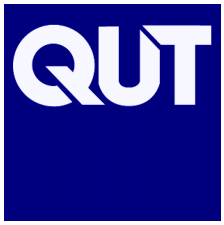

Queensland University of Technology
Discipline of Biogeosciences

Systematics of the *Ustilago-Sporisorium-Macalpinomyces* complex of smut fungi

A thesis submitted by Alistair Ross McTaggart (BSc, BEd, MSc) to the Discipline of Biogeosciences, Faculty of Science and Technology, Queensland University of Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Keywords

Systematics; phylogenetics; maximum likelihood; Bayesian analysis; fungi; Ustilaginaceae; taxonomy; character homology; synapomorphy; *Stollia*; *Langdonia*; *Mycosarcoma*; *Anthracocystis*.

Abstract

Smut fungi are important pathogens of grasses, including the cultivated crops maize, sorghum and sugarcane. Typically, smut fungi infect the inflorescence of their host plants. Three genera of smut fungi (*Ustilago*, *Sporisorium* and *Macalpinomyces*) form a complex with overlapping morphological characters, making species placement problematic. For example, the newly described *Macalpinomyces mackinlayi* possesses a combination of morphological characters such that it cannot be unambiguously accommodated in any of the three genera. Previous attempts to define *Ustilago*, *Sporisorium* and *Macalpinomyces* using morphology and molecular phylogenetics have highlighted the polyphyletic nature of the genera, but have failed to produce a satisfactory taxonomic resolution.

A detailed systematic study of 137 smut species in the *Ustilago-Sporisorium-Macalpinomyces* complex was completed in the current work. Morphological and DNA sequence data from five loci were assessed with maximum likelihood and Bayesian inference to reconstruct a phylogeny of the complex. The phylogenetic hypotheses generated were used to identify morphological synapomorphies, some of which had previously been dismissed as a useful way to delimit the complex. These synapomorphic characters are the basis for a revised taxonomic classification of the *Ustilago-Sporisorium-Macalpinomyces* complex, which takes into account their morphological diversity and coevolution with their grass hosts. The new classification is based on a redescription of the type genus *Sporisorium*, and the establishment of four genera, described from newly recognised monophyletic groups, to accommodate species expelled from *Sporisorium*. Over 150 taxonomic combinations have been proposed as an outcome of this investigation, which makes a rigorous and objective contribution to the fungal systematics of these important plant pathogens.

List of Publications

Papers in Preparation

McTaggart AR, Callaghan B, Geering ADW, Shivas RG, Scharaschkin T. Phylogenetic utility of molecular and morphological data for determining monophyletic groups in a complex of smut fungi. *Molecular Phylogenetics and Evolution*.

McTaggart AR, Shivas RG, Geering ADW, Scharaschkin T. Toward a systematic resolution of the *Ustilago-Sporisorium-Macalpinomyces* complex. *Persoonia*.

McTaggart AR, Shivas RG, Geering ADW, Callaghan B, Scharaschkin T. A reassessment of character homology in the *Ustilago-Sporisorium-Macalpinomyces* complex (Ustilaginaceae). *Persoonia*.

McTaggart AR, Shivas RG, Geering ADW, Scharaschkin T. A taxonomic revision of *Ustilago*, *Sporisorium* and *Macalpinomyces*. *Persoonia*.

Publications resulting from PhD

McTaggart AR, Shivas RG (2009). *Macalpinomyces mackinlayi*. *Persoonia* **23**: 186-187.

McTaggart AR, Shivas RG (2009). *Tilletia challinorae*. *Persoonia* **23**: 184-185. (Appendix 3)

Shivas RG, McTaggart AR (2009). Three new species of *Tilletia* on native grasses from northern Australia. *Australasian Plant Pathology* **38**: 128-131. (Appendix 4)

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Vánky K, Shivas RG, McTaggart AR, Vánky C, Arce WA (2009). Additions to the smut fungi (Ustilaginomycetes) of Bolivia. *Mycologia Balcanica* **6**: 99-105.

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List of Abbreviations

DNA	Deoxyribonucleic acid
rDNA	ribosomal DNA
ITS	Internal Transcribed Spacer (nuclear ribosomal DNA)
LSU	Large Subunit (nuclear ribosomal DNA)
COX3	Cytochrome oxidase subunit 3 (mitochondrial DNA)
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase (nuclear DNA)
EF1α	Elongation factor 1α (nuclear DNA)
PCR	Polymerase Chain Reaction

List of Terms

Clade: A branch of a phylogenetic tree; a monophyletic group of taxa sharing a closer common ancestry with one another than with members of other clades.

Columella: A structure within the sorus formed by fungal and host material, connecting the sorus to the host.

Emend: In taxonomy, to make corrections or improvements to a description.

Homologous: Characters that have common ancestry, but do not necessarily maintain the same structure, function or behaviour.

Homoplasy: Structural resemblance due to convergent evolution rather than common ancestry.

Monophyletic: A group of taxa descended from a single ancestor, that includes all the descendants of that ancestor.

Peridium: The outer covering of a smut sorus.

Polyphyletic: A group of taxa descended from two or more distinct ancestral taxa, usually grouped together based on a character of convergence rather than common ancestry.

Sorus: A structure of smut fungi where spores are produced.

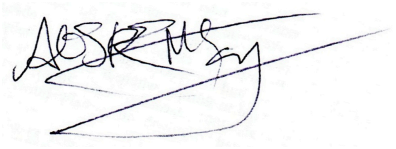
Sporogenous: Fungal tissue that develops into spores.

Synapomorphy: A shared, derived, homologous character.

Systematics: Classification with reference to phylogenetic relationships.

Statement of Original Authorship

The work contained in this thesis has not been previously submitted for a degree or diploma in any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by any other person, except where due reference is made.

Signed: 

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Chapter 1: Introduction

1.1 Description of the research problem investigated

Smut fungi are common pathogens of grasses (Poaceae), including staple agricultural crops, such as maize, wheat, sorghum and sugarcane. Smut fungi destroy the host flower structures, often resulting in heavy crop losses and a reduction in grain quality. The black spore masses resulting from smut infection have long been referred to as the "insidious black harvest" (Carefoot and Sprott 1969). Currently, smut fungi such as loose smut of sorghum (*Sporisorium cruentum*) and Karnal bunt (*Tilletia indica*) present a serious biosecurity risk to Australia. Despite the great need for an accurate identification system for these pathogens, the systematic classification of many smuts remains ambiguous.

The primary goal of systematics is to circumscribe taxa into groups based on shared, derived characters (synapomorphies) according to evolutionary relationships (Hennig 1966; Bremer and Wanntorp 1978; Baum 1989; de Queiroz and Gauthier 1992; Stevens 2006; Horandl 2007; Judd et al. 2008). Phylogenetic trees, when reconstructed from homologous morphological characters and molecular sequence data (Freeman and Herron 1998; Phillips 2006), can be used to represent and understand the evolution of organisms (Page and Holmes 1998; Delsuc et al. 2005), and determine characters that define monophyletic groups (a group of taxa descended from a single ancestor, that includes all the descendants of that ancestor).

Three genera of smut fungi (sub-phylum Ustilaginomycotina), *Ustilago*, *Sporisorium* and *Macalpinomyces*, contain about 530 described species that mostly infect grasses (Vánky in press). These three genera belong to the family Ustilaginaceae, a group of fungi that have intracellular hyphae and produce phragmobasidia after germination of the spore (Bauer et al. 2001; Begerow et al. 2006). *Ustilago, Sporisorium* and *Macalpinomyces* were shown by molecular phylogenetic analyses (Begerow et al. 1997; Stoll et al. 2003; Stoll et al. 2005; Begerow et al. 2006) and ultrastructural analysis of spores and host parasite interactions (Bauer et al. 1997) to form a monophyletic group within the Ustilaginaceae.

The original generic concepts of *Ustilago* and *Sporisorium*, established over 200 years ago, were not sufficiently robust to encompass the complete morphological diversity present in species that have been subsequently discovered. Four morphological characters that traditionally have been used to distinguish *Ustilago*, *Sporisorium* and *Macalpinomyces* are peridia, columellae, spore balls and sterile cells. However, species placement in these genera is problematic as many taxa share two or more of these characters. The four characters are currently considered to be non-homologous and have been dismissed as a means for defining the genera (Piepenbring 2004, Stoll et al. 2005). Consequently, species within *Ustilago, Sporisorium* and *Macalpinomyces* are part of an as yet systematically unresolved complex (Vánky 2002a; Stoll et al. 2003; Piepenbring 2004; Stoll et al. 2005; Vánky et al. 2006; Vánky and Shivas 2008).

Attempts to reconcile the taxonomy of the *Ustilago-Sporisorium-Macalpinomyces* complex using either morphology (Vánky 1991; Piepenbring et al. 1998b) or molecular approaches (Stoll et al. 2003; Stoll et al. 2005) have, to date, been unsuccessful. Vánky (2002a), Stoll et al. (2005) and Vánky et al. (2006) suggested that additional molecular loci should be analyzed to resolve the *Ustilago-Sporisorium-Macalpinomyces* complex. In order to reach a meaningful taxonomy, synapomorphic characters must be related to monophyletic groups (Mooi and Gill 2010). Therefore, resolution of the systematics of the complex will depend on a combined analysis of morphology and molecular characters.

1.2 Overall objective of the study

The objective of this study was to revise the taxonomy of the *Ustilago-Sporisorium-Macalpinomyces* complex in a systematic framework. A revision will enable monophyletic groups within the complex to be clearly defined and ameliorate the confusion surrounding taxonomic delimitation of taxa. It was hypothesised that morphological synapomorphies existed within the complex and, once identified, could be used to define the genera.

1.3 Specific aims of the study

So as to systematically resolve the *Ustilago-Sporisorium-Macalpinomyces* complex, this study aims to:

1. Clearly define the *Ustilago-Sporisorium-Macalpinomyces* complex, and trace previous attempts and outline future work required to reconcile the complex.
2. Examine a range of species from the *Ustilago-Sporisorium-Macalpinomyces* complex that represent the broad diversity across the group.
3. Reconstruct an accurate phylogeny of the complex using optimality criteria such as maximum likelihood and Bayesian inference. DNA sequences from different loci, and non-sequence data such as morphology and secondary structure folding of ribosomal RNA will be included in the analyses.
4. Identify morphological synapomorphies that could be used to define monophyletic groups within the *Ustilago-Sporisorium-Macalpinomyces* complex.
5. Describe new genera and incorporate taxonomic changes for reclassified species within the complex.

1.4 Account of research progress linking the research papers

This thesis contains five chapters, one of which has been published and the others are in an advanced stage of preparation for submission to journals. Chapter 2 is a literature review on sytematics, phylogenetic methods and higher-level classification of smut fungi. Chapter 3 is a comprehensive literature review of the systematics of the *Ustilago-Sporisorium-Macalpinomyces* complex. Chapter 3 chronologically traces the concepts of the genera and discusses reasons behind formation of the complex. It summarizes studies by Australian mycologists who sought to delimit the complex based on the development of smut fungi within their hosts and it discusses recent attempts to reconcile the complex with molecular phylogenetic analyses. In light of these studies, Chapter 3 identifies what remains to be done to resolve the *Ustilago-Sporisorium-Macalpinomyces* complex, thereby fulfilling the first aim of this study.

The polyphyletic nature of the complex, as discussed in Chapter 3, is exacerbated by the discovery of taxa displaying characters diagnostic of both *Ustilago* and *Sporisorium*. These taxa are often placed into *Macalpinomyces* for want of a more suitable genus. Consequently, *Macalpinomyces* contains many taxa that do not resemble the type species, *Macalpinomyces eriachnes*. An example is *Macalpinomyces mackinlayi*, which was discovered in a remote region of northern Western Australia during a survey for smut fungi undertaken as part of this thesis. This unique species infected a native grass, *Eulalia mackinlayi*, and was described formally in Chapter 4. This taxonomic description highlights the polyphyletic nature of *Macalpinomyces*. *Macalpinomyces mackinlayi* became an important taxon in resolving a monophyletic group that had been under-represented in previous phylogenetic analyses of the *Ustilago-Sporisorium-Macalpinomyces* complex. This contributed to the second aim of the study.

Monophyletic groups in the *Ustilago-Sporisorium-Macalpinomyces* complex were reconstructed in a phylogenetic analysis using four molecular loci in Chapter 5 of this thesis. The phylogenetic analyses incorporated DNA sequence data available in GenBank, in addition to 165 novel sequences for five loci obtained in this study. Variation at a mitochondrial locus was determined to be incompatible with results of four nuclear loci by an incongruence length difference test. Maximum likelihood and Bayesian inference were used in phylogenetic reconstruction because they incorporate a model of evolution, and are considered to be more appropriate phylogenetic criteria than parsimony and distance methods when using molecular data (Kosiol et al. 2006). Similar phylogenetic relationships for 137 taxa in the *Ustilago-Sporisorium-Macalpinomyces* complex were determined using both maximum likelihood and Bayesian inference. Ten monophyletic groups were identified within the reconstructed phylogeny of the complex, addressing the third aim of this research.

Chapter 6 discusses character homology within the *Ustilago-Sporisorium-Macalpinomyces* complex and the use of characters to define genera, addressing the fourth aim of the study. The recovery of monophyletic groups in Chapter 5 was followed by a detailed examination of morphological characters within the complex, which revealed synapomorphies that were useful in defining genera. This work refuted the view that morphology was inadequate to delimit the groups within the

Ustilago-Sporisorium-Macalpinomyces complex (Piepenbring 2004; Stoll et al. 2005).

Chapter 7 addresses the fifth aim of this study, using morphological synapomorphies identified in Chapter 6 to revise the systematics of the majority of the *Ustilago-Sporisorium-Macalpinomyces* complex. *Sporisorium* was redefined and four genera, *Langdonia*, *Lundquistia*, *Mycosarcoma* and *Stollia*, were established to accommodate species that form distinct monophyletic groups. Over 150 taxonomic changes were made to incorporate species into these new genera.

This thesis describes the *Ustilago-Sporisorium-Macalpinomyces* complex, and provides an example of the problems with generic placement of some taxa within the complex. A systematic approach, using morphological and molecular phylogenetic analyses, was employed to resolve the monophyletic groups within the complex. Character homology and morphological synapomorphies were identified and used to define current and new genera within the *Ustilago-Sporisorium-Macalpinomyces* complex.

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Chapter 2: Literature review

This review focuses on the importance of systematics and discusses phylogenetic reconstruction in terms of assessment criteria and data use. An introduction to the classification of smut fungi is also given. A comprehensive review identifying the knowledge gaps in the *Ustilago-Sporisorium-Macalpinomyces* complex is provided in Chapter 3.

2.1 The importance of systematics

The categorisation of living organisms has been an ongoing endeavour throughout human history and several classification schemes have been developed. Classification involves arranging organisms into groups and naming these groups to enable communication, understanding and identification (Constance 1964; Mayr and Bock 2002; Sarkar 2006; Judd et al. 2008). Linnaeus is generally credited with developing the binomial system, although philosophers before him, including Aristotle, Caesalpinus and Tournefort, had sought the same orderliness (Judd et al. 2008). Subsequent scientists have refined and progressed systematics from original Linnaean classification.

Systematics incorporates taxonomy in a phylogenetic framework, and is the study of the evolutionary history of all organisms. Phylogenetic classification has become conventional despite some early resistance to the incorporation of evolutionary theory with classification (Hull 1965). It is now accepted that taxonomy without reference to evolutionary history is meaningless (Hennig 1966; Bremer and Wanntorp 1978; Baum 1989; de Queiroz and Gauthier 1992; Stevens 2006; Horandl 2007; Judd et al. 2008).

Arranging organisms into ‘natural groups’ is the primary goal of systematics, although there has not been unanimous agreement on what constitutes a ‘natural group’. The creation of monophyletic groups is the approach taken by most systematists (Bremer and Wanntorp 1978; Eldredge and Cracraft 1980; Baum 1989; de Queiroz and Gauthier 1992; Ebach et al. 2006; Judd et al. 2008). Others have argued that paraphyly is also an acceptable approach to classification (Mayr and Bock

2002; Brummitt 2003; Horandl 2007; Zander 2007), as it truly represents evolutionary processes, such as speciation. Paraphyletic groups have single ancestry but exclude some descendants of the most recent common ancestor (Horandl 2007). Paraphyletic groups are considered by most systematists to be 'artificial conveniences' rather than products of evolution (Ebach et al. 2006). Polyphyletic groups, which are unintentionally based on homoplasious characters, are not natural groups, as they do not share common ancestry. Regardless of a monophyletic or paraphyletic approach, the consensus for circumscription of natural taxa should be based on shared and derived characters. The validity of these natural groups is tested when new taxonomic additions can be added without changing the topology and relationships of the tree.

2.1.1 Practical applications of systematics

A systematic understanding of monophyletic groups enables predictions to be made about the ecological impact or uses of an organism (Judd et al. 2008). For example, hypocrealean fungi (Ascomycota) can have symbiotic associations with plants, animals and fungi (Spatafora et al. 2007). These organisms have applications for biological control of insects and fungi (Shah and Pell 2003; Meyling and Eilenberg 2007) and could be used in mutualistic host/endophyte interactions to improve crop growth (Clay and Schardl 2002). Ecological interactions of these fungi could be predicted when Spatafora et al. (2007) systematically revised this paraphyletic group.

Australia's quarantine policies relating to grain crops rest in part on developing an understanding of the systematics of smut fungi. It is important to know which taxa must be excluded from Australia and which taxa may be beneficial as biological control organisms on invasive, weedy grass species. For example, Cunnington and Shivas (2006) constructed a phylogeny of smut fungi to predict whether an exotic smut fungus would be an effective biocontrol agent and also whether it posed a threat to native grass species. Similar studies on the co-phylogenies of fungi and their hosts by Jackson (2004), Matsuda and Takamatsu (2003) and Refregier et al. (2008) offered improvements in biological control and quarantine approaches, and provided a more detailed understanding of co-evolution between fungi and their hosts. Systematics contextualises our knowledge of organisms and their evolution, allowing us to infer

evolutionary relationships and then apply this knowledge to conservation, biodiscovery and ecology (Judd et al. 2008).

2.2 Phylogenetic reconstruction

Phylogenetic trees are created to model the evolution of organisms (Page and Holmes 1998; Delsuc et al. 2005; DeSalle 2006). Molecular or morphological data can be incorporated to construct phylogenies as long as the characters employed are independent, homologous and variable among the taxa (Freeman and Herron 1998; Phillips 2006). Phylogenetic analysis of molecular data should incorporate homologous alignments (Lee 2001; Talavera and Castresana 2007) from orthologous loci (Maddison 1997). Paralogous genes result from gene duplication events and are avoided in phylogenetic reconstruction as these genes are subject to different selection pressures (Maddison 1997). Homologous data can be analysed using a variety of phylogenetic criteria depending on the requirements of the phylogeny and the type of data used to reconstruct the phylogeny.

2.2.3 Phylogenetic criteria

Phylogenetic trees can be reconstructed using different assessment criteria, for example parsimony (Weber et al. 2006), maximum likelihood (Harrison and Langdale 2006; Kosiol et al. 2006) and Bayesian analysis (Huelsenbeck and Ronquist 2001). Distance methods, such as neighbor joining and UPGMA, are less frequently used because they do not incorporate a model of evolution and once the data has been converted to distances, all phylogenetic information contained within a sequence is collapsed to a single value (Page and Holmes 1998).

2.2.3.1 Parsimony

Cladists traditionally used parsimony for phylogenetic tree reconstruction (Sober 1983). Parsimony is nonparametric, as it does not incorporate a model of evolution (Kolaczkowski and Thornton 2004); rather it assumes that evolution occurs with the least amount of changes and generates the most parsimonious tree or the tree with the fewest evolutionary steps (Page and Holmes 1998). Parsimony can be used to resolve trees with non-sequence data, to which parametric models cannot be applied.

Felsenstein (1978) first observed that parsimony is subject to long-branch attraction when taxon sampling is low and the dataset contains many polymorphisms. Long-branch attraction can create spurious sister group relationships based on homoplasious characters within a phylogenetic tree (Bergsten 2005). Parsimony is particularly prone to long-branch attraction when molecular sequence data are analysed (Bergsten 2005).

2.2.3.3 Maximum likelihood

In maximum likelihood, molecular data are analysed using a specified model of evolution, for example GTR gamma, which sets parameters for nucleotide substitutions and generates the most likely tree and branch lengths for the dataset (Page and Holmes 1998; Kosiol et al. 2006). Maximum likelihood algorithms have been improved to be computationally inexpensive (Stamatakis 2006), to search tree space effectively. Maximum likelihood cannot be used to analyze non-sequence data, as the models of evolution incorporate nucleotide changes only.

2.2.3.4 Bayesian inference

Bayesian inference is similar to maximum likelihood, in that it uses a model of evolution to analyze molecular data. The approach generates a single tree by sampling many trees over a range of parameters and determines the probabilities of individual relationships within the final consensus tree (Huelsenbeck and Bollback 2004). Prior information, such as tree topologies or branch lengths, can be included to aid tree reconstruction and this is one of the strengths of Bayesian inference (Huelsenbeck and Ronquist 2001). Yang and Rannala (2005) argued that the inherent priors in Bayesian analyses create stronger support values for branches that may be less supported by other statistical methods. It is important for systematists to understand that posterior probabilities derived in Bayesian inference provide a different measure of support than bootstrap values obtained from pseudo-replicates of a dataset analysed with maximum likelihood or parsimony methods (Yang and Rannala 2005).

Different loci or genes evolve at different rates (Maddison 1997; Page and Holmes 1998). Data obtained from several molecular loci can be partitioned in phylogenetic programs that use both maximum likelihood and Bayesian analyses. This accounts for different rates of evolution among loci (Huelsenbeck and Ronquist 2001; Stamatakis

2006). Morphological data can also be included in a Bayesian analysis, as no model of evolution needs to be specified.

2.2.4 Use of multiple molecular loci in phylogenetic reconstruction

2.2.4.1 Does the gene tree reflect the species tree?

Phylogenetic trees should provide an accurate hypothesis of the evolution of the taxa being investigated. The choices of outgroup, the phylogenetic assessment criterion, and the molecular loci used in the study, can all have a bearing on the final topology of the species tree. Genes may be duplicated, have undergone convergent evolution or have been transferred between individuals through processes other than reproduction (horizontal gene transfer). These events can lead to discrepancies between gene and species trees (Bull et al. 1993; Maddison 1997; Rokas et al. 2003; Degnan and Rosenberg 2006; Liu and Pearl 2007; Rosenberg and Tao 2008).

Rokas et al. (2003) demonstrated that varying phylogenies are produced using a range of different genes. They recommended that at least 8-20 concatenated genes be incorporated to obtain an accurate phylogeny, depending on the statistical support required. Rokas et al. (2003) did not suggest how loci should be selected for an analysis, but recommended use of many loci.

2.2.4.2 The multi-locus approach in fungal studies

Studies prior to Rokas et al. (2003) had advocated using multiple loci and combining different forms of data to incorporate as much information as possible (Bull et al. 1993; de Queiroz 1993; Doyle 1997; Hillis and Weins 2000). Rokas et al. (2003) made multi-locus phylogenies standard practice. Brito and Edwards (2008) concluded that the move to concatenation of multiple loci was inevitable, as researchers found there were not enough phylogenetically informative sites when only a single locus was analysed.

Several fungal phylogenies have been constructed using multiple loci. Blair et al. (2008) used seven molecular loci in their study of *Phytophthora* de Bary. They were unable to infer the phylogeny of the entire genus in their study of 82 taxa, but they

resolved relationships between closely related species and identified potential loci for molecular diagnostic analysis. Munkacsi et al. (2007) analysed five nuclear and mitochondrial loci to determine the coevolution between smuts and some important agricultural crops. They chose 'House-keeping' genes (i.e. genes required for normal metabolic function), which contained conserved regions for primer design and spanned polymorphic regions for differentiation of species. Fitzpatrick et al. (2006) were able to take advantage of publicly available genomes for 42 fungal taxa and constructed a phylogeny based on the entire genome. This practice, termed phylogenomics, is not an option for most groups of organisms in the immediate future and has the obvious disadvantage of being available only to taxa with sequenced genomes.

Other studies have incorporated the use of multi-gene phylogenies, but have used fewer than five loci (O'Donnell et al. 2000; Froslev et al. 2005; Kurtzman et al. 2005; Schoch et al. 2006; Tooley et al. 2006). Adding more data to single locus phylogenies will give a better indication of the evolution of a group and provide support in areas where there is low resolution with a single gene. This can be seen between the phylogenetic analyses of Stoll et al. (2003) and Stoll et al. (2005), where addition of extra taxa and loci provided further resolution of clades in a phylogenetic reconstruction of smut fungi.

2.2.5 Complications with multi-locus phylogenies

It was widely accepted that concatenating datasets by including several loci in a single analysis would eliminate problems associated with analysis of single genes (such as paralogy and deep coalescence) and provide a utilitarian phylogeny of all the data (Brito and Edwards 2008). Rokas and Carroll (2005) argued that concatenating sequences in phylogenetic analyses is preferable to adding extra taxa when resolving a phylogeny. However, it became clear that concatenating large amounts of molecular data can be unreliable, and the most 'democratic' tree produced in a consensus is not necessarily the 'most correct' one (Gatesy and Baker 2005; Degnan and Rosenberg 2006; Jeffroy et al. 2006; Rosenberg and Tao 2008; Wiens et al. 2008). With the ability to partition data within an analysis (Huelsenbeck and Ronquist 2001; Stamatakis 2006), concatenating datasets can reduce the phylogenetic information

available as it ignores the different rates of evolution of different loci.

2.2.6 Addition of taxa in phylogenetic reconstruction

Adding more taxa to phylogenies, rather than increasing the molecular dataset, may improve phylogenetic accuracy i.e. recovering a topology close to the true tree (Graybeal 1998; Pollock et al. 2002; Zwickl and Hillis 2002; Hedtke et al. 2006; Wiens 2006; Heath et al. 2008). Hedtke et al. (2006) experimented with a dataset similar to Rokas and Carroll (2005), but with increased taxon sampling. They found that more genes were required to achieve high bootstrap support with a low number of taxa, but conversely that fewer genes were required to resolve a well supported phylogeny with increased number of taxa.

2.2.7 Phylogenetically informative molecular loci for smut fungi

Molecular loci should be orthologous rather than paralogous, to ensure that homologous molecular regions are analysed instead of duplicated genes. These regions should be suitably conserved so that the loci will be amplified in all of the taxa. Ideally they should be interspecifically polymorphic (nucleotide variation among taxa) to be phylogenetically informative. Loci that have effectively resolved fungal phylogenies or that may have phylogenetic value are discussed below.

2.2.7.1 The ITS region

The internal transcribed spacer (ITS) is a non-translated region of nuclear ribosomal DNA (rDNA). Ribosomal DNA codes for the formation of ribosomal RNA and is present in high copy number in the cell (Eickbush and Eickbush 2007). The ITS region has been used to develop phylogenetic hypotheses for all classification levels of plants and fungi. Feliner and Rossello (2007) attributed the widespread use of ITS sequences for phylogenetic analyses to the availability of universal PCR primers (White et al. 1990), the manageable length and high copy number of the sequence in the cell (which simplifies PCR amplification) and the significant variation within parts of the sequence that often allows for species-level differentiation. The ITS region is the most likely candidate for a molecular barcode in the fungal kingdom, as it has been widely used in molecular studies (Seifert 2009).

Several studies have used the ITS region alone to infer evolutionary relationships (Almaraz et al. 2002; Stoll et al. 2003) but this can have limited success. For example, in three studies of members of the Basidiomycota, the ITS region was insufficient to resolve phylogenies because of a lack of polymorphic sites (Lieckfeldt and Seifert 2000a; Stoll et al. 2003; Begerow et al. 2006). Begerow et al. (2006) excluded half of the ITS region (due to non-homologous alignment) for phylogenetic reconstruction of classes in the Ustilaginomycotina, and the ITS region was unhelpful to Stoll et al. (2003) for their distinction of genera and species in the Ustilaginaceae.

The ITS region has a conserved secondary structure and this can be incorporated as a homologous character in phylogenetic analyses (Coleman 2003; Wolf et al. 2005; Schultz and Wolf 2009; Koetschan et al. 2010). Two studies have used ITS secondary structure to resolve the phylogeny of groups of fungi (Lieckfeldt and Seifert 2000b; Mullineux and Hausner 2009), with limited success.

2.2.7.2 GAPDH

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a 'housekeeping' enzyme involved with the breakdown of glucose in eukaryotic and prokaryotic cells (Smith and Leong 1990; Templeton et al. 1992). Several studies have incorporated GAPDH sequence data into their phylogenetic reconstruction of fungal taxa (Berbee et al. 1999; Poggler 1999; Myllys et al. 2003; den Bakker et al. 2004; Myllys et al. 2005; Munkacsi et al. 2007) as it is orthologous, conserved and has suitable levels of interspecific polymorphism (Myllys et al. 2003; Munkacsi et al. 2007). Munkacsi et al. (2007) concluded that there were lower levels of polymorphisms in GAPDH compared with other protein coding genes in their analysis, though Berbee et al. (1999) and Myllys et al. (2003) found that there were abundant synonymous substitutions of nucleotides, which were phylogenetically informative.

2.2.7.3 Beta Tubulin

β-tubulin is a protein responsible for cell architecture and other cytoplasmic functions (Iwasaki 1998). It has been used with mixed results in a number of recent phylogenetic studies of fungi (Thon and Royse 1999a, b; de Jong et al. 2001;

Begerow et al. 2004b; Miller and Huhndorf 2005; Tooley et al. 2006; Spatafora et al. 2007; Blair et al. 2008). β-tubulin was the most phylogenetically informative locus in the studies of Blair et al. (2008) on *Phytophthora*, de Jong et al. (2001) on Ascomycete wood fungi and Begerow et al. (2004b) on higher order classification in the Basidiomycota. However, Miller and Huhndorf (2005) and Thon and Royse (1999a) found it less phylogenetically informative than other genes in their respective studies on Ascomycetes and Basidiomycetes. β-tubulin is known to have undergone a gene duplication event in the Ascomycetes (Thon and Royse 1999b; Miller and Huhndorf 2005), but is considered orthologous for Basidiomycota (Begerow et al. 2004b).

2.2.7.4 Pathogenicity loci (ubc2 gene)

Pathogenicity loci encode the proteins important for the infection process of smut fungi. The cascade pathway that leads to smut penetration of the host is controlled by a group of virulence genes (Kronstad 1997; Durrenberger and Kronstad 1999; Kahmann et al. 1999; Mayorga and Gold 2001; Klosterman et al. 2008). The ubc2 gene, which is involved in the pathogenicity cascade pathway, is conserved in Basidiomycota (Klosterman et al. 2008) and could potentially be phylogenetically informative for smut and other fungi.

2.2.7.5 Mating loci (b locus)

Mating loci encode proteins that are required for filamentous growth after smut spore germination and also contribute to the infection process. In some smut species, it is necessary for two compatible mating types of hyphae to meet and conjugate before infection of a host plant can occur (Banuett 1995; Gold et al. 1997; Andrews et al. 2000; Abramovitch et al. 2002; Martinez-Espinoza et al. 2002; Schirawski et al. 2005; Bakkeren et al. 2006; Scherer et al. 2006). Two mating loci occur in *Ustilago maydis*: 'a', which has two alleles, and 'b', which is multi-allelic (Banuett 1995). Mating loci are conserved among the Basidiomycota. *Malassezia globosa* Midgley, E. Guého & J. Guillot, a member of the Ustilaginomycotina responsible for dandruff in humans, has orthologous genes to *Ustilago maydis* mating loci (Xu et al. 2007). Mating loci can be polymorphic and have the potential to be highly useful for phylogenetic analysis. Schirawski et al. (2005) found that there was 60-70% difference between amino acids

in mating loci of *Ustilago maydis* and *Sporisorium reilianum* (J.G. Kühn) Langdon & Full. As yet, no phylogenetic studies have applied mating loci in fungi.

2.2.7.6 COX3

Mitochondrial DNA, which is present in mitochondria, is often included in phylogenetic studies because of its simple genetic structure, rapid rate of mutation, low recombination rate, uniparental inheritance and independence from the nuclear genome (Moore 1995; Funk and Omland 2003; Mattern 2004; Mueller 2006; Brito and Edwards 2008). A multitude of protein coding genes are available for analysis in the mitochondrial genome, and Mueller (2006) summarized the applications of each locus. Cytochrome oxidase subunit 3 (COX3) is a mitochondrial gene that has been used for phylogenetic analyses of fungi (Kretzer and Bruns 1999; Munkacsi et al. 2007). Munkacsi et al. (2007) found high levels of interspecific polymorphism in COX3 in their multi-gene study on smut fungi, as did Kretzer and Bruns (1999) in their study of the Basidiomycota, order Boletales.

Despite the apparent benefits of using mitochondrial loci, phylogenies of nuclear and mitochondrial gene loci have sometimes been shown to be discordant. The mitochondrial genome can be subject to introgression (Ballard and Whitlock 2004), recombination (Galtier et al. 2009) and the mode of inheritance is unknown for many fungal and plant species (Barr et al. 2005). Furthermore, pseudogenes of mitochondrial DNA may be present in the nuclear genome of fungi and other organisms (Wright and Cummings 1983).

2.2.7.7 Loci used in the AFToL project

A multi-gene phylogeny called the Assembling the Fungal Tree of Life (AFToL) project was undertaken by systematists and mycologists to trace the evolution of all fungal organisms (Hibbett et al. 2007). As part of this project, James et al. (2006) used six molecular loci to trace higher classification levels of fungi. They used the ITS region and two other rDNA genes, elongation factor 1-α, and two RNA polymerase subunits (RPB1 and RPB2). Future phylogenetic studies on fungi would benefit from use of some of these genes so that data generated can be incorporated into future Fungal Tree of Life projects.

RPB2 has been a popular gene used in fungal phylogenetic studies and was also used in the AFToL project (Liu and Hall 2004; Froslev et al. 2005; Miller and Huhndorf 2005; Bischoff et al. 2006; Schoch et al. 2006; Matheny et al. 2007). It is an orthologous gene (Miller and Huhndorf 2005) that can resolve major clades of Basidiomycota at high and low taxonomic levels (Matheny et al. 2007) and it also has potential to resolve smut fungal clades.

