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Molecular phylogeny of *Ustilago*, *Sporisorium*, and related taxa based on combined analyses of rDNA sequences*

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Combined analyses of ITS and LSU rDNA sequences were utilized to resolve the phylogenetic relationships of 98 members of the smut genera *Lundquistia*, *Melanopsichium*, *Moesziomyces*, *Macalpinomyces*, *Sporisorium*, and *Ustilago* (*Basidiomycota*: *Ustilaginales*). Minimum Evolution and Bayesian inference of phylogeny resolve three major groups of almost identical composition: *Sporisorium*, *Ustilago*, and a basal assemblage of both *Ustilago* and *Sporisorium* species. *Macalpinomyces* deserves generic rank regarding its type species *M. eriachnes*; all other *Macalpinomyces* species of our study clearly turn out to be part of *Ustilago* or *Sporisorium*. *Lundquistia* evidently belongs to *Sporisorium*. *Moesziomyces*, probably paraphyletic, stands basal to all other genera. Interestingly, *Melanopsichium* belongs to the *Ustilago* clade, being the only member of the ingroup not parasitizing on *Poaceae*. The patchy distribution of commonly used morphological characters along our phylograms points to their variability and dependence on the host's morphological traits instead of being valuable for resolving parasite phylogeny. The new combination: *Sporisorium fascicularis* comb. nov. (syn. *Lundquistia fascicularis*) is made.

INTRODUCTION

The smut genera *Ustilago*, *Macalpinomyces*, and *Sporisorium* (*Basidiomycota*: *Ustilaginales*: *Ustilaginaceae*) exhibit a great diversity on grasses (*Poaceae*). More than 600 species are known to date (Piepenbring 2003) developing sori in their host's inflorescences, leaves, or stems. Generic circumscriptions have hitherto been based mainly on soral characters such as those of the peridium (membrane of fungal and host origin covering the young spore mass), columella (hypertrophied host axial tissue), sterile cells, or teliospore balls. Obviously, these characters are highly dependent on the anatomy and morphology of the host (Savile 1954, Holton, Hoffmann & Durán 1968, Langdon & Fullerton 1975) or turn out to be rather variable or convergent (Vánky 1998b). Furthermore, intermediate character combinations have made it difficult to unequivocally assign certain species to a particular genus (Vánky 1985, 1998b, Piepenbring 2003). Consistent delimitation of *Ustilago*, *Macalpinomyces*, and *Sporisorium* from each other, in consequence, has been rather problematical (Piepenbring 2003).

We carried out a molecular study based on ITS sequences of 53 *Ustilago* and *Sporisorium* species which could address some of these problems by showing monophyly of *Sporisorium*, polyphyly of *Ustilago*, and suggesting possible subdivisions of these two genera (Stoll *et al.* 2003). However, no member of the closely related genera *Macalpinomyces*, *Moesziomyces*, and *Lundquistia* had been incorporated in that study, nor had morphological data been discussed in detail.

We sequenced the ITS and the LSU region of 98 species belonging to seven *Ustilaginaceae* genera to further elucidate their relationships and to broaden the basis for solving phylogenetic questions in these fungi. Morphological data from the literature are also discussed in the light of our new topologies in order to assess their usability in phylogenetic studies.

MATERIALS AND METHODS

DNA was isolated from the sori of 109 dried specimens (Table 1) utilizing the DNeasy™ Plant Mini Kit (Qiagen, Hilden) according to the manufacturer's protocol.

The ITS region was amplified utilizing PCR and the primers M-ITS 1 (Stoll *et al.* 2003) and ITS 4 (White

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et al. 1990). The LSU region was amplified with primers NL 1 and NL 4 (O'Donnell 1993). PCR products were purified using the QIAquick™ PCR Purification Kit (Qiagen). The dsDNA was sequenced directly with the ABI PRISM™ Dye-Terminator Cycle Sequencing Kit (Applied Biosystems, Weiterstadt) on an automated sequencer (ABI 373A; Applied Biosystems).

An alignment of 1611 base pairs was created with an iterative aligning method using ClustalX (Thompson *et al.* 1997). In few cases, the resulting alignment was corrected manually in SeAl (Rambaut 2002). 185 positions of ITS which could not be aligned unequivocally were omitted from the following analyses. Cloning experiments conducted with Topo Cloning® (Invitrogen, Carlsbad, CA) for two species (*Ustilago crameri* and *Sporisorium hwangense*) according to the manufacturer's protocol revealed a polymorphic region of ITS approximately 100 bp downstream of LSU. These 39 bp were omitted from the phylogenetic analyses as well. The appropriate model of DNA substitution was estimated with Modeltest 3.06 (Posada & Crandall 1998) separately for ITS, LSU, and the combined dataset. In order to determine whether ITS and LSU contain congruent information, the distance matrices of either gene were compared with each other, performing significance tests with 10 000 permutations using CADM (Legendre & Lapointe 2004).

PAUP* 4.0b10 (Swofford 2002) was used to construct a Minimum Evolution (ME) topology under the maximum likelihood model chosen by Modeltest with a heuristic search with 1000 random additions and TBR branch swapping. Out of the trees saved, we calculated a strict consensus tree. The branch lengths were estimated with ML using the model chosen by Modeltest. 500 replicates of bootstrap were performed, using ten random additions and TBR for each replicate.

Bayesian inference of phylogeny was performed using MrBayes 3.0b4 (Ronquist & Huelsenbeck 2003). Four incrementally heated simultaneous Monte Carlo Markov chains (MCMC) were run over five million generations. Trees were sampled every 100 generations leading to an overall sampling of 50 000 trees. This approach was repeated four times with random starting trees. The four runs were examined with Tracer (Rambaut & Drummond 2003) to check their convergence and to choose the adequate 'burn-in'. 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.

To determine the appropriate outgroup, a second alignment (1404 bp) containing a selection of 26 species was used as basis for a neighbour-joining topology applying the above mentioned procedures.

The sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/), and the alignment has been deposited in TreeBASE (http://www.treebase.org/).

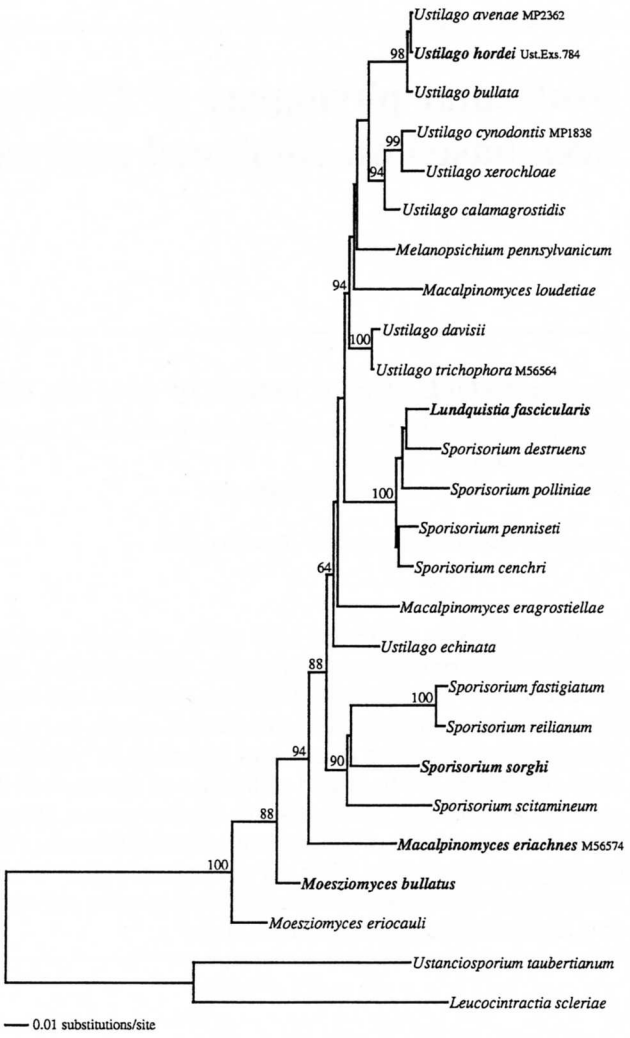

Fig. 1. Phylogram resulting from a neighbour-joining analysis (BioNJ) of 1404 bp of ITS and LSU rDNA sequences of 26 members of *Ustilaginales*. Bootstrap values (10 000 replicates) greater than 60% are given above the branches. Names of type species are printed in bold.

