* s. lat. into smaller genera as suggested by McTaggart *et al.* (2012a, 2016) is probably premature and might become obsolete due to the high degree of parallel evolution and associated homoplasy.

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