2.2.8 Relevance of morphological data in fungal phylogenetic reconstruction

2.2.8.1 Molecules versus Morphology

Many types of data can be used for phylogeny construction, provided that the characters used are homologous (Poe and Wiens 2000; Delsuc et al. 2005). Amino acid or nucleotide sequences are frequently used because many characters can be scored (Hillis and Weins 2000), although non-sequence data (morphological, biogeographical and ecological data) are also informative for phylogenetic reconstruction. Some consider that phylogenetic trees generated with morphological data may be redundant, primitive or incongruent when compared with phylogenetic trees derived solely from molecular data (Scotland et al. 2003; Wortley and Scotland 2006). Most research that has compared the two approaches concluded that a combination of the two produced better phylogenetic reconstructions and strengthened support for branches (Hillis and Weins 2000; Wortley and Scotland 2006; Pisani et al. 2007).

Those advocating use of morphological data in phylogenetic reconstructions have argued this for several reasons (de Queiroz 2000; Hillis and Weins 2000; McDade 2000; Wiens 2000, 2003; Jenner 2004; Wiens 2004; Pisani et al. 2007). Hillis and Weins (2000) and Weins (2004) explained the advantages of using phylogenies based on morphological data. The most appealing quality of morphological data is that a thorough sampling of taxa can be performed because the data are readily available, inexpensive to generate, and can be examined from older and fossilized specimens. Studies based on morphological data are also able to identify hybridization events among taxa (McDade 2000); an advantage when recovering the evolutionary history of organisms capable of forming hybrids like plants and fungi.

There are some caveats when using morphological data. Character selection (de Queiroz 2000; Hillis and Weins 2000; Poe and Wiens 2000) and the character scoring method (Wiens 2000) can greatly influence the outcome of phylogenies. These arbitrary aspects of morphological phylogenies are open to criticism, as different researchers may obtain different phylogenies depending on their mode of character selection and assessment (Scotland et al. 2003). The criticisms raised against use of morphological data, particularly in regard to character homoplasy, circularity when reasoning final character homology, and difficulty in resolving closely related groups, also apply to molecular data (Poe and Wiens 2000). Wiens (2000) outlined the most effective methods for scoring morphological characters and others (de Queiroz 2000; Poe and Wiens 2000) have helped to standardise data collection for phylogenies based on morphological characters.

Wortley and Scotland (2006) conducted a statistical analysis of congruence between morphological and molecular phylogenies. They concluded that molecular data were superior in resolution and support of phylogenetic trees, but could be enhanced when combined with morphological data. Wortley and Scotland (2006) excluded morphological phylogenies that included more taxa than molecular phylogenies. However, this is one of the advantages of using morphological data because characters can be scored for taxa that lack molecular data.

2.2.8.2 Mapping morphological characters onto molecular phylogenies

Scotland et al. (2003) argued that instead of using morphological data to generate phylogenies, the data could be mapped onto phylogenies obtained from molecular data. This approach has been quite popular for studies of the fungal kingdom and gives a good indication of whether the selected morphological characters are useful in a classification scheme (Piepenbring et al. 1998b; Piepenbring et al. 1999; Schroers 2000; Lanfranco et al. 2001; Castlebury et al. 2005; Letcher et al. 2005; Miller and Huhndorf 2005; Stoll et al. 2005; Kemler et al. 2006; Letcher et al. 2006; Garnica et al. 2007; Muggia et al. 2008). Despite wide use of this method, there is no enhancement of robustness or accuracy in the molecular phylogeny produced when morphological characters are merely mapped onto an existing phylogeny (Jenner

2004; Wiens 2004).

2.2.8.3 Phylogenies based on morphological data are rarely used for fungi

While morphological phylogenies have been generated for higher classification levels of fungi (McLaughlin et al. 1995; Tehler 1995; Hibbett and Binder 2002; Hibbett 2004), these studies are sparse when compared with the recent morphological phylogenies created for plants and animals. One reason for the limited number of morphological phylogenetic studies of fungi may be the limited number of phenotypic characters that can be used.

2.2.8.4 Potential morphological characters in the Ustilago-Sporisorium-Macalpinomyces *complex*

Smut fungi in the *Ustilago-Sporisorium-Macalpinomyces* complex are usually differentiated by host specificity, spore size, spore ornamentation and the presence or absence of spore balls or columellae (Vánky 2002). All of these characters can be visualized with the naked eye or via use of a light microscope. To date, no research has included a morphological phylogenetic analysis of smut fungi, although morphological characters have been scrutinized intensely and mapped onto reconstructed phylogenies (Vánky 1991; Bauer et al. 1997; Piepenbring et al. 1998c, a).

Characters such as spore ornamentation (viewed with a scanning electron microscope) and spore secondary ornamentation, growth in culture, *in situ* appearance on host and host plant ecology have not to date, been used for phylogenetic analysis of smut fungi. Castlebury et al. (2005) mapped spore ornamentation and host classification onto a molecular phylogenetic tree of *Tilletia* and observed that these characters could be synapomorphic. Stoll et al. (2005) mapped the presence or absence of columellae, peridia, sterile cells and spore balls, and the sorus structure and location on the host onto phylogenies for the *Ustilago-Sporisorium-Macalpinomyces* complex. They did not determine any morphological synapomorphies. Stoll et al. (2005) did not use morphological data in their phylogenetic reconstructions and so these characters have not been 'tested' systematically. A phylogeny generated using data that includes characters of the host-parasite relationship as well as details of smut structure could

provide important phylogenetic information to complement a molecular phylogeny.

2.3 An introduction to smut fungi

Smut fungi (class Ustilaginomycetes, phylum Basidiomycota) predominantly infect grasses, with some species posing a biosecurity threat to Australia and other grain-growing countries. Two examples are *Sporisorium scitamineum* (sugarcane smut) and *Tilletia indica* Mitra (Karnal bunt of wheat), which both have serious quarantine implications for Australia (Pascoe et al. 2005; Croft and Braithwaite 2006; Vánky and Shivas 2008). Murray and Brennan (1998) estimated that introduction of Karnal bunt to Australia would cost the wheat industry approximately $491 million per year through loss of markets from quarantine restrictions and a downgrading in the quality and value of exported grain.

Smuts are recognized primarily by the black spore mass that consume the inflorescence (flower cluster) of their hosts, although infections can also develop on leaves (*Entyloma* de Bary), roots (*Entorrhiza* C.A. Weber) and stems (*Pericladium* Pass.) (Vánky 2002). Smuts can cause fruit galls, swollen ovaries or form fungal-derived sori that can replace the entire host inflorescence (Piepenbring 2004). Infection may lead to the destruction of some or all seeds or fruits, as well as stunting and atrophy of the entire plant (Piepenbring 2004).

The biology of *Ustilago maydis* (DC.) Corda (corn smut), a model plant pathogen, has been studied extensively and its genome has now been fully sequenced (Martinez-Espinoza et al. 2002). The life cycle of *Ustilago maydis* is well known and can be generalised for other smut taxa, for example *U. cynodontis* (Pass.) Henn. (Figure 1). Other smut fungi classified in the family Ustilaginaceae can grow saprophytically in an anamorphic (mitotic) state (Figure 1), or parasitically in host plants and form a teleomorphic (meiotic), teliospore/spore forming stage (Figure 1).

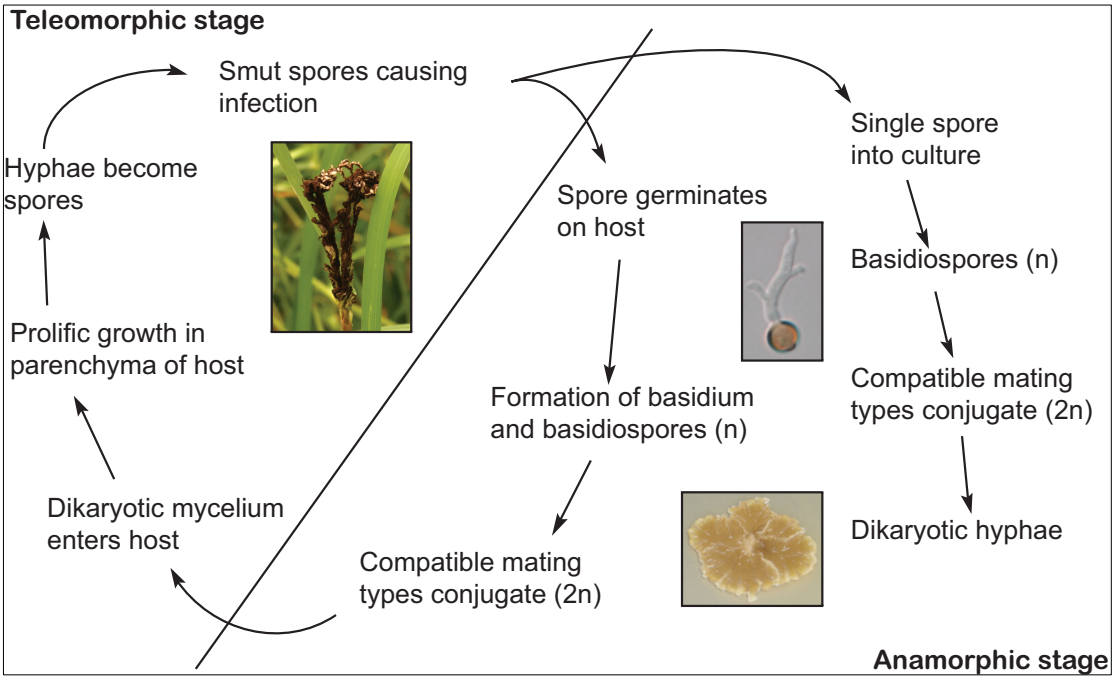

Fig. 1. The smut life cycle typified by *Ustilago cynodontis*.

2.3.1 The current understanding of smut systematics

2.3.1.1 Basidiomycota (Phylum)

The 'Assembling the Tree of Life' (AToL) project is a collaboration between many organizations around the world and aims to establish the evolutionary relationships of all living things (CIPRES 2007). Fungi are a significant, monophyletic clade in the 'tree of life' and the AFToL project aims to resolve higher and lower-order fungal classification (Lutzoni et al. 2004; Hibbett et al. 2007). Higher order fungal taxa such as the two largest phyla, Ascomycota and Basidiomycota, have been established as monophyletic groups (Bruns et al. 1992; Swann and Taylor 1993, 1995; Lutzoni et al. 2004; James et al. 2006; Hibbett et al. 2007). The Basidiomycota are further divided into three monophyletic subphyla; macroscopic mushrooms (Agaricomycotina), rusts (Pucciniomycotina) and smuts (Ustilaginomycotina) (Swann and Taylor 1993; Begerow et al. 2004b; Aime et al. 2006; Bauer et al. 2006; Begerow et al. 2006; Matheny et al. 2007).

2.3.1.2 Ustilaginomycotina (Sub-phylum)

The first classification of the Ustilaginomycotina by the Tulasne brothers in 1847 was based on light microscopic examination of teliospore germination and resulted in the

description of two smut families, Ustilaginaceae and Tilletiaceae (Vánky 2002). Bauer et al. (1997) and Bauer et al. (2006) expanded on this pioneering work by using transmission electron microscopy to examine hyphal septation and host-parasite interactions. They identified synapomorphies shared by all fungi included in the Ustilaginomycotina (namely a dominance of glucose in fungal cell walls and enlarged host interaction zones). Molecular phylogenetic analyses of the ribosomal Large Sub-Unit (LSU) coding region by Begerow et al. (1997), and later by Begerow et al. (2006) using a multi-gene systematic analysis of five protein coding genes, supported the classification of the 'smut' fungi (Ustilaginomycotina) by Bauer et al. (1997) based on ultrastructural characteristics.

Within the subphylum Ustilaginomycotina proposed by Begerow et al. (2006) there are three accepted classes (Entorrhizomycetes, Exobasidiomycota and Ustilaginomycetes) and 11 orders (Entorrhizales, Ceraceosorales, Georgefischeriales, Tilletiales, Malasseziales, Microstromatales, Entylomatales, Doassansiales, Exobasidiales, Urocystales and Ustilaginales). There is evidence that the Entorrhizomycetes may not belong within the Ustilaginomycotina (Matheny et al. 2006).

2.3.1.3 Ustilaginomycetes (Class)

The Ustilaginomycetes are an ecologically and morphologically diverse group. Numerous studies have demonstrated that the group is monophyletic (Swann and Taylor 1995; Bauer et al. 1997; Begerow et al. 2004b; Bauer et al. 2006; Begerow et al. 2006; Matheny et al. 2007). The orders Ustilaginales and Tilletiales are separated at class level (classified in the Ustilaginomycetes and Exobasidiomycota, respectively). Both orders contain taxa that parasitize the inflorescences of their Poaceae hosts and form black powdery spores. Piepenbring et al. (1998b; 1998c) suggested that this case of apparent convergent evolution has occurred more than once in the evolution of smut fungi. Another case of convergent evolution is observed between the Ustilaginales and the Microbotryales (sub-phylum: Pucciniomycotina). Taxa in both orders have similar life cycles and soral morphology, and were originally grouped together in the Ustilaginomycetes (Vánky 1998). The Microbotryales were shown by Bauer et al. (1997), Begerow et al. (1997), Roux et al.

(1998) and Bauer et al. (2006) to be more closely related to rust fungi than smuts and they were reclassified in Pucciniomycotina.

2.3.1.4 Ustilaginales (Order)

In the currently accepted higher-level classification of the Basidiomycota, the most taxon-rich group is the smut fungi included in the order Ustilaginales (Vánky 2002). Bauer et al. (1997) hypothesized monophyly of the Ustilaginales after ultrastructural examination. They concluded that the Ustilaginales shared synapomorphies in having enlarged host interaction zones, no septal pores and intracellular hyphae within the host, but did not examine these characteristics in a phylogenetic analysis. Begerow et al. (2006) were able to further discriminate taxa in the Ustilaginales with a systematic molecular analysis of rDNA, protein-coding DNA regions and ultrastructural analysis. They transferred *Mycosyrinx* G. Beck, a genus lacking septal pores that had been grouped by Bauer et al. (1997) in the Ustilaginales, to a sister group in the Ustilaginomycotina, the Urocystales.

2.3.1.5 Ustilaginaceae (Family)

Vánky (2001) discussed the taxonomic status of the 21 genera within the Ustilaginaceae that had been emended by Bauer et al. (1997) based on ultrastructure. Vánky (2001) proposed seven new families, which he deemed groups according to phenotype, to accommodate genera that did not conform to the type species of the family, *Ustilago*. This morphological classification was supplemented and revised by Begerow et al. (2006), who rejected six of Vánky's seven families and found support for a single family using phylogenetic molecular analysis of nuclear ribosomal and protein-coding DNA. The Ustilaginaceae now contains 10 teleomorphic (taxa with a sexual stage) genera and one anamorphic (taxa without a known sexual stage) genus, *Pseudozyma* Bandoni emend. Boekhout. The recently described genus, *Parvulago* (Bauer et al. 2007), is considered to belong to the Ustilaginaceae according to the classification scheme proposed by Bauer et al. (2001). Under the morphological classification proposed by Vánky (2001a), it would not be included in the Ustilaginaceae as it does not resemble *Ustilago*.

2.3.1.6 Designation of genera within the Ustilaginaceae

Typically smut genera are described on features that may be dependant on host

anatomy (Holton et al. 1968), as there are often more characteristics of "systematic value" found in hosts when compared with parasites in general (Begerow et al. 2004a). These features include characters of the smut sorus such as the presence or absence of a peridium (a combined fungal and host envelope around sorus) or a columella (a column of smut spores and host material within the sorus); spore ball formation, the colour and consistency of spore masses and the presence or absence of sterile cells (Vánky 2002; Piepenbring 2004). Sometimes the definitions of the sorus morphology have been open to interpretation, as discussed by Stoll et al. (2005), who found that three different authors had differing soral descriptions of the same organism.

Vánky (2001; 2002) recognized 14 genera in the Ustilaginaceae. Molecular studies have reduced some of the monotypic genera to synonymy with older genera, for example *Lundquistia* Vánky to *Sporisorium* (Stoll et al. 2005), and other monotypic genera have been erected, including *Anomalomyces* (Vánky et al. 2006) and *Parvulago* (Bauer et al. 2007).

Morphological features that were determined to be non-homologous (such as spore balls) or that are dependent on host anatomy are no longer considered legitimate characters for recognizing distinct genera (Vánky 2001; Begerow et al. 2004b). One taxonomic group of fungi that remains problematic to taxonomists is the *Ustilago-Sporisorium-Macalpinomyces* complex. The characters used to define the genera overlap and form a heterogeneous 'continuum' (Vánky 2002). Some smut species possess characteristics of all three genera and cannot be classified reliably. The chronological history of the systematics of the *Ustilago-Sporisorium-Macalpinomyces* complex is presented in Chapter 3.

This review has outlined the relevence of systematics for applications in biosecurity and biological control, and the importance of clear and communicable taxonomic classifications. It has discussed the priciples of different phylogenetic assessment criteria and the advantages of including a variety of data for phylogenetic reconstruction. Finally it has reviewed the systematics of smut fungi, and the different approches taken to obtain the current classification.

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Chapter 3: Toward a systematic resolution of the *Ustilago-Sporisorium-Macalpinomyces* complex

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Abstract

The fungal genera *Ustilago, Sporisorium* and *Macalpinomyces* represent an unresolved complex. Taxa within the complex often possess characters that occur in more than one genus, creating uncertainty for species placement. Previous studies have indicated that the genera cannot be separated on morphology alone. A combined molecular and morphological approach is required to identify the synapomorphic characters needed to define the new classification. We conclude that the type genera of *Ustilago*, *Sporisorium* and *Macalpinomyces* need to be redescribed explicitly and new genera, based on monophyletic groups, need to be established to accommodate taxa that no longer belong to *Ustilago* or *Sporisorium*.

Key Words

Ustilaginaceae, systematics, smut fungi

3.1 Introduction

Three genera of smut fungi (Ustilaginomycotina), *Ustilago* (Pers.) Roussel, *Sporisorium* Ehrnb. ex Link and *Macalpinomyces* Langdon & Full. contain about 540 described species (Vánky in press). The three genera belong to the family Ustilaginaceae, a group that mostly infect grasses (Begerow et al. 2006), have intracellular hyphae and produce phragmobasidia after germination of the teliospore (Bauer et al. 2001; Begerow et al. 2006). *Ustilago, Sporisorium* and *Macalpinomyces* were shown to form a monophyletic group within the Ustilaginaceae after molecular phylogenetic analysis (Begerow et al. 1997; Stoll et al. 2003; Stoll et al. 2005; Begerow et al. 2006), ultrastructural examination of spores and characterization of host parasite interactions (Bauer et al. 1997).

Many taxa within *Ustilago, Sporisorium* and *Macalpinomyces* share two or more morphological characters indicative of the different genera. This makes taxonomic placement of species within genera problematic. The original characters used to identify genera were not sufficiently robust to encompass the full morphological diversity of novel species that have since been discovered. Taxa within *Ustilago, Sporisorium* and *Macalpinomyces* are part of a systematically unresolved complex (Vánky 2002a; Stoll et al. 2003; Piepenbring 2004; Stoll et al. 2005; Vánky et al.

2006; Vánky and Shivas 2008). Two further genera, *Melanopsichium* Beck and *Anomalomyces* Vánky, M. Lutz & R.G. Shivas, are considered to be distinct, well-defined members of this complex.

Attempts to reconcile the taxonomy of this complex using either morphology (Vánky 1991; Piepenbring et al. 1998) or molecular phylogenetics (Stoll et al. 2003; Stoll et al. 2005) have been unsuccessful. This paper reviews chronologically changing generic concepts in the *Ustilago-Sporisorium-Macalpinomyces* complex and presents an approach for resolving systematic anomalies.

3.2 Taxonomic history

3.2.1 *Ustilago*

Ustilago, derived from the Latin *ustilare* (to burn), was named by Persoon (1801) for the blackened appearance of the inflorescence in infected plants, as seen in the type species *U. hordei* (Pers.) Lagerh. According to Clinton (1906), Persoon adopted the name *Ustilago* from Johann Bauhin's 1651 edition of *Historia plantaum universalis*. Persoon (1801) created *Ustilago* as a subgenus of *Uredo* in his *Synopsis Methodica Fungorum*. He described *Uredo*, now classified within the rust sub-phylum Pucciniomycotina (Aime et al. 2006), as lacking a peridium and having spores that were powdery, loose, uniform and mostly globose. *Ustilago*, now classified in the smut subphylum Ustilaginomycotina, was separated from *Uredo* by possessing black to brown powdery spores that parasitize plant inflorescences. *Ustilago* was promoted to the level of genus by Roussel (1806). *Ustilago* became a catch-all genus for a diversity of smut fungi. Many taxa currently regarded as belonging to *Microbotryum* Lév, *Tilletia* Tul. & C. Tul., *Cintractia* Cornu, *Anthracoidea* Bref., *Macalpinomyces* and *Sporisorium* were originally described as members of *Ustilago*.

Léveillé (1847) established *Microbotryum* for three species of *Ustilago* found in the inflorescence of hosts in the angiosperm family Caryophyllaceae. *Microbotryum* differed from *Ustilago* by possessing sori with branched filaments and swollen apices (Vánky 1998a). Ultrastructural (Bauer et al. 1997) and molecular data (Begerow et al. 1997) confirmed that smuts occurring in the inflorescences of the Caryophyllaceae

formed a monophyletic group, distinct from *Ustilago*. *Microbotryum* is now considered to be a member of the rust sub-phylum Pucciniomycotina (Begerow et al. 1997; Aime et al. 2006).

In 1847, the Tulasne brothers introduced a classification of smut fungi based on spore germination. They divided smut fungi into two groups, the Ustilaginaceae and Tilletiaceae (cited in Vánky 2002a). Taxa that were grouped by the Tulasne brothers in Tilletiaceae are now considered to belong to the Exobasidiomycetes, a class proposed by Bauer et al. (1997) as part of their classification of the Ustilaginomycotina. This division, which was originally based on germination pattern, separated *Tilletia* from *Ustilago*.

Cornu separated *Cintractia* from *Ustilago* in 1883 on the basis that it had firmly agglutinated spores that were liberated on maturity. *Cintractia* and other related smut genera found on Cyperaceae and Juncaceae are placed in a monophyletic group, the Anthracoideaceae, separate from *Ustilago* (Begerow et al. 2006).

Two attempts have been made to subdivide *Ustilago*, although the proposed classifications have not been widely accepted. Firstly, Brefeld (1912) proposed the genus *Mycosarcoma* for *Ustilago maydis*. Brefeld (1912) based *Mycosarcoma* on the structure of the peridium, incubation time in the host, localized infection and development of aerial conidia. Generic placement of *Ustilago maydis* within the complex is contentious (Piepenbring et al. 2002; Stoll et al. 2005) and until the complex is resolved, this taxon is best left within *Ustilago* because of its importance as a model organism.

Another attempt to subdivide *Ustilago* was made in 1949 by the mycologist Tchen Ngo Liou, who considered that the basidia of *U. esculenta* differed from the type species of *Ustilago* (cited in Piepenbring et al. 2002). Liou erected the genus *Yenia*, with *Y. esculenta* as the type, and transferred seven additional *Ustilago* species into the new genus (Liou 1949). Vánky (2002a) considered that the eight taxa Liou selected differed widely in their biology, soral structure, spore morphology and germination patterns, and he suggested that they did not constitute a natural group.

Piepenbring et al. (2002) sought to define the 'true' generic position of *U. esculenta* and in their single-locus phylogenetic analysis, *U. esculenta* was sister to 21 species of *Ustilago* and *Sporisorium*. Piepenbring et al. (2002) accepted that *U. esculenta* belonged in a separate genus to *Ustilago*. Stoll et al. (2005) did not support the separation of *U. esculenta* from *Ustilago* on the basis of a molecular phylogenetic analysis, which included this and 97 other *Ustilago, Sporisorium* and *Macalpinomyces* species.

Beck (1894) introduced the genus *Melanopsichium* for a taxon first described as *Ustilago austro-americanum* Speg. on *Polygonum*. The genus was characterised by compact, hard, irregularly lobed galls in the inflorescence, stems and leaves (Halisky and Barbe 1962; Vánky 2002a). Begerow et al. (2004) and Stoll et al. (2005) concluded that *Melanopsichium* represented an example of a host jump from Poaceae to Polygonaceae, as *M. pennsylvanicum* Hirschh. belonged to the *Ustilago* clade. Begerow et al. (2006) consequently rejected the family Melanopsichiaceae proposed by Vánky (2001a).

Langdon and Fullerton (1975) studied the soral ontogeny of six *Ustilago* species. Their revised concept of *Ustilago* included taxa that colonised host plants with hyphae that destroyed parenchymatous tissue to then become spores, without forming fungal peridia, columellae, sterile cells or spore balls (Langdon and Fullerton 1975).

The gross morphology of *Ustilago* is variable (Fig. 2). Piepenbring (2004) recorded 14 different soral morphologies for *Ustilago* in her treatise of the sori found in the Ustilaginomycotina. Some taxa, such as *U. sparsa* Underw. and *U. trichophora* (Link) Kunze, occurred as localised galls on the host plant, inducing hypertrophied ovaries rather than a burnt inflorescence. *Ustilago altilis* Syd. and *U. esculenta* Henn. infected the culms of the host, and some species occurred in the leaves, for example *U. striiformis* (Westend.) Niessl and *U. calamagrostidis* (Fuckel) G.P. Clinton. Vánky (2002a) considered *Ustilago* as occurring solely on hosts in the Poaceae, accepting 174 species (Vánky in press).

Fig. 2. Diversity of soral morphology in *Ustilago*. Photos b, c, e with permission from K. Vánky. a. *Ustilago spinificis* on *Spinifex lonifolius*. b. *Ustilago xerochloae* on *Xerochloa barbata*. c. *Ustilago drakensbergiana* on *Digitaria tricholaenoides*. d. *Ustilago tritici* on *Triticum astevium*. e. *Ustilago bouriqueti* on *Stenotaphrum dimidatum*. f. *Ustilago altilis* on *Triodia* sp. g. *Ustilago phragmitis* on *Phragmites karka*. h. *Ustilago cynodontis* on *Cynodon dactylon*.

3.2.2 *Sporisorium*

Ehrenberg described *Sporisorium* in a letter to Link, based on a collection he had made of *S. sorghi* Ehrenb. ex Link (Link 1825). *Sporisorium* was described as being unique because it possessed columellae of equal length with the glumes, formed from agglutinated spores and mutilated floral parts. It also had sterile partitioning cells in groups or chains and a peridium (Link 1825; Langdon and Fullerton 1978). Ehrenberg's original 1825 collection of *S. sorghi* from Egypt was lost (Langdon and Fullerton 1978) and a neotype of *S. sorghi* has since been established (Vánky 1990).

Four years after the description of *Sporisorium*, Rudolphi (1829) described the confusingly named *Sorosporium* from *Saponaria officinalis* in the Caryophyllaceae. Many authors subsequently chose *Sorosporium* for smut taxa with peridia and spore balls occurring on Poaceae. *Sporisorium* appears to have been overlooked for about 150 years after it was first used, until Langdon and Fullerton (1978) re-established the name. Mycologists continued to describe new species as members of *Ustilago* or

Sorosporium. Many of these species have since been reclassified in *Sporisorium*. For example, *Sporisorium* contains at least 60 taxa previously classified as *Ustilago*, and at least 170 taxa originally described as *Sorosporium* (Robert et al. 2005).

Lavrov (1936) and Ciferri (1938) divided the genus *Sorosporium* into two subgenera depending on whether they infected hosts in Poaceae or Caryophyllaceae (cited in Vánky 2002a). Langdon and Fullerton (1975) noted that *Sorosporium* species on Poaceae differed in soral ontogeny and structure to species on Caryophyllaceae, essentially because *Sorosporium* on Caryophyllaceae lacked a well-defined sorus. Langdon and Fullerton (1975) suggested that smuts occurring on Poaceae should be grouped in a separate genus, but did not make any taxonomic revisions at that stage. Vánky (1998b) considered *Sorosporium* to be a synonym of *Thecaphora* Fingerh. after an examination of the types of both genera revealed no essential differences.

Sphacelotheca de Bary was established in 1884 for *Sphacelotheca hydropiperis* (Schumach.) de Bary on *Polygonum*. de Bary (1884) defined *Sphacelotheca* as having a membrane or peridium enclosing the spores and a columella (cited in Langdon and Fullerton 1978). Clinton (1902) transferred ten taxa from *Ustilago* to *Sphacelotheca*, including *Sporisorium sorghi*, which he referred to as *Ustilago sorghi* Link. Clinton did not mention *Sporisorium*, but he attributed the authorship of *U. sorghi* to Link, indicating that he was aware of *Sporisorium* as an earlier described genus. Aside from a brief mention of the characters of *Sphacelotheca*, Clinton gave no reason why the ten taxa would be better suited to *Sphacelotheca*. Clinton's transferral of taxa in *Sporisorium* to *Sphacelotheca sensu* Clinton was precedent for over 110 subsequent descriptions of species of *Sphacelotheca* on grasses (Robert et al. 2005).

Langdon and Fullerton (1978) ascertained that the columellae formed in *Sphacelotheca* species on Polygonaceae and Poaceae were not homologous. *Sphacelotheca* formed a columella from fungal cells adhering to one another on hosts in the Polygonaceae, whereas columellae were derived from host material in the Poaceae. They also noted differences in the peridium and the development of the spore mass between *Sphacelotheca* in the Polygonaceae and Poaceae. *Sphacelotheca*

occurred only on hosts in the Polygonaceae and has been shown by Bauer et al. (1997) to belong to the Microbotryales in the Pucciniomycotina.

Fig. 3. Diversity of soral morphology in *Sporisorium*. a. *Sporisorium cenchri-elymoidis* on *Cenchrus elymoidis*. b. *Sporisorium cryptum* on *Yakirra* sp. c. *Sporisorium heteropogonicola* on *Heteropogon contortus*. d. *S. bothriochloae* on *Dichanthium sericium*. e. *Sporisorium tumefaciens* on *Chrysopon* sp. f. *Sporisorium iseilematis-ciliati* on *Iseilema* sp. g. *Sporisorium themedae* on *Themeda triandra*. h. *Sporisorium aristidicola* on *Aristida* sp. i. *Sporisorium likhitekerajae* on *Ischaemum* sp. j. *Sporisorium doidgeae* on *Capillipedium parviflorum*. k. *Sporisorium sacchari* on *Saccharum*, l. *Sporisorium scitamineum* on *Saccharum officinarum*. m. *Sporisorium caledonicum* on *Heteropogon contortus*. n. *Sporisorium ischaemi* on *Iscahemum indicum*. o. *Sporisorium holwayi* on *Andropogon bicornis*.

Langdon and Fullerton (1978) resurrected *Sporisorium* after showing that *Sphacelotheca* and *Sorosporium* were not suitable genera for smut fungi on grasses. They designated a new type specimen of *Sporisorium sorghi* from an Australian collection on *Sorghum leiocladum* Hack., which Vánky (1990) believed represented

S. cruentum (J.G. Kühn) Vánky. Vánky (1990) proposed a second neotype from an Egyptian collection of *S. sorghi*, and described *S. australasiaticum* Vánky & R.G. Shivas based on the neotype originally proposed by Langdon and Fullerton (Vánky and Shivas 2001).

Langdon and Fullerton (1978) outlined the characteristics of *Sporisorium* based on their neotype of *Sporisorium sorghi*. Characters of importance included a "hyphal peridium, columella composed of host tissues and hyphae, and spores intermixed with partitioning (sterile) cells". These characters are variable among other *Sporisorium* taxa (Fig. 3).

The varied appearances of the peridia, columellae, sterile cells, and dimorphic spores in *Sporisorium* led to different morphological interpretations by mycologists. For example, Langdon and Fullerton (1975) described the presence of a columella in *Sporisorium consanguineum* (Ellis & Everh.) Vánky, but it was later reported absent by Vánky and Shivas (2008). A columella was not described by Langdon (1962) in *Ustilago porosa*, but this species was regarded as having one by Vánky and Shivas (2001). The presence or absence of columellae, peridia, sterile cells and dimorphic spores has formed the taxonomic boundary between *Sporisorium* and *Ustilago*, and interpretations of these structures must be consistent before the complex can be resolved.

Another character used to define *Sporisorium* was that spores were compacted in permanent (or semi-permanent) spore balls (Vánky 2002a; Vánky and Shivas 2008). This character was regarded as analogous in the Ustilaginomycotina (Vánky 1998c) and does not occur across all taxa in *Sporisorium*. Vánky (in press) recognized 326 species of *Sporisorium*.

3.2.3 *Macalpinomyces*

Langdon and Fullerton (1977) erected *Macalpinomyces* to accommodate *M. eriachnes* (Thüm) Langdon & Full., which they described as distinct from *Sporisorium* and *Ustilago*. *Macalpinomyces* lacked columellae, produced sterile cells and the spores

were uniformly ornamented and polyangular or subpolyangular (Langdon and Fullerton 1977; Vánky 1996).

The nomenclatural history of *M. eriachnes* epitomizes the confusion caused by many taxa in the *Ustilago-Sporisorium-Macalpinomyces* complex. The original collection of *M. eriachnes* in Australia by the botanist Ferdinand von Mueller, was divided and sent to two mycologists, Mordecai Cooke in England and Felix von Thümen in Germany. Two new fungal taxa were described based on this single collection, *Sorosporium eriachnis* Thüm. (1878) and *Ustilago australis* Cooke (1879) (Langdon and Fullerton 1977). Langdon and Fullerton (1977) later erected *Macalpinomyces* to accommodate this species nearly a century after the specimen was first described.

Vánky (1996) broadened the concept of *Macalpinomyces* to include taxa that shared morphological features of both *Sporisorium* and *Ustilago*, notably taxa that lacked a columella but possessed sterile cells. This led to numerous taxonomic combinations, for example *M. bursus* (Berk.) Vánky, *M. neglectus* (Niessl) Vánky and *M. spinulosus* (L. Ling) Vánky. The broadened concept of *Macalpinomyces* allowed for a variety of gross morphologies to be included, ranging from localised or systemic galls in the ovaries, to longitudinally hypertrophied sori up to 16cm long in *M. chrysopogonicola* (Mundk. & Thirum.) Vánky (Fig. 3).