RESULTS

Congruence of distance matrices

Significance tests of ITS and LSU distance matrices with 10 000 permutations conducted with CADM (Legendre & Lapointe 2004) yielded an incongruence level of $p = 0.0001$. This value is well below a significance level of $\alpha = 0.001$ (0.1%), both matrices are congruent. Thus, ITS and LSU data were analysed together in a single alignment.

Outgroup selection

In order to verify the appropriate outgroup for subsequent analyses, a neighbour joining phylogram was constructed with 24 of the species in question together with *Ustanciosporium taubertianum and Leucocintractia scleriae*. In this phylogram, *Moesziomyces eriocauli* and *M. bullatus* are resolved as basal to the remaining species with bootstrap values of 88 and 94, respectively (Fig. 1).

Phylogenetic trees

The phylogenetic hypotheses are based on an analysis of 1387 base pairs of a combined alignment of ITS and LSU rDNA sequences. Altogether, 109 dried specimens belonging to 98 different species were sequenced.

One phylogenetic tree was obtained through Minimum Evolution (Fig. 2) and one from Bayesian inference of phylogeny using MCMC (Fig. 3). All methods of phylogenetic reconstruction yielded consistent results with only minor discrepancies (see below). The four runs of MCMC resulted in identical topologies as well. One run linked the group around *Ustilago davisii* to *Ustilago s. str.*, but with only a moderate support of 69, though. *Macalpinomyces eriachnes* is resolved as basal to the remaining ingroup species, which separate into three major clades. One clade contains a variety of *Ustilago* and *Sporisorium* species around *S. cordobensis* and *U. spermophora*. This clade is supported by ME and MCMC. A second clade around the type species *U. hordei*, in the following *Ustilago s. str.*, appears in all topologies with high support values, whereas the affiliation of a *Macalpinomyces* clade and *Melanopsichium* to it is only supported well by MCMC. Two small *Ustilago* groupings around *U. davisii* and *U. esculenta* are not resolved as part of a *Ustilago s. lat.* clade, but clearly do not belong to *Sporisorium* or the *Ustilago-Sporisorium*-clade. The *U. davisii* clade receives good support values by both methods applied. A clade including *U. esculenta*, *U. triodiae*, and relatives is only supported by MCMC. ME does not resolve its position.

Finally, the third clade, *Sporisorium*, is supported by MCMC but not by ME. Its division into two major groups ('*Sporisorium* 1' and '*Sporisorium* 2') and two small assemblages containing, among others, *U. maydis* and *S. bursum*, is resolved by all methods. The *U. maydis* group is supported well by ME and MCMC. One species, *S. consanguineum*, occupies changing positions in the topologies.

DISCUSSION

Outgroup selection

Leucocintractia scleriae and *Ustanciosporium taubertianum* belong to the family *Ustilaginaceae*, based on ultrastructural and molecular data (Bauer, Oberwinkler & Vánky 1997, Piepenbring, Begerow & Oberwinkler 1999, Bauer *et al.* 2001). Molecular evidence places both genera close to *Ustilago* and *Sporisorium* (Piepenbring *et al.* 1999). Consequently, it seems appropriate to choose members of these as outgroup species. Due to their large molecular distance in respect to *Ustilago* and *Sporisorium* (Fig. 1; Stoll *et al.* 2003), however, the *Leucocintractia* and *Ustanciosporium* species are only suitable as aids to determine the basal clades of the ingroup. In the NJ topology, both *Moesziomyces* species are placed basal with sufficient bootstrap support (Fig. 1), thus justifying their use as outgroup for subsequent phylogenetic analyses. Monophyly of *Moesziomyces* is not supported by our results, although *M. bullatus* and *M. eriocauli* share a very peculiar morphology with fragments of sterile cells adhering to the teliospores (Vánky 1986, 1998b).

Furthermore, the host range of *M. bullatus* is extraordinarily broad in comparison with its *Ustilago* and *Sporisorium* relatives (Vánky 1986, 1998b). If there is any evolutionary trend towards specialization in *Ustilago* and *Sporisorium* a comparatively unspecific relative would presumably occupy such a basal position.

Macalpinomyces

The separation of *Macalpinomyces* from other ustilaginacean genera proposed by Langdon & Fullerton (1977) is supported well by our data. The wide generic concept of *Macalpinomyces* that has been introduced and emended by Vánky (1995a, 1996a, 1997b) to accommodate smuts with intermediate *Ustilago* and *Sporisorium* characters, however, cannot be confirmed by any of our phylogenies. In fact, the *Macalpinomyces* species recognised by Vánky proved to be very difficult to separate from *Ustilago* and *Sporisorium* (Piepenbring 2003). Thus, *Macalpinomyces* is most probably monotypic, although the unusually large molecular distance between the specimen collected on *Eriachne aristidea* and one on *E. helmsii* may even point to two distinct species. Undoubtedly, all other *Macalpinomyces* species examined in our study are members of either *Ustilago* or *Sporisorium*.

Ustilago-Sporisorium *clade*

This well-supported clade, appearing in all topologies and in Stoll *et al.* (2003), contains a mixture of species placed in *Ustilago* and *Sporisorium*. Our methods do not support a connection to either *Ustilago* or *Sporisorium*, and verify its separate position as well as its monophyly. The members of this group exhibit combinations of *Ustilago* and *Sporisorium* characters: *S. neglectum*, for example, shows all characters typically assigned to *Sporisorium*. *U. crameri*, in contrast, is a typical *Ustilago* species while *U. ixophori* exhibits *Sporisorium* traits (Vánky 1994a, Piepenbring 2003). Almost all members of this clade parasitize on panicoid grasses, with the exception of *U. austro-africana* and *U. spermophora* which are found on chloridoid grasses. The clades around *U. syntherismae* and *U. affinis* even parasitize on members of a single grass genus, the first on *Digitaria*, the latter on *Setaria*. Whether or not *S. consanguineum* must be considered part of this clade, as shown by MCMC only, will be discussed below.

