

Molecular phylogeny of *Ustilago* and *Sporisorium* species (Basidiomycota, Ustilaginales) based on internal transcribed spacer (ITS) sequences¹

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Abstract: DNA sequence data from the internal transcribed spacer (ITS) region of the nuclear rDNA genes were used to determine a phylogenetic relationship between the graminicolous smut genera *Ustilago* and *Sporisorium* (Ustilaginales). Fifty-three members of both genera were analysed together with three related outgroup genera. Neighbor-joining and Bayesian inferences of phylogeny indicate the monophyly of a bipartite genus *Sporisorium* and the monophyly of a core *Ustilago* clade. Both methods confirm the recently published nomenclatural change of the cane smut *Ustilago scitaminea* to *Sporisorium scitamineum* and indicate a putative connection between *Ustilago maydis* and *Sporisorium*. Overall, the three clades resolved in our analyses are only weakly supported by morphological characters. Still, their preferences to parasitize certain subfamilies of Poaceae could be used to corroborate our results: all members of both *Sporisorium* groups occur exclusively on the grass subfamily Panicoideae. The core *Ustilago* group mainly infects the subfamilies Pooideae or Chloridoideae.

Key words: basidiomycete systematics, ITS, molecular phylogeny, Bayesian analysis, Ustilaginomycetes, smut fungi.

Résumé : Afin de déterminer la relation phylogénétique des genres *Ustilago* et *Sporisorium* (Ustilaginales), responsables du charbon chez les graminées, les auteurs ont utilisé les données de séquence de la région espaceur transcrit interne (ITS) des gènes nucléiques ADN. Ils ont analysé 53 membres de ces genres, ainsi que trois genres apparentés. Les liens avec les voisins et l'inférence bayésienne de la phylogénie indiquent la monophylie d'un genre *Sporisorium* bipartite et la monophylie d'un clade *Ustilago* central. Les deux méthodes confirment le changement de nomenclature récemment publié faisant passer le charbon du roseau d'*Ustilago scitaminea* à *Sporisorium scitamineum*, et indiquent un lien possible entre l'*Ustilago maydis* et le genre *Sporisorium*. Dans l'ensemble, les trois clades résolus dans ces analyses ne sont que faiblement supportés par des caractères morphologiques. Tout de même, leurs préférences comme parasites de certaines familles de Poaceae pourraient être utilisées pour corroborer les résultats obtenus : tous les membres des deux groupes de *Sporisorium* se retrouvent exclusivement dans la sous-famille Panicoideae. Le groupe central *Ustilago* infecte les sous-familles Pooideae ou Chloridoideae.

Mots clés : systématique des basidiomycètes, ITS, phylogénie moléculaire, analyse bayésienne, Ustilaginomycètes, champignon du charbon.

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Introduction

The basidiomycetous order Ustilaginales Clinton emend. R. Bauer et Oberwinkler (Bauer et al. 1997) comprises about 35 genera and more than 1000 species of phytopathogenic smut fungi (Bauer et al. 2001). *Ustilago* (Pers.) Roussel, its largest genus, encompasses approximately 350 species and can be characterized by single teliospores and sori lacking

peridium and columella (Vánky 1987). Because of the high frequency of these morphological traits, many smut species on various families of host plants have been erroneously included in this genus. However, according to Bauer et al. (2001), "true" *Ustilago* species occur exclusively on grasses (Poaceae), which has been shown by ultrastructural and molecular data.

The genus *Sporisorium* Ehrenb. ex Link was established by Ehrenberg (Link 1825) to accommodate a smut fungus on *Sorghum* spp. *Sporisorium* has not been used in literature until Langdon and Fullerton (1978) reinstated this genus, which includes, among others, all graminicolous smuts formerly cited as *Sorosporium*, *Sphacelotheca*, and *Thecaphora* (Vánky and Berbee 1988; Vánky 1998). Its morphological characteristics include teliospores in more or less persistent spore balls along with groups of sterile cells between the spores. The sorus usually consists of a teliospore mass permeated by one to several columellae, which are remnants of vascular bundles. The sorus is covered by a peridium, a membrane consisting of host tissue, which in some cases is

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interwoven with sterile fungal cells. To date, more than 300 species of *Sporisorium* are known.

Consistent delimitation of these two closely related genera proved to be very difficult, in particular because of intermediate morphological characters and character combinations (Vánky 1985, 1998). Supplementary ultrastructural research revealed no marked differences between *Ustilago* and *Sporisorium* species (Piepenbring et al. 1998b). Moreover, commonly used characters like the sorus structure are, to a great extent, dependent on the host's morphology or even determined by it (Savile 1954; Fullerton and Langdon 1968; Holton et al. 1968).

Molecular data contributed in many ways to a new system of Ustilaginales (e.g., Begerow et al. 1997; Bauer et al. 2001). One approach utilizing nuclear large subunit (LSU) ribosomal DNA (rDNA) sequences has not resolved the close relationship of *Ustilago* to *Sporisorium* in a satisfactory way (Piepenbring et al. 2002). Owing to the limited genetic variability of the LSU region, internal transcribed spacer (ITS) sequences appear to provide higher resolution at a species or subspecies level (Bruns 1991; Gardes and Bruns 1993). To date, ITS sequence data are available for only few economically important species on crops (Roux et al. 1998; Bakkeren et al. 2000), therefore providing only a limited understanding of the relationships within and between *Ustilago* and *Sporisorium*.