Molecular phylogenetic analysis has shown that *Macalpinomyces* is polyphyletic, with the type species (*M. eriachnes*) sister to all other taxa in the complex, and forming a monotypic genus within the Ustilaginaceae (Stoll et al. 2005). Begerow et al. (2006) in their phylogenetic study of the Ustilaginomycotina, proposed that *M. eriachnes* might not belong to the Ustilaginaceae as it did not occur in the clade containing *Sporisorium*, *Ustilago* and *Moesziomyces* Vánky.

Species of *Macalpinomyces* have sterile cells, a peridium derived from host material, and lack true spore balls (Vánky in press). Vánky (in press) accepted 46 species of *Macalpinomyces*.

Fig. 4. Diversity of soral morphology in *Macalpinomyces*. a. *Macalpinomyces ewartii* on *Sorghum timorense*. b. *Macalpinomyces arundinellae-setosae* on *Arundinella setosa*. c. *Macalpinomyces mackinlayi* on *Eulalia mackinlayi*. d. Spores of *Macalpinomyces mackinlayi*. e. *Macalpinomyces siamensis* on *Coelorachis striata* (photo with permission of K. Vánky). f. *Macalpinomyces eriachnes* on *Eriachne helmsii*. g. Spores of *Macalpinomyces eriachnes*. Scale d, f = 10 µm.

3.2.4 Relationships within the *Ustilago-Sporisorium-Macalpinomyces* complex

Taxa within the *Ustilago-Sporisorium-Macalpinomyces* complex often possess morphological characters that occur in more than one genus. Overlapping characters create uncertainty for species placement, as illustrated by *Macalpinomyces eriachnes*, which was independently placed in both *Ustilago* and *Sporisorium*. In a comprehensive taxonomic study over the course of eight years, Vánky (1996, 1997, 1998d, 2001c, 2002b, 2003b, a, 2004b, a) and Vánky and Shivas (2001, 2003) combined over 30 smut species that possessed a combination of *Sporisorium* and *Ustilago* characters into *Macalpinomyces*. Taxonomic shuffling occurred later with many species described before 1978 as *Ustilago* and that were subsequently moved to either *Macalpinomyces* or *Sporisorium*. The result is that many taxa have been moved back and forth among genera without systematic assessment that they constituted natural, monophyletic groups.

New genera have been raised for some smuts that differed subtly from the type descriptions of *Ustilago, Sporisorium* and *Macalpinomyces*. *Endosporisorium* Vánky (1995), *Lundquistia* Vánky (2001b), *Anthracocystis* Brefeld (1912) and *Yenia* (Liou 1949) are examples of genera that were proposed to subdivide *Ustilago* and *Sporisorium*. The description of new genera or placement of taxa in poorly defined genera, has contributed to systematic confusion within the complex.

Vánky (1995) described *Endosporisorium* to accommodate *Macalpinomyces chrysopogonicola* and three other smut taxa. This genus differs from *Ustilago* in having sterile cells and ephemeral spore balls, and from *Sporisorium* in lacking columellae and a fungal derived peridium. The sori of *Endosporisorium* were described as occurring in the stems rather than the inflorescence. After Vánky (1996) had emended *Macalpinomyces* to encompass more taxa, he subsequently synonymised *Endosporisorium* with *Macalpinomyces*, preferring to have a large, well-delimited genus, rather than many monotypic and closely related genera (Vánky 1997).

Vánky (2001b) erected *Lundquistia* for five taxa, three of which were renamed from either *Sporisorium* or *Ustilago*. *Lundquistia* differed from *Ustilago* in having spore balls; from *Sporisorium* in lacking sterile cells, a peridium and columellae, and from *Macalpinomyces* in lacking sterile cells and having spore balls. Molecular phylogenetic analyses showed that *Lundquistia* was a synonym of *Sporisorium* as it occurred in the *Sporisorium* clade (Cunnington et al. 2005; Stoll et al. 2005). Cunnington et al. (2005) included four *Lundquistia* species in their phylogenetic analysis using the ITS region and demonstrated that it was a polyphyletic group. Vánky (2001) described *Lundquistia* as lacking true columellae, whereas, Piepenbring (1999) considered the fascicular vascular bundles mixed with fungal material as columellae in *Sporisorium panici-leucophaei* (Bref.) M. Piepenbr. (= *Lundquistia panici-leucophaei* Vánky).

Brefeld (1912) described *Anthracocystis* for a smut on *Panicum miliaceum*, which is now known as *Sporisorium destruens* (Schltdl.) Vánky. He considered it different from *Ustilago* due to the peculiar formation of its soral peridium, which developed

from the floral envelopes. Soral structures such as columella and spore balls were not included in the diagnosis by Brefeld (1912). Vánky (2002) erroneously considered *Anthracocystis* as *nomen nudum*, which is an invalid name according to the *International Code of Botanical Nomenclature*, Vienna Code (available at http://ibot.sav.sk/icbn/main.htm). *Anthracocystis* is a validly published name, as it contained a diagnosis and was described in 1912, which is before 1935 when Latin was required in taxonomic descriptions.

Vánky et al. (2006) described *Anomalomyces* as a monotypic genus with shared characters of *Ustilago*, *Sporisorium* and *Macalpinomyces*, but with a unique partitioning of the sorus and two types of sterile cells. They established a new genus based on the peculiar morphology and a phylogenetic analysis that placed *Anomalomyces* in a polytomy with the *Sporisorium* groups and the *Ustilago* group occurring on pooid grasses. *Anomalomyces* differed from *Ustilago* by possessing a peridium, spore balls and sterile cells, but did not fit into *Sporisorium* as it lacked a columella. It differed from *Macalpinomyces* by possessing genuine spore balls.

Some species fit unambiguously into *Sporisorium* and *Ustilago*. Molecular phylogenetic analysis has shown many morphologically similar smut species to be sister to the types of *Sporisorium* and *Ustilago* (Stoll et al. 2005). *Macalpinomyces* was resolved as a monotypic genus containing the type species (Stoll et al. 2005). The difficulty with the *Ustilago-Sporisorium-Macalpinomyces* complex has been that many species do not fit strictly within the boundaries of the genera as defined by the types. To resolve this problem, the genera *Ustilago* and *Sporisorium* must be redescribed explicitly and new genera, based on monophyletic groups, must be established to accommodate taxa not included by the emended genera.

3.3 Determining a natural classification for the *Ustilago-Sporisorium-Macalpinomyces* complex

Studies based on spore and ultrastructural morphologies were unable to resolve fully the *Ustilago-Sporisorium-Macalpinomyces* complex (Vánky 1991; Piepenbring et al. 1998). Langdon and Fullerton (1975) studied soral ontogeny to separate *Sporisorium* (as *Sorosporium*) and *Ustilago*. Molecular phylogenetic analyses based on rDNA

showed that there were several monophyletic groups within the *Ustilago-Sporisorium-Macalpinomyces* complex, but there was no correlation between these groups and their morphological traits (Stoll et al. 2003; Stoll et al. 2005). Stoll et al. (2005) noted strong evidence that smuts had co-evolved with their grass hosts, as sister taxa usually occurred on closely related grasses.

Stoll et al. (2005) examined the columellae, peridia, sterile cells, spore balls and host plant tribe or sub-tribe of the taxa included in their molecular phylogenetic analysis of the *Ustilago-Sporisorium-Macalpinomyces* complex. They mapped the characters onto the hypothesized phylogeny, but none appeared consistently within the monophyletic groups. They concluded that soral morphology was unsuitable for delimiting genera and for resolving groups in the *Ustilago-Sporisorium-Macalpinomyces* complex. Stoll et al. (2005) were unable to propose a classification based on the monophyletic groups observed within the complex.

3.3.1 Taxa in the *Ustilago-Sporisorium-Macalpinomyces* complex should not be unified under *Ustilago*: a case study with smuts on *Themeda*

Themeda belongs to the grass tribe Andropogoneae in the subfamily Paniceae. It is parasitized by 17 species in the *Ustilago-Sporisorium-Macalpinomyces* complex, which represent four types of soral morphology (Fig. 4). Several taxa such as *Sporisorium themedae* (Duke) Vánky (Fig. 2g), *S. exsertum* (McAlpine) L. Guo and *S. benguetense* (Zundel) L. Guo (Fig. 4a), infect all the spikelets in an inflorescence, but leave the inflorescence architecture otherwise intact. These species also possess stout or woody columellae. *Sporisorium anthistiriae* (Cobb) Vánky (Fig. 4b) and *S. holstii* (Henn.) Vánky infect individual spikelets in an inflorescence. Species such as *Sporisorium enteromorphum* (McAlpine) Vánky (Fig. 4c) and *S. langdonii* Vánky, destroy entire racemes with sori that consist of several filiform columellae. *Macalpinomyces bursus* (Berk.) Vánky (Fig. 4d) occurs localised in hypertrophied ovaries.

Fig. 5. Four smuts that occur on *Themeda*. a. *Sporisorium benguetense*. b. *Sporisorium anthistiriae*. c. *Sporisorium enteromorphum*. d. *Macalpinomyces bursus*.

Vánky (2001a, 2002a) and Piepenbring (2004) believed one of two approaches were needed to resolve the *Ustilago-Sporisorium-Macalpinomyces* complex. The first was to synonymise all of the genera under the earliest name, *Ustilago*, and the second was to split the three genera into smaller genera and subgenera. To unify the smuts on *Themeda* under one genus would provide a natural classification, albeit not a very useful one. To put them into groups based on what appear to be convergent characters would exacerbate taxonomic problems within the complex.

There has been a view that host anatomy dictates the soral morphology of smut taxa (Piepenbring 2004; Stoll et al. 2005). Holton et al. (1968) argued that gross morphology was determined by genotypic or 'inherently permanent' factors. To an extent, the gross morphology of an infection will be influenced by environmental factors (Fullerton 1975), but as in the case of the smuts on *Themeda*, the morphology of the sorus will be distinctive for different species rather than dependant on the structure of the grass.

A diverse range of soral morphologies occur in taxa in the *Ustilago-Sporisorium-Macalpinomyces* complex on many other andropogonoid grasses, for example in *Bothriochloa*, *Sorghum* and *Heteropogon*, which are host to 15, nine and eight smuts, respectively. If soral morphology is synapomorphic, it will be possible to distinguish genera based on soral characteristics. We consider that this diversity necessitates the recognition of new genera or subgenera, rather than unification of current genera in the complex.

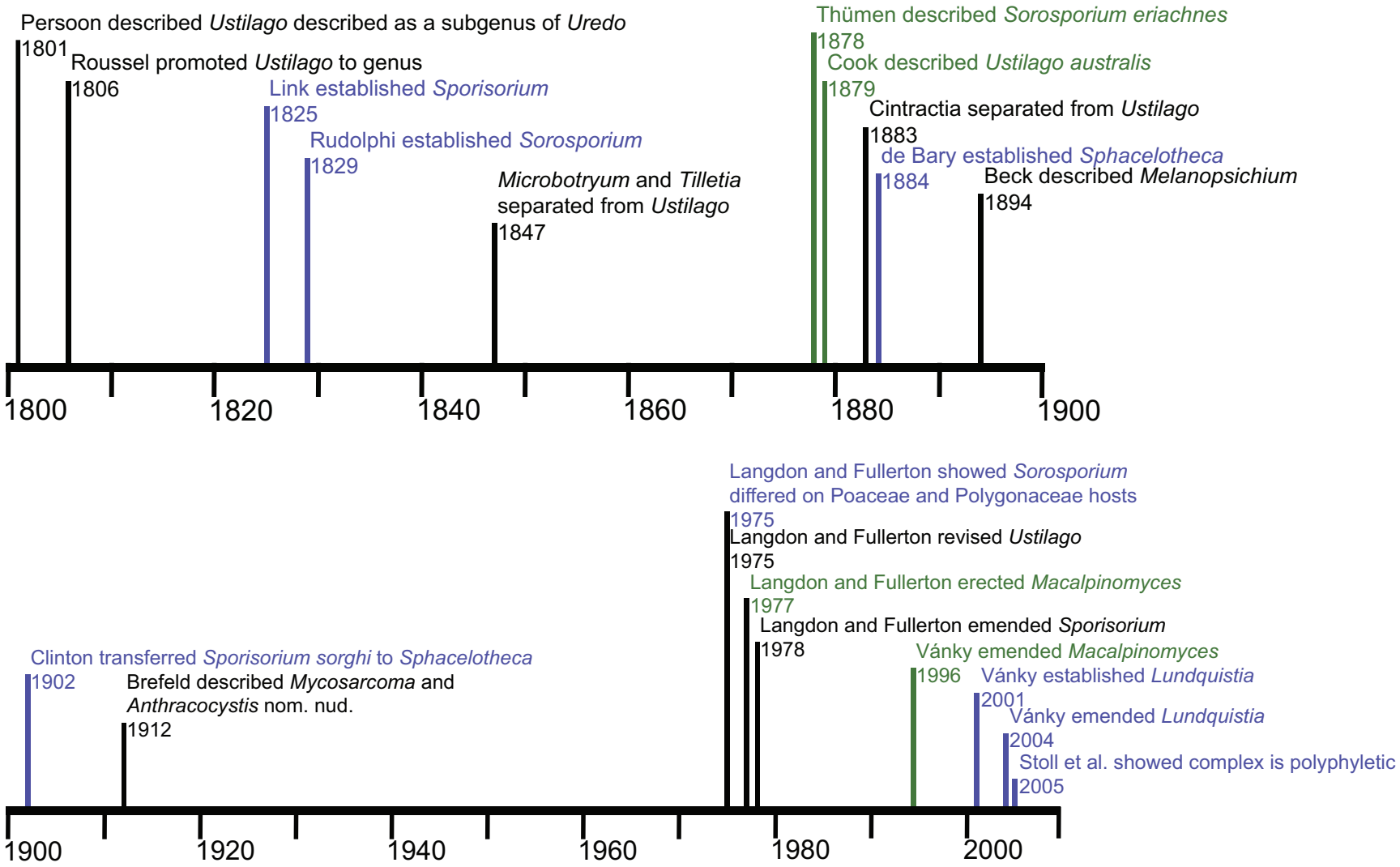

Fig. 6. Timeline of work on the *Ustilago-Sporisorium-Macalpinomyces* complex. Black = *Ustilago*; blue = *Sporisorium*; green = *Macalpinomyces*.

3.4 Conclusion

3.4.1 Solutions to the *Ustilago-Sporisorium-Macalpinomyces* complex

It has been approximately 200 years since the genera *Ustilago* and *Sporisorium* were first described (Fig. 5). These genera contain a diversity of taxa that do not strictly conform to the original genus descriptions. In particular, the genus *Macalpinomyces* contains many species that have specific characters from both *Sporisorium* and *Ustilago*. The taxonomic confusion within the *Ustilago-Sporisorium-Macalpinomyces* complex represents the first knowledge gap addressed in the current study.

Vánky (2002a), Stoll et al. (2005) and Vánky et al. (2006) suggested that analysing additional molecular loci could resolve the *Ustilago-Sporisorium-Macalpinomyces* complex. To create a meaningful taxonomy it is important to relate synapomorphic characters to monophyletic groups (Mooi and Gill 2010). Resolution of the complex will depend on a combined analysis of morphology and molecular characters.

Inclusion of morphological data will help to determine synapomorphies that can be used to define groups within the *Ustilago-Sporisorium-Macalpinomyces* complex. To accomplish this, a more detailed examination of the soral structures and their development is warranted. Langdon and Fullerton (1975) identified different soral development patterns in several *Sporisorium* species, but lacked the advantage of molecular phylogenetic analysis on which to base a new classification. Stoll et al. (2005) examined the presence or absence of columellae and peridia. The structure of these characters may prove to be synapomorphic. Dismissing characters considered to be homoplasious, for example spore balls, as a means to delimit genera in the Ustilaginaceae is premature. It is possible that spore balls have evolved independently within monophyletic groups in the *Ustilago-Sporisorium-Macalpinomyces* complex. Because there are limited morphological characters that can be examined it is necessary to include all the available characters to determine their systematic potential. The unknown state of character homology within the *Ustilago-Sporisorium-Macalpinomyces* complex is the second knowledge gap addressed by the current study.

Generic concepts of *Ustilago*, *Sporisorium* and *Macalpinomyces* have been refined over the last 30 years, although they still remain polyphyletic genera. The diversity of taxa within the complex requires further delimitation rather than unification of all smuts under *Ustilago*. *Ustilago*, *Sporisorium* and *Macalpinomyces* need to be revised and a new classification established based on the synapomorphic characters found in monophyletic groups.

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Chapter 4: *Macalpinomyces mackinlayi*

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Statement of Joint Authorship

Title: *Macalpinomyces mackinlayi*

Authors: Alistair McTaggart and Roger Shivas

Alistair McTaggart: Collected, analysed and interpreted data and wrote the manuscript.

Roger Shivas: Provided mentoring role for description of new species and offered editorial comments during development of the manuscript.

Fig. 7. Drysdale River floodplain, Western Australia; sori in ovaries of *Eulalia mackinlayi*; spores and sterile cells; spore wall and sterile cells seen in SEM. Scale bars (from top to bottom) = 1 cm, 10 µm, 5 µm.

Abstract

Macalpinomyces mackinlayi is described from *Eulalia mackinlayi*, an endemic grass from north-western Australia. This study highlighted that *Macalpinomyces* is polyphyletic.

4.1 *Macalpinomyces mackinlayi* McTaggart & R.G. Shivas, sp. nov.

Sori in nonnullis ovariis inflorescentiae, longe cylindrici, 10 – 35 × 1.0 –1.5 µm, primo virides tum cinerei. Sporae globosae, subglobosae vel late ellipsoideae, 9 –13 × 8 –12 µm, luteobrunneae; paries aequalis, dense opertus conicis spi- nis 1–2 µm altis. Cellulae steriles in catervis irregularibus, cellulae singulae globosae, subglobosae, ellipsoideae, 5.5–10.0 × 4.5–8.0 µm, hyalinae; paries aequalis, ca. 0.3 µm, levis.

Etymology. Derived from the host epithet.

Sori in some ovaries of an inflorescence, hypertrophied, long- cylindrical, sometimes twisted, 10–35 × 1.0–1.5 mm wide, initially green becoming grey from the apex downwards, with reddish brown remnants about 2 mm long of the host pericarp at the apex, rupture longitudinally exposing the powdery spore mass mixed with sterile cells. Spores globose, subglobose or broadly ellipsoidal, 9–13 × 8–12 µm, yellowish brown; wall even, densely covered in conical spines 1–2 µm high. Sterile cells in large, loose, irregular groups; individual cells globose, subglobose, ellipsoidal or slightly irregular, 5.5 –10.0 × 4.5 – 8.0 µm, hyaline; wall even, c. 0.3 µm thick, smooth.

Typus. AUSTRALIA, Western Australia, c. 35 km north of Drysdale River, alt. c. 380 m, 15° 23' 13" S, 126° 16' 58" E, *Eulalia mackinlayi*, 10 May 2009, A.R. McTaggart, V.L. Challinor, M.J. Ryley, C.E. Gambley, T. Scharaschkin, M.D.E & R.G. Shivas, BRIP 52549, holotype; ITS sequence GenBank GU014817, MycoBank MB515252. Paratypus, Western Australia, between King Edward River crossing and Mitchell Falls, 10 May 2009, A.R. McTaggart, V.L. Challinor, M.J. Ryley, C.E. Gambley, T. Scharaschkin, M.D.E & R.G. Shivas, BRIP 52546.

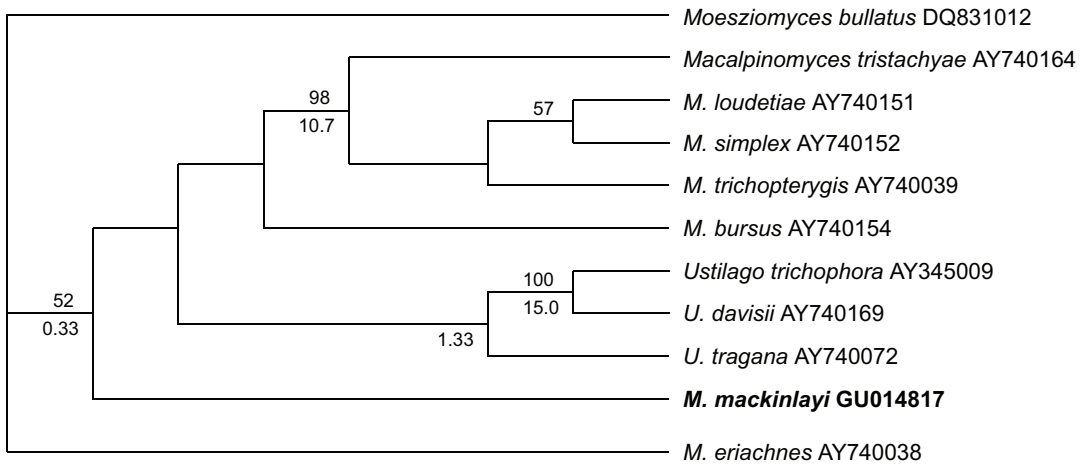
4.2 Notes on *Macalpinomyces mackinlayi*

Macalpinomyces is a polyphyletic genus comprised of many species referable to either *Ustilago* or *Sporisorium* (Stoll et al. 2005). *Macalpinomyces* is represented in Australia by 12 taxa (Vánky & Shivas 2008). *Macalpinomyces mackinlayi* is best placed in *Macalpinomyces* until the *Ustilago-Sporisorium-Macalpinomyces* complex can be resolved. It lacks columellae, typically present in *Sporisorium* and has sterile cells, which are not diagnostic of *Ustilago*. It is morphologically similar to other *Macalpinomyces* species that have sterile cells, hypertrophied sori derived from host material, and densely echinulate spores, e.g. *M. arundinellae-setosae*, *M. tubiformis* and *M. siamensis*. *Macalpinomyces mackinlayi* occurs on *Eulalia mackinlayi*, which is only known from the Mitchell Plateau region in north-western Australia. Eight *Sporisorium* species have been recorded on *Eulalia*, seven of which destroy the entire inflorescence or all the spikelets in an inflorescence. *Sporisorium trispicate* has localised sori and can be distinguished from *M. mackinlayi* by the white sorus derived from fungal cells, the presence of spore balls and the verrucose rather than echinulate spores.

BLASTn results of an ITS sequence from *Macalpinomyces mackinlayi* (GU014817) had high identity with sequences from *M. tristachyae* on *Loudetiopsis chrysothrix* (GenBank: AY740164, 96 % identical over 90 % query coverage), *M. bursus* (as *Sporisorium bursum*) on *Themeda quadrivalvis* (GenBank: AY740154, 94 % identical over 90 % query coverage), *Ustilago trichophora* on *Echinochloa colona* (GenBank: AY345009, 94 % identical over 83 % query coverage) and *M. loudetiae* on *Loudetia flavida* (GenBank: AY740151, 91 % identical over 90 % query cover- age). Genomic DNA of *M. mackinlayi* (holotype) is stored in the Australian Biosecurity Bank (http://www.padil.gov.au/pbt/).

Analysis of the ITS region from *Macalpinomyces mackinlayi* and some closely related taxa from GenBank in an exhaustive parsimony search using PAUP v4.0b4 yielded a single tree (TL = 696; CI = 0.838; RI = 0.552; RC = 0.462). Bootstrap values from 1000 replicates are shown above nodes and decay indices shown below nodes. The species described here is printed in **bold** face. The tree was rooted to *Moesziomyces*

bullatus (GenBank DQ831012), a known outgroup of the *Ustilago-Sporisorium-Macalpinomyces* complex (Stoll et al. 2005). A maximum likelihood analysis resolved a similar tree topology, except that *M. mackinlayi* was sister to the *M. tristachyae* clade. This tree highlights that *Macalpinomyces* is a non-monophyletic group.



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Chapter 5: Phylogenetic utility of molecular and morphological data for determining monophyletic groups in a complex of smut fungi

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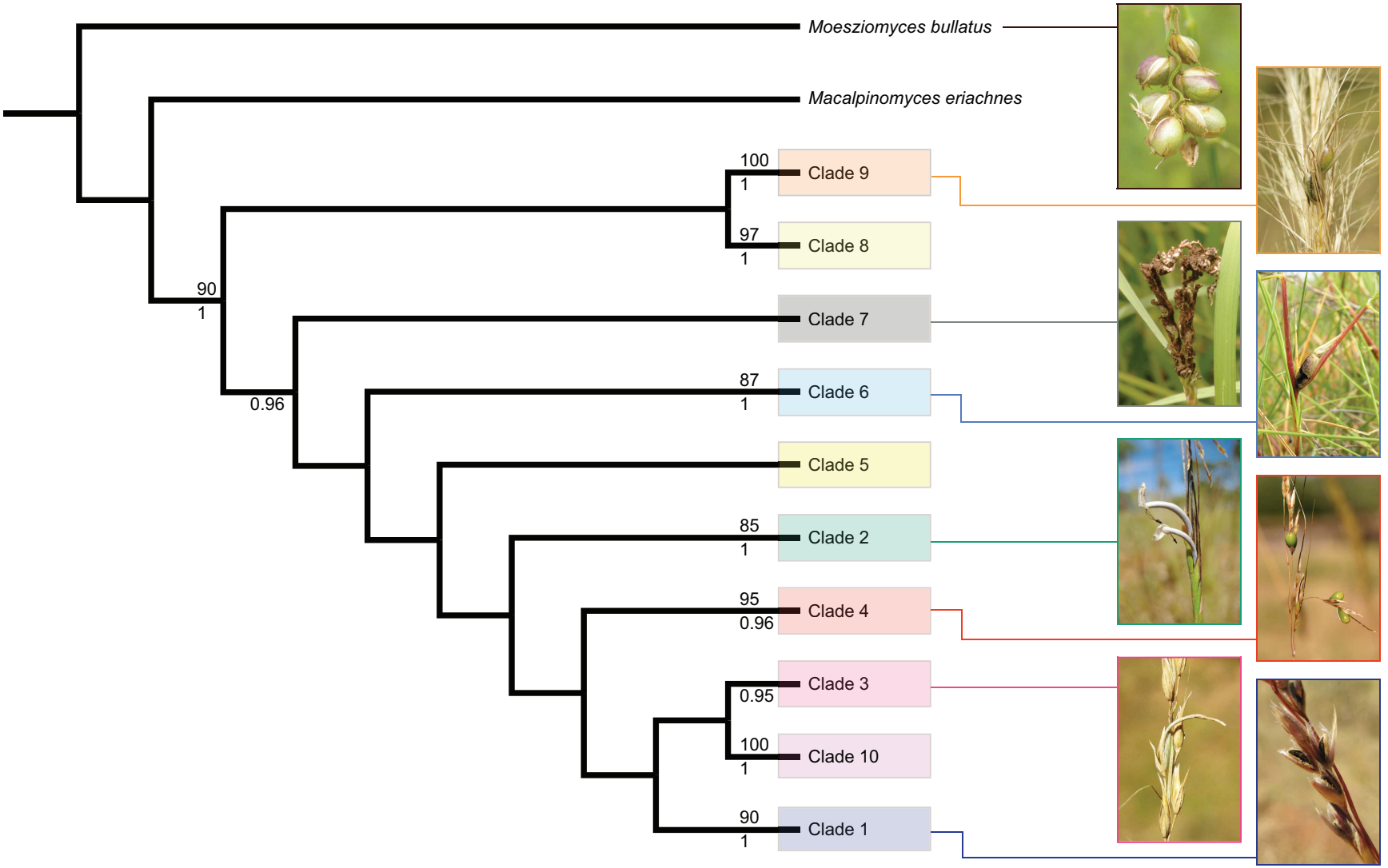
Andrew Geering: Contributed to development of manuscript.

Ben Callaghan: Provided laboratory assistance and provided editing of the manuscript.

Tanya Scharaschkin: Provided mentorship for phylogenetic methods and contributed to the writing and editing of the manuscript.

Image abstract



Abstract

Sequences of four nuclear (internal transcribed spacer, large subunit, elongation factor and glyceraldehyde 3-phosphate dehydrogenase) and one mitochondrial locus (cytochrome c oxidase subunit 3), morphological data and inferred internal transcribed spacer region 2 (ITS2) rRNA secondary structures were used in a super-matrix to reconstruct the phylogenetic relationships of 137 taxa in a monophyletic complex of smut fungi. Phylogenetic trees derived using maximum likelihood and Bayesian inference consistently recovered ten monophyletic groups within the complex. Relationships among the ten clades were not defined. Relationships among taxa generated from the mitochondrial locus were incongruent with results from the nuclear loci, and the morphological and secondary structure data did not add further support to the inferred phylogenetic relationships. Morphological homology within the *Ustilago-Sporisorium-Macalpinomyces* complex can be re-evaluated in light of this study, thereby enabling a reclassification of the genera based on morphological synapomorphies within the recovered groups.

Key Words

Ustilaginaceae, systematics, polytomy, secondary-structure, alignment curation

5.1 Introduction

Smut fungi (Ustilaginomycotina) such as corn smut (*Ustilago maydis*), sorghum smut (*Sporisorium sorghi*) and sugarcane smut (*Sporisorium scitamineum*), are important pathogens of agricultural crops. Economic losses to farmers are caused by colonisation of the inflorescence by the fungus and replacement of the grain with a powdery, black spore mass. Three genera of smut fungi, *Ustilago*, *Sporisorium* and *Macalpinomyces*, comprise approximately 530 species, which together form a monophyletic group within the family Ustilaginaceae (Bauer et al., 1997; Begerow et al., 1997; Begerow et al., 2006; Stoll et al., 2003; Vánky, 2002, in press). Morphological characters are inadequate in delimiting the three genera, as many taxa possess characters that overlap generic descriptions (Vánky, 2002). Taxa that possess one or more characters usually associated with each of *Ustilago*, *Sporisorium* or *Macalpinomyces* cannot be placed confidently into a genus. The *Ustilago-*

Sporisorium-Macalpinomyces clade remains an unresolved complex due to these insufficiently defined genera.

Attempts to resolve the *Ustilago-Sporisorium-Macalpinomyces* complex using morphological and developmental studies have been unsuccessful in identifying robust, unambiguous characters to define the genera (Langdon and Fullerton, 1975; Piepenbring et al., 1998; Vánky, 1991). Molecular phylogenetic studies that sought to determine groups within the *Ustilago-Sporisorium-Macalpinomyces* complex have essentially highlighted the polyphyly of the three genera and the problematic nature of the morphological characters used to delimit them (Cunnington et al., 2005; Piepenbring et al., 2002; Stoll et al., 2005; Stoll et al., 2003; Vánky et al., 2006). These studies utilised nuclear ribosomal DNA sequences (rDNA) analysed with Bayesian inference or parsimony as phylogenetic assessment criteria. The most comprehensive examination of the complex included 97 smut taxa in a Bayesian analysis of two nuclear rDNA loci (Stoll et al. 2005). Morphological characters were then mapped onto the inferred phylogenetic tree, but were considered homoplasious as they varied greatly within and among monophyletic groups. Morphology was considered inadequate to define systematic groups within the *Ustilago-Sporisorium-Macalpinomyces* complex (Piepenbring, 2004; Stoll et al., 2005).

Morphological phylogenetic analyses have not been widely used in systematic studies of fungal genera and species. There are few phenotypic characters to incorporate (Taylor et al., 2000) and they can be subject to convergent evolution (Berbee and Taylor, 1992). To date, morphological data have not been included in phylogenetic analyses of the *Ustilago-Sporisorium-Macalpinomyces* complex.

The present investigation sought to identify well-supported monophyletic groups within the *Ustilago-Sporisorium-Macalpinomyces* complex through combined phylogenetic analyses of five molecular loci, rRNA secondary structure and morphological data in a super-matrix of 137 taxa. The effects of increased taxon sampling and using different loci and phylogenetic assessment criteria were explored. The monophyletic groups recovered in the final topology will be able to be used to identify morphological synapomorphies and delimit current genera, and establish new genera or sub-genera within the complex.

5.2 Materials and Methods

5.2.1 Taxon selection

Taxa were selected to represent the main groups recovered in previous studies (Stoll et al. 2003; Stoll et al. 2005), with increased sampling of under-represented groups, for example *Macalpinomyces* and smut fungi occurring on *Aristida*. In total, this study included 137 species (14 species of *Macalpinomyces*, 81 species of *Sporisorium* and 39 species of *Ustilago*), 35 of which had not previously been evaluated in other phylogenetic analyses (Appendix 1). Two additional taxa included in the dataset were *Anomalomyces panici* and *Melanopsichium pennsylvanicum*, both distinctive members of the complex. *Moesziomyces bullatus* was included as an outgroup to the complex based on a relationship reported in Stoll et al. (2005).

5.2.2 Morphological characters

Character and character state selection were based on taxonomic descriptions in monographs of the Ustilaginomycotina (Vánky 1994; Vánky and Shivas 2008; Vánky in press) and from direct observation of 61 Australian species. A thorough examination of seven characters was included in the morphological analysis. The character states were scored according to Wiens (2000). Characters were all unweighted; missing and ambiguous characters or characters showing intraspecific variation between descriptions were scored as polymorphic. Nine characters were considered originally, two of which were excluded in the final dataset. The excluded characters were ornamentation of spore walls and growth in culture. Ornamentation was assessed using SEM images of spores and scored according to the classification of spore morphology proposed by Vánky (1991). Ornamentation of the spores was variable within the sori and could not be accurately scored. Growth in culture was obtained for less than 20 taxa and also could not be scored accurately. The final data matrix consisted of seven characters. These characters and their states are presented in Appendix 2.

5.2.3 DNA Extraction

DNA was extracted from 120 smut specimens, representing 92 taxa, by a combination of enzymatic and mechanical lysis. Smut sori or spores were mechanically lysed in a Qiagen Tissue Lyser with 0.5 mm beads, then shaken at 55ºC overnight in SNES buffer (0.01 M sodium phosphate pH 7.6, 0.15 M sodium chloride, 0.005 M EDTA, 1% SDS) containing proteinase K at a final concentration of 0.8 μg/ml. The purification was then completed using a Gentra Puregene kit according to the manufacturer's instructions.