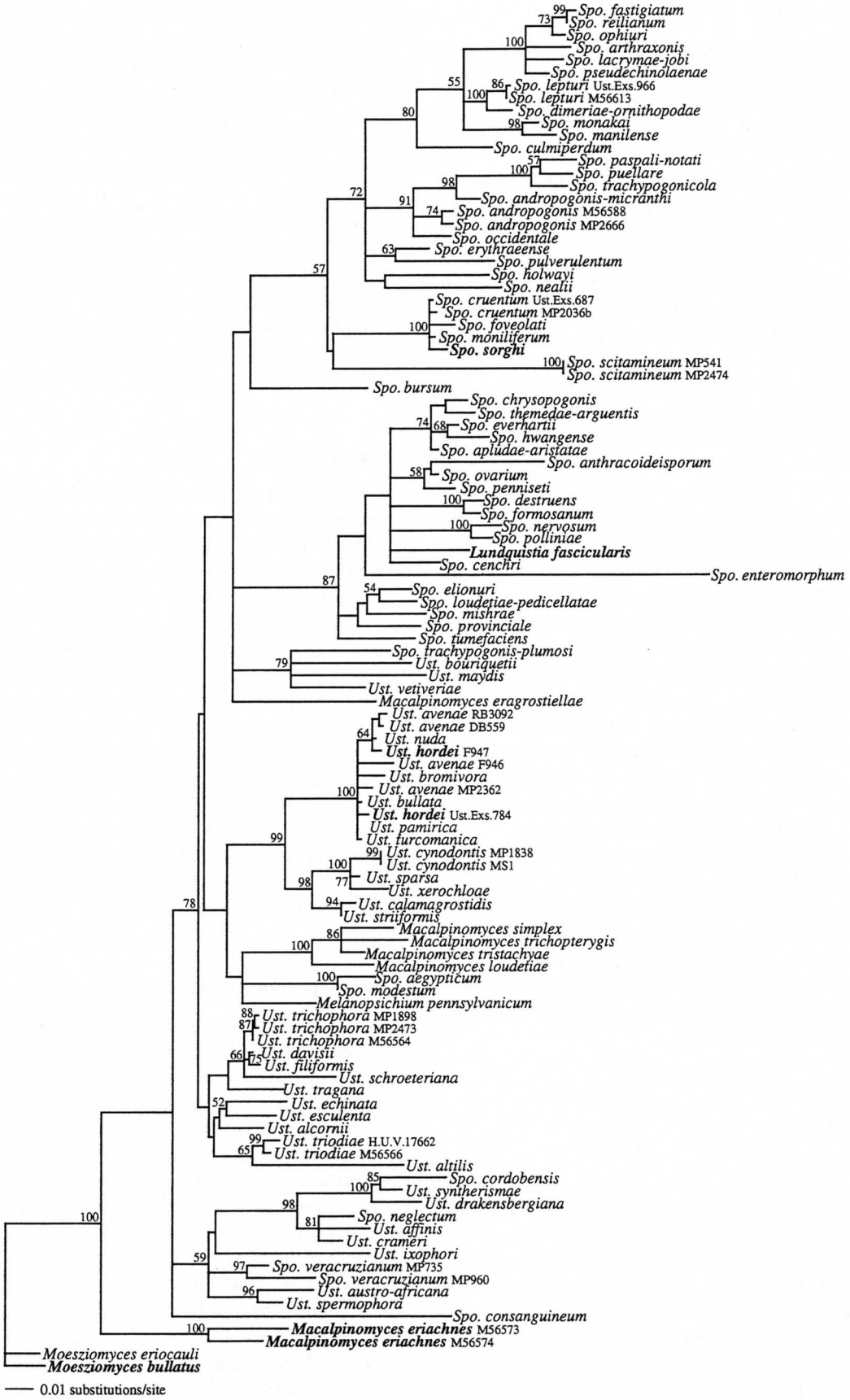

Fig. 2. Strict consensus topology of 84 trees resulting from a Minimum Evolution analysis of 1387 bp of ITS and LSU rDNA sequences of 109 members of *Ustilaginales*. Bootstrap values (500 replicates) greater than 50 % are given above the branches. Names of type species are printed in bold.

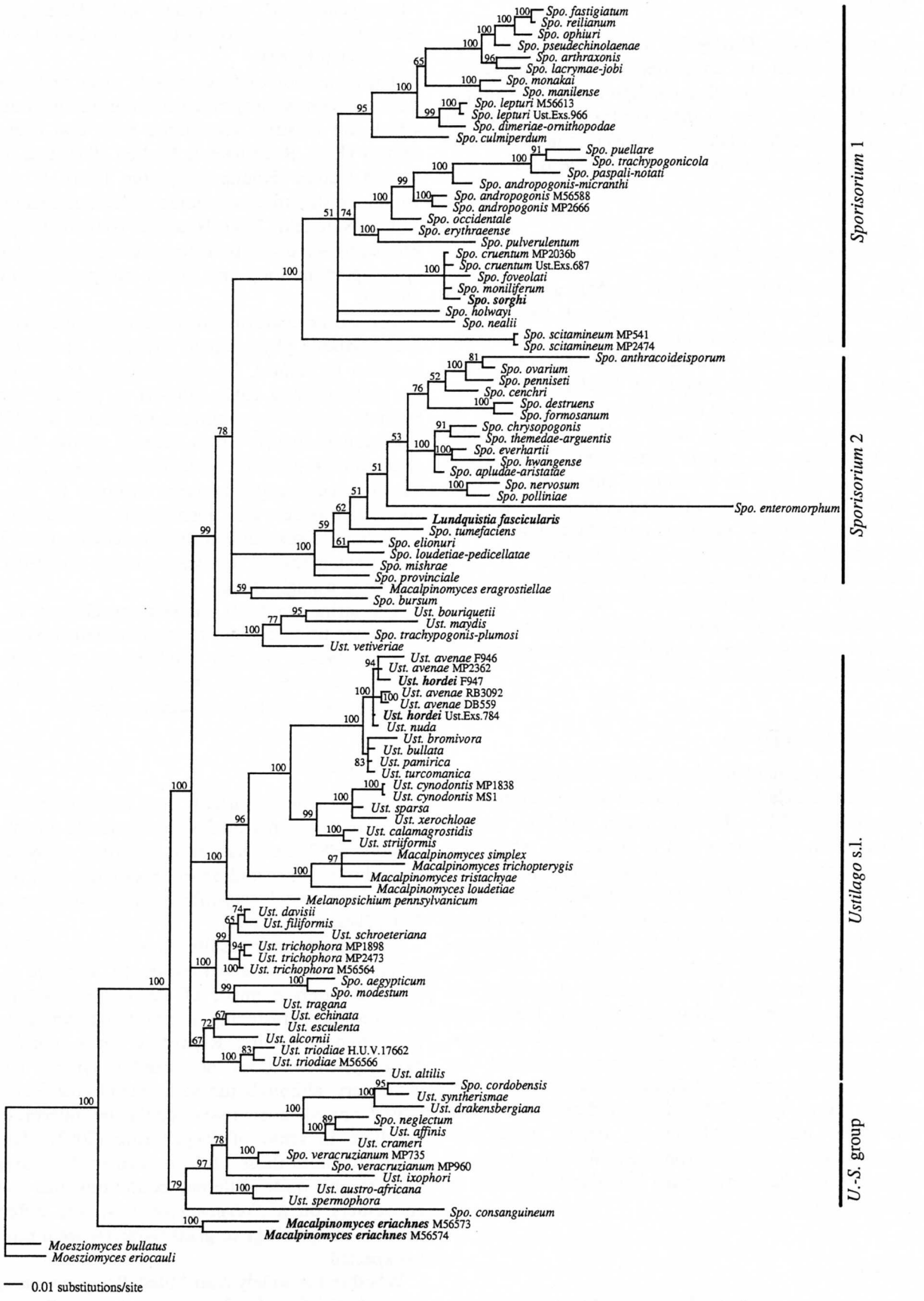

Fig. 3. Phylogram resulting from a Bayesian Monte Carlo Markov chains (MCMC) analysis of 1387 bp of ITS and LSU rDNA sequences of 109 members of *Ustilaginales*. Majority-rule consensus tree of 30 000 trees, numbers above the branches indicate *a posteriori* probabilities. Duplicate sequences are marked with their respective collection numbers. Name of type species are printed in bold.

Ustilago s. lat.

A monophyletic *Ustilago s. lat.* appears in the ME topology, albeit not supported by bootstrap values. MCMC does not resolve *Ustilago s. lat.* as a monophyletic group. Due to these contradictory results, it cannot be clarified whether *Ustilago* is monophyletic or not. However, *Ustilago s. lat.* could be divided into three subgroups.

Ustilago esculenta group

This clade which is resolved by ME and MCMC, receives low to moderate nodal support. Its members attack mainly chloridoid grasses, the atypical *U. esculenta* is found on *Zizania latifolia* (*Ehrhartoideae*). Soral characters are typical of *Ustilago*. The peculiarities of *U. esculenta* are addressed in Nagler *et al.* (1990) and Piepenbring, Stoll & Oberwinkler (2002) even applying the generic name *Yenia* for it. These special traits may be a result of the ecological conditions under which the attacked grass lives, the taxonomic rank of the *U. esculenta* group cannot be verified by our data. The relatives of *U. esculenta* occur on wetland grasses, whereas *U. altilis* and *U. triodiae* are found in arid surroundings on the closely related *Triodia* or *Plectrachne* spp. (*Triodiinae*) which could be another example for an 'evolution with ecosystems' as described in Bauer *et al.* (2001).