To resolve the phylogenetic relationship between *Ustilago* and *Sporisorium*, we sequenced the ITS region of 43 species of both genera, one *Sorosporium*, and three outgroup species and analysed the data together with nine sequences already published in GenBank.

Materials and methods

DNA was isolated from the sori of 47 herbarium specimens utilizing DNeasy™ Plant Mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol.

The ITS region was amplified utilizing the polymerase chain reaction (PCR) and the primers ITS 1 and ITS 4 (White et al. 1990). In some cases, we were able to increase PCR yields with a slightly modified primer M-ITS 1 (5'-GGTGAACCTGCAGATGGATC-3'). PCR products were purified using the QIAquick™ PCR purification kit (QIAGEN GmbH). This double-strand DNA was sequenced directly with the ABI PRISM™ Dye-Termination Cycle Sequencing kit (Applied Biosystems, Weiterstadt, Germany) on an automated sequencer (ABI 373A, Applied Biosystems). An alignment of 728 base pairs was created manually with Se-Al 2.0a7b (Rambaut 2001), of which 113 bp were excluded from the following analyses because of their tentative positional homologies.

PAUP* 4.0b10 (Swofford 2002) was used to construct a neighbor-joining (NJ) topology under the Kimura 2-parameter model. Bootstrap values were computed for 1000 replicates. Bayesian inference of phylogeny was performed using MrBayes 2.01 (Huelsenbeck et al. 2001). Four incrementally heated simultaneous Monte Carlo Markov chains (MCMC) were run over 1 000 000 generations. Trees were sampled every 100 generations leading to an overall sampling of 10 000 trees. Out of those trees that were sampled after the process had reached stationarity, a majority rule

consensus was calculated to obtain estimates for the a posteriori probabilities. This Bayesian approach was repeated four times with random-starting trees to assess reproducibility of the resulting topologies.

All phylograms were rooted with *Cintractia axicola* (Berk.) Cornu, *Farysia chardoniana* Zundel, and *Tolyposporium junci* (J. Schröt.) Woronin as outgroup species. The sequences have been deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>); the alignment has been deposited in TreeBase (<http://www.treebase.org/>).

Results

The phylogenetic trees presented here are based on a 615-bp ITS rDNA analysis of 56 members of the smut order Ustilaginales (see Table 1). One phylogram was obtained through Bayesian inference of phylogeny using MCMC (Fig. 1), the other via NJ analysis (Fig. 2).

Four runs of Bayesian phylogeny resulted in consistent topologies. Minor discrepancies appeared in one run, with respect to the weakly supported grouping around *Sporisorium destruens*, *Sporisorium catharticum*, and *Sporisorium cenchri*. The MCMC reached stationarity after the sampling of approximately 1000 trees. We therefore discarded the first 1000 trees and included the remaining 9000 trees in the majority rule consensus tree of each run.

Overall, the topologies of both phylograms corresponded; *Ustilago* and *Sporisorium* are part of a well-supported monophyletic ustilaginalean subgroup divided into three clades with adequate statistical support. The genus *Ustilago* appears to be paraphyletic in the MCMC dendrogram. NJ indicates its monophyly, lacking bootstrap support, though.

On the other hand, *Sporisorium* is evidently resolved as monophyletic by MCMC analysis, whereas NJ, though not supported by bootstrap, points to paraphyly. Furthermore, both genera are split into two groups designated here as *Ustilago* 1, "Ustilago 2", *Sporisorium* 1, and *Sporisorium* 2, respectively. Three out of these four subgeneric groups are clearly supported by a posteriori probability and bootstrap. No more than two *Sporisorium* species are assigned to the *Ustilago* clades, and clearly, *Sporisorium scitamineum* appears to be a true *Sporisorium*. The corn smut, *Ustilago maydis*, occupies a solitary position and cannot be included unequivocally within either assemblage. All *Sporisorium* species analysed here parasitize members of the grass subfamily Panicoideae. The species of *Ustilago* 1 occur primarily on pooid grasses, while those of "Ustilago 2" exhibit a broader host range.

Discussion

Suprageneric *Ustilago*–*Sporisorium* clade

Both ITS phylograms presented here illustrate the close relationship between *Ustilago* and *Sporisorium*. The monophyly of this group has never been seriously questioned (Vánky 1985, 1987; Bauer et al. 2001) and was further corroborated by LSU data (Begerow et al. 1997, 2000) and likewise by teliospore wall ultrastructure (Piepenbring et al. 1998a, 1998b, 1998c). Moreover, the comparatively short branch lengths in the NJ topology indicate a low molecular diversity, hence corroborating the close relationship between

Table 1. List of species studied.