5.2.4 Selection of molecular loci

Five loci were targeted for amplification and sequencing. A mitochondrial gene, cytochrome c oxidase subunit 3 (COX3), two nuclear housekeeping genes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and elongation factor 1α (EF1α), and two nuclear rDNA genes, internal transcribed spacer (ITS) and the large subunit (LSU) region. Nuclear rDNA loci have been used widely in fungal systematics (White et al. 1990). ITS and LSU loci were used by Stoll et al. (2005) in their study of the *Ustilago-Sporisorium-Macalpinomyces* complex. COX3 and GAPDH were both used by Munkacsi et al. (2007). COX3 and GAPDH were chosen due to their high and low rates of interspecific polymorphism respectively observed by Munkacsi et al. (2007). EF1α was included as it has been used for phylogenetic reconstruction in the Assembling the Fungal Tree of Life project (http://aftol.org/).

5.2.5 PCR and sequencing

Genomic DNA was amplified by PCR with high fidelity Phusion® DNA Polymerase (Finnzymes) using the manufacturer-specified cycling and reaction conditions. PCR primers and annealing temperatures are shown in Table 1. PCR products were purified by ethanol precipitation using standard methods (Maniatis et al. 1982). Purified PCR product was sent to Macrogen Korea and AGRF Queensland for sequencing using the forward and reverse primers from amplification. ABI sequence trace files were assembled using ContigExpress® (Invitrogen™). The 165 novel sequences have been deposited in GenBank (Appendix 1).

Table 1. PCR amplification and sequencing primers used in this study

Locus	Primer name	Region	Annealing temp °C	Sequence 5'	Source
ITS	MITS	rDNA	58	GGTGAACCTGCAGATGGATC	(Stoll et al. 2003)
	ITS4			TCCTCCGCTTATTGATATGC	(White et al. 1990)
LSU	LR0R	rDNA	58	ACCCGCTGAACTTAAGC	AFTOL
	LR5			TCCTGAGGGAAACTTCG	AFTOL
COX3	COX3F	Mitocho-	60	WGTTACACCKAGYCCWTGGC	This study
	COX3R	ndrial		TAGGAATAGCCAAACWACATC	This study
GAPDH	GAPDHF	Nuclear	65	CGGTCGTATCGGMCGTATC	This study
	GAPDHR			GTARCCCCACTCGTTGTCGTA	This study
EF1α	EF1αF	Nuclear	62	GCCCTMTGGAAGTTCGAGACYCCCA	This study
	EF1αR			GAYACCGACAGCRACGGTCTG	This study

5.2.6 Alignment of sequences

Sequence alignments were undertaken in the MEGA software package (Kumar et al. 2008) using the Muscle algorithm (Edgar 2004). Alignments of protein coding loci (COX3, GAPDH and EF1α) were converted to amino acid sequences in MEGA. The original and curated nucleotide alignments have been deposited as Nexus files in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11013). The super-matrix consisted of 134 ITS sequences, 91 LSU sequences, 52 COX3 sequences, 32 EF1α sequences and 35 GAPDH sequences.

5.2.6.1 Curation of alignments

Alignments were uploaded to Phylogeny.fr (available at http://www.phylogeny.fr/) (Dereeper et al. 2008) and curated in Gblocks to remove poorly aligned positions and divergent regions (Talavera and Castresana 2007). All alignments were trimmed as follows: ITS from 1140 nucleotides, including gaps, to 448 nucleotides with no gaps; LSU from 609 to 593 nucleotides; COX3 from 680 to 649 nucleotides; EF1α from 935 to 926 nucleotides; GAPDH from 1158 to 769 nucleotides. The final curated super-matrices consisted of 3385 nucleotides for the nucleotide data set, and 1041 nucleotides and 748 amino acids for the protein coding data set. The final nucleotide super-matrix was composed of approximately 55% missing data.

5.2.6.2 Secondary structure alignment of ribosomal RNA

Alignment of secondary structure folding in the ITS2 region was undertaken using the method described by Schultz and Wolf (2009). ITS2 secondary structure was predicted for all taxa in the study using the ITS2 database (available at:

http://its2.bioapps.biozentrum.uni-wuerzburg.de/), which compares ITS2 sequences with the known structure of a homologous ITS2 sequence (Wolf et al. 2005; Koetschan et al. 2010). The 4SALE program (Seibel et al. 2008) was used to align the secondary structure models obtained from the ITS2 database. ITS2 secondary structure alignment was then incorporated into the curated ITS dataset and included as a separate partition.

5.2.7 Phylogenetic analyses

Two phylogenetic assessment criteria were implemented, Bayesian inference using MrBayes (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) and maximum likelihood using RAxML (Stamatakis 2006). Resulting trees were observed with FigTree (available at http://www.tree.bio.ed.ac.uk/software/figtree/). Data and command files for both Bayesian and RAxML analyses and the resulting trees, are available at TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11013).

5.2.7.1 Partitions

The five loci were included as separate partitions in the maximum likelihood and Bayesian analyses so that each locus could be run under different optimal model parameters. Analyses were conducted on both curated and non-curated alignments. The ITS2 region was removed from the ITS dataset so that the secondary structure alignment could be included as a separate partition in a maximum likelihood analysis. Amino acid data from protein coding loci (COX3, GAPDH and EF1α) were included as separate partitions in maximum likelihood, but combined as a single partition in the Bayesian analysis. Morphological data were added as a distinct partition in the Bayesian analyses, but excluded from the maximum likelihood analysis. A summary of the analyses conducted and the bootstrap support for the recovered clades is presented in Table 2.

5.2.7.2 Bayesian analysis

MrBayes was used to conduct a Markov Chain Monte Carlo (MCMC) search in a Bayesian analysis. Four runs, each consisting of four chains, were implemented until the standard deviation of split frequencies were 0.02. The cold chain was heated at a

temperature of 0.25. Substitution model parameters were sampled every 50 generations and trees were saved every 5000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (Nylander et al. 2008) (available at: ceb.csit.fsu.edu/awty/). Convergence was not reached even after 40 million generations with all datasets. A user-defined tree obtained from the maximum likelihood analyses was used as a starting point for all of the Bayesian analyses, which helped to improve convergence of the four runs. A burn-in was not used to summarize the values that were created with a user-defined tree.

5.2.7.3 Maximum likelihood analysis

RAxML (Stamatakis 2006) was used to search for the best-scoring likelihood tree with a rapid Bootstrap analysis (command -f a). The model of evolution specified was GTRMIX, which uses an accelerated algorithm to calculate maximum likelihood Bootstrap values and uses a GTRGAMMA model of evolution to calculate the final tree topology (Stamatakis et al. 2008). An amino acid model of evolution was selected for each protein-coding locus in MEGA5. The analyses were run with a random starting tree and with 1000 maximum likelihood bootstrap replicates.

5.2.7.4 Incongruence length difference test

An incongruence length difference test was run in PAUP* v4.0b10 (Swofford 2000) to examine the level of phylogenetic disagreement between the mitochondrial locus and nuclear rDNA loci, and nuclear protein-coding and rDNA loci. A heuristic search was used with a random starting tree and 1000 repetitions. The validity of the incongruence length difference test has been questioned by several systematists (Barker and Lutzoni 2002; Ramirez 2006), but it remains the most widely used computerized method for determining congruence between datasets (Sanderson and Shaffer 2002; Planet 2006).

Table 2. The phylogenetic analyses run in the course of the study. I = ITS; L = LSU; E = EF1α; G = GAPDH; C = COX3; ITS2 = ITS2 secondary structure alignment; pro = amino acid alignment of protein coding loci; bayes = Bayesian inference; ml = maximum likelihood. Not recovered indicates that the clade was not present in the phylogeny. Clade 5 represents a single taxon (*Anomalomyces panici*), which always occurred by itself.

Analyses run	**Clade support**									
Gblocks curated	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6	Clade 7	Clade 8	Clade 9	Clade 10
IL ml	88	78	0	80	n/a	70	0	79	100	43
IL bayes	1	1	0.95	1	n/a	0.99	0	1	1	not included
IL morphology bayes	1	1	0	1	n/a	1	not recovered	1	1	1
IL, ITS2 ml	90	39	11	95	n/a	87	5	84	100	not included
ILC ml	not recovered	73	12	not recovered	n/a	not recovered	not recovered	not recovered	100	not recovered
ILCpro ml	not recovered	4	1	not recovered	n/a	not recovered	not recovered	not recovered	100	94
ILEG ml	88	85	63	81	n/a	79	22	97	100	82
ILEG bayes	1	0.98	0.84	1	n/a	1	0.68	1	1	not included
ILEGpro ml	90	85		81	n/a	80		97	100	not included
ILEGpro bayes	1	1	0.74	0.96	n/a	0.99	0.77	1	1	not included
Non-curated										
IL ml	95	99	90	99	n/a	28	not recovered	76	100	99
ILCEG ml	not recovered	97	8	not recovered	n/a	not recovered	not recovered	not recovered	100	95
ILCEGpro ml	not recovered	7	2	not recovered	n/a	not recovered	not recovered	not recovered	100	96
ILEG ml	97	96	92	99	n/a	not recovered	5	96	100	98
ILEGpro ml	97	95	90	100	n/a	not recovered	5	95	100	98

5.3 Results

5.3.1 Phylogenetic relationships obtained with nuclear rDNA loci

Ten monophyletic groups were recovered consistently in maximum likelihood and Bayesian analyses of combined ITS and LSU sequence data (Fig. 8). Clades 1, 2, 3, 7, 8 and 10 were recovered by Stoll et al. (2005) in a Bayesian analysis of 97 smut species.

Inclusion of ITS2 secondary structure alignment in the curated ITS and LSU dataset recovered ten clades, but with reduced nodal support for some of the monophyletic groups recovered by nuclear and rDNA loci. The ITS2 phylogenetic tree supported the relationship of Clade 2 as sister to Clades 1, 3 and 4. A maximum likelihood analysis of the ITS2 alignment alone recovered similar species relationships to those obtained with all loci, but failed to resolve the ten monophyletic groups. Gblocks-curation of ITS and LSU reduced the datasets by more than half. The curated alignment recovered Clade 2 as sister to Clades 1 and 3. In the non-curated dataset, Clade 3 was sister to Clades 1 and 2. The Gblocks-curated datasets reduced support for Clade 3 but did not change support for other clades. The bootstrap support for Clade 3 dropped from around 90% to below 75% when the ITS1 region was removed by Gblocks.

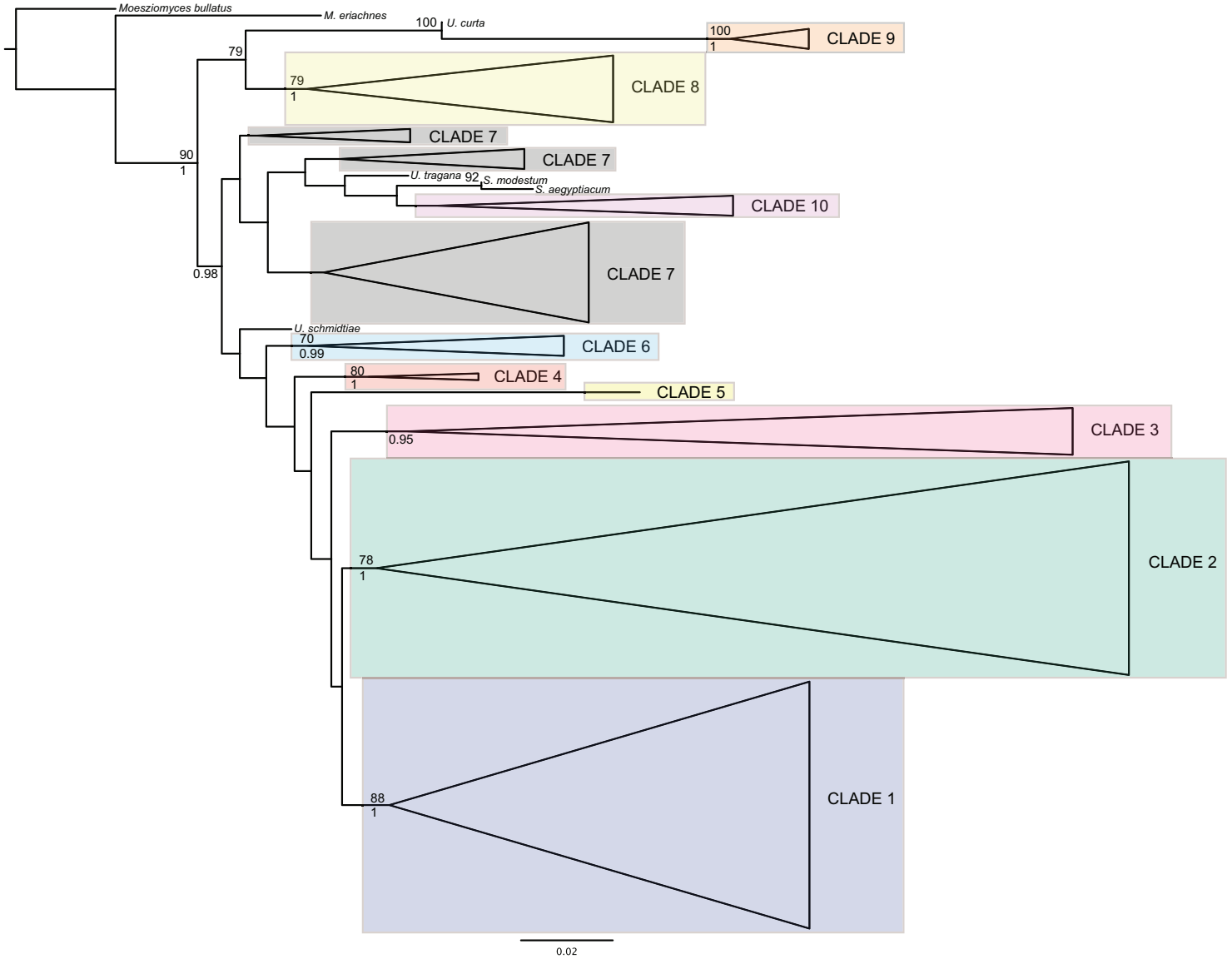


Fig. 8. Phylogram obtained from maximum likelihood analysis of rDNA loci, ITS and LSU. Bootstrap support values (>70%) from a maximum likelihood search with 1000 replicates shown above the nodes. Posterior probabilities (>0.95) obtained from Bayesian inference shown below the nodes

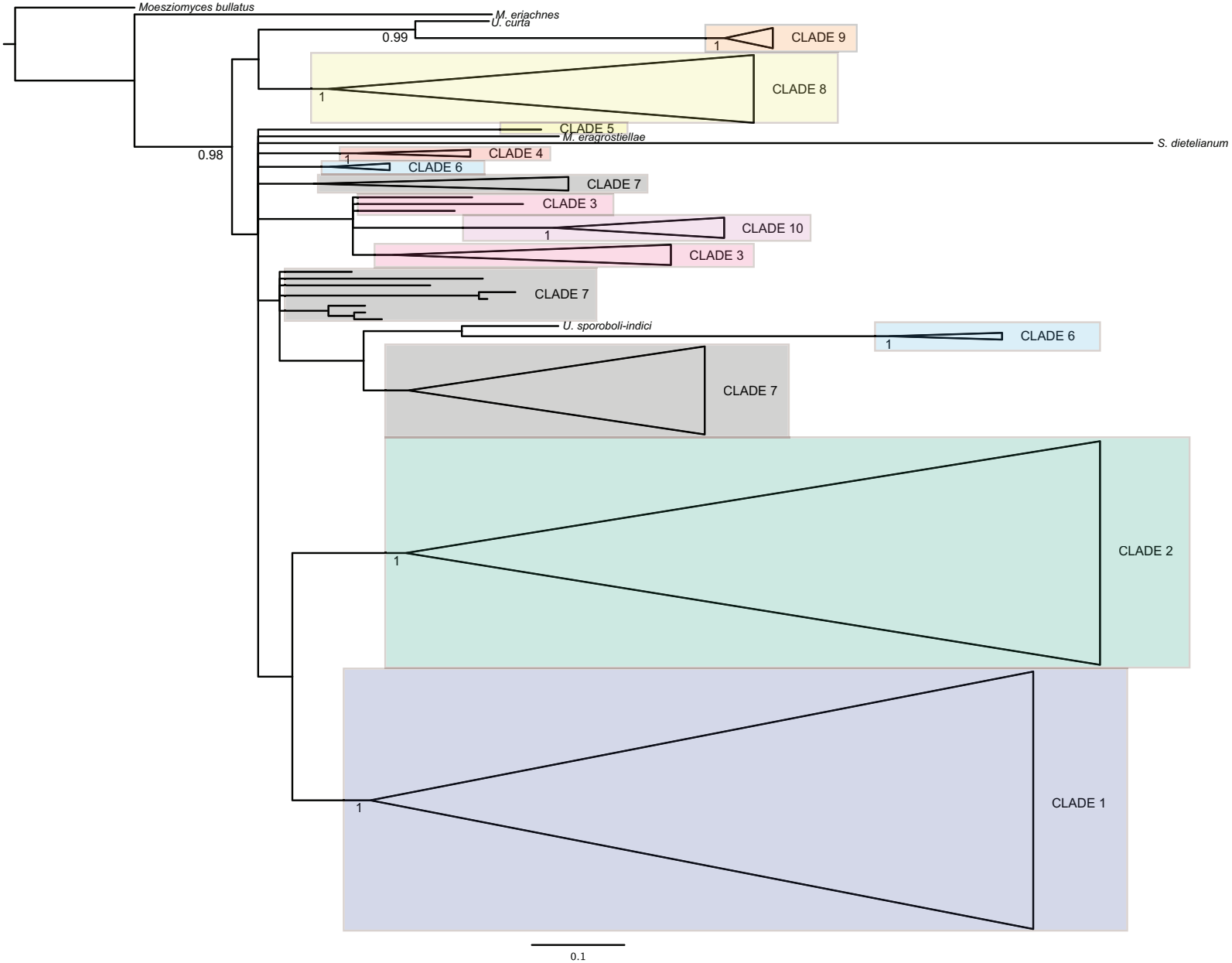


Fig. 9. Phylogram obtained from Bayesian inference using rDNA loci ITS and LSU, and morphological data. Posterior probabilities (>0.95) below nodes.

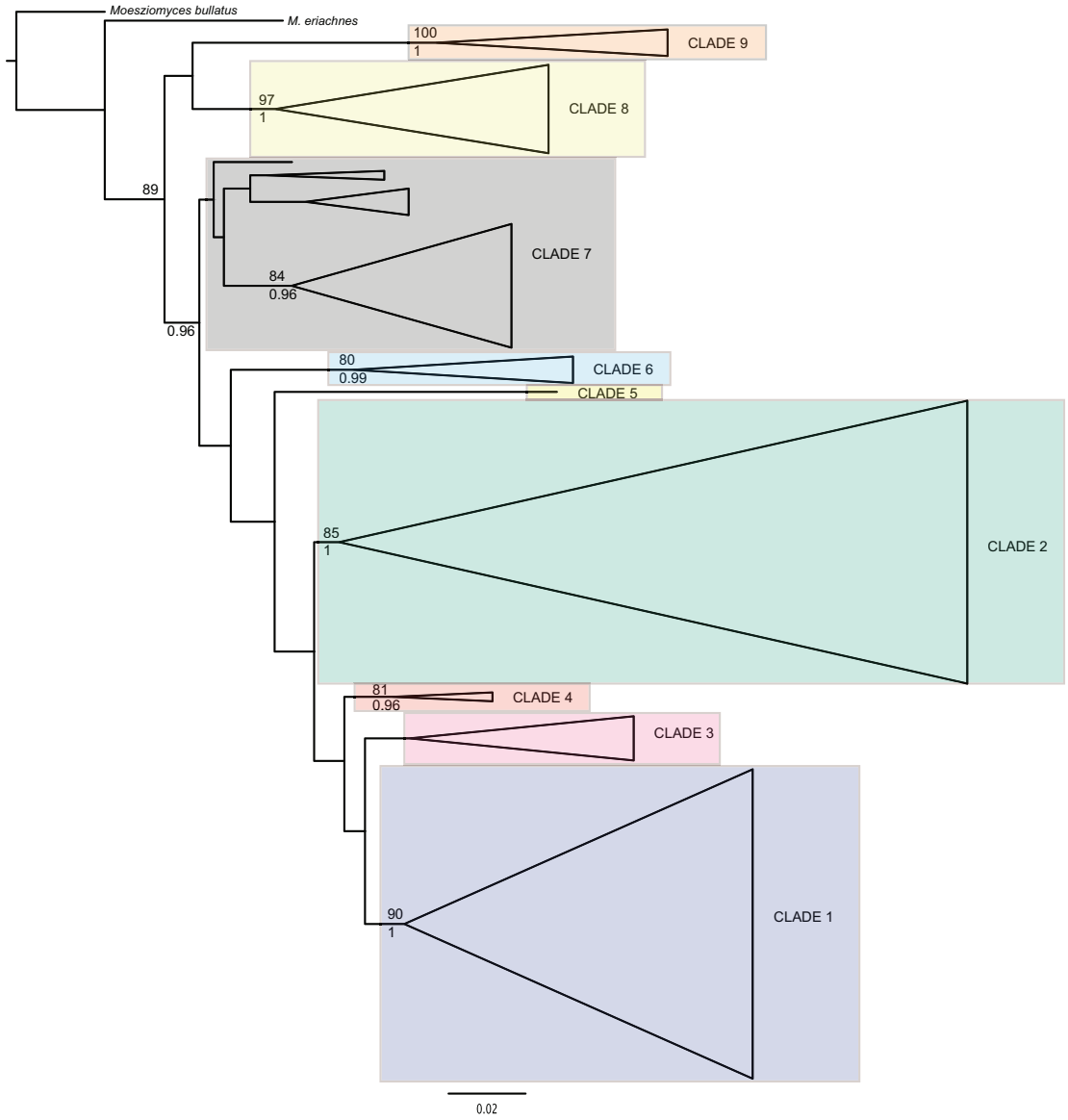


Fig. 10. Phylogram obtained from maximum likelihood analysis of nuclear rDNA loci, ITS and LSU, and nuclear loci, GAPDH and EF1α. Bootstrap support values (>70%) from a maximum likelihood search with 1000 replicates shown above the nodes. Posterior probabilities (>0.95) obtained from Bayesian inference shown below the nodes.

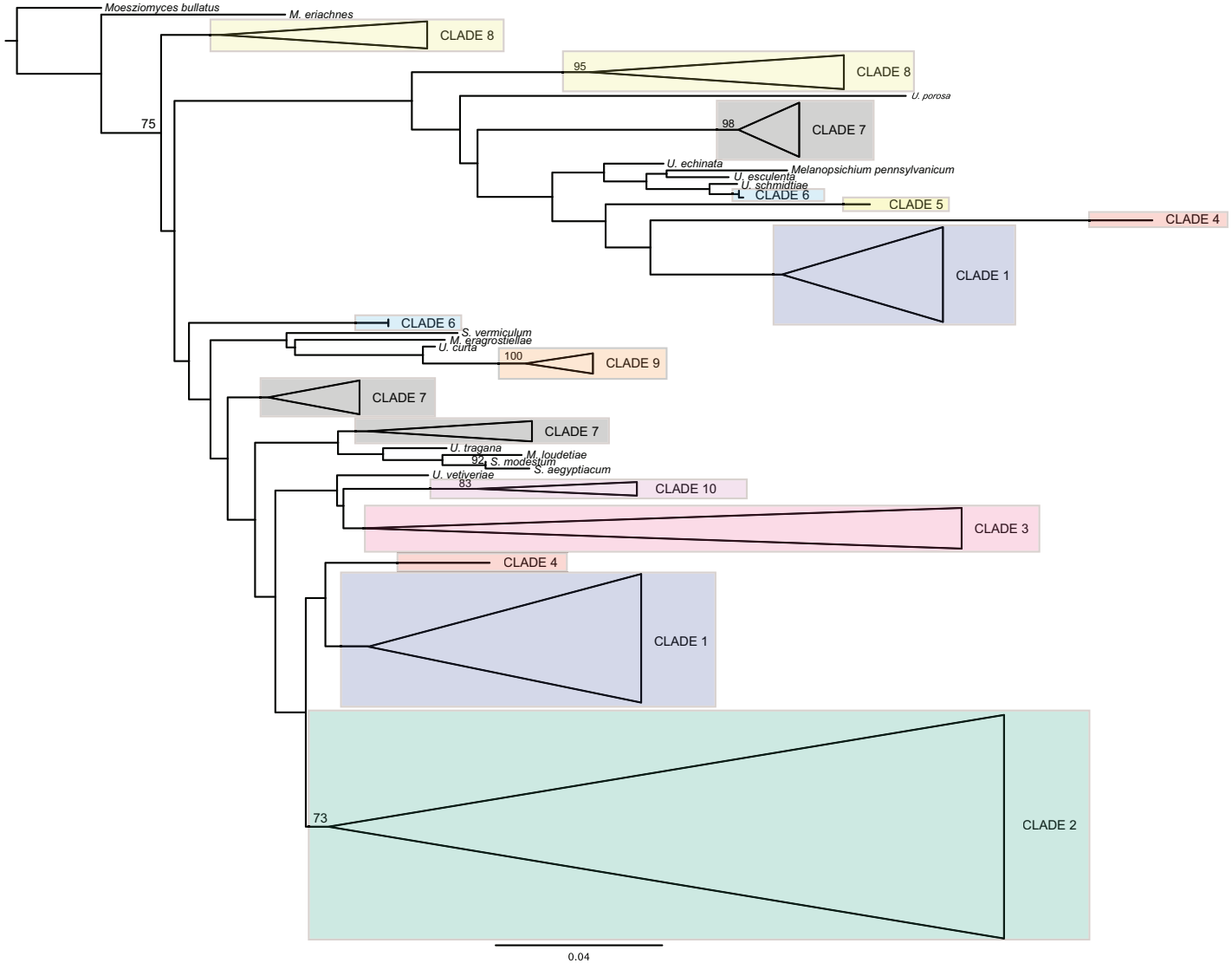


Fig. 11. Phylogram obtained from maximum likelihood analysis of nuclear rDNA loci, ITS and LSU, and the mitochondrial locus COX3. Bootstrap support values (>70%) from a maximum likelihood search with 1000 replicates shown above the nodes.

5.3.2 Phylogenetic relationships obtained with nuclear rDNA and morphological data

The ITS and LSU regions were combined in a Bayesian phylogenetic analysis with morphological characters (Fig. 9). The combined molecular and morphological analysis recovered ten clades, but the relationships of the clades were reduced to polytomies and Clades 3, 6 and 7 were divided. Morphological data were also included in Bayesian analyses with protein coding loci, but these runs did not converge in MrBayes.

5.3.3 Phylogenetic relationships obtained with nuclear rDNA and nuclear loci

The GAPDH and EF1α nucleotide alignments were converted to amino acid sequences and run under a protein model of evolution with the rDNA loci in maximum likelihood and Bayesian analyses (Fig. 10). The topologies obtained from amino acid data were similar to those obtained using nucleotide data. The addition of nuclear loci supported the relationship of Clade 2 as sister to Clades 1, 3 and 4 in both maximum likelihood and Bayesian analysis. The topology recovered in Bayesian analysis differed from that obtained with maximum likelihood in that Clade 9 was a subgroup of Clade 7 (not shown). This relationship was not supported by posterior probabilities.

5.3.4 Phylogenetic relationships obtained with nuclear rDNA and mitochondrial loci

The mitochondrial locus reduced support for the ten major clades. Clades 1, 7 and 8 were divided when the mitochondrial locus was incorporated with nuclear rDNA or nuclear protein-coding loci in maximum likelihood analyses (Fig. 11). Divided clades were distributed to new relationships in the reconstructed trees. The COX3 nucleotide data were converted to amino acid sequence and run under the JTT protein model of evolution in an attempt to reduce any noise caused by synonymous substitutions. This had no effect on tree topology and a similar tree was reconstructed to the nucleotide data. Incongruence between the mitochondrial and rDNA loci was hence not caused by synonymous substitutions. Significant heterogeneity was observed between results

of the mitochondrial locus and the rDNA loci when assessed with an incongruence length difference test (PAUP*: P = 0.001).

5.3.5 Monophyletic groups recovered in analyses

Ten monophyletic groups were consistently recovered in the Bayesian and maximum likelihood analyses.

Clade 1: Stoll et al. (2005) recovered this group and named it *Sporisorium* 1. Clade 1 contains the type specimen of *Sporisorium*, *S. sorghi*, and 39 other taxa.

Clade 2: Stoll et al. (2005) also recovered this group and named it *Sporisorium* 2. It contains 34 taxa that were described as *Sporisorium*.

Clade 3: This group contained 12 taxa described as *Ustilago*, *Sporisorium* or *Macalpinomyces*, and included the model organism *Ustilago maydis*. The position of *U. maydis* has been unclear in other phylogenetic analyses that included fewer taxa (Piepenbring et al. 2002; Stoll et al. 2003; Stoll et al. 2005). Clade 3 was recovered in all the Bayesian and maximum likelihood analyses.

Clade 4: *Macalpinomyces bursus* and *M. ewartii* always occurred as sister to one another and separate from other groups. *Macalpinomyces ewartii* was not included in the analysis by Stoll et al. (2005), and the relationship of *M. bursus* to other smut fungi was unknown.

Clade 5: *Anomalomyces* is a monotypic genus, distinct from *Ustilago*, *Sporisorium* and *Macalpinomyces* because of its unique soral characters. It is a member of the complex and did not occur in any of the robust clades. It usually occurred as sister to Clades 1, 2, 3 and 4.

Clade 6: The four smuts that occur on the arid grass *Triodia* usually formed a monophyletic group. This clade was not supported in all analyses. Stoll et al. (2005) included two of the smut fungi that infect *Triodia* in their analysis. These two taxa formed a part of Clade 7 (Stoll et al. 2005).

Clade 7: This clade comprises three sub-groups with varied support. Stoll et al. (2005) broadly defined the three groups within Clade 7 as *Ustilago s. lat.* The three groups were (1) *Ustilago s. str.*, which was well supported in our analysis and consisted of taxa that are closely related to the type species of *Ustilago*, *U. hordei*. (2) The *Ustilago davisii* group, which was not represented by additional taxa in our analysis. (3) The *Ustilago esculenta* group, which contained *Melanopsichium pennsylvanicum* in our analysis, though Stoll et al. (2005) recovered this taxon as sister to the *Ustilago s. str*. sub-group.

Clade 8: The 12 taxa in this group share morphological characters from both *Sporisorium* and *Ustilago*. The group was well supported by maximum likelihood and Bayesian inference with all loci.

Clade 9: The four smuts that occur on *Aristida* form a monophyletic group. Stoll et al. (2005) included one taxon from *Aristida* in their analysis, but were unable to determine whether it formed a monophyletic group or was part of Clade 8.

Clade 10: Four taxa, *Macalpinomyces loudetiae*, *M. trichopterygis*, *M. simplex* and *M. tristachyae*, form a monophyletic group, sister to Clade 3 or Clade 7. These four taxa occur on the grass subfamily Arundinoideae (Stevens 2001). The position of these four taxa in the phylogeny remains uncertain.

5.4 Discussion

The utility of different phylogenetic criteria and data will be discussed in relation to the reconstruction of a well-supported phylogeny of the *Ustilago-Sporisorium-Macalpinomyces* complex. Increased taxon sampling and addition of nuclear protein-coding loci helped to further delimit clades within the complex. The potential applications of this revised phylogeny will be discussed in future studies.

5.1 Utility of phylogenetic reconstruction methods

5.1.1 Increased taxon sampling, nuclear loci and alignment curation

Addition of taxa to a phylogenetic study has often been demonstrated to improve resolution in phylogenetic studies (Graybeal 1998; Hedtke et al. 2006; Wiens 2006; Heath et al. 2008). Here 35 taxa were added to the ITS and LSU dataset of Stoll et al. (2005). Our taxon selection aimed to increase the sampling of groups that were poorly resolved in previous analyses. For example, we included smut fungi that occur on *Aristida* and several taxa that were classified as *Macalpinomyces*, which were ambiguous clades in the molecular phylogeny recovered by Stoll et al. (2005). The 35 additional taxa helped to define clades that previously contained fewer taxa, for example Clades 2, 3, 4, 5, 6 and 9. Increased membership of these clades will enable taxonomic decisions to be made about monophyletic groups (Heath et al. 2008).

Quality of a nucleotide alignment can affect tree topology, especially in the case of large datasets (Jeffroy et al. 2006; Rodriguez-Ezpeleta et al. 2007). Alignments that have non-homologous sites are biased and can give support to spurious clades (Susko et al. 2005). In this study, different topologies were reconstructed with Gblocks-curated datasets and non-curated datasets. Tree topology was most influenced by computerized curation of the ITS region. Computerized alignment-curation programs remove ambiguity from difficult alignments and generally increase support for phylogenetic relationships (Talavera and Castresana 2007).

The position of several taxa varied between clades depending on the phylogenetic criterion and loci employed. For example, *Macalpinomyces eragrostiellae*, *Ustilago schmidtiae* and *Sporisorium aegyptiacum* were often sister to or a part of Clade 7 and the four taxa in Clade 10 occurred as a part of either Clade 3 or Clade 7, depending on which phylogenetic assessment criterion and which loci were used. In most cases, these taxa were represented by only one or two molecular loci. Removal of these rogue taxa (Thomson and Shaffer, 2010) increased convergence in Bayesian inference and improved bootstrap support for monophyletic groups in maximum likelihood, but did not resolve the relationship of the clades to each other.

5.1.2 Mitochondrial loci, secondary structure and morphology

The mitochondrial locus was incongruent with results of nuclear rDNA and nuclear protein-coding loci. Other fungal systematic studies have encountered difficulties with mitochondrial loci. Sung et al. (2007) observed incongruence between a mitochondrial locus and nuclear protein-coding loci in their phylogenetic analysis of fungi in the Clavicipitaceae. Wedin et al. (2005) found that results of sequence evolution from a mitochondrial locus were incongruent with results from nuclear rDNA loci in their study on the Lecanoromycetes. Separate analyses of these two loci gave less resolved trees than the combined analysis.