Ustilago davisii group

The species around *Ustilago davisii* are members of a monophyletic clade with moderate to good support by ME and MCMC. To this clade, MCMC assigns *Sporisorium aegypticum* and *S. modestum*, which are closer to the *Macalpinomyces loudetiae* group in ME.

U. davisii and *U. filiformis* both attack *Glyceria* spp. (*Pooideae*). They only differ in teliospore size and sorus appearance (Vánky 1994a), and might be representatives of a single species according to our data. *U. trichophora* and *U. schroeteriana* diversified on *Setariinae* grasses (Clayton & Renvoize 1986), and the adjacent *U. tragana* occurs on a chloridoid genus. *S. aegypticum* and *S. modestum* parasitize on *Danthonioideae* and *Chloridoideae*, respectively. Both species show 'typical' *Sporisorium* characters, where the other members of the *U. davisii* group are intermediate in sorus morphology. We do not try to interpret this clade any further here due to the lack of non-molecular traits.

Ustilago s. str.

In accordance with Stoll *et al.* (2003), this clade consists of the type species *U. hordei* and its relatives on crops or pooid grasses, as well as a subgroup around *U. cynodontis* on chloridoid grasses. *Ustilago s. str.* can be widened through the inclusion of a '*Macalpinomyces*' grouping and *Melanopsichium pennsylvanicum*, as strongly confirmed by MCMC and not rejected by ME.

The separation of *U. avenae*, *U. nuda*, and *U. hordei* does not seem appropriate when considering our data, which are in strict accordance with that chemotaxonomy (Kim, Rohringer & Nielsen 1984) and hybridisation studies (Huang & Nielsen 1984). *U. segetum* would be the adequate name to accommodate these smuts (Nannfeldt 1959, Huang & Nielsen 1984) which are barely separable by teliospore ornaments, basidiospore germination, and sorus morphology (Vánky 1994a).

The second clade on crops around *U. bullata* exhibits a comparable phylogenetic structure. The differences between *U. bullata*, *U. pamirica*, and *U. turcomanica* are small (Vánky 1988a, 1994a). A position separate from *U. segetum* is confirmed by Kim *et al.* (1984) in resolving *U. tritici*, which belongs to the *U. bullata* clade (Stoll *et al.* 2003), as distinct species. *U. bromivora* shows a comparatively large distance to the other species, whether this justifies its separation from *U. bullata* (Vánky 2001b) has to remain open. In the ME phylogram all *Ustilago* species on crops even appear in a single clade.

The stripe smuts *U. calamagrostidis* and *U. striiformis* are being distinct by spore ornamentation only (Vánky 1994a). In our phylograms, the molecular distance between both species is very small, thus questioning their status as separate species.

Melanopsichium

Melanopsichium has usually been defined clearly by its exclusive occurrence on *Polygonaceae* and morphology (Vánky 1987) and has been regarded to be closely related to *Ustilago* and *Sporisorium* (Begerow, Bauer & Oberwinkler 1997, Piepenbring, Bauer & Oberwinkler 1998, Bauer *et al.* 2001).

Interestingly, our results show a close affiliation of *Melanopsichium pennsylvanicum* to *Ustilago s. str.*, being the only member of the *Ustilago-Sporisorium* relationship parasitizing a non-pooacean and even non-monocot family. Regarding sorus morphology, *Melanopsichium* does not exhibit typical *Ustilago* characters, although the sorus membrane formed by hypertrophied host tissue could be interpreted as peridium in sense of Piepenbring (2003). The gall itself resembles those of *U. maydis* or *U. bouriquetii*. Due to the quite different conditions this parasite encounters on a *Polygonaceae* host, the differences in sorus morphology to grass parasites seem not to be unexpected.

Whether the widely distributed *Polygonaceae* served as a basis for *Ustilago* to colonise *Poaceae* or *Melanopsichium* is the result of a jump from grasses to *Polygonum* species cannot be answered from our data without co-evolutionary studies or incorporation of molecular clock hypotheses.

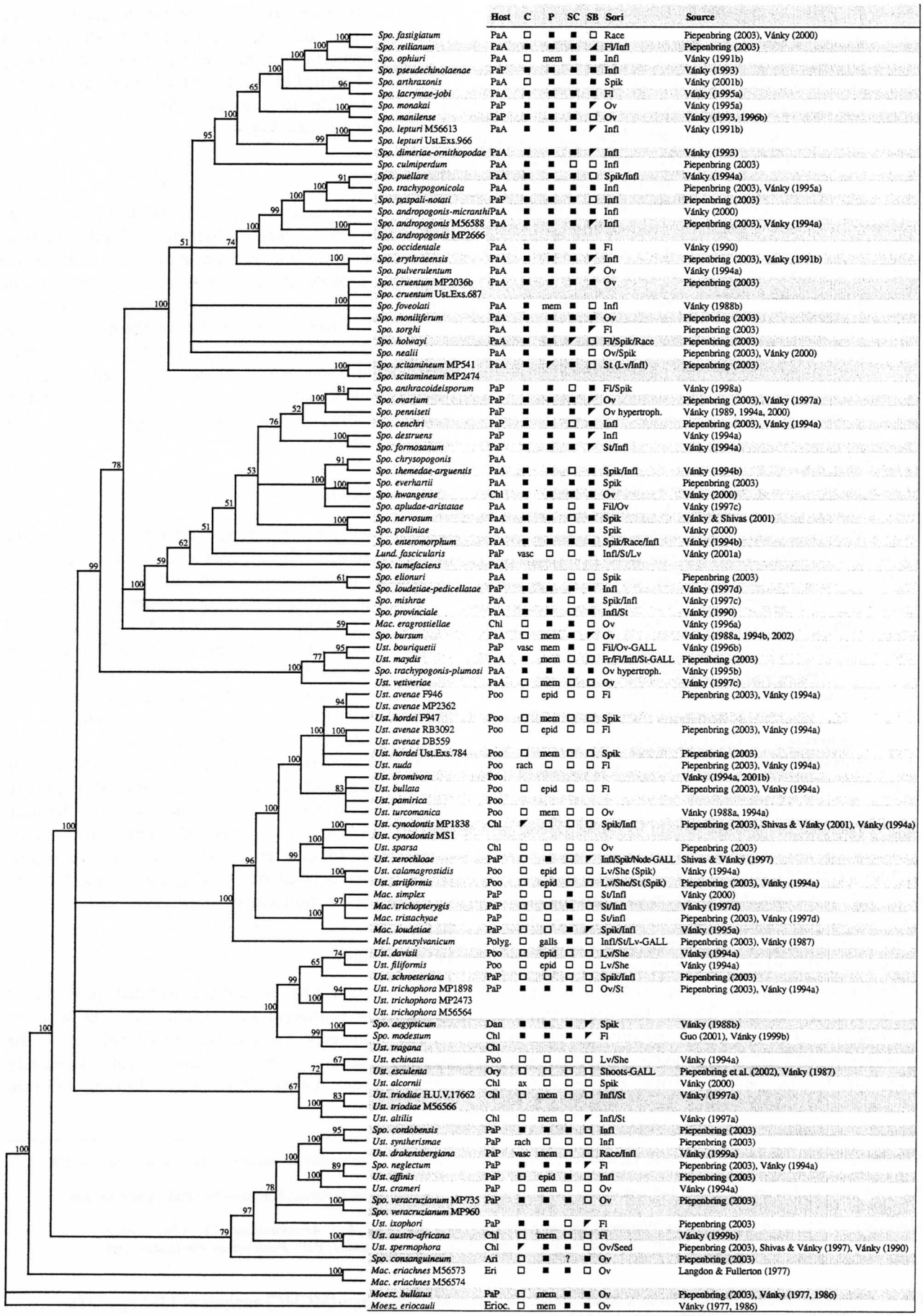

Fig. 4. For legend see opposite page.