Species	Host	GenBank accession No.	Source
<i>Cintractia axicola</i> (Berk.) Cornu	<i>Fimbristylis tetragona</i> R. Br.	AY344967	H.U.V. 17460
<i>Farysia chardoniana</i> Zundel	<i>Carex polystachya</i> Sw. ex Wahlenb.	AY344968	MP 2062
<i>Sorosporium tumefaciens</i> McAlpine ^a	<i>Chrysopogon aciculatus</i> (Retz.) Trin.	AY344969	Ust. Exs. 231 (M)
<i>Sporisorium aegypticum</i> (Fischer v. Waldh.) Vánky	<i>Schismus arabicus</i> Nees	AY344970	Ust. Exs. 756 (M)
<i>Sporisorium catharticum</i> (Maire) Vánky	<i>Pennisetum setaceum</i> (Forssk.) Chior.	AY344971	MP 2367
<i>Sporisorium cenchri</i> (Lagerh.) Vánky	<i>Cenchrus pilosus</i> Kunth	AY344972	MP 1974
<i>Sporisorium chrysopogonis</i> Vánky	<i>Chrysopogon fulvus</i> (Spreng.) Choiv.	AY344973	Ust. Exs. 407 (M)
<i>Sporisorium cruentum</i> (Kühn) Vánky	<i>Sorghum halepense</i> (L.) Pers.	AY344974	Ust. Exs. 687 ex H.U.P.
<i>Sporisorium culmiperdum</i> (J. Schröter) Vánky	<i>Andropogon gerardii</i> Vitman	AY344975	MP 2060
<i>Sporisorium destruens</i> (Schlecht.) Vánky	<i>Panicum miliaceum</i> L.	AY344976	Ust. Exs. 472 (M)
<i>Sporisorium destruens</i> (Schlecht.) Vánky	Host not cited, presumably <i>Panicum</i> sp. (Vánky 1994)	AF045871	Roux et al. 1998
<i>Sporisorium dimeriae-ornithopodae</i> Vánky & Menge	<i>Dimeria ornithopoda</i> Trin.	AY344977	Ust. Exs. 848 (M)
<i>Sporisorium fastigiatum</i> Vánky	<i>Andropogon angustatus</i> (Presl.) Steud.	AY344978	MP 1976
<i>Sporisorium formosanum</i> (Sawada) Vánky	<i>Panicum repens</i> L.	AY344979	Ust. Exs. 688 ex H.U.P.
<i>Sporisorium holwayi</i> (G.P. Clinton & Zundel) Vánky	<i>Andropogon bicornis</i> L.	AY344980	MP 1271
<i>Sporisorium lepturi</i> (Thüm.) Vánky	<i>Hemarthria uncinata</i> R. Br.	AY344981	Ust. Exs. 966 (M)
<i>Sporisorium mishrae</i> Vánky	<i>Apluda mutica</i> L.	AY344983	Ust. Exs. 967 (M)
<i>Sporisorium moniliferum</i> (Ell. & Ev.) Guo	<i>Heteropogon contortus</i> (L.) P. Beauv. ex Roem. et Schult.	AY344984	Ust. Exs. 851 (M)
<i>Sporisorium occidentale</i> (Seym. ex G.P. Clinton) Vánky & Snets.	<i>Andropogon gerardii</i> Vitman	AY344985	Ust. Exs. 758 (M)
<i>Sporisorium panici-leucophaei</i> (Bref.) M. Piepenbr.	<i>Digitaria insularis</i> (L.) Fedde	AY344986	MP 2461
<i>Sporisorium paspali-notati</i> (Henn.) M. Piepenbr. (cited as <i>Sporisorium microsporium</i>)	<i>Paspalum notatum</i> Fluegge	AY344982	MP 2101
<i>Sporisorium pollinae</i> (Magnus) Vánky	<i>Andropogon distachyos</i> L.	AY344987	Ust. Exs. 690 (M)
<i>Sporisorium provinciale</i> (Ell. & Galloway) Vánky & Snets.	<i>Andropogon gerardii</i> Vitman	AY344988	Ust. Exs. 759 (M)
<i>Sporisorium pseudechinolaenae</i> Vánky & C. Menge	<i>Pseudechinolaena polystachya</i> (Kunth) Stapf	AY344989	Ust. Exs. 853 (M)
<i>Sporisorium puellare</i> (Syd.) Deml	<i>Hyparrhenia hirta</i> (L.) Stapf	AY344990	MP 2364
<i>Sporisorium reilianum</i> (Kühn) Langdon & Fullerton	<i>Sorghum</i> sp.	AF135432	Bakkeren et al. 2000
<i>Sporisorium scitamineum</i> (Syd.) M. Piepenbr., M. Stoll & Oberw.	<i>Saccharum</i> sp. cultivar	AY345007	MP 2474
<i>Sporisorium scitamineum</i> (Syd.) M. Piepenbr., M. Stoll & Oberw.	<i>Saccharum</i> sp. cultivar	AF135433	Bakkeren et al. 2000
<i>Sporisorium sorghi</i> Ehrenb. ex Link	<i>Sorghum bicolor</i> (L.) Moench	AF038828	Roux et al. 1998
<i>Sporisorium themedae-arguentis</i> Vánky	<i>Themeda arguens</i> (L.) Hack.	AY344991	Ust. Exs. 855 (M)
<i>Sporisorium trachypogonicola</i> K. & C. Vánky	<i>Trachypogon plumosus</i> (H. & B. ex Willd.) Nees	AY344992	MP 2463
<i>Sporisorium veracruzianum</i> (Zundel & Dunlap) M. Piepenbr.	<i>Dichantherium viscidellum</i> (Scribn.) Gould	AY344993	MP 960
<i>Tolyposporium junci</i> (J. Schröt.) Woronin	<i>Juncus bufonius</i> L.	AY344994	H.U.V. 17169
<i>Ustilago affinis</i> Ell. & Everh.	<i>Stenotaphrum secundatum</i> (Walter) Kuntze	AY344995	G. Rivera s.n. ex H.U.P.
<i>Ustilago avenae</i> (Pers.) Rostrup	<i>Avena barbata</i> Pott ex Link	AY344997	MP 2362
<i>Ustilago avenae</i> (Pers.) Rostrup	<i>Avena sativa</i> L.	AY344996	F 946
<i>Ustilago bullata</i> Berk.	<i>Bromus diandrus</i> Roth	AY344998	MP 2363
<i>Ustilago bullata</i> Berk.	Host not cited, most probably a pooid grass genus (Vánky 1994)	AF135423	Bakkeren et al. 2000
<i>Ustilago crameri</i> Körn.	<i>Setaria italica</i> (L.) P. Beauv.	AY344999	Ust. Exs. 995 (M)
<i>Ustilago cynodontis</i> (Henn.) Henn.	<i>Cynodon dactylon</i> (L.) Pers.	AY345000	MP 1838

