

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

Matthias STOLL, Dominik BEGEROW and Franz OBERWINKLER

*Spezielle Botanik und Mykologie, Botanisches Institut, Universität Tübingen, Auf der Morgenstelle 1, D-72076 Tübingen, Germany.*

*E-mail: matthias.stoll@uni-tuebingen.de*

*Received 28 June 2004; accepted 27 November 2004.*

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

\* Part 219 of the series: 'Studies in Heterobasidiomycetes'.

*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).

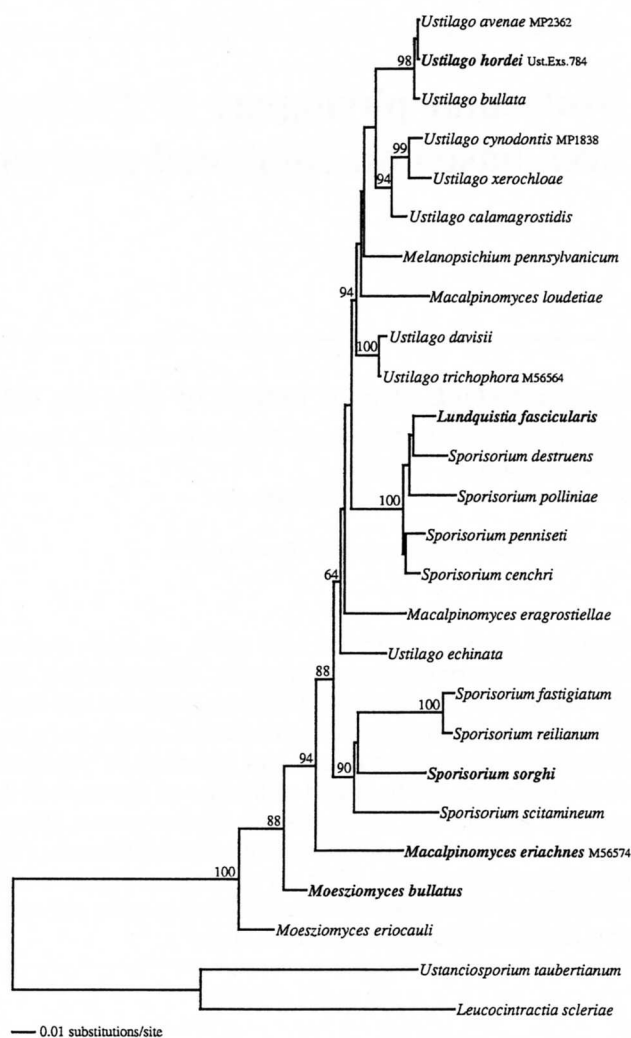
An alignment of 1611 base pairs was created with an iterative aligning method using ClustalX (Thompson *et al.* 1997). In few cases, the resulting alignment was corrected manually in SeAl (Rambaut 2002). 185 positions of ITS which could not be aligned unequivocally were omitted from the following analyses. 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).