Mitochondrial loci are considered a useful tool in evolutionary analysis; the mode of inheritance is uniparental, the genome is clonal and there is ample mitchondrial DNA within the cell that is easily amplified (Moore 1995). The mitochondrial genome can be subject to introgression (Ballard and Whitlock 2004) and recombination (Galtier et al. 2009), which also occur in nuclear loci. The mode of inheritance of the mitochondrion is unknown for many fungi and plants (Barr et al. 2005). We do not speculate why reults from the COX3 locus were incongruent with the nuclear rDNA and nuclear protein-coding loci. In the present study, COX3 recovered the relationships of closely related taxa, but not relationships within or between clades.

Inclusion of morphological data in the phylogenetic analysis did not further resolve the phylogeny. The characters assessed may be homologous and will be mapped later onto the resolved molecular phylogeny to infer character homology within the complex.

The secondary structure alignment of the ITS2 region did not resolve clades in this study. The ITS2 region has been less useful than the ITS1 region for phylogenetic reconstruction of fungi in the Basidiomycetes (Mullineux and Hausner 2009) and the Ascomycetes (Lieckfeldt and Seifert 2000). Within the *Ustilago-Sporisorium-Macalpinomyces* complex the ITS1 region could not be aligned and was removed from the curated alignment.

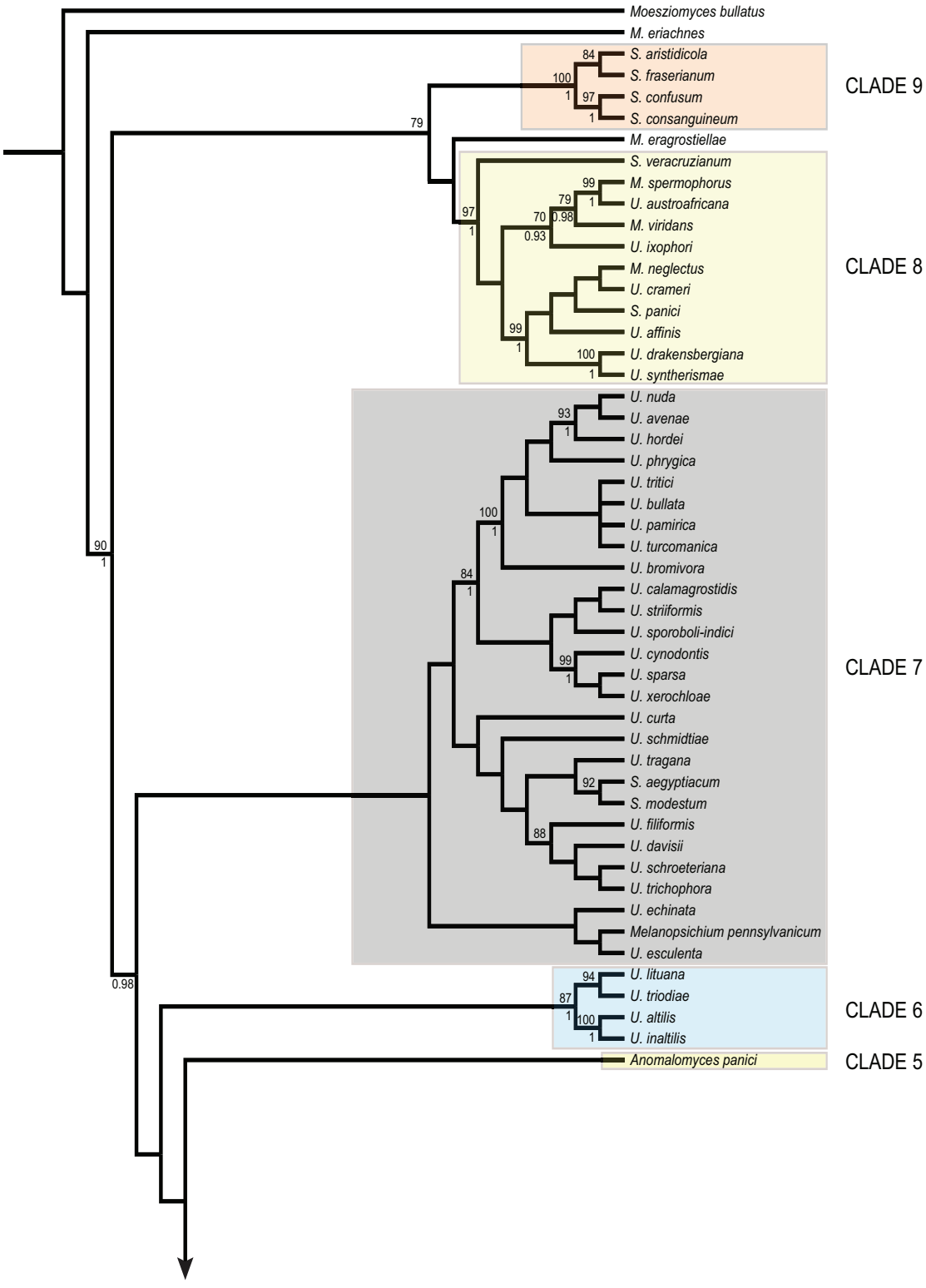

Moesziomyces bullatus
M. eriachnes
S. aristidicola
S. fraserianum
S. confusum
S. consanguineum
CLADE 9
M. eragrostiellae
S. veracruzianum
M. spermophorus
U. austroafricana
M. viridans
U. ixophori
M. neglectus
U. crameri
S. panici
U. affinis
U. drakensbergiana
U. syntherismae
CLADE 8
U. nuda
U. avenae
U. hordei
U. phrygica
U. tritici
U. bullata
U. pamirica
U. turcomanica
U. bromivora
U. calamagrostidis
U. striiformis
U. sporoboli-indici
U. cynodontis
U. sparsa
U. xerochloae
U. curta
U. schmidtiae
U. tragana
S. aegyptiacum
S. modestum
U. filiformis
U. davisii
U. schroeteriana
U. trichophora
U. echinata
Melanopsichium pennsylvanicum
U. esculenta
CLADE 7
U. lituana
U. triodiae
U. altilis
U. inaltilis
CLADE 6
Anomalomyces panici
CLADE 5

Fig. 12. Cladogram depicting the common topology obtained from Bayesian and maximum likelihood analysis of rDNA loci, ITS and LSU, and nuclear loci, GAPDH and EF1α. Bootstrap support values (>70%) obtained from maximum likelihood searches with 1000 replicates shown above the nodes. Posterior probabilities (>0.95) obtained from Bayesian inference shown below the nodes.

5.2 Relationships of clades

The topology that we have selected to represent the ten monophyletic groups of the *Ustilago-Sporisorium-Macalpinomyces* complex is a cladogram with support values obtained from a combined analysis of four molecular loci using maximum likelihood and Bayesian analyses (Fig. 12). The addition of 35 taxa resolved three additional clades compared with previous studies, and provides a potential basis for the reinterpretation of character homology. The final ten clades are well supported, although the relationship of the clades to each other remains unclear.

We recovered the same tree topologies using both maximum likelihood and Bayesian inference with different datasets, but with low support for the position of clades relative to one another. Typically, nodes that receive low bootstrap support in parsimony analyses are collapsed to form polytomies in a majority rule consensus tree; whether this should be the case for trees obtained with maximum likelihood will be discussed later.

Polytomies are either considered to be hard, as a result of non-dichotomous branching events (Walsh et al. 1999), or soft, which are potentially resolved by the inclusion of additional data (Coddington and Scharff 1996). It is plausible that hard polytomies exist within the *Ustilago-Sporisorium-Macalpinomyces* complex. The coevolution of smut fungi with their Poaceae hosts is complex and there have been cospeciation and host jumping events (Begerow et al. 2004), which could result in a non-dichotomously branching lineage.

We consider that low support for relationships of the clades with each other could be resolved by the inclusion of additional taxa. The creation of a soft polytomy of weakly supported nodes is not required in this case. The relationships were recovered as the most likely tree in maximum likelihood analyses from a combination of different molecular loci. The same topology was also recovered in the consensus of Bayesian analyses.

In summary, addition of more taxa and the incorporation of nuclear protein-coding loci assisted in resolving monophyletic groups that were ambiguous in the *Ustilago-*

Sporisorium-Macalpinomyces complex of smut fungi. Ten monophyletic groups were recovered in maximum likelihood and Bayesian analyses that were well supported by bootstrap and posterior probabilities.

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Chapter 6: A reassessment of character homology in the *Ustilago-Sporisorium-Macalpinomyces* complex (Ustilaginaceae)

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Alistair McTaggart: Collected, analysed and interpreted data and wrote the manuscript.

Roger Shivas: Contributed to discussion of morphology within the *Ustilago-Sporisorium-Macalpinomyces* complex and editing of manuscript.

Andrew Geering: Contributed to editing of manuscript.

Ben Callaghan: Provided laboratory assistance and editing of the manuscript.

Tanya Scharaschkin: Provided mentorship for phylogenetic methods, discussion of character evolution and contributed to the editing of the manuscript.

Abstract

The genera *Ustilago*, *Sporisorium* and *Macalpinomyces* form a monophyletic complex of fungi. The four main morphological characters used to define these genera have been considered homoplasious and are not useful for resolving the complex. This study re-evaluates character homology and discusses the use of the four characters for defining monophyletic groups within the complex. Generic delimitation of smut fungi based on their hosts is also discussed as a means for identifying genera within this group. Morphological characters and host plant can be used to circumscribe genera within the *Ustilago-Sporisorium-Macalpinomyces* complex.

Key Words

systematics, smut fungi, morphology, homology, columella, partitioning cells, spore balls, peridium

6.1 Introduction

Three genera of smut fungi (sub-phylum Ustilaginomycotina), *Ustilago*, *Sporisorium* and *Macalpinomyces*, contain about 530 described species that mostly infect grasses (Vánky in press). Several phylogenetic studies have demonstrated that *Ustilago*, *Sporisorium* and *Macalpinomyces* collectively form a monophyletic group within the Ustilaginomycotina (Swann and Taylor 1995; Bauer et al. 1997; Begerow et al. 1997; Stoll et al. 2003; Begerow et al. 2004b; Stoll et al. 2005; Begerow et al. 2006). Despite the undisputed monophyly of the group, morphological characters have proven inadequate for placement of species within the three genera (Chapter 3, this thesis). The three genera have been shown to be polyphyletic (Stoll et al. 2003; Stoll et al. 2005), and collectively form an unresolved complex. Studies on morphology (Langdon and Fullerton 1975; Vánky 1991; Piepenbring et al. 1998) and molecular phylogenetic analyses (Stoll et al. 2003; Stoll et al. 2005) have not identified characters that define monophyletic groups amongst species within this complex.

Smut fungi in the *Ustilago-Sporisorium-Macalpinomyces* complex either partially or completely destroy the inflorescence of grasses, forming a sorus that contains fungal

spores. Four characteristics of the sorus, namely columellae, sterile cells, spore balls and peridia, have been used traditionally to separate *Ustilago*, *Sporisorium* and *Macalpinomyces* (Vánky 2002). Within the sorus, columellae form a central axis of fungal and host origin (Vánky 2002); sterile cells, either derived from non-sporogenous hyphae or a fungal peridium, are found with the spores (Langdon and Fullerton 1975, 1978); spore balls appear as either an ephemeral or permanent agglomeration of spores (Vánky 2002). A peridium is the outer layer of the sorus and can be composed of host or fungal material (Vánky 2002). The soral characters have been interpreted differently by various mycologists (Stoll et al. 2005; Chapter 3, this thesis). For example, the columella in *Ustilago porosa* was considered absent by Langdon (1962) but present by Vánky and Shivas (2001). Similarly, *Sporisorium consanguineum* was considered to have a columella by Langdon and Fullerton (1975), but not by Vánky and Shivas (2008). Subsequently, soral morphology has been considered too variable to serve as a reliable character that can separate *Ustilago*, *Sporisorium* and *Macalpinomyces* (Piepenbring 2004; Stoll et al. 2005).

The current study evaluated the distribution of morphological characters in the *Ustilago-Sporisorium-Macalpinomyces* complex based on well-supported monophyletic groups reconstructed in a multi-locus molecular phylogeny, and discusses the utility of these characters as criteria for taxonomic revision. A detailed reinterpretation of the soral characters and a re-evaluation of their homology is provided in light of the phylogenetic results. The merits of using host specificity and the redefined soral characters are discussed as a basis for delimiting taxa.

6.2 Materials and methods

A detailed description of taxon selection, acquisition of molecular data and phylogenetic methods used in this study can be found in Chapter 5.

6.2.1 Taxon selection

Taxa were selected to represent the main groups recovered in previous studies (Stoll et al. 2003; Stoll et al. 2005), with increased sampling of under-represented groups, for example *Macalpinomyces* and smut fungi occurring on *Aristida*. In total, 137 taxa

were included in the analysis (Appendix 1), of these, 35 were Australian species that have not been included in any prior phylogenetic study.

6.2.2 Scoring of morphological characters

Character and character state selection were based on taxonomic descriptions in monographs of the Ustilaginomycotina (Vánky 1994; Vánky and Shivas 2008; Vánky in press) and from direct observation of 61 Australian species. A thorough examination of the four soral characters was included in the morphological analysis. Character states were scored according to Wiens (2000) and were all unweighted; missing and ambiguous characters, or characters with intraspecific variation between descriptions were scored as polymorphic. Columellae were scored as either absent, stout (Fig. 17a, b) or filiform (Fig. 18a, c). Spore states were classified as single spores, permanent spore balls, ephemeral spore balls or dimorphic spores. Sterile cells were scored as present or absent. The peridium was classified as either host derived, hypertrophied-host derived or fungal derived. These characters were included in the phylogenetic analysis and also mapped onto the final tree topology.

6.3 Results and Discussion

Ten clades were consistently recovered in a phylogenetic analysis of four molecular loci (Fig. 13). The structure of columellae (Fig. 14), the presence or absence of partitioning or sterile cells (Fig. 15) and the presence or absence of spore balls (Fig. 16) were traced onto the topology. Evolution and homology of characters were also assessed according to the relationships from the tree topology (Fig. 17). A discussion of the homology of these characters and their use in identifying the clades of the *Ustilago-Sporisorium-Macalpinomyces* complex follows.

6.3.1 Characters associated with monophyletic groups

The major clades recovered here are similar to those obtained in previous molecular phylogenetic analyses using different assessment criteria (Stoll et al. 2003; Cunnington et al. 2005; Stoll et al. 2005; Vánky et al. 2006). For example, several phylogenetic studies have reconstructed two monophyletic groups in *Sporisorium* (Stoll et al. 2003; Cunnington et al. 2005; Stoll et al. 2005; Vánky et al. 2006), but the

studies were not able to separate the two groups using morphological characters. Our reconstructed phylogeny revealed both synapomorphic and apomorphic characters within ten clades in the *Ustilago-Sporisorium-Macalpinomyces* complex. A discussion of the nature and use of characters for generic delimitation of ten main clades within the complex follows.

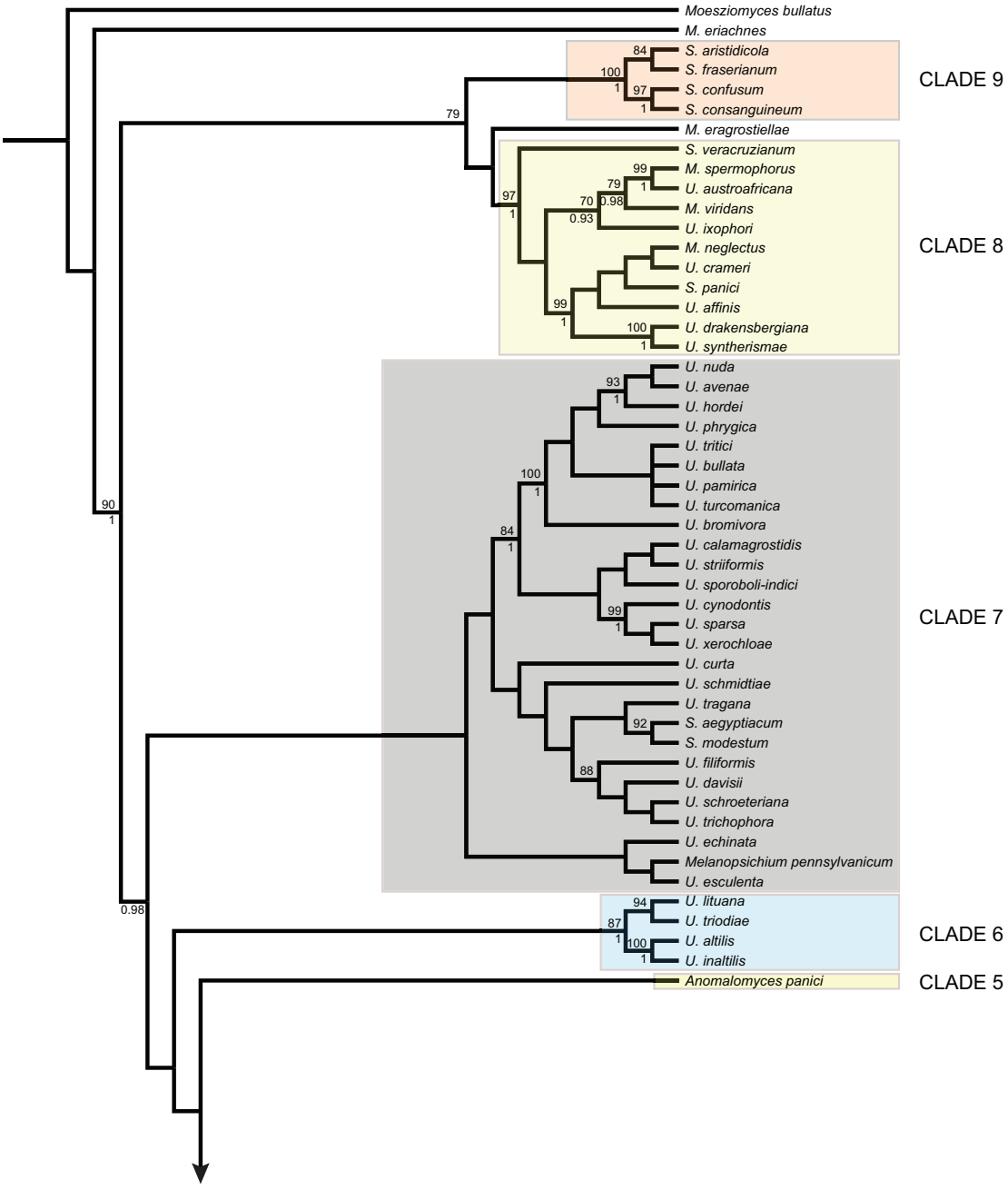
Moesziomyces bullatus
M. eriachnes
S. aristidicola
S. fraserianum
S. confusum
S. consanguineum
CLADE 9
M. eragrostiellae
S. veracruzianum
M. spermophorus
U. austroafricana
M. viridans
U. ixophori
M. neglectus
U. crameri
S. panici
U. affinis
U. drakensbergiana
U. syntherismae
CLADE 8
U. nuda
U. avenae
U. hordei
U. phrygica
U. tritici
U. bullata
U. pamirica
U. turcomanica
U. bromivora
U. calamagrostidis
U. striiformis
U. sporoboli-indici
U. cynodontis
U. sparsa
U. xerochloae
U. curta
U. schmidtiae
U. tragana
S. aegyptiacum
S. modestum
U. filiformis
U. davisii
U. schroeteriana
U. trichophora
U. echinata
Melanopsichium pennsylvanicum
U. esculenta
CLADE 7
U. lituana
U. triodiae
U. altilis
U. inaltilis
CLADE 6
Anomalomyces panici
CLADE 5

Fig. 13. Cladogram depicting the common topology obtained from Bayesian and maximum likelihood analysis of rDNA loci, ITS and LSU, and nuclear loci, GAPDH and EF1α. Bootstrap support values (>70%) obtained from maximum likelihood searches with 1000 replicates shown above the nodes. Posterior probabilites (>0.95) obtained from Bayesian inference shown below the nodes.

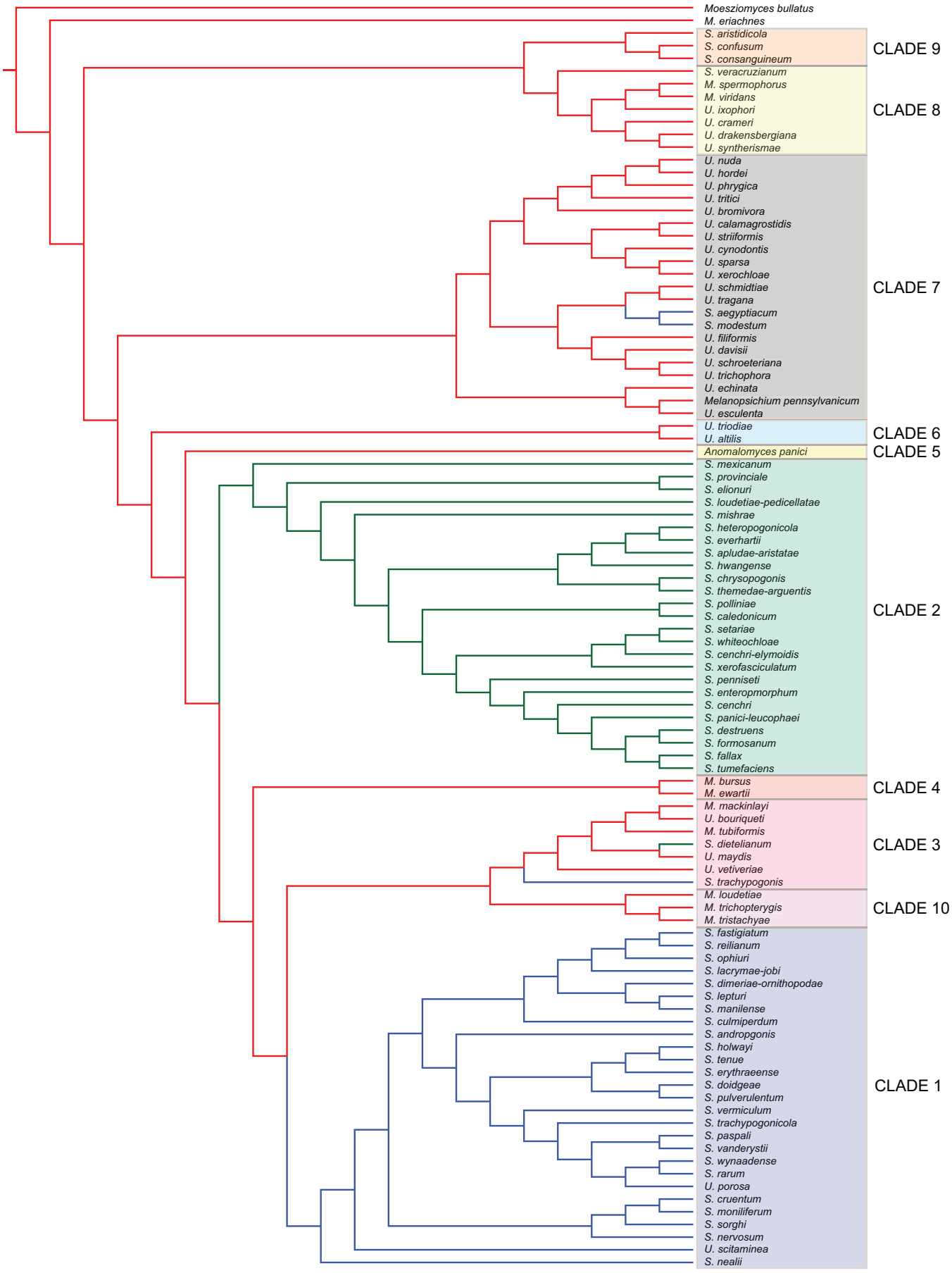

Fig. 14. Structure of columellae mapped onto a subset of the final topology. Red = columella absent; Green = filiform columellae; Blue = stout columellae.

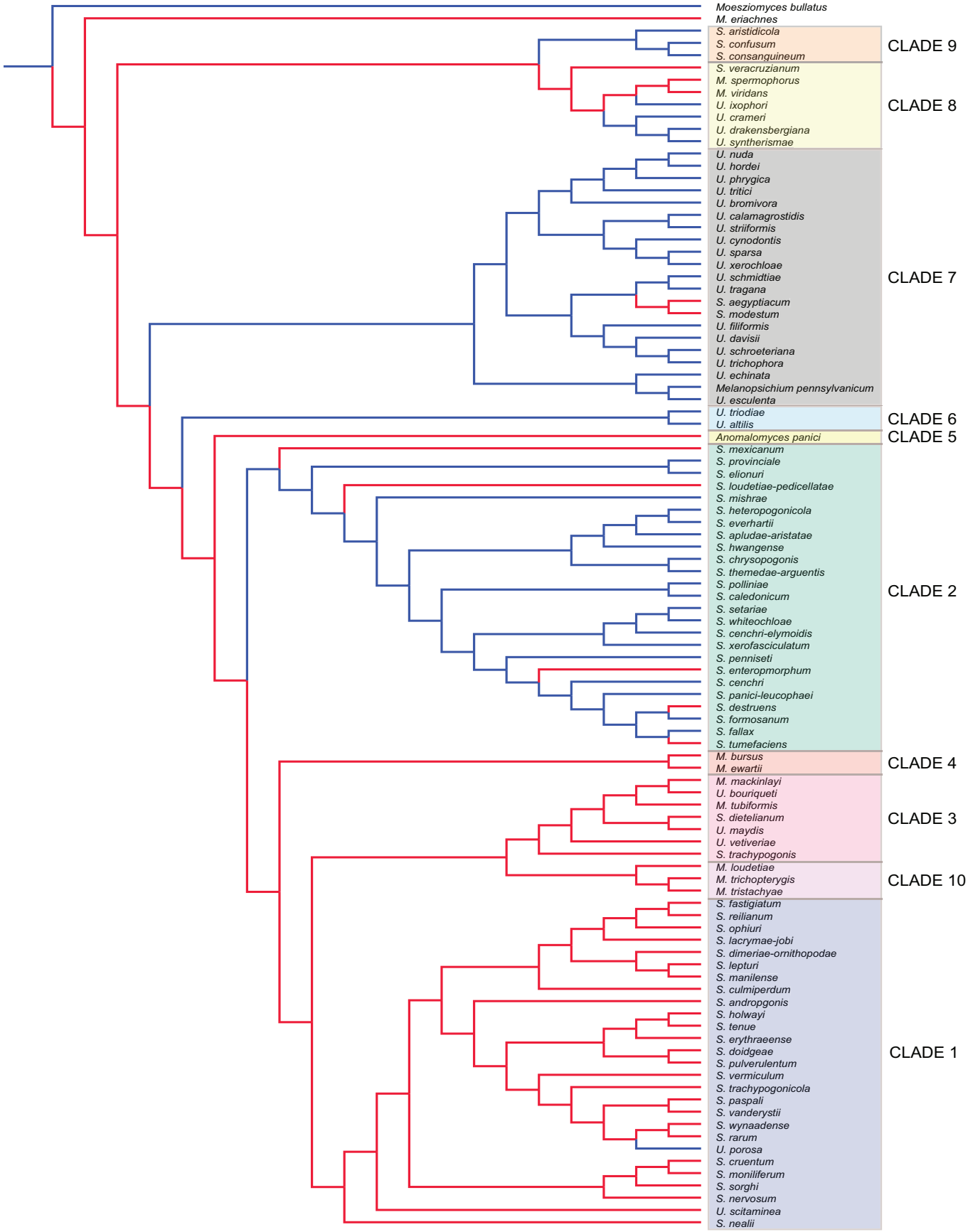

Fig. 15. Distribution of partitioning or sterile cells within clades of the *Ustilago-Sporisorium-Macalpinomyces* complex. Red = partitioning cells present; Blue = partitioning cell absent.

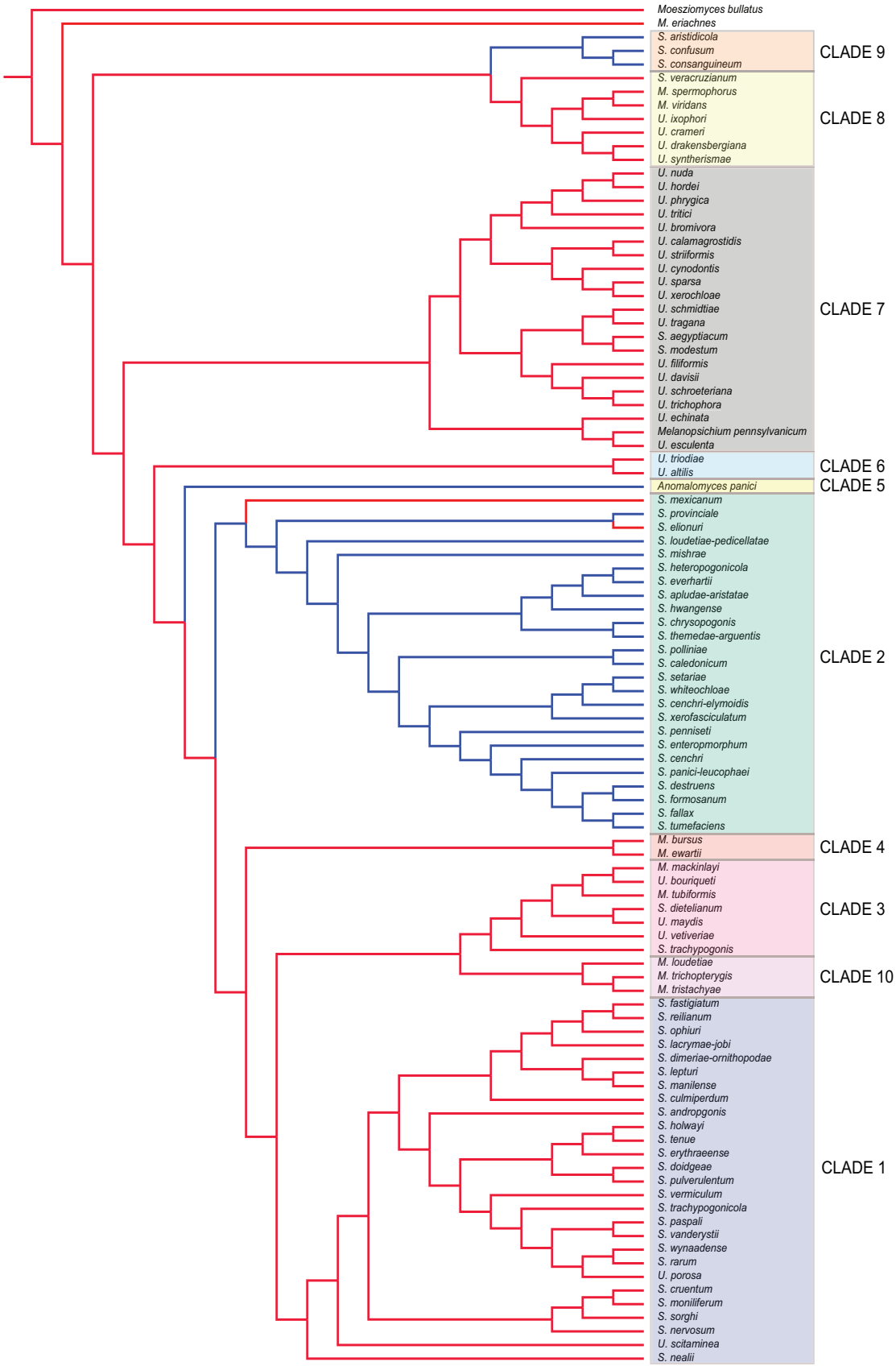

Fig. 16. Presence of spore balls within clades of the *Ustilago-Sporisorium-Macalpinomyces* complex. Red = Spore balls absent; Blue = spore balls present**.**

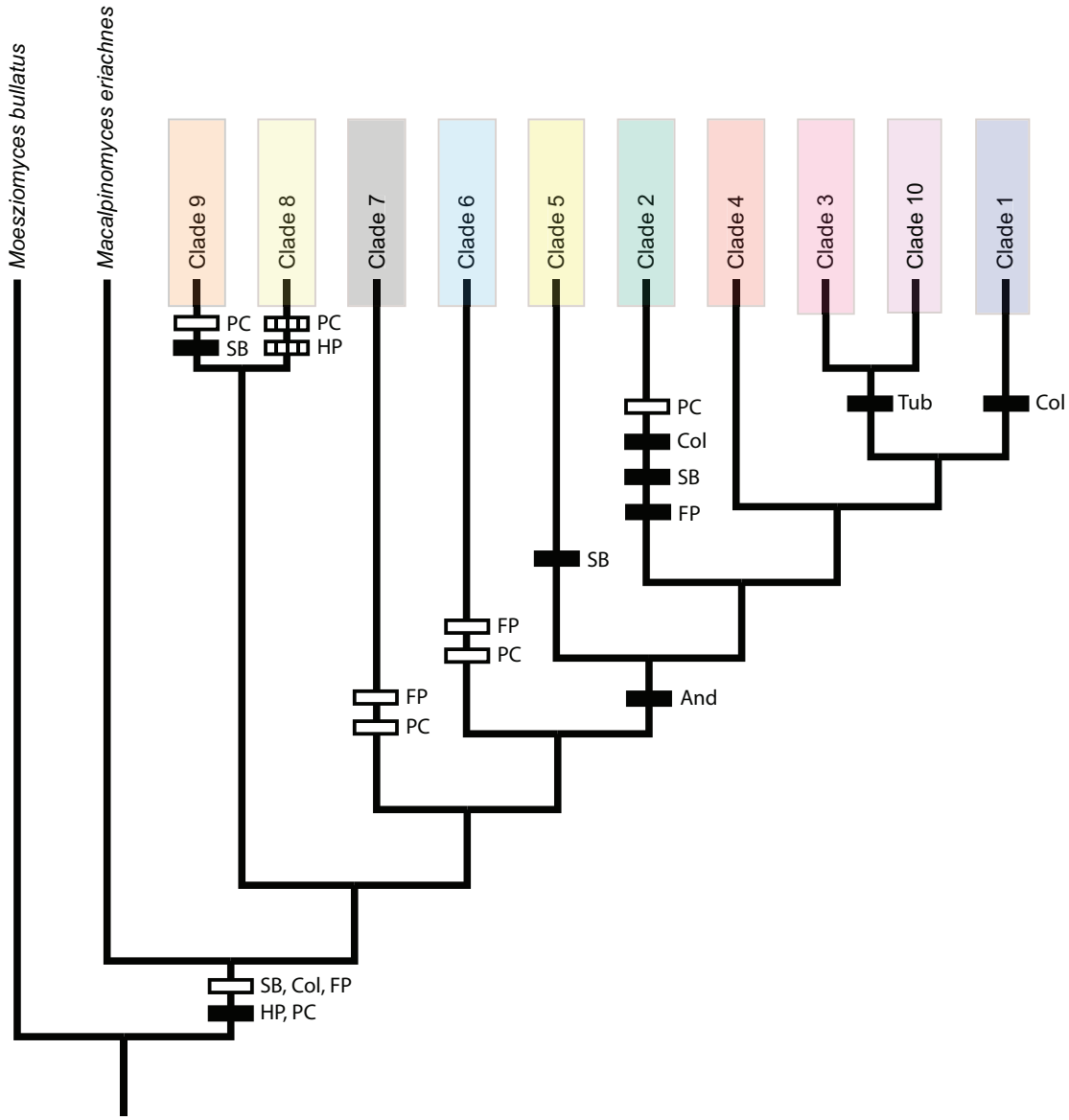

Fig. 17. Evolution of characters within the *Ustilago-Sporisorium-Macalpinomyces* complex, based on the tree topology obtained by in Chapter 5. Filled in boxes represent character gains; light boxes represent character losses; lined boxes represent equivocal states. HP = host peridium; PC = partitioning cells; SB = spore balls; Col = columella; And = andropogonoid host; FP = fungal derived peridium; Tub = tubular sorus.