Table 1 (concluded).

Species	Host	GenBank accession No.	Source
<i>Ustilago cynodontis</i> (Henn.) Henn.	<i>Cynodon dactylon</i> (L.) Pers.	AF038825	Roux et al. 1998
<i>Ustilago echinata</i> J. Schröt.	<i>Phalaris arundinacea</i> L.	AY345001	Ust. Exs. 540 (M)
<i>Ustilago esculenta</i> Henn.	<i>Zizania latifolia</i> (Griseb.) Turcz. ex Stapf	AY345002	Ust. Exs. 590 (M)
<i>Ustilago hordei</i> (Pers.) Lagerh.	<i>Hordeum vulgare</i> L.	AY345003	Ust. Exs. 784 (M)
<i>Ustilago hordei</i> (Pers.) Lagerh.	<i>Hordeum vulgare</i> L.	AF105224	Willits and Sherwood 1999
<i>Ustilago maydis</i> (DC.) Corda	<i>Zea mays</i> L.	AY345004	RB s.n. (TUB)
<i>Ustilago maydis</i> (DC.) Corda	<i>Zea mays</i> L.	AF135431	Bakkeren et al. 2000
<i>Ustilago nuda</i> (Jens.) Rostrup	<i>Hordeum</i> sp.	AF135430	Bakkeren et al. 2000
<i>Ustilago pamirica</i> Golovin	<i>Bromus gracillimus</i> Bunge	AY345005	Ust. Exs. 789 (M)
<i>Ustilago schroeteriana</i> Henn.	<i>Paspalum paniculatum</i> L.	AY345006	Ust. Exs. 887 (M)
<i>Ustilago sparsa</i> L. Underw.	<i>Dactyloctenium aegyptium</i> (L.) P. Beauv.	AY345008	Ust. Exs. 892 (M)
<i>Ustilago trichophora</i> (Link) Körn.	<i>Echinochloa colona</i> (L.) Link	AY345009	MP 2473
<i>Ustilago tritici</i> (Pers.) Rostrup	<i>Triticum</i> sp. cultivar	AF135424	Bakkeren et al. 2000
<i>Ustilago turcomanica</i> Tranzschel ex Vánky	<i>Eremopyrum distans</i> (C. Koch) Nevski	AY345010	F 585 ex H.U.V. 23 (M)
<i>Ustilago vetiveriae</i> Padwick	<i>Vetiveria zizanioides</i> (L.) Nash	AY345011	H.U.V. 17954
<i>Ustilago xerochloae</i> Vánky & Shivas	<i>Xerochloa imberbis</i> R. Br.	AY345012	Ust. Exs. 1000 (M)

Note: H.U.V., Herbarium Ustilaginales Vánky; MP, Meike Piepenbring; Ust. Exs., Kálmán Vánky; Ustilaginales Exsiccata; H.U.P., Herbarium Ustilaginales Piepenbring; F, Franz Oberwinkler; RB, Robert Bauer. The abbreviations of herbaria are given in brackets: M, München, Germany; TUB, Tübingen, Germany.

^aNomenclatural change to *Sporisorium* has not been published yet.

the two genera. This close relationship also appeared in LSU analyses, in which many species were virtually indistinguishable at a molecular level, thus leading to insufficient bootstrap support (Piepenbring et al. 2002).

Ustilago

A monophyly of only *Ustilago* results from the NJ topology; however, this is only weakly supported. Apart from the already mentioned sorus and teliospore characters, no reliable and unique morphological characters could be used to uphold this hypothesis of monophyly, which is also contradicted by the Bayesian approach. As a result of our analyses, the genus itself could be divided into a core group around *Ustilago hordei* and a heterogeneous and paraphyletic assemblage of smut species with uncertain affiliation. These subgroups are distinguishable primarily by their host preferences as explained below.

Ustilago subgroup 1 (core *Ustilago*)

Because of high bootstrap values in NJ and probabilities in MCMC analyses, this cluster containing the type species *U. hordei* can be regarded with some certainty as monophyletic. The analysed species of this clade exhibit typical *Ustilago* characters, such as single teliospores and the lack of peridium and columella. The teliospores are usually quite small, smooth or covered with warts of one size, and flattened on one side because of the presence of a germ area.