Macalpinomyces loudetiae group

The cluster around *Macalpinomyces loudetiae* is a monophyletic clade with good support values of all methods. Its members are found exclusively on the panicoid tribe *Arundinelleae*. Three out of four species transform their host's culms into tube-like sori. This peculiar appearance, together with the lack of *Sporisorium* characters lead to their placement into *Macalpinomyces* (Vánky 1995a, 1997d, 2000). Regarding our data, there is no connection to the type species of *Macalpinomyces*. This clade seems to be highly specialised in host selection and morphology. Two species might even occupy the same ecological niche in developing their sori in the culms of *Loudetia* spp.; a possible competitor, *S. loudetiae-pedicellatae*, however, is in the inflorescence of the same grass genus.

Sporisorium

Ustilago maydis group

A close relationship of *Ustilago maydis* to *Sporisorium* had already been assumed by (Piepenbring *et al.* 2002, Stoll *et al.* 2003) and is verified by the phylogenies presented here. *U. maydis* does not share common characters with the other species of its clade, except the hypertrophied sori in *U. bouriquetii* and *S. trachypogonis-plumosi*. The four species examined bear a variety of *Ustilago* and *Sporisorium* sorus characters. Regarding non-molecular traits, *U. maydis* occupies an isolated position (Stoll *et al.* 2003). Whether this in conjunction with our data could lead to another generic name being proposed for the *U. maydis* clade cannot be answered without studying additional characters of its members.

'*Sporisorium 2*'

Supported by ME and MCMC, '*Sporisorium* 2' consists of *Sporisorium, Macalpinomyces*, and *Lundquistia* species, corresponding with the analyses of Stoll *et al.* (2003). About 50 % of its species parasitize *Panicodae* grasses, and the other half occurs on *Andropogonodae*. *S. hwangense* on *Sporobolus* sp. is the only representative of '*Sporisorium* 2' on the grass subfamily *Chloridoideae*. The well-supported subclade containing *S. destruens* and allies exclusively parasitizes grasses belonging to the panicoid tribe *Paniceae*. Among these, *S. destruens* and *S. formosanum* are found on *Panicum* spp. and can only be distinguished by size and ornamentation of their teliospores (Vánky 1994a). No shared morphological or ecological character is known to date to separate '*Sporisorium* 2' from '*Sporisorium* 1' (see Fig. 4).

Lundquistia

Based on the lack of 'typical' *Sporisorium*, *Macalpinomyces*, or *Ustilago* characters, Vánky (2001a) introduced this new generic name for a smut on *Digitaria brownii*. According to our phylograms, the monotypic *Lundquistia fascicularis* belongs to '*Sporisorium* 2'. From our point of view, the morphological traits used by Vánky (2001a) do not justify its separation from *Sporisorium*. Incorporating all species of '*Sporisorium* 2' into *Lundquistia* would also be rather arbitrary, due to the lack of support from non-molecular data.

'*Sporisorium 1*'

Sporisorium 1, incorporating the type species, *S. sorghi*, is well supported by all methods we applied. These results are in congruence with Stoll *et al.* (2003), and confirm the transfer of *Ustilago scitaminea* to *S. scitamineum* (Piepenbring *et al.* 2002). The *Sporisorium* 1 species almost exclusively parasitize *Andropogonodae* grasses, five of them occurring only on *Andropogon* spp. (*S. andropogonis, S. andropogonis-micranthi, S. occidentale, S. holwayi, and S. fastigiatum*).

The clade around *S. sorghi* exhibits the same phenomenon as does the *U. avenae* group (Stoll *et al.* 2003) where morphologically distinguishable individuals appear to belong to the same species at the molecular level. Their occurrence on different host species may account for differences in their appearance. Again, no character beyond our phylogenies is available to corroborate the monophyly of *Sporisorium* 1.

Incertae sedis

The uncertain and somewhat isolated position of *Sporisorium consanguineum* might correlate with its unique occurrence on *Aristida*, which belongs to the peculiar grass tribe *Aristideae* (or subfamily *Aristidioideae*; Grass Phylogeny Working Group

Morphological data: ■, character present; □, character absent; ◤, intermediate; C, columella; P, peridium; SC, sterile cells; and SB, spore balls.
Sorus location: Fl, flowers, Fr, fruits; Infl, inflorescences; Lv, leaves; Ov, ovaries; Race, racemes; She, sheaths; Spik, spikelets; and St, stems.
Abbreviations of host subfamilies/tribes (Watson & Dallwitz 1992 on): Ari, *Aristideae*; Aru, *Arundinoideae*; Chl, *Chloridoideae*; Dan, *Danthonieae*; Eri, *Eriachneae*; Ory, *Oryzoideae*; PaA, *Panicoideae-Andropogonodae*; PaP, *Panicoideae-Panicodae*; and Poo, *Pooideae*.

Fig. 4. Phylogenetic tree resulting from a Bayesian Monte Carlo Markov chains (MCMC) analysis of 1387 bp of ITS and LSU rDNA sequences of 109 members of Ustilaginales. Majority-rule consensus tree of 30 000 trees, numbers above the branches indicate *a posteriori* probabilities.

2001). Both methods place *S. consanguineum* at the base of the *Ustilago-Sporisorium* clade. Although *Macalpinomyces eragrostiellae* and *S. bursum* are of unclear affiliation too, their placement in *Sporisorium* seems to be stable. At present we are not able to explain this situation further without the inclusion of other data.

Additional characters

As depicted on Fig. 4, sorus characters such as the type of peridium, columella, and sterile cells, commonly used to delimit *Ustilago*, *Macalpinomyces*, and *Sporisorium*, are dispersed along our phylogenies. Some distribution patterns are easily visible, but especially in *Ustilago s. lat.* and in the *Ustilago-Sporisorium*-clade, intermediate character combinations predominate. Even in the quite homogeneous *Sporisorium* or *Ustilago* clades, some species exhibit atypical characters in a 'classical' sense. In the following, we discuss the characters of *Sporisorium*, for commonly *Ustilago* is defined by their absence (Vánky 1987, Piepenbring 2003).

Sorus characters

The distribution pattern of sorus characters is obscured by their inconsistent interpretation in the literature. In the description of *Sporisorium* (Langdon & Fullerton 1978) the peridium, for instance, was defined as consisting of host tissue interwoven with fungal hyphae. Later, this concept was extended by Vánky (1985, 1987) to 'fungal elements overlaid by host tissue'. To this definition, Piepenbring (2003) even added 'peridia formed ... or only by more or less hypertrophied host tissue'. Hence, the interpretation of this trait depended largely on the particular author's concept, which might be illustrated by the following case. With reference to the sorus of *Ustilago spermophora*, Vánky (1994a) speaks of a 'membrane of fungal and host origin' but does not mention a 'peridium', whereas Shivas & Vánky (1997) and Piepenbring (2003) clearly assign a peridium to this species.

Another main character, the columella, an '(...) integrated structure composed of host tissue and hyphae (...)' (Langdon & Fullerton 1978), or '(...) composed of host tissues permeated by hyphae (...)' (Vánky 1987) was later described as '(...) formed by hypertrophied axial host tissue (...)' (Piepenbring 2003), thus significantly extending the previous definitions. Unlike the narrow definition of columellae, Vánky explicitly cites columellae 'of host origin' in the cases of *S. dimeriae-ornithopodae* (Vánky 1993), *S. lepturi* (Vánky 1991b), *S. pseudechinolaenae* (Vánky 1997b), and *S. themedae-arguentis* (Vánky 1994b). In the above mentioned *U. spermophora*, Shivas & Vánky (1997) report a columella, and Vánky (1994a) a 'columella with hyphae', while Piepenbring (2003) does not mention any columella-like structures.