6.3.1.1 Clade 1

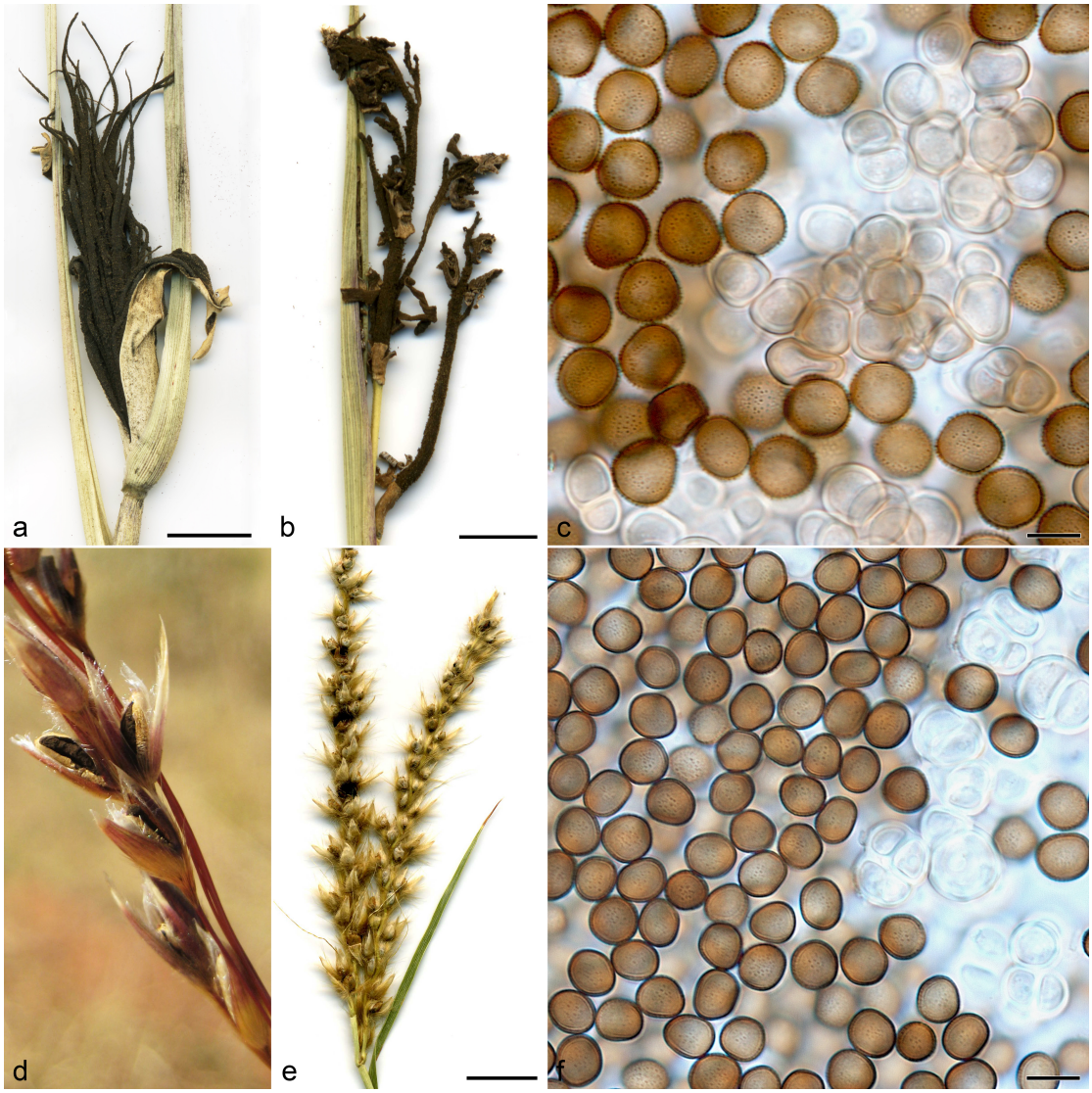

Fig. 18. Clade 1 character states. a. Stout columellae in *Sporisorium reilianum*. b. Branched columella destroying entire inflorescence in *S. doidgeae*. c. Spores and partitioning cells of *S. themedae*. d. All ovaries of the inflorescence infected in *S. ryleyi*. e. All spikelets of the inflorescence infected in *S. rarum*. f. Spores and partitioning cells of *S. rarum*. Scale bars a-b, e = 1 cm; c, f = 10 µm.

Clade 1 includes *S. sorghi*, the type of *Sporisorium*. The members of this clade share a number of characters.

1. A hardened or stout columella that either replaces the entire inflorescence, for example in *Sporisorium scitamineum*, *Sporisorium andropogonis* and *S. doidgeae* (Fig. 18b), or that occurs in all of the ovaries or spikelets of an inflorescence, for example in *S. sorghi*, *S. ryleyi* (Fig. 18d) and *S. rarum* (Fig. 18e).
2. Sterile cells formed from non-sporogenous hyphae are intermixed with spores in the sorus (Figs. 18c, f), except in *Ustilago porosa* and *Sporisorium culmiperdum*.

3. A peridium derived mainly from host tissue, either from leaf sheaths or the ovary wall.

Taxa in Clade 1 mainly infect grasses belonging to the sub-family Panicoideae, in one of two tribes, Paniceae or Andropogoneae. The infection is usually systemic and destroys either the entire inflorescence or all of the ovaries or spikelets.

Langdon and Fullerton (1978) examined the soral ontogeny of several species included in Clade 1, namely *Sporisorium sorghi*, *S. andropogonis* and *S. vanderystii*. They observed that the columella began to form after intercellular hyphae became confluent and caused the host cells to proliferate. Hyphae at the periphery of the columella formed a sheath of elongated, thick-walled, vacuolate cells. Other hyphae were present inter- and intracellularly in the tissue of the columella.

Columellae of species in Clade 1 are stout and woody due to the peripheral formation of thick-walled, vacuolate cells (Fig. 14). These columellae are cylindrical and grow vertically. Occasionally, more than one columella is present in a sorus, for example in *S. reilianum* (Fig. 18a). Sometimes columellae are branched, for example in *S. doidgeae* (Fig. 18b). Stout columellae are a synapomorphy for species in Clade 1 (Fig. 17)

Langdon and Fullerton (1978) observed that non-sporogenous hyphae partitioned the sporogenous hyphae in the sorus of *Sporisorium sorghi*. The partitioning hyphae formed groups of partitioning cells that mixed with the spores as the sorus matured. This pattern of development can explain the chains of sterile cells found in many species of *Sporisorium* (Fig. 15), for example *S. rarum* (Fig. 18f), *S. themedae* (Fig. 18c), *S. ophiuri* and *S. vermiculum*. Langdon and Fullerton (1978) termed these 'partitioning cells', though subsequent descriptions of smut fungi referred to them as sterile cells. We prefer to use the term partitioning cells to differentiate between the cells formed by sterile, partitioning hyphae, as determined by Langdon and Fullerton (1978), and the sterile cells formed from the peridium or within spore balls.

6.3.1.2 Clade 2

Species in Clade 2 either destroy the entire inflorescence, as in *S. caledonicum* (Fig. 19c) and *S. tumefaciens*; whole racemes, as in *S. enteromorphum*; or are localized in the inflorescence, as in *S. heteropogonicola* (Fig. 19a), *S. anthistiriae* and *S. bothriochloae*. Species in Clade 2 exhibit a number of common morphological characters.

1. Filiform or slender columellae (Fig. 19a, c).
2. Persistent spore balls (Fig. 19d). Two distinct spore types are usually present within the spore ball, namely inner and outer spores. Outer spores are often ornamented and are darker than the inner spores (Fig. 19b).
3. A sorus surrounded by a peridium composed mostly of fungal tissue.
4. Partitioning cells derived from non-sporogenous hyphae are rarely present within the sorus.

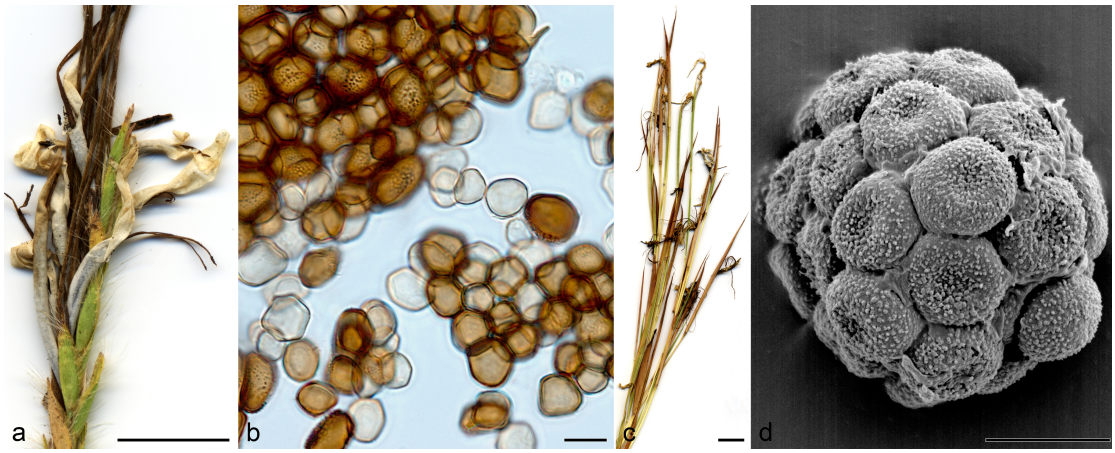

Fig. 19. Clade 2 character states. a. Localized spikelets infected in *Sporisorium heteropogonicola*. b. Dimorphic spores of *S. heteropogonicola*. c. Entire inflorescence destroyed in *S. caledonicum*. d. Permanent spore balls of *S. caledonicum*. Scale bars a, c = 1 cm; b, d = 10 µm.

Langdon and Fullerton (1978) examined the soral ontogeny of two species found in Clade 2, namely *S. caledonicum* and *S. anthistiriae*. They described the columella of *Sporisorium caledonicum* as a vascular bundle surrounded by host parenchyma, with tissues permeated by inter- and intracellular hyphae. Multiple columellae were formed by growth of hyphae in the parenchyma between the vascular bundles, which separate the central column into five to seven columellae. Host cells close to intercellular hyphae in some instances were distorted but there was little destruction of host tissue. Langdon and Fullerton (1975) also studied the soral ontogeny of *Sporisorium cryptum* (McAlpine) Vánky, which had a single columella made of several vascular bundles of parenchyma and mycelium that did not separate.

Species within Clade 2 have filiform or slender columellae (Fig. 13). These columellae are typically flattened in one plane and are never cylindrical. They are flexuous and do not grow vertically without support from the sorus as there are no thickened cells to sustain vertical growth. Many columellae are present in the sorus, for example in *Sporisorium caledonicum*, *S. fallax* and *S. enteromorphum*. A single, filiform columella comprised of several vascular bundles is sometimes present, for example in *Sporisorium cryptum* and *S. bothriochloae*. The columellae formed in this fashion are not hardened or woody, although they are sufficiently robust to persist in the sorus.

The presence of a columella was the defining character of *Sporisorium* (Link 1825; Langdon and Fullerton 1978; Vánky 2002). Members of Clades 1 and 2 that were examined by Langdon and Fullerton (1975, 1978) possessed two differences in development and structure of columellae. The first difference was that peripheral cells of Clade 2 species were not distorted or hardened in contrast to the thickened, vacuolated peripheral cells in Clade 1 species. The second difference was that the central columns was separated into several columellae in *Sporisorium caledonicum* or were made of numerous vascular bundles, as in *S. cryptum*; the columellae of Clade 1 members, *S. sorghi* and *S. andropogonis* were not separated into vascular bundles. Filiform columellae composed of vascular bundles constitute a synapomorphy in species in Clade 2 (Fig. 17).

Many species of *Sporisorium* that possess permanent spore balls were originally described as members of *Sorosporium* (Chapter 5, this thesis). Interestingly, most of these species belong to Clade 2 (Fig. 16). Langdon and Fullerton (1975) observed spore balls in several *Sporisorium* (as *Sorosporium*) species and described their formation. Coils of sporogenous hyphae were produced among mycelium that grew from the columellae as the sorus elongated. Coils consisted of two or three intertwined hyphae. Non-sporogenous hyphae, present between the spore balls, disintegrated and did not form partitioning cells. Spores that were formed in spore balls were dimorphic. The peripheral spores developed surface ornamentation in the form of warts or spines and the internal spores were smooth.

Sporisorium panici-leucophaei has spore balls and occurred in Clade 2. According to Vánky (2001) the spore balls of *S. panici-leucophaei* differentiate from non-concentric, sporogenous hyphae. This differed from the mode of formation described for *Sporisorium* by Langdon and Fullerton (1975), and was one reason Vánky (2001) established *Lundquistia*. The images Vánky (2001) used to illustrate how "spore balls differentiate within the mass of sporogenous hyphae, without concentric hyphae around them", depicted mature spores and did not show the state of the sporogenous hyphae. The mode of spore ball development in *S. panici-leucophaei* cannot be determined from the images provided by Vánky (2001). They are not agglutinated by sterile cells, as in *Moesziomyces*, and if the sporogenous hyphae are intertwined, as for species in Clade 1, then it is unlikely that the spores would form balls. It is unknown how spore balls are formed in *Sporisorium panici-leucophaei*.

Langdon and Fullerton (1975) observed that non-sporogenous hyphae in *Sporisorium caledonicum*, and three other species that occurred in Clade 2, disintegrated after the spores had matured. Often sterile cells derived from the fungal peridium were reported for species in Clade 2, for example *Sporisorium loudetiae-pedicellatae* Vánky & C. Vánky. Sterile cells were not formed by non-sporogenous, partitioning hyphae.

Species within Clade 2 possess a peridium made of fungal cells surrounded by a layer of host cells. Langdon and Fullerton (1975) discussed the formation of this peridium in *Sporisorium caledonicum* and three other smut fungi that occurred in Clade 2. They observed that hyphae adjacent to the peripheral host tissues became enlarged, with vacuolate cells and thickened cell walls. These hyphae were orientated in the direction of the long axis of the sorus and formed a sheath inside the peripheral layer of host tissue. This fungal sheath and the host cells external to it constituted the soral peridium, which surrounded the soral contents.

Members of Clade 2 mostly occurred on grasses in the tribes Andropogoneae or Paniceae in the subfamily Panicoideae. *Sporisorium hwangense* infects *Sporobolus* in the subfamily Chloridoideae. It shared characters with other taxa in Clade 2, namely filiform columellae, spore balls and dimorphic spores, and lacked partitioning cells.

Other examples of smut fungi that shared characters in Clade 2 but occurred on chloridoid grasses were *S. normanensis* R.G. Shivas & Vánky, *S. cynodontis* (L. Ling) R.G. Shivas & Vánky, *S. parodii* (Hirschh.) Vánky, and *S. saharianum* (Trotter) Karatygin.

6.3.1.3 Clade 3

Species within Clade 3 have been described as *Ustilago*, *Sporisorium* and *Macalpinomyces*. They share two common morphological characters.

1. Some, though never all, ovaries in the inflorescence are infected. The sori are relatively long, twisted and cylindrical, and are derived from hypertrophied host material, as in *Macalpinomyces tubiformis* (Fig 20a), *M. mackinlayi* and *Sporisorium dietelianum*.
2. Partitioning cells are found in the sori.

Fig. 20. Clade 3 character states. a. Localized spikelets infected in *Macalpinomyces tubiformis*. b. Spores and partitioning cells in *M. tubiformis*.

Clade 3 contains *Ustilago maydis*, which Piepenbring et al. (2002) and Stoll et al. (2005) determined to be more closely related to *Sporisorium* than to *Ustilago*. Other taxa that may belong to Clade 3, based on soral characters, are *Macalpinomyces elionuri-tripsacoidis* Vánky, *M. flaccidus* S.H. He & L. Guo, *M. nodiglumis* Vánky, *M. siamensis* R.G. Shivas, Vánky & Athipunyakom and *M. zonotriches* Vánky. Localised, tubular, host-derived sori are a synapomorphy for species within Clade 3 (Fig. 17).

Sporisorium trachypogonis and *S. dietelianum*, which are members of Clade 3, were both described as having columellae (Fig. 13). It is unlikely that these structures are homologous to the stout and filiform columellae in Clades 1 and 2, which are synapomorphies for these clades. Vánky (2004) combined *Sporisorium dietelianum* into *Lundquistia* because he did not consider the fascicles of host tissue as true columellae. Vánky (in press) later re-considered this view, equating these fascicles with columellae. The columellae of *Sporisorium dietelianum* are filiform and similar to the columellae of species in Clade 2. *Sporisorium dietelianum* can be distinguished from species in Clade 2 because it does not form either a fungal peridium or spore balls, and it possesses partitioning cells.

The columella of *Sporisorium trachypogonis* was described by Vánky (1995b) as a well-formed central columella. This description was similar to the columellae that are formed in the taxa of Clade 1. *Sporisorium trachypogonis* can be distinguished from other species in Clade 1 by the presence of a localized tubular sorus, rather than a systemic infection.

6.3.1.4 Clade 4

Macalpinomyces bursus and *M. ewartii* occur in a strongly supported clade separate from other clades recovered in the analysis. *Macalpinomyces bursus* and *M. ewartii* are morphologically very similar in appearance and occur on *Themeda* and *Sorghum* respectively, which are members of the tribe Andropogoneae. The sori form hypertrophied galls in the host ovaries. Partitioning cells are present in the sori, which never have a columella. The spores are prominently echinulate. These characters are similar to those of the smuts that infect grasses in the sub family Chloridoideae and the tribe Paniceae in Clade 8. Host classification is the simplest character to separate these two clades. Other smut taxa that may occur in this clade are *Macalpinomyces bothriochloae* (L. Ling) Vánky, *M. ovariicolopsis* (Vánky) Vánky and *M. pseudanthistiriae* A.R. Patil, T.M. Patil & M.S. Patil.

6.3.1.5 Clade 5

Anomalomyces panici is sister to the Clades 1, 2, 3 and 4. In terms of soral morphology, this species is similar to *M. bursus* and *M. ewartii* as it forms globose hypertrophied sori localized in the host ovaries. *Anomalomyces* infects a *Panicum trachyrachis* in the tribe Paniceae. The sorus is filled with hardened spore balls formed by coiled sporogenous hyphae (Vánky et al. 2006), dimorphic spores and partitioning cells. *Anomalomyces* possessed a unique combination of characters that warrants a monotypic genus within the *Ustilago-Sporisorium-Macalpinomyces* complex.

6.3.1.6 Clade 6

Four taxa that occur on the arid grass *Triodia* form a clade supported in maximum likelihood and Bayesian inference. The Bayesian analysis conducted by Stoll et al. (2005) grouped two *Triodia* taxa with the *Ustilago esculenta* group within Clade 7.

Ustilago altilis and *U. inaltilis* infect the host plant culms, while *U. triodiae* and *U. lituana* destroy the host inflorescence. Near identical ITS sequences for *U. altilis* and *U. inaltilis* (99% identical over 98% query coverage in a BLAST search), and *U. triodiae* and *U. lituana* (98% identical over 88% query coverage in a BLAST search) demonstrate their very close relationships.

A synapomorphy for these four taxa is that they infect species of *Triodia*. They have similar characters to species in Clade 7, in that they do not possess soral structures such as spore balls, columellae or partitioning cells.

6.3.1.7 Clade 7

Stoll et al. (2005) recovered Clade 7 as a weakly supported clade, which included *Melanopsichium pennsylvanicum*. They designated this clade as *Ustilago sensu lato* and defined three subgroups within the clade, (i) *Ustilago sensu stricto*, (ii) the *Ustilago davisii* group and (iii) the *Ustilago esculenta* group. Additional taxa included in this study were underrepresented in Clade 7 and further loci were only sequenced for six taxa in this group. Host and morphological synapomorphies have not been resolved for Clade 7 in our analysis.

6.3.1.7.1 *Ustilago sensu stricto* clade

Ustilago species that infect grasses in the tribe Pooideae formed a well-supported group that included the type species, *U. hordei*. Stoll et al. (2005) also recovered this group with strong support after Bayesian analysis. The stripe smuts *U. calamagrostidis* and *U. striiformis*, as well as *U. sporoboli-indici* (Chloridoideae) were sister to the smuts that destroy the inflorescence of pooid grasses. Stoll et al. (2005) included a subgroup in *Ustilago s. str.* that contained *Ustilago cynodontis*, *U. sparsa* and *U. xerochloae*. These three taxa occur on panicoid and chloridoid grasses. Inclusion of this subgroup and the stripe smuts in *Ustilago s. str.* was supported by both maximum likelihood and Bayesian inference. Taxa within the *Ustilago s. str.* clade lacked three characters that were found in most other clades.

1. Absence of partitioning or sterile cells in the sorus.
2. Absence of spore balls formed by coiled sporogenous hyphae.
3. Absence of a columella derived from host and fungal material.

6.3.1.7.2 *Ustilago davisii* group

Stoll et al. (2005) recovered a strongly supported but unresolved clade containing seven species, *Sporisorium aegypticum*, *S. modestum*, *Ustilago davisii*, *U. filiformis*, *U. schroeteriana*, *U. tragana* and *U. trichophora*. We recovered the same clade but it was not well supported by bootstrap values (< 70%) in maximum likelihood or posterior probabilities (< 0.95) after Bayesian inference. *Sporisorium aegypticum*, *S. modestum* and *Ustilago trichophora* were described as having columellae. It is

doubtful whether the columellae of *Sporisorium aegypticum* and *S. modestum* are homologous to columellae in Clades 1 and 2.

Fullerton and Langdon (1968) examined the soral development of *Ustilago trichophora* and concluded that a columella was present, however no columella was mentioned by Vánky and Shivas (2008) or Vánky (in press). The sori of *Ustilago trichophora* are deciduous and are easily removed from the host plant. These columellae are not a continuation of the host meristem and are not homologous to the columellae formed in Clades 1 and 2.

6.3.1.7.3 *Ustilago esculenta* group

Stoll et al. (2005) recovered a weakly supported group that contained several smut fungi found on chloridoid grasses together with the atypical *Ustilago esculenta*, which occurs in the subfamily Ehrhartoideae. *Ustilago curta*, which infects *Tripogon* of the Chloridoideae sub-family, either occurred in the *Ustilago esculenta* group, or as sister to Clade 6 or 9. Stoll et al. (2005) also recovered *Ustilago curta* (as *U. alcornii*) in the *Ustilago esculenta* group. We were unable to resolve any synapomorphies for this group, but indicated that the smuts on *Triodia* occurred in a separate monophyletic clade.

Stoll et al. (2005) demonstrated a close relationship between *Melanopsichium pennsylvanicum* and the *Ustilago s. str.* group. Our maximum likelihood analyses placed *Melanopsichium* in the *Ustilago esculenta* group rather than sister to the *Ustilago s. str.* group. Only the two nuclear rDNA loci obtained by Stoll et al. (2005) were included for *Melanopsichium* in the combined analysis of molecular loci. Begerow et al. (2004a) discussed the complicated coevolution between smut fungi and their hosts. *Melanopsichium pennsylvanicum* may represent a jump from Poaceae to a distantly related host, although this cannot be corroborated without further evidence.

6.3.1.8 Clade 8

This clade was recovered in studies by Stoll et al. (2003) and Stoll et al. (2005) and was strongly supported by both maximum likelihood and Bayesian inference here. Stoll et al. (2005) noted that taxa in this clade had a combination of characters observed in *Sporisorium* and *Ustilago*. Taxa in this group have often been described as *Macalpinomyces* because of the mixed soral characteristics associated with both *Sporisorium* and *Ustilago* (Chapter 5, this thesis). They occur on grasses in the tribe Paniceae and the subfamily Chloridoideae.

Partitioning cells are present in *Macalpinomyces spermophorus*, *M. viridans*, *M. neglectus* and *Ustilago affinis*, but are absent in the other members of this clade. Several taxa formed galls in the host ovaries, while *U. drakensbergiana*, *U. syntherismae* and *U. affinis* destroyed the entire inflorescence similar to taxa in *Ustilago s. str*. Columellae were described in several of the species in this clade, including *Ustilago drakensbergiana*, *Macalpinomyces spermophorus*, *M. viridans* and *M. neglectus*.

The columellae of *U. drakensbergiana* were formed from the remnants of the destroyed inflorescence and were not homologous with the columellae of Clades 1 and 2. Vánky (in press) observed that the sori of species of *Macalpinomyces* were deciduous and separated from the host plant at maturity, whereas species of *Sporisorium* had sori that remained attached to the inflorescence because the columella was connected to the host plant. The sori of *M. viridans* and *M. spermophorus* were deciduous and easily removed from the host plant. These columellae were not formed from the host meristem and were not homologous to the columellae of the Clades 1 and 2.

A synapomorphic character for Clade 8 was not identified. Subdivision of Clade 8 based on morphology is impractical at this stage, because the characters are highly variable in the group.

6.3.1.9 Clade 9

Four taxa that destroy the ovaries of *Aristida* formed a well-supported monophyletic group. Stoll et al. (2005) included *Sporisorium consanguineum* in their study, but were unable to determine whether it was sister to, or part of Clade 8. The inclusion of three additional smuts that infected *Aristida* has resulted in a separate, monophyletic group. The smuts on *Aristida* share several morphological characters.

1. Formation of galls in the ovaries of their hosts. They can infect all of the ovaries in an inflorescence (*Sporisorium confusum*, *S. consanguineum*) or be localized in the inflorescence (*S. aristidicola*).
2. The spores are commonly compacted into spore balls formed by coiled sporogenous hyphae, for instance in *Sporisorium consanguineum* (Langdon and Fullerton 1975).
3. Absence of partitioning cells within the sorus.

6.3.1.10 Clade 10

Macalpinomyces loudetiae, *M. simplex*, *M. trichopterygis* and *M. tristachyae* formed a well-supported monophyletic group either within Clade 7, which was also recovered by Stoll et al. (2005) using nuclear rDNA loci, or sister to Clade 3, using nuclear rDNA and protein-coding loci. Stoll et al. (2005) noted that these species infected grasses in the subfamily Arundinoideae. These four species shared more characters with Clade 3 than with Clade 7. They all have partitioning cells within tubular sori formed from hypertrophied host material. Another species within Clade 3, *M. arundinellae-setosae*, infected hosts in Arundinoideae. The four taxa in Clade 10 destroy the entire inflorescence or occur in the culms of the host. This is similar to the mode of infection in *Macalpinomyces chrysopogonicola* (Mundk. & Thirum.) Vánky, which Vánky (1995a) included in *Endosporisorium* Vánky. Systemic infection of hosts by species in Clade 10 is the main difference from species in Clade 3, which are localised in the inflorescence.

The phylogenetic position of Clade 10 is ambiguous, as there was only weak support for its placement as sister to Clade 3. We prefer to keep Clade 10 as a monophyletic group separate from Clade 3 because of the differences between the method of infection and uncertain phylogenetic relationships. Several other taxa not included in the phylogenetic analysis have a similar appearance to the taxa in Clade 10, namely

M. chrysopogonicola, *M. effusus* (Syd. & P. Syd) Vánky, *M. magicus* Vánky & T. Vánky and *M. ugandensis* Vánky.

6.3.1.11 Macalpinomyces eriachnes

Macalpinomyces eriachnes is the sister taxon to the *Ustilago-Sporisorium-Macalpinomyces* complex. We agree with Stoll et al. (2005), who were first to indicate that *Macalpinomyces* was a monotypic genus, with *M. eriachnes* the sole representative. *Macalpinomyces eriachnes* has giant partitioning cells formed from non-sporogenous hyphae (Langdon and Fullerton 1977; Vánky 1996) and a peridium, but lacks a columella. Giant partitioning cells do not occur in any other taxa in the complex. The spore balls of *Macalpinomyces eriachnes* were not formed from coiled sporogenous hyphae (Langdon and Fullerton 1977).

6.3.1.12 Taxa of uncertain placement

A few taxa frequently moved between clades in trees reconstructed using different datasets and different phylogenetic assessment criteria. These taxa were not supported in any group, although previous analyses have grouped most of these taxa in Clade 7 (Stoll et al. 2005). These taxa were only represented by data from two molecular loci in most cases.

Ustilago tragana, *U. schmidtiae*, *Sporisorium aegypticum* and *S. modestum* often grouped together after maximum likelihood analysis. These taxa, except for *Ustilago schmidtiae*, were included with taxa now assigned to Clade 7 by Stoll et al. (2005).

Maximum likelihood analyses placed *Ustilago curta* in a number of clades. Stoll et al. (2005) recovered *U. curta* (as *U. alcornii*) in the *Ustilago esculenta* group of *Ustilago s. lat.* after Bayesian analysis of data from two nuclear rDNA loci. With the addition of nuclear loci, *U. curta* was often placed as sister to the *Aristida* group or as sister to the *Triodia* group. It is currently not known to which group *Ustilago curta* really belongs.

6.3.2 Can host classification delimit smut genera?

Taxa within the *Ustilago-Sporisorium-Macalpinomyces* complex infect hosts in the Poaceae, with the exception of *Melanopsichium*, which occurs on Polygonaceae. The systematics of Poaceae has been well resolved and the relationships of the sub-families and tribes are well understood (Hsiao et al. 1999; Kellogg 2000; Stevens 2001; Bouchenak-Khelladi et al. 2008).

Host classification has often been used in the classification of smut fungi. Within *Ustilago*, *Sporisorium* and *Macalpinomyces*, host specificity is used to differentiate morphologically indistinguishable species (Bauer et al. 2001). Many of the keys to these genera are based on host taxonomy (Begerow et al. 2004a). Higher-level host taxonomy has been used to delimit smut genera, for example *Ustilago* is restricted to members of Poaceae (Bauer et al. 2001).

Begerow et al. (2004a) concluded that the phylogenetic relationships between smut fungi and their hosts were not straightforward. While species of *Ustilago* and *Sporisorium* showed evidence for co-speciation, it was considered more likely that smut fungi evolved after their hosts had speciated (Begerow et al. 2004a). Host jumps are evident in Clade 2, which contains taxa that infect grasses in two subfamilies, the Paniceae and the Chloridoideae.

The phylogenetic analyses of the *Ustilago-Sporisorium-Macalpinomyces* complex recovered several monophyletic groups that share similar morphological characters and are restricted to hosts in a specific genus, tribe or subfamily. Four smuts that occur on *Aristida* in the subfamily Aristidoideae (Stevens 2001) form a monophyletic group in Clade 9. They had similar morphological characters but there were no unique synapomorphies that separate them unambiguously from other species in the *Ustilago-Sporisorium-Macalpinomyces* complex. The fact that they infect hosts in the subfamily Aristidoideae implies this is a synapomorphy that distinguishes this clade from other clades.

Macalpinomyces bursus and *M. ewartii*, which are members of Clade 4, infect hosts in the tribe Andropogoneae. They possess morphological characteristics that are

similar to some species of Clade 8 occurring on hosts in Chloridoideae or Paniceae. The occurrence of Clade 4 members on hosts in the tribe Andropogoneae is a synapomorphy that can be used to distinguish *Macalpinomyces bursus* and *M. ewartii* from taxa in Clade 8.

Macalpinomyces eriachnes infects hosts in the grass subfamily Micrairoideae (Stevens 2001), tribe Eriachneae (Bouchenak-Khelladi et al. 2008), from which no other smut species have been recorded to date. Morphological characters in *M. eriachnes* differentiate it from other members of the *Ustilago-Sporisorium-Macalpinomyces* complex. The giant cells formed from partitioning hyphae, the polyangular spores and the character combination of a peridium and no columella make it unique. That it occurs exclusively on *Eriachne* is a synapomorphy that defines this monotypic genus.

Where morphological characteristics prove inadequate for recognizing smut taxa, we propose that delimitation of smut genera can be based on host range, provided monophyletic groups are resolved after molecular phylogenetic analyses. In the absence of contradictory evidence, host subfamily or tribe is a legitimate criterion for generic delimitation in the *Ustilago-Sporisorium-Macalpinomyces* complex.

6.3.3 Conclusion

A detailed examination of morphology is required to determine homology and to improve classification (Mooi and Gill 2010). Synapomorphies outlined here enable delimitation of clades and allow confident placement of new taxa into clades within the *Ustilago-Sporisorium-Macalpinomyces* complex with respect to gross morphology and host coevolution. Although there are some morphological anomalies, the monophyletic groups were mainly robust and well supported.