A further separation of *Ustilago* 1 into two lineages is supported by either analysis. The first clade encloses pathogens on crops of temperate regions like barley, wheat, and oats, or pooid grasses in general. On the other hand, the species close to *Ustilago cynodontis* are found predominantly on chloridoid grasses.

Because of their economic importance (see Thomas 1989a), the taxonomy and systematics of crop-infesting *Ustilago* species have been the subject of numerous studies. The underlying species concept was primarily based on host specificity and teliospore morphology. *Ustilago pamirica* and *Ustilago bullata* usually occur on *Bromus* species, *Ustilago turcomanica* on *Eremopyrum*, and *Ustilago tritici* on wheat. *Ustilago pamirica* and *U. bullata* differ in teliospore ornamentation and symptomology; *U. turcomanica* and *U. bullata* are separable only by teliospore size (Vánky 1988, 1994). The present phylograms indicate a very close relationship among these four smuts. The host range of *U. tritici* extends to many species of the grass tribe Triticeae (Nielsen 1978a). Owing to similar morphology, *U. tritici* has often been synonymized with *Ustilago nuda*, a smut on *Hordeum* or *Agropyron* species (Langdon et al. 1976; Nielsen 1978a). Our topologies, together with chemotaxonomical data (Kim et al. 1983), point to a difference (though small) between *U. tritici* and *U. nuda*.

In our phylograms, *Ustilago avenae*, *U. hordei*, and *U. nuda* form another branch of *Ustilago* species on crops. *Ustilago avenae* and *U. hordei* differ in sorus (loose vs. covered smut) and teliospore morphology, whereas *U. avenae* and *U. nuda* can only be distinguished on the basis of germination and host preferences (Vánky 1994). A very short distance between all three can be read from the present topologies, indicating their close relationship.

Moreover, no differences at the molecular level can be determined among *U. avenae*, *U. bullata*, *U. hordei*, *U. nuda*, and *U. turcomanica* in LSU phylograms presented by Begeerow et al. (1997) and Piepenbring et al. (2002). These results are in accordance with Nannfeldt (1959) and Huang and Nielsen (1984), who both proposed a single species *Ustilago segetum* (Pers.) Roussel with two morphologically different varieties, *U. segetum* var. *avenae* (Pers.) Brun. and *U. sege-*

Fig 1. Topology resulting from a Bayesian Monte Carlo Markov chains (MCMC) analysis of 615 bp of internal transcribed spacer (ITS) rDNA of 56 members of Ustilaginales. Data shows a majority rule consensus tree of 9000 trees; numbers above branches indicate a posteriori probabilities. Duplicate sequences are marked with their respective collection numbers or GB for sequences obtained from GenBank. The systematic positions of the host genera following Watson and Dallwitz (1992 onwards) are indicated by the following symbols: ■, Panicoideae–Panicodae; □, Panicoideae–Andropogonodae; ★, Arundinoideae; ❖, Chloridoideae; +, Pooideae; °, Oryzoideae (Ehrhartoideae).