Teliospore balls, which do not appear in Langdon & Fullerton's (1978) description of *Sporisorium*, were later considered to be a typical *Sporisorium* character (Vánky 1987). Vánky (1998b) subsequently questioned the value of spore-balls for phylogenetic research owing to their frequently ephemeral nature. Consequently, they do not appear as unique characters in Piepenbring (2003). This also applies to teliospore ornamentation, whose variabilities and convergencies have been documented by Vánky (1991a).

Sterile cells between the teliospores, or 'partitioning cells', play a part during teliosporogenesis (Langdon & Fullerton 1978). Their presence seems to be rather arbitrary in light of our phylograms. Only detailed studies of teliosporogenesis might assess their systematic value in the future.

Embedded teliospore initials in a hyaline matrix are cited by Piepenbring (2003) as characteristic for *Sporisorium*. Due to missing data, we were not able to incorporate this trait into the table on Fig. 4 and assess its value for phylogenetic purposes.

The majority of sorus data had been sampled only from the study of dried specimens. As Langdon & Fullerton (1975, 1978) showed, the use of dried specimens with mature sori may be misleading unless sorus development is studied in detail, which has not been done extensively since then. In their studies, the process of soral development largely depended on the rapidity of hyphal growth, the stage of host development, and the susceptibility of host tissues.

Furthermore, a general relationship of sorus morphology with the affected host organ or the affected host species has been demonstrated by Fullerton & Langdon (1968), Holton *et al.* (1968), and Savile (1954). In our phylograms this view is confirmed by the following examples:

S. cenchri and *S. penniseti* attacking the same host genera (*Cenchrus* and *Pennisetum*) can only be distinguished by the location of their sori and teliospore size, not by any other morphological character (Piepenbring 2003), although they most probably represent two separate species in our phylograms.

S. ovarium and *S. neglectum* both parasitize species of the grass subtribe *Setariinae*, they closely resemble each other by means of soral structure (Piepenbring 2003).

S. andropogonis on *Andropogon*, *Dichantium*, and *Heteropogon* spp., and *S. puellare* on *Hyparrhenia hirta*, are morphologically indistinguishable (Vánky 1994a). *S. foveolati* on *Eremopogon* sp. is very similar to both species (Vánky 1988b), and Clayton & Renvoize (1986) treat *Eremopogon* as a synonym of *Dichantium*. In our phylograms, all three species turn out to be only distantly related to each other, whereas their hosts are closely related or even belong to the same genus.

U. ixophori and *U. trichophora* exhibit a similar sorus morphology and parasitize the closely related panicoid genera *Ixophorus* and *Echinochloa*, respectively (Piepenbring 2003). Our data show their large

Table 1. List of species studied.

Species	Host	Origin	GenBank[a] accession no.	Source[b]
Leucocintractia scleriae	*Rhynchospora triflora*	Honduras	I: AY740025 L: AJ236154	MP 2074 (USJ)
Lundquistia fascicularis	*Digitaria brownie*	Australia	I: AY740035 L: AY740088	58832a (DAR)
Macalpinomyces eragrostiellae	*Eragrostiella bifaria*	India	I: AY740036 L: AY740089	Ust. Exs. 960 (M)
M. eriachnes	*Eriachne aristidea*	Australia	I: AY740037 L: AY740090	56573 (M)
M. eriachnes	*Eriachne helmsii*	Australia	I: AY740038 L: AY740091	56574 (M)
M. loudetiae	*Loudetia flavida*	South Africa	AY740151	56576 (M)
M. simplex	*Loudetia simplex*	Zimbabwe	AY740152	56577 (M)
M. trichopterygis	*Trichopteryx dregeana*	South Africa	I: AY740039 L: AY740092	56578 (M)
M. tristachyae as *Sporisorium tristachyae*	*Loudetiopsis chrysothrix*	Bolivia	AY740164	MP 2630 (LPB)
Melanopsichium pennsylvanicum	*Polygonum glabrum*	India	I: AY740040 L: AY740093	H.U.V. 17548 (TUB)
Moesziomyces bullatus	*Paspalum distichum*	India	AY740153	Ust. Exs. 833 (M)
M. eriocauli	*Eriocaulon cinereum*	India	I: AY740041 L: AY740094	56580 (M)
Sporisorium aegypticum	*Schismus arabicus*	Iran	I: AY344970 L: AY740129	Ust. Exs. 756 (M)
S. andropogonis	*Bothriochloa saccharoides* (as *Andropogon saccharoides*)	Ecuador	I: AY740042 L: AY740095	56588 (M)
S. andropogonis	*Bothriochloa cf saccharoides*	Bolivia	I: AY740043 L: AY740096	MP 2666 (LPB)
S. andropogonis-micranthi (as *Sporisorium capillipedii*)	*Capillipedium spicigerum*	Australia	I: AY740047 L: AY740100	56595 (M)
S. anthracoideisporum	*Pseudoraphis spinescens*	Papua New Guinea	I: AY740044 L: AY740097	H.U.V.18350 (BRIP, BPI)
S. apludae-aristatae	*Apluda mutica*	India	I: AY740045 L: AY740098	56590 (M)
S. arthraxonis	*Arthraxon lanceolatus*	China	I: AY740046 L: AY740099	56592 (M)
S. bursum	*Themeda quadrivalvis*	India	AY740154	Ust. Exs. 844 (M)
S. cenchri	*Cenchrus pilosus*	Nicaragua	I: AY344972 L: AF453943	MP 1974 (TUB)
S. chrysopogonis	*Chrysopogon fulvus*	Sri Lanka	I: AY344973 L: AY740131	Ust. Exs. 407 (M)
S. consanguineum	*Aristida urugayensis*	Argentina	I: AY740048 L: AY740101	H.U.V. 19145 (TUB)
S. cordobense	*Digitaria insularis*	Bolivia	AY740155	MP 2634 (LPB)
S. cruentum	*Sorghum halepense* Pers.	USA	I: AY344974 L: AF453939	Ust. Exs. 687 (M)
S. cruentum	*Sorghum bicolor*	Nicaragua	AY740156	MP 2036b (USJ)
S. culmiperdum	*Andropogon gerardii*	Honduras	I: AY344975 L: AF133580	MP 2060 (TUB)
S. destruens	*Panicum miliaceum*	Romania	I: AY344976 L: AY747077	Ust. Exs. 472 (M)
S. dimeriae-ornithopodae	*Dimeria ornithopoda*	India	I: AY344977 L: AY740132	Ust. Exs. 848 (M)
S. elionuri	*Elionurus muticus*	Bolivia	AY740157	MP 2601 (LPB)
S. enteromorphum	*Themeda triandra*	South Africa	AY740158	56602 (M)
S. erythraeense	*Hackelochloa granularis*	India	I: AY740049 L: AY740102	Ust. Exs. 849 (M)
S. everhartii	*Andropogon virginicus*	Cuba	AY740159	MP 2270 (HAJB)
S. fastigiatum	*Andropogon angustatus*	Nicaragua	I: AY344978 L: AY740133	MP 1976 (USJ)
S. formosanum	*Panicum repens*	Taiwan	I: AY344979 L: AY740134	Ust. Exs. 688 (M)
S. foveolati	*Eremopogon foveolatus*	Canary Islands	I: AY740050 L: AY740103	MP 2365 (TUB)
S. holwayi	*Andropogon bicornis*	Panama	I: AY344980 L: AF453941	MP 1271 (PMA, USJ)
S. hwangense	*Sporobolus panicoides*	Zimbabwe	I: AY740051 L: AY740104	56607 (M)
S. lacrymae-jobi	*Coix lacryma-jobi*	India	I: AY740052 L: AY740105	56611 (M)

Table 1. (*Cont.*)