After inclusion of Australian taxa, use of nuclear protein coding loci and a thorough study of morphological diversity, we have identified morphological synapomorphies within the *Ustilago-Sporisorium-Macalpinomyces* complex. The determination of monophyletic groups and synapomorphic characters within the complex necessitates taxonomic reassessment of some genera and the creation or resurrection of others in

future studies. The major outcomes of our phylogeny and explanation of character homology in the *Ustilago-Sporisorium-Macalpinomyces* complex are:

1. *Sporisorium* can be subdivided based on soral characteristics. *Sporisorium sensu stricto* needs to be described explicitly to prevent ambiguity for future taxonomic placement.
2. New genera are required for the placement of taxa that form monophyletic groups and no longer fit the definition of *Sporisorium sensu stricto*.
3. *Ustilago maydis* and other taxa with localized tubular sori and sterile cells form a monophyletic group that represents a separate genus.
4. *Macalpinomyces bursus*, *M. ewartii* and similar taxa belong to a monophyletic group that can be differentiated by soral characteristics and host tribe, representing a new genus.
5. The monophyletic group of smut fungi that infects *Aristida* represents a new genus delimited by soral characteristics and host subfamily.
6. *Macalpinomyces* is a monotypic genus, sister to all other taxa in the *Ustilago-Sporisorium-Macalpinomyces* complex (Stoll et al. 2005).
7. Until Clades 8 and 10 are resolved, *Macalpinomyces* will remain a polyphyletic genus.

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Chapter 7: A taxonomic revision of *Ustilago*, *Sporisorium* and *Macalpinomyces*

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Alistair McTaggart: Collected, analysed and interpreted data and wrote the manuscript.

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Andrew Geering: Contributed to editing of manuscript.

Tanya Scharaschkin: Contributed to editing of manuscript.

Abstract

Morphological characters within the *Ustilago-Sporisorium-Macalpinomyces* complex are defined explicitly. The genera *Sporisorium*, *Anthracocystis* and *Mycosarcoma* are emended to reflect identified morphological synapomorphies within the *Ustilago-Sporisorium-Macalpinomyces* complex. Two new genera, *Stollia* and *Langdonia* are described based on their host classification. The new classification presented incorporates 152 new taxonomic combinations.

Key Words

Ustilaginaceae, systematics, *Anthracocystis*, *Mycosarcoma*, *Stollia*, *Langdonia*

7.1 Introduction

The three genera of smut fungi, *Ustilago*, *Sporisorium* and *Macalpinomyces*, form a monophyletic complex that has eluded resolution using morphology (Langdon and Fullerton 1975; Vánky 1991; Piepenbring et al. 1998) or molecular phylogenetic analysis (Stoll et al. 2003; Stoll et al. 2005). Two suggestions to reconcile the taxonomy of the complex have been proposed. The first was to break up the current taxa into several smaller genera and subgenera (Vánky 2002; Piepenbring 2004). The second was to unify the three genera into a single genus, *Ustilago* (Vánky 2002; Piepenbring 2004). The former solution is dependent on finding morphological synapomorphies that can delimit the genera, and the latter solution neglects a more pragmatic and precise taxonomy.

Previous studies (Chapter 6, this thesis) argued that the *Ustilago-Sporisorium-Macalpinomyces* complex would best be broken into smaller, monophyletic generic groups based on synapomorphic morphological characters and host plant classification that reflected morphological diversity within the complex. As a first step towards deconstruction of the complex, synapomorphic characters were identified that could be used to define monophyletic groups within the complex (Chapter 6, this thesis). The current study proposes a new classification for species currently placed in *Ustilago*, *Sporisorium* and *Macalpinomyces*.

7.2 Phylogeny

Phylogenetic analyses (Chapter 5, this thesis) resolved ten clades within the *Ustilago-Sporisorium-Macalpinomyces* complex (Fig. 1). Six of the clades can be delimited by morphology or host. *Sporisorium* and *Anomalomyces* are accepted genera. Here two genera are reinstated, *Mycosarcoma* and *Anthracocystis*, and two new genera, *Langdonia* and *Stollia*, are proposed to accommodate newly resolved clades (Fig. 22).

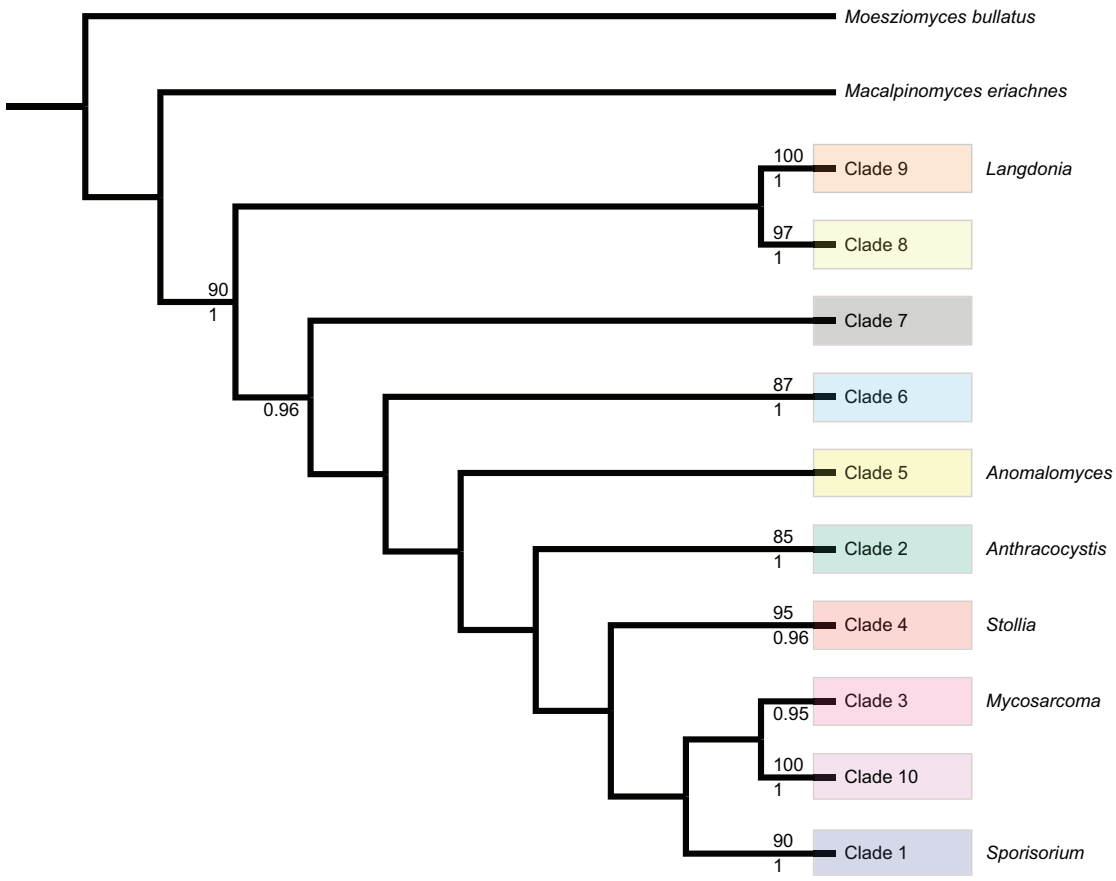

Fig. 21. Summary of clades resolved in the molecular phylogenetic analysis in Chapter 5, and the names proposed for the clades in this study. Clades 6, 7 and 8 belong to the unresolved *Ustilago*. The taxa in Clade 10 belong to *Macalpinomyces s.lat*.

7.2.1 Definitions of soral characters in the *Ustilago-Sporisorium-Macalpinomyces* complex

The interpretation of the soral morphology is inconsistent for many descriptions of smut species. An example is description of columellae in *Sporisorium consanguineum*, *Macalpinomyces spermophorus* and *M. viridans*, which were not homologous with columellae in Clades 1 and 2 (Chapter 6, this thesis). In light of the

character homology revealed earlier, the soral characters can now be defined accurately to prevent misinterpretation of these characters.

7.2.1.1 Columellae

We define a columella as a structure formed by both fungal and host material, which proliferates after hyphal-induced growth of the host meristem, and connects the sorus to the host. The columella is invariably the same length or slightly shorter than the length of the sorus. There are two types of true columellae within the complex. Stout columellae are a synapomorphy of Clade 1, and filiform, flexuous columellae are a synapomorphy of Clade 2. The non-homologous columella-like structures found in other clades have different origins and do not satisfy our definition of columellae. In particular, care should be taken not to confuse the columella with remnants of the inflorescence, such as in *Ustilago drakensbergia*.

7.2.1.2 Partitioning cells

We define partitioning cells as the cells formed from non-sporogenous hyphae within the sorus. Partitioning cells are a synapomorphic character of the complex, present in *Macalpinomyces eriachnes* and Clades 1, 3, 4, 5, 8 and 10. Partitioning cells also occur in other groups of smut fungi, including *Tilletia* in the Exobasidiomycetes, and are not a valuable character for higher levels of classification. Cells that occur in the peridium or between spore balls are not formed from partitioning hyphae and are referred to as sterile cells.

Partitioning cells formed from non-sporogenous hyphae within the sorus are a useful character for delimitation of genera within the *Ustilago-Sporisorium-Macalpinomyces* complex. Taxa that lack partitioning cells occur in two monophyletic groups, namely Clade 2 and the subgroup, *Ustilago s. str.*, of Clade 7. Absence of partitioning cells is a synapomorphy for these groups.

7.2.1.3 Spore balls

Spore balls were considered to be a convergent character within the Ustilaginomycotina (Vánky 2001). However, spore balls produced by coiled sporogenous hyphae are a synapomorphic character within Clades 2, 5 and 9 of the

Ustilago-Sporisorium-Macalpinomyces complex. Although spore balls are a homoplasious character within the Ustilaginomycotina, they can be used for generic delimitation within the complex.

7.2.1.4 Sorus structure

Swollen ovaries or galls are a convergent soral characteristic in the Ustilaginomycotina, as they occur in distantly related genera, for example *Tilletia*, *Thecaphora* (Fingerh.), and *Microbotryum* (Lév.), as well as several groups of the *Ustilago-Sporisorium-Macalpinomyces* complex (Piepenbring 2004). *Macalpinomyces eriachnes*, *Moesziomyces* and members of Clades 4, 5 and 9 induce swollen ovaries in their host plants. Although swollen ovaries are a convergent character, combined with other synapomorphic characters, swollen ovaries are useful for generic delimitation of these groups. Only some taxa in Clades 7 and 8 produce swollen ovaries on the host. Soral morphology is unsuitable for delimiting Clades 7 and 8 because of presence of diverse soral morphology.

7.3 Taxonomy

Earlier analyses (Chapter 5, this thesis) revealed ten monophyletic groups within the *Ustilago-Sporisorium-Macalpinomyces* complex (Fig. 22). Character homology was assessed (Chapter 6, this thesis) and morphological synapomorphies were identified that defined monophyletic groups within the complex. Here we propose a new classification for taxa within *Ustilago*, *Sporisorium* and *Macalpinomyces*.

7.3.1 *Sporisorium sensu stricto* (Clade 1)

Sporisorium Link was originally described as having columellae and partitioning cells (Link 1825; Langdon and Fullerton 1978). Many species were described in *Sporisorium* even though they lacked partitioning cells, for example *Sporisorium absconditum* Vánky, *S. cenchri* Vánky and *S. glutinosum* (Zundel) Vánky. Spore balls became a *de facto* defining character of *Sporisorium* (Vánky 2002), although spore balls were not mentioned in the type description. Earlier analysis (Chapter 6, this thesis) resolved the synapomorphies of the clade that contained *Sporisorium sorghi*, the type species of *Sporisorium*. It is now possible to define *Sporisorium* in a strict

sense, according to the descriptions by Link (1825) and Langdon and Fullerton (1978). Emended or additional characters have been placed in bold font.

Sporisorium Ehrenb. ex Link, in Link, Linné's Species Plantarum, Ed. 4, 6(2): 86. 1825 **emend.** McTaggart & R.G. Shivas

Sori replacing inflorescences or florets. Peridium of interwoven hyphae overlain by several layers of host tissue. Columella composed of host tissues permeated by inter- and intra-cellular hyphae, **cylindrical, stout or woody, branched or unbranched, peripheral cells thick-walled and vacuolated**. Hyphae growing from columella of young sori differentiating as pockets of sporogenous hyphae enclosed by non-sporogenous partitioning hyphae. **Sporogenous hyphae uncoiled**. Spores at first somewhat agglutinated, later pulverulent, dark, single, globose to subglobose. Partitioning cells hyaline, subglobose to globose, in groups or chains, intermixed with the spores, **formed from non-sporogenous partitioning hyphae**. Germination of *Ustilago* type.

Type species: *Sporisorium sorghi* Ehrenb. ex Link
Neotype (design. by Vánky 1990: 275) on *Sorghum bicolor*, Egypt, Cairo, VI.1876, G. Schweinfurth, HUV 1672 ; isoneotypes in Thümen, Mycoth. univ. no. 725 (as '*Ustilago reiliana* J.G. Kühn f. *Sorghi cernui* on *Sorghum cernuum*). Paraneotype on *Sorghum bicolor*, Romania, Transylvania, near Odorhei [Székelyudvarhely], alt. c. 480 m, 5.IX.1963, K. Vánky, HUV 2027 ; isoparaneotypes in Vánky, Ust. exs. no. 50 (as *Sphacelotheca sorghi* on *Sorghum vulgare*). For comments on neotypifying see Vánky 1990: 275.

7.3.1.1 New combinations for Sporisorium s. str.
The following new combination of *Sporisorium* is based on a molecular phylogenetic analysis conducted in the current study[1] and examination of type specimens or specimens from the exsiccata *Herbarium Ustilaginales Vánky* [2].

[1,2]***Sporisorium porosum*** (Langdon) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Ustilago porosa* Langdon, Proc. Linn. Soc. New South Wales 87: 48. 1962.

Specimens examined: AUSTRALIA, Western Australia, Kununurra, Sewerage treatment plant, *Sarga timorense* (Kunth) Spangler, 07 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51811 a; Western Australia, Kununurra, Mulligan Lagoon Road, *Sarga timorense*, 09 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51842a; Northern Territory, NE of Anthony Lagoon, *Sarga timorense*, 15 Apr. 1947, S.T. Blake, BRIP 7803a, holotype.

Sporisorium porosum does not have partitioning cells, which are usually present in other species of *Sporisorium*.

7.3.2 *Anthracocystis* (Clade 2)

Many of the taxa recovered in Clade 2 in the molecular phylogenetic analysis (Chapters 5, this thesis) were previously regarded to belong to *Sorosporium*. Langdon and Fullerton (1975) described soral differences between *Sorosporium*, which occurred on hosts in the *Polygonaceae*, and *Sorosporium*, which occurred on hosts in the Poaceae. The name *Sorosporium* was considered a synonym of *Thecaphora* Fingerh. (Vánky 2002) and is not suitable for species in Clade 2. Another name that has been applied to a species in Clade 2 is *Anthracocystis*, which was published by Brefeld (1912) to accommodate *Sporisorium destruens* (Schltdl.) Vánky. Brefeld (1912) diagnosed *Anthracocystis* as a separate genus due to presence of smut galls or sori, and the peculiar formation of its peridium that develops from floral envelopes.

A third name applied to the species in Clade 2 was *Lundquistia*, which Vánky (2001a) established for taxa possesing spore balls embedded in the host tissue, and sori that lacked sterile cells, peridia and columellae. Vánky (2001a) initially transferred one species, *Lundquistia panici-leucophaei* (= *Sporisorium panici-leucophaei*), which occurs on *Digitaria brownii*, into *Lundquistia*. Three years later, Vánky (2004) emended *Lundquistia* to include species that had either permanent or ephemeral spore balls, with or without sterile cells between the spore balls. He included three South American taxa, *L. mexicana* (= *Sporisorium mexicanum*), *L. duranii* (Vánky) Vánky (= *S. duranii* (Vánky) Vánky & Cunnington), and *L. deiteliana* (Henn.) Vánky (= *S. dietelianum* Vánky), which possessed combinations of soral characters that were not typical of *Sporisorium*. Vánky (2004) stated that the characters he used to establish

Lundquistia were "not strong enough to differentiate two genera", but retained *Lundquistia* as a genus.

The first two descriptions of *Lundquistia* by Vánky (2001a, 2004) made some mistaken conclusions about the soral morphology of species included in this genus (Chapter 6, this thesis). *Lundquistia* was originally described as lacking columellae (Vánky 2001a). The presence of filiform columellae is a synapomorphy for taxa in Clade 2 (Chapter 6, this thesis). The combination of fungal and host material in the shredded fascicles of vascular bands described in *Sporisorium panici-leucophaei* are considered columellae under our definition. Vánky (2001a) reported that the spore balls in *Lundquistia* were not formed from coiled sporogenous hyphae, which is an apomorphic character for taxa in the Clade 2. The method of formation of spore balls in *Sporisorium panici-leucophaei* is currently unknown (Chapter 6, this thesis). For other taxa in Clade 2, spore ball formation is caused by the formation of coiled sporogenous hyphae, as outlined by Langdon and Fullerton (1975).

Cunnington et al. (2005) demonstrated that the emended *Lundquistia* (Vánky 2004) was polphyletic. Stoll et al. (2005) and Cunnington et al. (2005) independently synonymized *Lundquistia* with *Sporisorium*. They were unable however, to determine any morphological characters that could separate the two genera.

Sporisorium panici-leucophaei and *S. mexicanum*, which were both combined into *Lundquistia* (Vánky 2001a, 2004), sit in Clade 2. Taxa incuded in Clade 2 following molecular phylogenetic analysis (Chapter 5, this thesis), represent a genus separate from *Sporisorium*. *Lundquistia* and *Anthracocystis* are valid names to accommodate these species. *Anthracocystis* was described in 1912 and takes priority over *Lundquistia*.

Characters that can be used to separate *Anthracocystis* from *Sporisorium* are the presence of filiform colulmellae and spore balls, and the absence of partitioning cells. *Anthracocystis* is emended to accommodate taxa with these characters. Emended or additional characters have been placed in bold font.

Anthracocystis Bref., Unters. Gesammtgeb. Mykol. 15: 53. 1912. **emend.** McTaggart & R.G. Shivas.

= *Lundquistia* Vánky, Mycotaxon 77: 371. 2001, emend. Vánky, Fungal Diversity, 17: 160. Type: *Lundquistia panici-leucophaei* (Bref.) Vánky on *Digitaria brownii*.

Sori replacing inflorescences, **all of the racemes or localised in spikelets of an inflorescence**. **Peridium of vacuolated fungal cells surrounded by a single layer of host cells**. **Columella composed of vascular bundles surrounded by host parenchyma, the tissues being permeated by inter- and intracellular hyphae, often separated into several columellae each around a vascular bundle surrounded by parenchyma, filiform, flexuous, flattened. Sporogenous hyphae coiled** or **unknown**. Spores compacted in spore balls, globose to subglobose, often outer spores darker than inner spores. **Partitioning cells formed from non-sporogenous partitioning hyphae few or absent**.

Type species: *Anthracocystis destruens* Bref.

Neotype (design. by Vánky 1985:116) on *Panicum miliaceum*, Germany, Bunzlau [Poland, Bolesławiec], J. Kühn, HUV 1895; isoneotypes in Rbh., Herb. viv. myc., ed. 2, no. 400 (as *'Ustilago destruens'*).

7.3.2.1 New combinations for Anthracocystis

The following taxa are new combinations of *Anthracocystis* based on either results of the molecular phylogenetic analysis[1], examination of either type specimens or specimens from the exiccata *Herbarium Ustilaginales Vánky*[2], or from the type descriptions in the protologues[3].

[3]***Anthracocystis abramoviana*** **(**Lavrov) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Sorosporium abramovianum* Lavrov, Trudy Tomsk. Gosud. Univ. 86: 85. 1934.

≡ *Sporisorium abramovianum* (Lavrov) I.V. Karatygin, in Karatygin & Azbukina, Definitorum fungorum URSS, etc.: 72. 1989.

[1,2]***Anthracocystis abscondita*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Sporisorium absconditum* Vánky, Mycotaxon 85: 36. 2003.

Specimens examined: AUSTRALIA, Queensland, Mount Garnet, Forty Mile Scrub National Park, *Schizachyrium fragile* (R. Br.) A. Camus, 06 Apr. 1998, C. & K. Vánky, BRIP 43880, isotype; Northern Territory, 26 km S of Tennant Creek, *Schizachyrium fragile*, 26 Apr. 2007, A.R. McTaggart, J.R. Liberato, R.G. Shivas, BRIP 49648.

[3]***Anthracocystis anadelphiae*** (G. Viennot-Bourgin) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium anadelphiae* Viennot-Bourgin, Bull. Soc. Bot. France 104: 266. 1957 (as '*ananelphiae*').
≡ *Sporisorium anadelphiae* (Viennot-Bourgin) Vánky, Mycotaxon 85: 58. 2003.

[3]***Anthracocystis andropogonis-aciculati*** (Petch) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago andropogonis-aciculati* Petch, Ann. Roy. Bot. Gard. (Peradeniya) 4: 303. 1909.
≡ *Sorosporium andropogonis-aciculati* (Petch) Petch, Ann. Roy. Bot. Gard. (Peradeniya) 5: 227. 1912.
≡ *Sporisorium andropogonis-aciculati* (Petch) Vánky, Mycotaxon 18: 328. 1983.

Specimen examined: CHINA, Yunnan, Jinghong, *Chrysopogon aciculatus* (Retz.) Trin., 24 Sep. 1985, T. & K. Vánky, BRIP 26282 = Vánky, Ust. exs. no. 522.

[3]***Anthracocystis andropogonis-chinensis*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium andropogonis-chinensis* (Vánky), Mycotaxon 95: 5. 2006.

[3]***Anthracocystis andropogonis-eucomi*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium andropogonis-eucomi* Vánky, Mycotaxon 95: 5. 2006.

Specimen examined: SOUTH AFRICA, Mpumalanga Prov., 9 km NE of Grascop, 1 km along road R534, *Andropogon eucomus* Nees, 22 Jan. 1997, C. & K. Vánky, BRIP 47128, isotype.

[2,3]***Anthracocystis andropogonis-finitimi*** (Maubl.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago andropogonis-finitimi* Maubl., Bull. Soc. Mycol. France 22: 74. 1906.
≡ *Sporisorium andropogonis-finitimi* (Maubl.) Vánky & Mouch., Mycol. Res. 104: 382. 2000.

Specimens examined: ZAMBIA, Southern Province, 22 km NE of Pemba, *Hyparrhenia filipendula* (Hochst.) Stapf., 12 Apr. 2001, C., T. & K. Vánky, BRIP 39626; Southern Province, 10 km NW of Monze, *Hyparrhenia filipendula*, 15 Apr. 2001, C., T. & K. Vánky, BRIP 39634.

[3]***Anthracocystis andropogonis-gabonensis*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium congoense* L. Ling
≡ *Sporisorium andropogonis-gabonensis* Vánky Mycotaxon 95: 7. 2006.

[3]***Anthracocystis andropogonis-pumili*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium andropogonis-pumili* Vánky, Mycotaxon 95: 7. 2006.

[1,2]***Anthracocystis anthistiriae*** (Cobb) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Tolyposporium anthistiriae* Cobb, Agric. Gaz. New South Wales 3: 1006. 1892.
≡ *Sorosporium anthistiriae* (Cobb) L. Ling, Mycol. Pap. 11: 9. 1945.
≡ *Sporisorium anthistiriae* (Cobb) Vánky, in Vánky & Guo, Acta Mycol. Sinica, Suppl. I: 230. 1987.

Specimens examined: CHINA**,** Beijing, Botanical Garden, *Themeda triandra* Forssk., 08 Oct. 1985, L. Guo & K. Vánky, BRIP 27351 = Vánky, Ust. exs. no. 579; AUSTRALIA, Western Australia, between Wyndham and Kununurra, *Themeda triandra*, 13 Apr. 2007, A.R. McTaggart, M.J. Ryley & R.G. Shivas, BRIP 49775.

[1,2]***Anthracocystis anthracoideispora*** (Vánky & R.G. Shivas) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium anthracoideisporum* Vánky & R.G. Shivas, in Vánky, Mycotaxon 68: 335. 1998.

Specimen examined: PAPUA NEW GUINEA, Western Province, Bensbach River, *Pseudoraphis spinescens* (R. Br.) Vickery, 13 Apr. 1997, A.A. Mitchell & R.G. Shivas, BRIP 39176, isotype.

[1,2]***Anthracocystis apludae-aristatae*** (B.V. Patil & Thirum.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium apludae-aristatae* B.V. Patil & Thirum., Sydowia 20: 48. 1968
≡ *Sporisorium apludae-aristatae* (B.V. Patil & Thirum.) Vánky, Mycotaxon 65: 135. 1997.

Specimen examined: INDIA, Uttar Pradesh, Varanasi, *Apluda mutica* L., 07 Oct. 1992, K. Vánky, BRIP 26326 = Vánky, Ust. exs. no. 916.

[2,3]***Anthracocystis azmatii*** (Mundk.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium azmatii* Mundk., Trans. Brit. Mycol. Soc. 23: 115. 1939.
≡ *Sporisorium azmatii* (Mundk.) Vánky, Fungal Diversity 18: 180. 2005.

Specimen examined: INDIA, Karnataka, Mysore, Bilikere, *Chrysopogon caeruleus* (Steudel) Watson, 19 Sept. 1903, C.A. Barber, BRIP 8052, isotype.

[2,3]***Anthracocystis berndtii*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Sporisorium berndtii* Vánky, Mycotaxon 85: 37. 2003.

Specimen examined: THAILAND, Chiang Mai, Mae Taeng District, Mae Ngad Dam, *Schizachyrium sanguineum* (Retz.) Alston, 19 Dec. 2007, P. Athipunyakom, S. Likhitekaraj, V.L. Challinor, A.R. McTaggart, T.S. Marney, M.D.E & R.G. Shivas, BRIP 51559.

[2,3]***Anthracocystis blakeana*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium blakeanum* Vánky, Mycotaxon 89: 74. 2004.

Specimen examined: AUSTRALIA, Queensland, N of Hughenden, Poison Creek, *Schizachyrium fragile* (R. Br.) A. Camus, 10 Apr. 1935, S.T. Blake, BRIP 7804, holotype.

[1,2]***Anthracocystis bothriochloae*** (L. Ling) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium bothriochloae* L. Ling, Lloydia 16: 186. 1953.
≡ *Sporisorium bothriochloae* (L. Ling) Vánky, Fungal Diversity 15: 229. 2004.

Specimens examined: AUSTRALIA, Northern Territory, on Stuart Highway, 209 km SE of Katherine, *Dichanthium fecundum* S.T. Blake, 14 Mar. 2000, R.G. Shivas, I.T. Riley, C. & K. Vánky, BRIP 44251 = Vánky, Ust. exs. no. 1196; Western Australia, between Wyndham and Kununurra, *Dichanthium sericeum* (R. Br.) A. Camus, 08 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51819.

[1,2]***Anthracocystis caledonica*** (Pat.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium caledonicum* Pat., Bull. Soc. Mycol. France 3: 173. 1887.
≡ *Sporisorium caledonicum* Vánky, Mycotaxon 40: 165. 1991.
= *Sorosporium heteropogonis-contorti* Bacc., Ann. Bot. (Rome) 14: 132. 1917.

Specimens examined: INDIA, Utter Pradesh, Tehri, Garwal Himalaya Mt., *Heteropogon contortus* (L.) Roem. & Schult., 17 Sept. 1992, T. & K. Vánky, BRIP 27446 = Vánky, Ust. exs. no. 1053; AUSTRALIA, Northern Territory, Timber Creek, Policeman's Lookout, *Heteropogon contortus*, 10 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51854.

[1,2]***Anthracocystis cenchri*** (Lagerh.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago cenchri* Lagerh., in Patouillard & Lagerheim, Bull. Herb. Boissier 3: 62. 1895.
≡ *Sporisorium cenchri* Vánky, Symb. Bot. Upsal. 24: 114. 1985.
= *Sorosporium cenchri* Henn., Hedwigia 35: 221. 1896.
= *Tolyposporium cenchri* Bref., Unters. Gesammtgeb. Mykol. 12: 156. 1895.
= *Sorosporium chardonianum* Zundel, Mycologia 34: 125. 1942.
= *Sorosporium texanum* Zundel, Mycologia 36: 409. 1944.
= *Sorosporium cenchri* Henn. var. *levis* Vörös & Ubrizsy, Acta Phytopathol. Acad. Sci. Hung. 3: 269. 1968.

Specimen examined: MEXICO, 56 km NE of Durango, *Cenchrus pauciflorus* Benth., 18 Nov. 2003, T. & K. Vánky, BRIP 45311: Vánky Ust. exs. no. 1214.

[1,2]***Anthracocystis cenchri-elymoidis*** (Vánky & R.G. Shivas) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium cenchri-elymoidis* Vánky & R.G. Shivas, in Vánky, Mycotaxon 81: 392. 2002.

Specimens examined: AUSTRALIA, Western Australia, Corneille Island, *Cenchrus elymoides* F. Muell. var. *brevisetosus* B.K. Simon, 18 May 1998, A.A. Mitchell, BRIP 26491, holotype; Western Australia, Mitchell Plateau, Surveyor's Pool, *Cenchrus elymoides* var. *brevisetosus*, 12 May 2009, A.R. McTaggart, V.L. Challinor, M.J. Ryley, C.F. Gambley, T. Scharaschkin, M.D.E. & R.G. Shivas, BRIP 52532.

[2,3]***Anthracocystis chamaeraphis*** (Syd.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium chamaeraphis* Syd., in Sydow & Petrak, Ann. Mycol. 26: 431. 1928.
≡ *Sporisorium chamaeraphis* Vánky, Mycotaxon 68: 330. 1998.
= *Ustilago confusa* Massee, in Cooke, Grevillea 20: 65. 1892.
= *Sporisorium shivasii* Vánky, Mycotaxon 89: 104. 2004.

Specimen examined: AUSTRALIA, Northern Territory, Daly River, Fish Lagoon, *Pseudoraphis spinescens* (R. Br.) Vickery, 12 Sept. 1996, I.G. Pascoe, BRIP 26795.

[3]***Anthracocystis chrysopogonicola*** (A.R. Patil, T.M. Patil & M.S. Patil) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium chrysopogonicola* A.R. Patil, T.M. Patil & M.S. Patil J., Mycol. Pl. Pathol. 34: 779. 2004.

[3]***Anthracocystis chrysopogonis*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium chrysopogonis* Vánky, Mycotaxon 18: 327. 1983.

[3]***Anthracocystis chrysopogonis-fulvi*** (A.R. Patil, T.M. Patil & M.S. Patil) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium chrysopogonis-fulvi*, A.R. Patil, T.M. Patil & M.S. Patil, J. Mycol. Pl. Pathol. 34: 839. 2004 (as '*chrysopogonis-fulviis*').
≡ *Sporisorium chrysopogonis-fulvi* (A.R. Patil, T.M. Patil & M.S. Patil) Vánky & A.R. Patil, in Vánky, Mycotaxon *99*: 50. 2007.

[3]***Anthracocystis compacta*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium compactum* Vánky, Mycotaxon 85: 23. 2003.

[2,3]***Anthracocystis congensis*** (Syd. & P. Syd.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago congensis* Syd. & P. Syd., in Wildeman, Études sur la flore du Bas- et Moyen-Congo 3: 9. 1909.
≡ *Sphacelotheca congensis* (Syd. & P. Syd.) Wakef., in Zundel, Mycologia 22: 140. 1930.
≡ *Sporisorium congense* (Syd. & P. Syd.) Vánky, Fungal Diversity 12: 186. 2003.

Specimen examined: UGANDA, Wakiso, Entebbe, Zizka Forest, *Hyparrhenia diplandra* Staf, 15 Feb. 2002, M. Namaganda, T., C. & K. Vánky, BRIP 44088 = Vánky, Ust. exs. no. 1179.

[3]***Anthracocystis contorta*** (Griffiths) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium contortum* Griffiths, Bull. Torrey Bot. Club 31: 83. 1904.
≡ *Sporisorium contortum* (Griffiths) Vánky, Mycotaxon 40: 165. 1991.

[3]***Anthracocystis cryptica*** (Cooke & Massee) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Cintractia cryptica* Cooke & Massee, in Cooke, Grevillea 18: 34. 1889.
≡ *Sorosporium crypticum* (Cooke & Massee) Ling, Sydowia 3: 131. 1949.
≡ *Sporisorium crypticum* (Cooke & Massee) Vánky & M.S. Patil, in Vánky, Mycotaxon 74: 183. 2000.

[2,3]***Anthracocystis crypta*** (McAlpine) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago crypta* McAlpine, Proc. Linn. Soc. New South Wales 32: 42. 1897.
≡ *Sorosporium cryptum* (McAlpine) McAlpine, The smuts of Australia: 176. 1910.
≡ *Sporisorium cryptum* (McAlpine) Vánky, Mycotaxon 74: 173. 2000.
= *Sorosporium turneri* McAlpine, The smuts of Australia: 185. 1910.

Specimens examined: AUSTRALIA, Queensland, ca. 100 km SW of Mareeba, *Brachiaria holosericea* (R.Br.) Hughes, 03 Mar. 2000, C. & K. Vánky, BRIP 44094 = Vánky, Ust. exs. no. 1185; Western Australia, Drysdale River, Kalumburu Rd., *Yakirra* sp., 12 May 2009, A.R. McTaggart, V.L. Challinor, M.J. Ryley, C.F. Gambley, T. Scharaschkin, M.D.E. & R.G. Shivas, BRIP 52536.

[2,3]***Anthracocystis cymbopogonis*** (Mundk.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Tolyposporium cymbopogonis* Mundk., Indian J. Agric. Sci. 14: 51. 1944.
≡ *Sorosporium cymbopogonis* (Mundk.) Thirum. & Neerg., Friesia 11: 183. 1978.
≡ *Sporisorium cymbopogonis* (Mundk.) Vánky, Mycotaxon 85: 25. 2003.
= *Tolyposporium christensenii* Raghunath, Mycopathol. Mycol. Appl. 34: 120. 1968

Specimen examined: INDONESIA, Bali, Lake Batur, ca. 2 km SW of Hot Springs, *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson, 04 Apr. 1992, C. & K. Vánky, BRIP 39635.