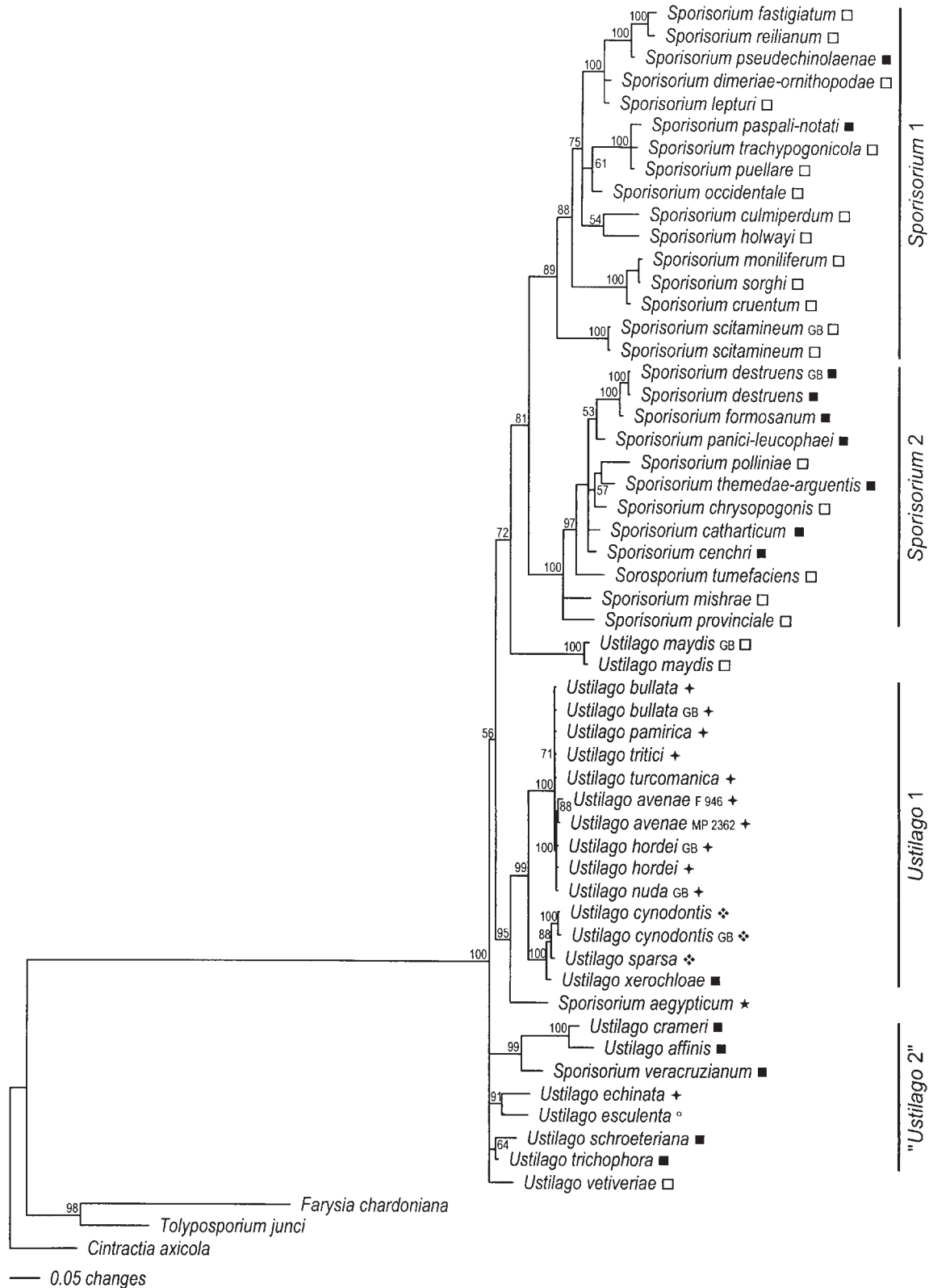
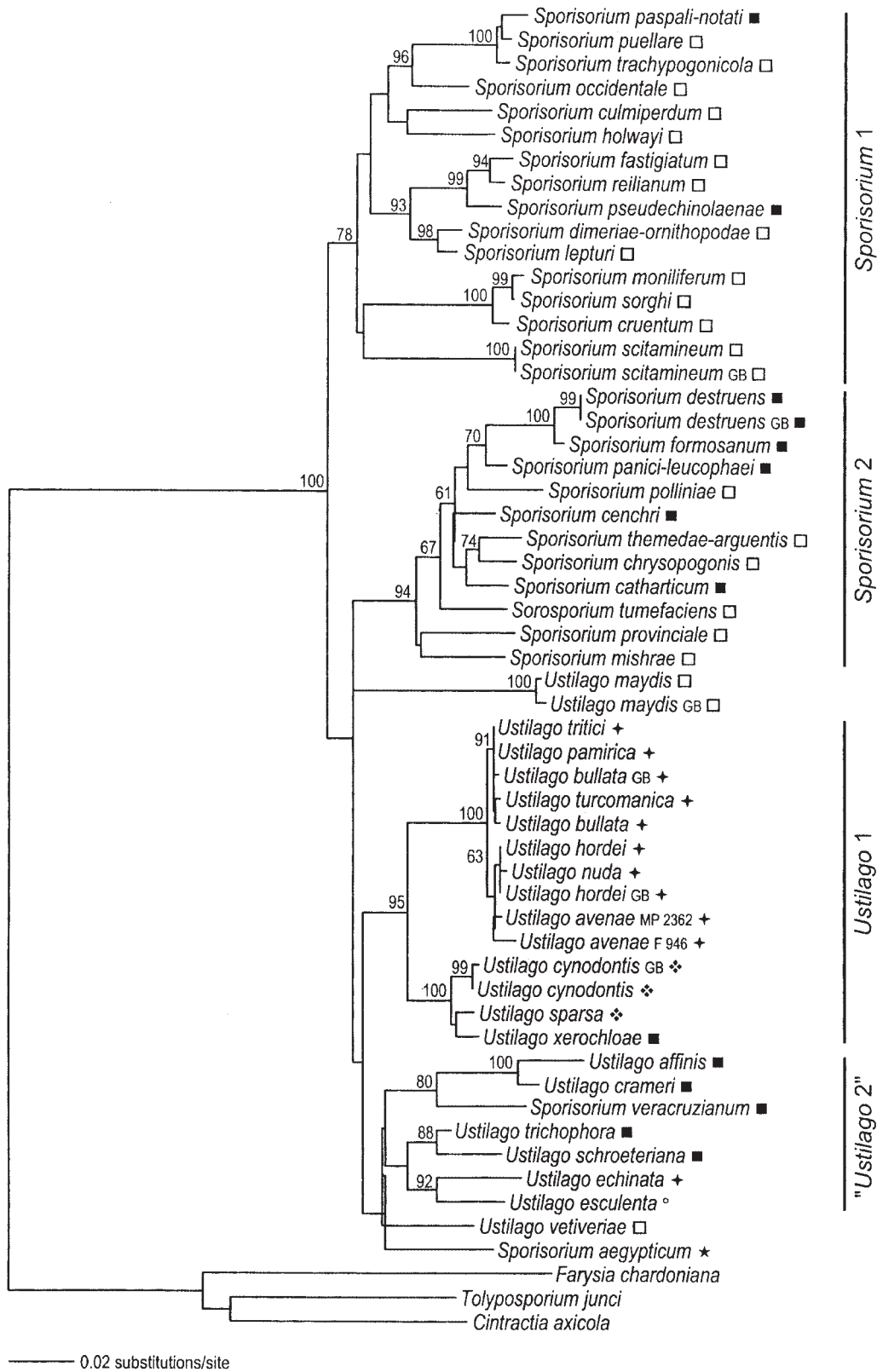