Species	Host	Origin	GenBank[a] accession no.	Source[b]
S. lepturi	*Hemarthria uncinata*	Australia	I: AY344981 L: AY740135	Ust. Exs. 966 (M)
S. lepturi	*Hemarthria uncinata*	Australia	AY740160	56613 (M)
S. loudetiae-pedicellatae	*Loudetia pedicellata*	South Africa	I: AY740053 L: AY740106	56615 (M)
S. manilense (as *Sporisorium sacciolepidis*)	*Sacciolepis indica*	India	I: AY740059 L: AY740112	Ust. Exs. 854 (M)
S. mishrae	*Apluda mutica*	India	I: AY344983 L: AY740136	Ust. Exs. 967 (M)
S. modestum	*Enneapogon avenaceus*	Australia	I: AY740054 L: AY740107	56617 (M)
S. monakai	*Isachne globosa*	India	AY740161	56618 (M)
S. moniliferum	*Heteropogon contortus*	Indonesia	I: AY344984 L: AF453940	Ust. Exs. 851 (M)
S. nealii	*Heteropogon melanocarpus*	India	I: AY740055 L: AY740108	56621 (M)
S. neglectum	*Setaria pumila*	Germany	I: AY740056 L: AY740109	RB 2056 (TUB)
S. nervosum	*Sehima nervosum*	Australia	I: AY740057 L: AY740110	56622 (M)
S. occidentale	*Andropogon gerardii*	USA	I: AY344985 L: AY740137	Ust. Exs. 758 (M)
S. ophiuri	*Rottboellia cochinchinensis*	Unkown	I: AY740019 L: AJ236136	HB 20
S. ovarium	*Urochloa fasciculata* (Sw.)	Mexico	I: AY740020 L: AJ236137	MP 1871 (XAL)
S. paspali-notati	*Paspalum notatum*	Cuba	I: AY344982 L: AF453944	MP 2101 (HAJB)
S. penniseti (as *Sporisorium catharticum*)	Pennisetum setaceum	Canary Islands	I: AY344971 L: AY740130	MP 2367 (TUB)
S. polliniae	*Andropogon distachyos*	Greece	I: AY344987 L: AY740138	Ust. Exs. 690 (M)
S. provinciale	*A. gerardii*	USA	I: AY344988 L: AY747076	Ust. Exs. 759 (M)
S. pseudechinolaenae	*Pseudechinolaena polystachya*	Indonesia	I: AY344989 L: AY740139	Ust. Exs. 853 (M)
S. puellare	*Hyparrhenia hirta*	Canary Islands	I: AY740058 L: AY740111	MP 2372 (TUB)
S. pulverulentum	*Saccharum strictum*	Yugoslavia	AY740162	56627 (M)
S. reilianum	*Sorghum halepense*	Greece	AY740163	Ust. Exs. 527 (M)
S. scitamineum (as *Ustilago scitaminea*)	*Saccharum* sp. cult	Cuba	I: AY345007 L: AY740147	MP 2474 (HAJB)
S. scitamineum (as *Ustilago scitaminea*)	*Saccharum* sp. cult	Costa Rica	I: AY740070 L: AJ236138	MP 541 (USJ)
S. sorghi	*Sorghum bicolor*	Nicaragua	I: AY740021 L: AF009872	MP 2036a (USJ)
S. themedae-arguentis	*Themeda arguens*	Indonesia	I: AY344991 L: AY740140	Ust. Exs. 855 (M)
S. trachypogonicola	*Trachypogon plumosus*	Cuba	I: AY344992 L: AY740141	MP 2463 (HAJB)
S. trachypogonis-plumosi	*T. plumosus*	Venezuela	I: AY740060 L: AY740113	56635 (M)
S. tumefaciens (as *Sorosporium tumefaciens*)	*Chrysopogon aciculatus*	Sri Lanka	I: AY344969 L: AY740128	Ust. Exs. 231 (M)
S. veracruzianum	*Panicum viscidellum*	Costa Rica	I: AY344993 L: AY740114	MP 960
S. veracruzianum	*P. viscidellum*	Costa Rica	I: AY747075 L: AY740142	MP 735 (USJ)
Ustanciosporium taubertianum	*Rhynchospora tenuis*	Cuba	I: AY740024 L: AJ236156	MP 2276 (HAJB)
Ustilago affinis	*Stenotaphrum secundatum*	Costa Rica	I: AY344995 L: AF133581	Rivera s.n. (USJ)
U. alcornii	*Tripogon loliiformis*	Australia	AY740165	56514 (M)
U. altilis	*Triodia pungens*	Australia	AY740166	Ust. Exs. 418 (M)
U. austro-africana	*Enneapogon cenchroides*	Zimbabwe	I: AY740061 L: AY740115	56516 (M)
U. avenae	*Arrhenaterum elatius*	Germany	I: AY740063 L: AY740117	DB 559 (TUB)

Table 1. (*Cont.*)

Species	Host	Origin	GenBank[a] accession no.	Source[b]
U. avenae	*A. elatius*	Germany	I: AY740062 L: AY740116	RB 3092 (TUB)
U. avenae	*Avena barbata*	Canary Islands	I: AY344997 L: AF453933	MP 2362 (TUB)
U. avenae	*Avena barbata*	Italy	I: AY344996 L: AJ236140	F 946/GD 1292 (TUB)
U. bouriquetii	*Stenotaphrum dimidiatum*	La Réunion	AY740167	56517 (M)
U. bromivora	*Bromus catharticus*	Argentina	I: AY740064 L: AY740118	H.U.V. 19322
U. bullata Berk	*B. diandrus*	Canary Islands	I: AY344998 L: AF453935	MP 2363 (TUB)
U. calamagrostidis	*Calamagrostis epigeios*	Bulgaria	I: AY740065 L: AY740119	56518 (M)
U. crameri	*Setaria italica*	India	I: AY344999 L: AY740143	Ust. Exs. 995 (M)
U. cynodontis	*Cynodon dactylon*	Mexico	I: AY345000 L: AF009881	MP 1838 (XAL)
U. cynodontis	*C. dactylon*	Taiwan	AY740168	MS 1 (TUB)
U. davisii	*Glyceria multiflora*	Argentina	AY740169	H.U.V. 19252
U. drakensbergiana	*Digitaria tricholaenoides*	South Africa	AY740170	56523 (M)
U. echinata	*Phalaris arundinacea*	Germany	I: AY345001 L: AY740144	Ust. Exs. 540 (M)
U. esculenta	*Zizania latifolia* cult	Taiwan	I: AY345002 L: AF453937	Ust.Exs. 590 (M)
U. filiformis	*Glyceria fluitans*	Germany	I: AY740066 L: AY740120	RB 3011 (TUB)
U. hordei (as *U. kolleri*)	*Avena sativa*	Spain	I: AY740068 L: AY740122	F 947/GD 1300
U. hordei	*Hordeum vulgare*	Iran	I: AY345003 L: AF453943	Ust. Exs. 784 (M)
U. ixophori	*Ixophorus unisetus*	Costa Rica	I: AY740067 L: AY740121	MP 2194 (USJ)
U. maydis	*Zea mays* L.	Germany	I: AY345004 L: AF453938	RB 3093 (TUB)
U. nuda	*Hordeum leporinum*	Unknown	I: AY740069 L: AJ236139	H.U.V. 17782
U. pamirica	*Bromus gracillimus*	Iran	I: AY345005 L: AY740145	Ust. Exs.789 (M)
U. schroeteriana	*Paspalum paniculatum*	Costa Rica	I: AY345006 L: AY740146	Ust. Exs.887 (M)
U. spermophora	*Eragrostis ferruginea*	n.a	AY740171	F 565/ H.U.V.13634
U. striiformis	*Alopecurus pratensis*	Germany	AY740172	H.U.V. 18286
U. syntherismae	*Digitaria ternata*	India	I: AY740071 L: AY740123	Ust. Exs. 998 (M)
U. tragana	*Tragus berteronianus*	Zimbabwe	I: AY740072 L: AY740124	56562 (M)
U. trichophora	*Echinochloa colona*	Cuba	I: AY345009 L: AY740148	MP 2473 (HAJB)
U. trichophora	*E. colona*	Mexico	I: AY740023 L: AJ236141	MP 1898 (XAL)
U. trichophora	*E. colona*	India	I: AY740073 L: AY740125	56564 (M)
U. triodiae	*Triodia microstachya*	Australia	I: AY740074 L: AY740126	H.U.V. 17662
U. triodiae	*T. microstachya*	Australia	I: AY740075 L: AY740127	56566 (M)
U. turcomanica	*Eremopyrum distans*	Iran	I: AY345011 L: AF453936	F 585/ H.U.V. 23
U. vetiveriae	*Vetiveria zizanioides*	Unknown	I: AY345011 L: AY740149	H.U.V. 17954
U. xerochloae	*Xerochloa imberbis*	Australia	I: AY345012 L: AY740150	Ust. Exs. 1000 (M)

[a] Accession numbers: I, ITS sequence; and L, LSU sequence. Contiguous sequences (ITS and LSU) bear single accession numbers.