Fig. 2. Topology resulting from a neighbor-joining analysis of 615 bp of internal transcribed spacer (ITS) rDNA of 56 members of Ustilaginales. Bootstrap values (1000 replicates) greater than 60% are given above the branches. Duplicate sequences are marked with their respective collection numbers or GB for sequences obtained from GenBank. The systematic positions of the host genera following Watson and Dallwitz (1992 onwards) are indicated by the following symbols: ■, Panicoideae–Panicoideae; □, Panicoideae–Andropogonodae; ★, Arundinoideae; ❖, Chloridoideae; +, Pooideae; °, Oryzoideae (Ehrhartoideae).



tum var. *hordei* (Pers.) Rbh. With respect to the short molecular distances, the ability to hybridize (Nielsen 1968, 1978a; Thomas 1989b) and the common hosts (Nielsen 1978a, 1978b, 1993) of the studied species of *Ustilago* on crops may provide the justification to merge them into *U. segetum* despite their differences in morphology. This assemblage of putative biotypes or subspecies may have undergone a radiation on closely related host species. Further research utilizing highly variable genome regions such as the intergenic spacer (Fell et al. 2000) in conjunction with population or infection studies could address these issues, which at present cannot be answered by ITS.

Remaining species ("Ustilago 2")

The second *Ustilago* assemblage contains a variety of morphologically distinct species of *Ustilago* and *Sporisorium*. Its monophyly is neither supported by a posteriori probability nor by bootstrap values. The Bayesian analyses do not even resolve this group as a single clade; hence a connection to *Ustilago* 1 has to remain speculative. In contrast to the aforementioned subgroup, the analysed species of "Ustilago 2" mainly occur on panicoid grasses.

Ustilago echinata and *Ustilago esculenta* are resolved as a monophyletic group in both analyses. Unlike the vast majority of *Ustilago* and *Sporisorium* species, these two species parasitize wetland grasses: *U. esculenta* on *Zizania latifolia* and *U. echinata* on *Phalaris* sp., *Glyceria* sp., or *Scolochloa* sp. Both species infect their host's stems and leaves, leaving them sterile (Vánky 1987, 1994). Together with the present molecular data, this peculiar ecology and the similarity of their appearance indicate the evolution of a collateral *Ustilago* clade of parasites on wetland grasses.

The remaining species exhibit intermediate morphological characters. Sori of *Ustilago trichophora*, for example, contain a columella and are covered by a peridium (Fullerton and Langdon 1968; Piepenbring 2003), which are classical *Sporisorium* characters. The similar species *Sporisorium veracruzianum* is considered a good species of *Sporisorium* by Piepenbring (2003), but in view of our dendrograms, is of rather uncertain affiliation. These few examples underline the necessity of reliable morphological studies for both genera to support newly emerging phylogenetic hypotheses from molecular data. Whether or not parts of this *Ustilago* assemblage should be named *Yenia* Liou, as proposed by Piepenbring et al. (2002), cannot be answered from the present data.

Sporisorium

Regarding the monophyly of this genus, the performed analyses yield two contrary hypotheses. The high a posteriori probability from MCMC confirms the monophyly of *Sporisorium*, which is not the case in the NJ dendrogram. However, the apparent lack of bootstrap support for the hypothesis derived from NJ does not convincingly reject the MCMC topology. The identical manner in which *Sporisorium* is split into two well-supported subgroups could lead to its further division into subgenera. Nevertheless, this has to remain putative until reliable morphological traits in conjunction with extensive molecular studies support a monophyly of *Sporisorium*.

Sporisorium subgroup 1

The first *Sporisorium* subgroup containing the type species *Sporisorium sorghi* results from both analyses and may well represent a natural grouping. Apart from the position of *Sporisorium scitamineum*, its terminal clades are of almost identical composition. The members of this cluster show typical *Sporisorium* features like columella and peridium. All but two species (*Sporisorium paspali-notati* and *Sporisorium pseudechinolaenae*) parasitize andropogonoid grasses.

Interestingly, the economically important smut on sugarcane, *Ustilago scitaminea*, seems to be part of this *Sporisorium* clade as well. Indeed, *U. scitaminea* shows morphological traits typical of *Sporisorium* species (e.g., whip-like columella), as pointed out previously by Vánky (1991, 2000). Moreover, a recently published analysis of LSU and morphological data showed the affiliation of *U. scitaminea* with *Sporisorium* (Piepenbring et al. 2002). The new combination *S. scitamineum* proposed therein is verified by our ITS data.

Sporisorium cruentum, *Sporisorium moniliferum*, and *S. sorghi* represent a well-supported monophyletic group in both analyses. The close relationship among these species is visible in LSU phylogenies as well (Piepenbring et al. 2002). *Sporisorium cruentum* and *S. sorghi*, in particular, are similar with respect to their morphology and host preference (Vánky 1994). This could demonstrate determination of both parasites' soral morphology by the morphology of their common host *Sorghum*.