[b] DB, Dominik Begerow; F, Franz Oberwinkler; GD, Günter Deml; HB, Hansjörg Prillinger; MP, Meike Piepenbring; MS, Matthias Stoll; RB, Robert Bauer; Ust. Exs., Kálmán Vánky: *Ustilaginales Exsiccata*. The private herbarium of K. Vánky is abbreviated as H.U.V., Herbarium Ustilaginales Vánky.

evolutionary distance, which is corroborated by the obvious inability of *U. ixophori* to infect *Echinochloa* (Piepenbring 2003).

In contrast, the species around *S. sorghi*, mostly attacking different hosts, are virtually indistinguishable on a molecular level, but can be separated from each other morphologically (Piepenbring 2003). The same applies to the situation in *U. avenae* and *U. hordei*, whose soral morphology and teliospore ornamentation differ from each other, although their sequences are almost identical, thus even questioning their rank as species (Stoll *et al.* 2003). Finally, characters like peridia, sterile cells, columellae, or spore balls already appear in basal ustilaginacean clades like *Anthracoidea*, *Dermatosorus*, or *Tolyposporium* (Vánky 1987) and are not suitable for phylogeny due to their plesiomorphic nature. Thus, soral characters are not suitable for delimiting genera nor resolving phylogeny of *Ustilago* and *Sporisorium* in general.

Ultrastructural characters

Bauer *et al.* (1997) impressively showed the importance of ultrastructural data for resolving phylogeny of smut fungi in general. Unfortunately, the data used (hyphal septation and host-parasite interaction zones) do not show any differences amongst *Ustilago* species and their close relatives. The characters of teliospores, as described by Piepenbring *et al.* (1998), did not reveal marked differences between species of *Ustilago*, *Macalpinomyces*, nor *Sporisorium*. Still, additional ultrastructural data might be valuable on a subgeneric level due to their possible independence of host morphology.

Hosts

Considering the distribution of *Ustilago* and *Sporisorium* species on their hosts, two complementary observations can be made. On the one hand, closely related parasites attack closely related hosts, such as *U. altilis*/*U. triodiae* on *Triodia* spp., *S. cordobensis*/*U. syntherismae*/*U. drakensbergiana* on *Digitaria* spp., or *Macalpinomyces simplex*/*M. loudetiae* on *Loudetia* spp. On the other hand, the distantly related *S. apludae-aristatae* and *S. mishrae* parasitize the same grass species, *Apluda mutica*. Likewise, *U. schroeteriana*, *S. paspali-notati*, and *Moesziomyces bullatus* are found on a single genus, *Paspalum*. *S. andropogonis*, *S. moniliferum*, and *S. nealii* infect the ovaries of *Heteropogon* species, the above mentioned grass genus *Loudetia* is attacked by *S. loudetiae-pedicellatae* as well.

These examples illustrate that only general assumptions regarding host subfamilies can be drawn from our data, which are not suitable for resolving the phylogeny of *Ustilago* and *Sporisorium* around the species level. Further implications on host distribution, host morphology, and co-evolutive phenomena are addressed in Begerow *et al.* (2004).

The aforementioned uncertainties regarding the delimitation of the grass-parasitizing relatives of *Ustilago* and *Sporisorium* have been discussed by Langdon & Fullerton (1975, 1978), Vánky (1985), and Piepenbring (2003). In contrast to the introduction of new genera by Vánky (1995a, 1996a, 1997b, 2001a), Vánky (1985) already recommended mycologists not to base monotypic genera or genera with only few species on morphological characters, but rather to include intermediate species with uncertain affiliation into *Ustilago*. This broad generic concept is strongly supported by our molecular data. In the light of these phylogenies, it seems to be very problematical to assign a species to either *Ustilago* or *Sporisorium* solely based on soral characters.

Conclusions and taxonomic consequences

- *Moesziomyces* species are basal and well-separated from *Ustilago* and *Sporisorium*. Most probably, the genus *Moesziomyces* is paraphyletic. However, its detailed position could be clarified by including more species of *Ustilaginaceae* like *Farysia* or *Dermatosorus* into future analyses.
- *Macalpinomyces* is a monophyletic clade, containing the type species *M. eriachnes*. The remaining *Macalpinomyces* species examined in our study evidently belong either to *Sporisorium* or to *Ustilago*. Regarding the *M. loudetiae* clade, we refrain from proposing a new combination *here* as any taxonomic changes would be premature before the phylogeny of *Ustilago s. lat.* was resolved in detail. *S. bursum* clearly belongs to *Sporisorium*, as already proposed by (Vánky 1988a).
- *Melanopsichium* is a well-supported member of *Ustilago s. str.*, being the only representative of the group not parasitizing *Poaceae*, thus illustrating the probability of host-jumps. Despite this affiliation to *Ustilago*, we do not yet propose a new combination due to missing additional data.
- As we were able to show that the morphological characters used to separate *Lundquistia* from *Sporisorium* are not reliable phylogenetic markers. Thus, we propose the new combination: **Sporisorium fascicularis** (Vánky) M. Stoll, Begerow & Oberw., **comb. nov.** (basionym: *Lundquistia fascicularis* Vánky, *Mycotaxon* **77**: 373, 2001; type: **Australia**: N.S.W.: NW Hermidale, on *Digitaria brownii*, 1971, *D. A. Campbell s. n*' (DAR 58832 – holotype).
- A well-supported monophyletic clade around *S. veracruzianum* and *U. affinis* contains a variety of both *Ustilago* and *Sporisorium* species with intermediate character combinations ('*Ustilago-Sporisorium* clade'), predominantly parasitizing species of the grass subfamily *Panicoideae*.
- The monophyly of *Ustilago s. lat.* cannot be clarified by our data, its is split into three major clades, all receive good support values. The clade containing

the crop-infecting *Ustilago s. str.* could be expanded by the inclusion of the '*Macalpinomyces loudetiae*' group. Soral characters typical to *Sporisorium* are absent. Many species are found on pooid grasses. The connection of the remaining groups to *Ustilago s. str.* remains unclear, although they are obviously not associated with *Sporisorium*.

- *Sporisorium* is a monophyletic clade, divided into two major subgroups: '*Sporisorium* 1' and '*Sporisorium* 2'; and an assemblage around *U. maydis*. The majority of species parasitizes on panicoid grasses and exhibits classical *Sporisorium* sorus characters. No further characters are available to support its division nor the affiliation of *U. maydis* to it.

As we showed, no consistent non-molecular markers are available to corroborate our rDNA sequence results. Thus, only a broad generic concept of *Ustilago* and *Sporisorium* seems to reflect their phylogeny in an adequate manner. Our data emphasize the need for additional research incorporating the host's morphological characters, parasite population data, physiology, or soral development, to further explain the extraordinary diversity these smut fungi developed on *Poaceae*.

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