Although adequately supported by a posteriori probability and bootstrap, the remaining subdivisions of *Sporisorium* subgroup 1 show no morphological or ecological features that could supplement our molecular data. As an exception to this, the clade containing *Sporisorium lepturi* and *Sporisorium dimeriae-ornithopoda* differs from the other clades in the palaeotropical distribution of its hosts.

Sporisorium subgroup 2

The second *Sporisorium* clade is supported by both methods applied in our study. The internal structure of this presumably natural group is virtually identical in both phylogenies. Approximately 50% of this group's hosts are members of Panicodae, which is in contrast to the majority of the remaining *Sporisorium* species of our analysis. To date, no additional morphological or ecological characters are known to support this clade.

Sporisorium destruens and *Sporisorium formosanum* parasitize members of the grass genus *Panicum*, the latter only known on *Panicum repens*. These two species can only be distinguished by size and teliospore ornamentation. In particular, their sorus morphology is quite similar (Vánky 1994). The destruction of complete host inflorescences leads to the formation of long filiform columellae, which also occurs in the closely related species *Sporisorium paniculeucophaei* on *Digitaria* sp. Thus, *S. destruens* and *S. formosanum* provide another example for the determination of smut sorus structure by the host's morphology.

Sorosporium tumefaciens is part of *Sporisorium* subgroup 2 as well. Based on their spore-ball formation, many graminicolous smuts have been mistakenly included into *Sorosporium*. As demonstrated by Vánky and Berbee (1988), true *Sorosporium* or *Thecaphora* species occur exclusively on di-

cots, whereas all graminicolous *Sporisorium* species are undoubtedly members of the genus *Sporisorium* (Vánky 1985, 1998). Our data confirm this view, at least in the present case. Further systematic interpretation of subgroup 2 clades would be highly speculative, as to the above-mentioned lack of morphological traits.

Taxa of uncertain position (*U. maydis*, *Ustilago vetiveriae*, and *Sporisorium aegypticum*)

Ustilago maydis on *Zea mays* is one of the most conspicuous species of smut fungi. Its ability to induce a wide variety of soral galls in different host organs is unique among the Ustilaginales. The isolated systematic position of the corn smut is evident from a variety of morphological (Tulasne and Tulasne 1847; Brefeld 1883; Vánky 1994; Piepenbring et al. 2002), physiological (Bradford et al. 1975), ultrastructural (Ramberg and McLaughlin 1980), and molecular studies (Begerow et al. 1997; Bakkeren et al. 2000; Piepenbring et al. 2002). LSU analyses put *U. maydis* next to *S. scitamineum* (Piepenbring et al. 2002). In our phylogenetic hypotheses, it is placed either as a basal member of *Sporisorium* or as part of a *Ustilago*–*Sporisorium* clade (NJ). Both dendrograms point to an affiliation of the corn smut with *Sporisorium*; however this is yet to be confirmed with morphological and ongoing molecular research.

To date, no corn variety resistant to *U. maydis* has been found, in contrast to all other crops, where cultivars not susceptible to *Ustilago* smuts are known. This apparent ability to overcome the defence of *Zea mays* may indicate a special genetic composition of the corn smut. Whether the cultivation of corn for centuries leads to a special selection pressure on its parasite has to remain speculative, unless a close relative of *U. maydis* with comparable genetic background is found.

Ustilago vetiveriae and *S. aegypticum* exhibit similar uncertainties in relation to their position in our topologies. *Ustilago vetiveriae* has special hyaline cells among the teliospores, the only known character that separates this smut from other *Ustilago* species (Vánky 1997). The present phylograms indicate a basal position of *U. vetiveriae* to either the *Ustilago*–*Sporisorium* clade or to “*Ustilago 2*”. The basal position of *S. aegypticum* to *Ustilago* subgroup 1 (MCMC) may correlate with its occurrence on an arundinoid grass genus (*Schismus*), which is very uncommon for both smut genera in question.

Conclusion

Ustilago and *Sporisorium* form a complex of closely related ustilaginalean parasites on Poaceae. Three major lineages can be distinguished in our ITS phylograms:

- *Ustilago 1* (core *Ustilago*), a morphologically and ecologically homogeneous and monophyletic smut cluster on pooid grasses and crops of temperate regions or chloridoid grasses, respectively.
- *Sporisorium 1* and *Sporisorium 2*, two well-supported lineages, parts of a possibly monophyletic genus, occurring almost exclusively on (sub)tropical grasses belonging to Andropogonodae or Panicodae, and

- “*Ustilago 2*”, a heterogeneous and clearly paraphyletic assemblage of species with intermediate morphological traits, mainly on nonpooid grasses.

The taxonomic rank of the groupings presented here cannot be determined by our data, although rDNA data usually exhibit sufficient resolution on a generic level in Ustilaginales taxa (Piepenbring et al. 1999). Further studies using multiple genes or highly variable regions will have to deal with these uncertainties.

Nevertheless, facing a lack of reliable and host-independent morphological traits, molecular data can significantly contribute to resolving the putative phylogeny of both genera. A reproducible morphological matrix similar to the one presented by Piepenbring et al. (1999) for the smut species belonging to *Cintractia* s.l. might aid in obtaining a better understanding of the evolution of *Ustilago* and *Sporisorium*.

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