[1,2]***Anthracocystis cymbopogonis-bombycini*** (R.G. Shivas & Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium cymbopogonis-bombycini* R.G. Shivas & Vánky, Mycol. Balcan. 1: 163. 2004.

Specimens examined: AUSTRALIA, Western Australia, Wyndham, *Cymbopogon bombycinus* (R.Br.) Domin, 03 Mar. 1989, R.G. Shivas, BRIP 26809, holotype; Western Australia, Mt. Hart Wilderness Lodge, *Cymbopogon bombycinus*, 14 May 2009, A.R. McTaggart, V.L. Challinor, M.J. Ryley, C.F. Gambley, T. Scharaschkin, M.D.E. & R.G. Shivas, BRIP 52511.

[3]***Anthracocystis cymbopogonis-distantis*** (L. Ling) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium cymbopogonis-distantis* L. Ling, Farlowia 4: 341. 1953.
≡ *Sporisorium cymbopogonis-distantis* (L. Ling) L. Guo, Mycosystema 17: 1. 1998.

[3]***Anthracocystis cynodontis*** (L. Ling) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium cynodontis* L. Ling, Sydowia 3: 131. 1949.
≡ *Sporisorium cynodontis* (L. Ling) R.G. Shivas & Vánky, Fungal Diversity 8: 150. 2001.

Anthracocystis cynodontis is one of five known *Anthracocystis* species that infects a chloridoid grass. The majority of *Anthracocystis* taxa infect andropogonoid grasses.

[3]***Anthracocystis decorsei*** (Har. & Pat.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Tolyposporium decorsei* Har. & Pat., Bull. Mus. Hist. Nat. (Paris) 15: 197. 1909.
≡ *Sorosporium decorsei* (Har. & Pat.) L. Ling, Lloydia 16: 187. 1953.
≡ *Sporisorium decorsei* (Har. & Pat.) Vánky, Mycotaxon 65: 160. 1997.

[2,3]***Anthracocystis dembianensis*** (Baccarini) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium dembianense* Baccarini, Ann. Bot. (Rome) 14: 132. 1917.
≡ *Sporisorium dembianense* (Baccarini) Vánky

Specimens examined: ZIMBABWE, North Province, Matabeland, Victoria Falls, *Hyparrhenia tamba* (Steudel) Stapf, 02 Dec. 1999, C. & K. Vánky, BRIP 39649; Zambia, Eastern Province, 230 km NE of Lusaka, *Hyparrhenia filipendula* (Hochst.) Stapf, 18 Apr. 2001, T., C. & K. Vánky, BRIP 39689; SOUTH AFRICA, Mpumalanga, 2 km E of Waterfall-Bowen, *Hyparrhenia filipendula*, 20 Dec. 2002, A. Witt, R.G. Shivas & K. Vánky, BRIP 39657; ETHIOPIA, Gojam Region, 24 km NE of Bahar Dahr, *Hyparrhenia hirta* (L.) Stapf, 23 Oct. 2004, T. & K. Vánky, BRIP 47130.

Anthracocystis dembianensis possesses several synapomorphic characters of *Anthracocystis*, namely permanent spore balls, dimorphic spores and no partitioning cells. The columella is described by Vánky (2003a) as flagelliform, often with a shortly bi- or trifurcate apex. Examination of four specimens of *L. dembianensis* confirmed that the columella is flagelliform. It is woody at the base of the sorus and tapers into a flattened, filiform apex.

[3]***Anthracocystis densiflora*** (L. Ling) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium densiflorum* L. Ling, Lloydia 16: 188. 1953.
≡ *Sporisorium densiflorum* (L. Ling) Vánky, Mycotaxon 85: 27. 2003.

[1,2]***Anthracocystis destruens*** (Schltdl.) Bref. MycoBank

Basionym: *Caeoma destruens* Schltdl., Fl. Berol., Pars 2. Cryptogamia: 130. 1824.

≡ *Uredo destruens* (Schltdl.) Duby, *Botanicon Gallicum*, *Ed*. *2*, *Pars 2*:901, 1830.

≡ *Tilletia destruens* (Schltdl.) Lév., *Ann*. *Sci*. *Nat*. *Bot*., *Sér*. *3*, *8*:372, 1847.

≡ *Ustilago destruens* (Schltdl.) Rabenh., Herb. viv. myc., ed. 2, no. 400, 1857.

≡ *Sphacelotheca destruens* (Schltdl.) J.A. Stev. & Aar.G. Johnson, Phytopathology 34: 613. 1944.

≡ *Sporisorium destruens* (Schltdl.) Vánky, Symb. Bot. Upsal. 24: 115. 1985.

= *Uredo segetum* Pers. *δ Uredo Panici-miliacei Pers.,* Syn. meth. fung. 1: 224. 1801.

≡ *Uredo carbo δ panici-miliacei* (Pers.) De Candolle, Fl. franç., ed. 3, 6: 76. 1815.

≡ *Erysibe panicorum β panici-miliacei* (Pers.) Wallroth, Flora Cryptogamica Germaniae 2: 216. 1833.

≡ *Ustilago panici-miliacei* (Pers.) G. Winter, Rabenh. Krypt.-Fl., 2 Aufl., 1: 89. 1881.

≡ *Sorosporium panici-miliacei* (Pers.) Takahashi, Bot. Mag. (Tokyo) 16: 184 & 247. 1902.

≡ *Sphacelotheca panici-miliacei* (Pers.) Bubák, Houby Ceské 2: 27. 1912.

= *Sorosporium manchuricum* S. Ito, Trans. Sapporo Nat. Hist. Soc. 14: 93. 1935.

≡ *Sphacelotheca manchurica* (S. Ito) Y.C. Wang, Acta Bot. Sinica 10: 134. 1962.

= *Sphacelotheca lioui* W.Y. Yen, Contr. Inst. Bot. Natl. Acad. Peiping 4: 193. 1937.

Specimens examined: ROMANIA, Dobrogea, delta Danubii, pr. brachium Sf. Gheorghe, *Panicum miliaceum* L., 19 Sep. 1982, G.A., Negrean, BRIP 27193 = Vánky, Ust. exs. no. 472; AUSTRALIA, Queensland, Dalby, *Panicum miliaceum*, 08 Apr. 1958, T. McKnight, BRIP 8221.

There are a few partitioning cells reported in *Anthracocystis destruens* (Vánky 1994b). These are most likely remnants of non-sporogenous hyphae.

[3]***Anthracocystis duranii*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Ustilago duranii* Vánky, Mycotaxon 89: 77. 2004.

≡ *Sporisorium duranii* (Vánky) Vánky & Cunnington, in Cunnington, Vánky & Shivas, Mycol. Balcan. 2: 96. 2005.

≡ *Lundquistia duranii* (Vánky) Vánky, Fungal Diversity 17: 165. 2004.

[2,3]***Anthracocystis dichanthii*** (Vánky & N.D. Sharma) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Sporisorium dichanthii* Vánky & N.D. Sharma, in Vánky, Fungal Diversity 15: 230. 2004.

Specimen examined: INDIA, Madhya Pradesh, Jabalpur, 200 km SW of Pachmarhi, *Dichanthium aristatum* (Poiret) C.E. Hubbard, 30 Oct. 1992, R. Sharma, S. Raich, BRIP 51777, HUV 20263, isotype.

[3]***Anthracocystis ehrenbergii*** (Kühn) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Sorosporium ehrenbergii* Kühn, Mitth. Vereins. Erdk. Halle 1877: 87.

≡ *Tolyposporium ehrenbergii* (Kühn) Pat., Bull. Soc. Mycol. France 19: 254. 1903.

≡ *Sporisorium ehrenbergii* (Kühn) Vánky, Mycotaxon 38: 270. 1990.

= *Tolyposporium filiferum* Busse, Arbeiten Biol. Abt. Landw.-Forstw. Kaiserl. Gesundheit. 4: 383. 1905.

≡ *Sorosporium filiferum* (Busse) Zundel, Mycologia 22: 148. 1930.

= *Sorosporium andropogonis-sorghi* S. Ito, Trans. Sapporo Nat. Hist. Soc. 14: 93. 1935.

[1,2]***Anthracocystis elionuri*** (Henn. & Pole-Evans) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Ustilago elionuri* Henn. & Pole-Evans, in Hennings, Bot. Jahresb. Syst. 41: 270. 1908.

≡ *Sphacelotheca elionuri* (Henn. & Pole-Evans) Viennot-Bourgin (nom. herb.?).

≡ *Sporisorium elionuri* (Henn. & Pole-Evans) Vánky, Mycotaxon 73: 155. 1999.

= *Ustilago elionuri* Speg., Anales Mus. Nac. Buenos Aires, Ser. 3, 12: 288. 1909. (later homonym, not Henn. & Pole-Evans 1908).

≡ *Ustilago elionuri-candidi* Speg., in Saccardo & Trotter, in Saccardo, Syll. fung. 21: 501. 1912. (nom. nov. pro *U. elionuri* Speg.).

≡ *Sphacelotheca elionuri-candidi* (Speg.) Hirschh., Ustil. Fl. Argent.: 119. 1986.

Specimen examined: SOUTH AFRICA, Eastern Cape, Lady Grey, *Elionurus muticus* (Spreng.) Kunth, 21 July 1996, C. & K. Vánky, BRIP 26429 = Vánky, Ust. exs. no. 1019.

[1,2]***Anthracocystis enteromorpha*** (McAlpine) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Ustilago enteromorpha* McAlpine, Agric. Gaz. New South Wales 7: 154. 1896.

≡ *Sorosporium enteromorphum* (McAlpine) McAlpine, The smuts of Australia: 177. 1910.

≡ *Sporisorium enteromorphum* (McAlpine) Vánky, Mycotaxon 51: 161. 1994.

Specimens examined: SOUTH AFRICA, KwaZulu-Natal, Drakensberg Mountains, *Themeda triandra* Forssk., 03 Jan. 1997, K. Vánky, BRIP 26430 = Vánky, Ust. exs. no. 1020; AUSTRALIA, Queensland, Carnarvon National Park, Carnarvon Gorge, *Themeda triandra*, 28 Jun. 2010, R.G. Shivas, BRIP 53624.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis*

enteromorpha (Vánky 1994b), which are most likely remnants of the non-sporogenous hyphae. McAlpine (1910) did not record partitioning cells in the type description.

[3]***Anthracocystis eriochloae*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium eriochloae* Vánky, Mycotaxon 74: 174. 2000.

[2,3]***Anthracocystis eulaliae*** (L. Ling) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium eulaliae* L. Ling, Sydowia 7: 155. 1953.
≡ *Sporisorium eulaliae* (L. Ling) Vánky, Mycotaxon 62: 137. 1997.

Specimen examined: AUSTRALIA, Queensland, Pindi Pindi, *Eulalia trispicata* (Schult.) Henr., 21 Aug. 1941, R.F.N. Langdon, BRIP 7929, isotype.

[1,3]***Anthracocystis everhartii*** (Ellis & Galloway) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium everhartii* Ellis & Galloway, J. Mycol. 6: 32. 1890.
≡ *Tolyposporium everhartii* (Ellis & Galloway) Dietel, in Engler & Prantl, Die Natürl. Pflanzenfam. I, 1: 14. 1897.
≡ *Sporisorium everhartii* (Ellis & Galloway) M. Piepenbr., Mycol. Res. 103: 462. 1999.

[1,2]***Anthracocystis fallax*** (R.G. Shivas & Cunningt.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium fallax* R.G. Shivas & Cunningt., in Shivas, Cunnington & Vánky, Fungal Diversity 16: 149. 2004.

Specimen examined: AUSTRALIA, NT, 268 km SE of Katherine, *Chrysopogon fallax* S.T. Blake, 15 Mar. 2000, R.G. Shivas, I.T. Riley, C. & K. Vánky, BRIP 27687, holotype.

[3]***Anthracocystis filiformis*** (Henn.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago filiformis* Henn., Bot. Jahrb. Syst. 30: 254. 1901 (not *U. filiformis* (Schrank) Rostrup 1890).
≡ *Sorosporium filiforme* (Henn.) Zundel, Mycologia 22: 153. 1930 (as '*filiformi*s').
≡ *Sporisorium filiforme* (Henn.) Vánky, Mycotaxon 74: 180. 2000.

[1,2]***Anthracocystis formosana*** (Sawada) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago formosana* Sawada, J. Formosan Nat. Hist. Soc. 34: 6. 1918 (in Japanese, n.v.); in Tanaka, Mycologia 14: 89. 1922.
≡ *Sorosporium formosanum* (Sawada) Sawada, Descriptive catalogue of the Formosan fungi. 4: 29. 1928.
≡ *Sporisorium formosanum* (Sawada) Vánky, Publ. Herb. Univ. Uppsala 11: 12. 1983.
= *Ustilago digitariae* Rabenh. f. *panici-repentis* Kühn, Hedwigia 15: 5. 1876 (nom. conf., comp. Vánky 1990:274).

= *Sorosporium panici* Beeli, Bull. Jard. Bot. État 8: 7. 1922 (later homonym, not MacKinnon 1912:201).

≡ *Sorosporium beelii* Zundel, Bothalia 3: 307. 1938.

= *Sorosporium panici* Beeli var. *kinshasaensis* Beeli, Bull. Jard. Bot. État 8: 8. 1922.

≡ *Sorosporium kinshasaensis* (Beeli) Zundel, Mycologia 29: 590. 1937.

≡ *Sorosporium beelii* Zundel var. *kinshasaensis* (Beeli) Hendrix, Publ. Inst. Nat. Etude Agron. Congo Belge, Ser. Sci. 35: 8. 1948.

= *Ustilago amadelpha* Syd. & P. Syd. & Butler var. *glabriuscula* Cif., Nuovo Giorn. Bot. Ital. 40: 255. 1933.

= *Ustilago overeemii* Cif., Nuovo Giorn. Bot. Ital. 40: 254. 1933 (as '*overeeimi*').

≡ *Sorosporium overeemii* (Cif.) Malençon, Rev. Mycol. (Paris), N.S., 10: 121. 1945.

≡ *Sporisorium overeemii* (Cif.) Rifai, Reinwardtia 9: 400. 1980.

= *Sorosporium punctatum* Malençon & W.Y. Yen, Rev. Mycol. (Paris), N.S. 2: 130. 1937.

= *Sorosporium trichophorum* (Tul. & C. Tul.) Zundel, Mycologia 31: 583. 1939

Specimen examined: TAIWAN, Taichung, University campus, *Panicum repens* L., 12 July 1988, F. Oberwinkler, BRIP 19637 = Vánky, Ust. exs. no. 688.

[2,3]***Anthracocystis gayana*** (Vánky & C. Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Sporisorium gayanum* Vánky & C. Vánky , in Vánky, Mycotaxon 74: 205. 2000.

Specimen examined: ZIMBABWE, North Province, Matabeleland, 12 km north of Lusulu, *Andropogon gayanus* Kunth, 16 Mar. 1999, C. & K. Vánky, BRIP 27435, Vánky, Ust. exs. no. 1064, isotype.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis gayana* (Vánky 2000), which are most likely remnants of the non-sporogenous hyphae.

[2,3]***Anthracocystis glutinosa*** (Zundel) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Sorosporium glutinosum* Zundel, Mycologia 36: 407. 1944.

≡ *Sporisorium glutinosum* (Zundel) Vánky, Mycotaxon 74: 180. 2000.

= *Tolyposporium andropogonis* Patel & N.B. Kulk., in Patel, Gokhale & Kulkarni, Indian Phytopathol. 4: 65. 1951.

≡ *Sorosporium andropogonis* (Patel & N.B. Kulk.) Thirum. & Neerg., Friesia 11: 182. 1978.

Specimen examined: AUSTRALIA, Queensland, Lakeland, 16 km from Lakeland to Cooktown Road, *Heteropogon triticeus* (R. Br.) Stapf, 24 Mar. 2005, T.S. Marney, R.G. Shivas, BRIP 46153.

[2,3]***Anthracocystis guaranitica*** (Speg.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank

Basionym: *Ustilago guaranitica* Speg., Anales Soc. Ci. Argent. 17: 87. 1884.
≡ *Sphacelotheca guaranitica* (Speg.) Zundel, Mycologia 22: 135. 1930.
≡ *Sorosporium guaraniticum* (Speg.) L. Ling, Lloydia 16: 190. 1953.
≡ *Sporisorium guaraniticum* (Speg.) Vánky, Mycotaxon 35: 155. 1989.

Specimen examined: ECUADOR, Pichincha, Quito, 30km E of inter pagg. Pifo et Yaruqui, *Schizachyrium condensatum* Nees, 21 Mar. 1993, H. Bauch, C. & K. Vánky, BRIP 43883 = Vánky, Ust. exs. no. 1168.

[2,3]***Anthracocystis henningsii*** (Sacc. & P. Syd.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago henningsii* Sacc. & P. Syd, in Saccardo, Syll. fung. 16: 368. 1902.
≡ *Sporisorium henningsii* (Sacc. & P. Syd.) Vánky, Mycotaxon 59: 106. 1996.
= *Ustilago stenotaphri* Henn., Hedwigia 37: 293. 1898.

[1,2]***Anthracocystis heteropogonicola*** (Mundk. & Thirum.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium heteropogonicola* Mundk. & Thirum., in Thirumalachar & Mundkur, Mycol. Pap. 40: 5. 1951.
≡ *Sporisorium heteropogonicola* (Mundk. & Thirum.) Vánky, in Shivas & Vánky, Mycol. Res. 101: 839. 1997.

Specimens examined: INDIA, Nainital, Utter Pradesh, *Heteropogon contortus* (L.) Roem. & Schult., 07 Sep. 1992, K. Vánky, BRIP 26329 = Vánky, Ust. exs. no. 919; AUSTRALIA, Western Australia, between Wyndham and Kununurra, *Heteropogon contortus*, 08 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51822.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis heteropogonicola* (Shivas and Vánky 1997), which were most likely remnants of the non-sporogenous hyphae.

[2,3]***Anthracocystis hodsonii*** (Zundel) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium hodsonii* Zundel, Mycologia 22: 152. 1930.
≡ *Sporisorium hodsonii* (Zundel) Vánky, Mycotaxon 91: 225. 2005.
= *Sorosporium harrismithense* Zundel, Mycologia 22: 154. 1930.
= *Sorosporium flanaganianum* Zundel, Mycologia 22: 155, 1930.
= *Ustilago versatilis* Syd., Ann. Mycol. 33: 231. 1935.
= *Sorosporium afrum* Syd., Ann. Mycol. 33: 232. 1935.

Specimen examined: SOUTH AFRICA, Limpopo, Naboomspruit, 20km S of Nylvsley Nature Reserve, *Panicum schinzii* Hack., 15 Mar. 1998, C. & K. Vánky, BRIP 45333 = Vánky, Ust. exs. no. 1236.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. Vánky (2005) included partitioning cells as sparse or absent in his description of *Anthracocystis hodsonii*.

[2,3]***Anthracocystis holstii*** (Henn.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium holstii* Henn., in Engler, Pflanzenwelt Ost-Afrikas, etc., C,: 49. 1895.
≡ *Sporisorium holstii* (Henn.) Vánky, Mycotaxon 51: 162. 1994.

Specimen examined: THAILAND, *Themeda triandra* Forssk., 20 Dec. 2005, R.G. Shivas, P. Athipunyakom, BRIP 47758.

[3]***Anthracocystis horsfallii*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium horsfallii* Vánky, Mycotaxon 78: 297. 2001.

[1,2]***Anthracocystis hwangensis*** (Vánky & C. Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium hwangense* Vánky & C. Vánky in Vánky, Mycotaxon 74: 194. 2000.

Specimen examined: ZIMBABWE, Matabeleland North, Hwange National Park, Main Camp, Sedina Waterhole, *Sporobolus panicoides* A. Rich, 06 Mar. 1999, C. & K. Vánky, BRIP 27441 = Vánky, Ust. exs. no. 1059, isotype.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *L. hwangensis*, (Vánky 2000), which are most likely remnants of the non-sporogenous hyphae. *Anthracocystis hwangensis* is one of five known *Anthracocystis* species that infect a chloridoid grass. The majority of *Anthracocystis* taxa infect andropogonoid grasses.

[3]***Anthracocystis ischaemiana*** (A.R. Patil, T.M. Patil & M.S. Patil) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium ischaemianum* A.R. Patil, T.M. Patil & M.S. Patil, J. Mycol. Pl. Pathol. 34: 783. 2004.

[2,3]***Anthracocystis ischaemoides*** (Henn.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago ischaemoides* Henn., in Wildeman, Ann. Mus. Congo Bot., Sér. 5, Bot. 2: 86. 1907.
≡ *Sorosporium ischaemoides* (Henn.) Zundel, Mycologia 29: 587. 1937.
= *Sorosporium wildemanianum* Henn., in Wildeman, Ann. Mus. Congo, Sér. 5, Bot. 2: 87. 1907.
= *Sorosporium austroafricanum* Zundel, Mycologia 22: 147. 1930 (as '*austro-africanum*').
= *Sorosporium hansfordii* Ainsw., Proc. Linn. Soc. London 153: 93. 1941.

Specimen examined: SOUTH AFRICA, Western Cape, Gordon Bay, 10km S of Somerset West, *Hyparrhenia anamesa* Clayton, 03 Dec. 1996, C. & K. Vánky, BRIP 43875 = Vánky, Ust. exs. no. 1162.

[2,3]***Anthracocystis langdonii*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium langdonii* Vánky, Mycotaxon 51: 156. 1994.

Specimen examined: AUSTRALIA, Queensland, Dalby, *Themeda avenacea* (F. Muell.) Hack ex Maiden & Betche, 27 May 1941, R.F.N. Langdon, BRIP 7865, holotype.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis langdonii* (Vánky 1994b), which are most likely remnants of the non-sporogenous hyphae.

[3]***Anthracocystis leersiae-hexandrae*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium leersiae-hexandrae* Vánky, Mycotaxon 89: 103. 2004 (nom. nov.).
= *Sorosporium leersiae* Mishra, Mycologia 48: 876. 1956 (not *Sporisorium leersiae* Bag & D.K. Agarwal 2001).
= *Sporisorium leersiae* Bag & D.K. Agarwal, Indian Phytopathol. 54: 221. 2001.

Leersia is a member of the grass tribe *Oryzeae* in the sub-family *Ehrhartoideae* (Stevens 2001). *Anthracocystis leersiae-hexandrae* is the only known species to occur on a grass in this sub-family.

[3]***Anthracocystis leucostachys*** (Henn.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago leucostachys* Henn., in Pazschke, Hedwigia 35: 50. 1896.
≡ *Sphacelotheca leucostachys* (Henn.) Zundel, Mycologia 22: 144. 1930.
≡ *Sporisorium leucostachys* (Henn.) M. Piepenbr., Flora Neotropica Monograph 86: 110. 2003.

[2,3]***Anthracocystis likhitekarajae*** (R.G. Shivas, Athipunyakom, McTaggart & Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium likhitekarajae* R.G. Shivas, Athipunyakom, McTaggart & Vánky, in Shivas, Athipunyakom & McTaggart, Mycol. Balcan. *5*: 103. 2008.

Specimen examined: THAILAND, Nakhon Phanom, 31 km west of Sri Songkram, *Ischaemum* sp., 12 Dec. 2007, P. Athipunyakom, S. Likhitekaraj, V.L. Challinor, T.S. Marney, A.R. McTaggart, M.D.E. & R.G. Shivas, BRIP 51521, holotype.

[2,3]***Anthracocystis livingstoneanum*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium livingstoneanum* Vánky, Mycotaxon 95: 17. 2006.

Specimen examined: ZAMBIA, Southern Province, 10 km N of Livingstone, *Andropogon gayanus* Kunth, 14 Apr. 2001, T., C. & K. Vánky, BRIP 47134, isotype.

[2,3]***Anthracocystis lophopogonis*** (Thirum. & Pavgi) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium lophopogonis* Thirum. & Pavgi, Sydowia 20: 23. 1968.
≡ *Sporisorium lophopogonis* (Thirum. & Pavgi) Vánky, Mycotaxon 48: 40. 1993.

Specimen examined: INDIA, Maharashtra, Pune, *Lophopogon tridentatus* Hack., 18 Oct. 1992, K. Vánky, BRIP 26331 = Vánky, Ust. exs. no. 921.

[3]***Anthracocystis loudetiae-pedicellatae*** (Vánky & C. Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium loudetiae-pedicellatae* Vánky & C. Vánky, in Vánky, Mycotaxon 65: 165. 1997.

[3]***Anthracocystis loudetia-superbae*** (L. Ling) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium loudetiae-superbae* L. Ling, Lloydia 16: 190. 1953.
≡ *Sporisorium loudetiae-superbae* (L. Ling) Vánky Mycotaxon 65: 162. 1997.

[3]***Anthracocystis maranguensis*** (Henn.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium maranguense* Henn., in Engler, Pflanzenwelt Ost-Afrikas, etc., C, p. 49, 1895 (as '*maranguensis*').
≡ *Sporisorium maranguense* (Henn.) Vánky, Fungal Diversity 12: 193. 2003.

[3]***Anthracocystis masseeana*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium masseeanum* Vánky, Australas. Pl. Pathol. 29: 160. 2000.

[3]***Anthracocystis megaloprotachnes*** (Vánky & T. Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank MB
Basionym: *Sporisorium megaloprotachnes* Vánky & T. Vánky, in Vánky, Mycotaxon 81: 389. 2002.

[1,3]***Anthracocystis mexicana*** **(**Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank MB
Basionym: *Lundquistia mexicana* Vánky, Fungal Diversity 17: 161. 2004.
≡ *Sporisorium mexicanum* (Vánky) Vánky & Cunningt., in Cunnington, Vánky & Shivas, Mycol. Balcan. 2: 98. 2005.

Vánky (2004a) described *Anthracocystis mexicana* with sterile cells mixed within the sorus. Partitioning cells derived from sterile partitioning hyphae are an apomorphic character of *Sporisorium*. *Anthracocystis mexicana* was sister to all other taxa in *Anthracocystis* (McTaggart et al. submitted 2010a). Whether these are actual partitioning cells that were lost subsequently in *Anthracocystis* or sterile cells present in spore balls is unknown.

[1,2]***Anthracocystis mishrae*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium mishrae* Vánky, Mycotaxon 65: 135. 1997 (nom. nov.).
Replacing *Sorosporium apludae* Mishra, Mycologia 48: 875. 1956 (not *Sporisorium apludae* (Syd. & P. Syd.) L. Guo).

Specimen examined: INDIA, Karnataka, Belgaum, *Apluda mutica* L., 16 Jun 1995, Sharma, K. Vánky, BRIP 26377 = Vánky, Ust. exs. no. 967.

[3]***Anthracocystis mixta*** (Massee) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Tilletia mixta* Massee, Bull. Misc. Inform. 1899: 145. 1899.
≡ *Sorosporium mixtum* (Massee) McAlpine, The Smuts of Australia: 178. 1910.
≡ *Sporisorium mixtum* (Massee) Vánky, Mycotaxon 56: 214. 1995.
= *Sorosporium eriochloae* Griffiths, Bull. Torrey Bot. Club 31: 84. 1904 (syn. by McAlpine 1910:178).

[1,2]***Anthracocystis mutabilis*** (Syd.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sphacelotheca mutabilis* Syd., Ann. Mycol. 35: 24. 1937.
≡ *Sorosporium mutabile* (Syd.) L. Ling, Lloydia 14: 107. 1951.
≡ *Sporisorium mutabile* (Syd.) Vánky, Mycotaxon 85: 29. 2003.

Specimens examined: AUSTRALIA, Western Australia, Morowa, *Cymbopogon bombycinus* (R.Br.) Domin, 26 Sep. 1993, R.J. Cranfield, BRIP 28994; New South Wales, 11km N of Coonabarabran, *Cymbopogon refractus* (R.Br.) A. Camus, 16 Apr. 2004, M.D.E. & R.G. Shivas, BRIP 44111.

[3]***Anthracocystis muticae*** (Vánky & A.R. Patil) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium apludae-muticae* A.R. Patil, T.M. Patil & M.S. Patil, J. Mycol. Pl. Pathol. 34: 839. 2004 (not *Sporisorium apludae-muticae* L. Guo 1999).
≡ *Sporisorium muticae* Vánky & A.R. Patil, in Vánky, Mycotaxon 99: 47. 2007 (nom. nov.).

[3]***Anthracocystis myosuroidis*** (Hirschh.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium myosuroidis* Hirschh., Revista Mus. La Plata, N.S., Bot. 3: 343. 1941.
≡ *Sporisorium myosuroidis* (Hirschh.) Vánky, Mycotaxon 81: 396. 2002.

[3]***Anthracocystis nardi*** (Syd. & P. Syd) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago nardi* Syd. & P. Syd., in H. & P. Sydow & Butler, Ann. Mycol. 4: 425. 1906.
≡ *Sphacelotheca nardi* (Syd. & P. Syd.) Zundel, Mycologia 22: 137. 1930.
≡ *Sorosporium nardi* (Syd. & P. Syd.) L. Ling, Sydowia 5: 47. 1951.
≡ *Sporisorium nardi* (Syd. & P. Syd.) Vánky, Mycotaxon 85: 30. 2003.

[2,3]***Anthracocystis normanensis*** (R.G. Shivas & Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium normanense* R.G. Shivas & Vánky, Fungal Diversity 8: 150. 2001 (as 'normanensis').

Specimen examined: AUSTRALIA, Queensland, Normanton, 18 km SSE of Norman River Bridge, *Cynodon dactylon* (L.) Pers., 10 Jul. 1999, R.G. Shivas, M. Gunther, BRIP 25751, holotype.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis normanensis* (Shivas and Vánky 2001), which are most likely remnants of the non-sporogenous hyphae. *Anthracocystis normanensis* is one of five known *Anthracocystis* species that infect a chloridoid grass. The majority of *Anthracocystis* taxa infect andropogonoid grasses.

[3]***Anthracocystis nyasalandicum*** (L. Ling) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium nyasalandicum* L. Ling, Sydowia 7: 156. 1953.
≡ *Sporisorium nyasalandicum* (L. Ling) Vánky, Mycotaxon 91: 226. 2005.

[2,3]***Anthracocystis operculata*** (Vánky, C. Vánky & R.G. Shivas) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium operculatum* Vánky, C. Vánky & R.G. Shivas, in Vánky & Shivas, Fungal Diversity 7: 154. 2001.

Specimen examined: AUSTRALIA, Queensland, Chillagoe, *Mnesithea formosa* (R.Br.) de Koning & Sosef, 04 Mar. 2000, K. & C. Vánky, BRIP 27015, holotype.

[1,2]***Anthracocystis ovaria*** (Griffiths) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium ovarium* Griffiths, Bull. Torrey Bot. Club 34: 209. 1907.
≡ *Sporisorium ovarium* (Griffiths) Vánky, Mycotaxon 65: 138. 1997.
= *Sphacelotheca diplospora* (Ellis & Everh.) Clinton var. *verruculosa* Clinton, North American Flora 7: 27. 1906.
= *Ustilago verecunda* Syd., Ann. Mycol. 33: 231. 1935.
≡ *Sorosporium verecundum* (Syd.) Zundel, Bothalia 3: 304. 1938.
= *Sorosporium brachiariae* J.C.F. Hopkins, Trans. Rhodesia Sci. Assoc. 35: 126. 1938.
= *Ustilago urochloana* Zundel, Mycologia 35: 166. 1943.
= *Sorosporium brachiariae-ramosae* T.S. Ramakr., Proc. Indian Acad. Sci. 35: 113. 1952.

Specimen examined: AUSTRALIA, NT, Alice Springs, 393km N of Devils Marbles, *Brachiaria piligera* (F. Muell.ex Benth.) Hughes, 15 Mar. 2000, R.G. Shivas, I.T. Riley, C. & K. Vánky, BRIP 44093 = Vánky, Ust. exs. no. 1184.

[2,3]***Anthracocystis panicicola*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium panicicola* Vánky, Mycotaxon 91: 229. 2005.

Specimen examined: REUNION, St Benoit, 14km SW of Lacus Le Grand Etang, *Panicum coloratum* L., 02 Dec. 1994, C. & K. Vánky, BRIP-45335, Vánky, Ust. exs. no. 1238, isotype.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *L. panicicola* (Vánky 2005), which are most likely remnants of the non-sporogenous hyphae.

[3]***Anthracocystis panici-fasciculati*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium panici-fasciculati* Vánky, Mycotaxon 91: 229. 2005.

[1,2]***Anthracocystis panici-leucophaei*** (Bref.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank *
Basionym: *Ustilago panici-leucophaei* Bref., Unters. Gesammtgeb. Mykol. 12: 114. 1895.
≡ *Sphacelotheca panici-leucophaei* (Bref.) Clinton, North American Flora 7: 28. 1906.
≡ *Sporisorium panici-leucophaei* (Bref.) M. Piepenbr., Mycol. Res. 103: 465. 1999.
≡ *Lundquistia panici-leucophaei* (Bref.) Vánky, Fungal Diversity 17: 167. 2004.
= *Ustilago insularis* Henn., in Pazschke, Hedwigia 35: 51. 1896.
= *Ustilago bonariensis* Speg., Anales Mus. Nac. Buenos Aires, Ser. 3, 12: 287. 1909.
≡ *Sphacelotheca bonariensis* (Speg.) Ciferri, Ann. Mycol. 29: 56. 1931.
≡ *Sorosporium bonariense* (Speg.) Zundel, Ustil. World: 54. 1953 (as '*bonariensis*').
≡ *Sporisorium bonariense* (Speg.) Vánky, nom. herb.
= *Sphacelotheca viegasiana* Zundel, Mycologia 31: 588. 1939.
= *Sorosporium lindmanii* Zundel, Mycologia 35: 173. 1943.
= *Ustilago garcesii* Zundel, Mycologia 37: 372. 1945 (as '*Garcesi*').
= *Lundquistia fascicularis* Vánky, Mycotaxon 77: 373. 2001.
≡ *Sporisorium fasciculare* (Vánky) M. Stoll, Begerow & Oberw., Mycol. Res. 109: 354. 2005 (as '*fascicularis*').

Specimens examined: AUSTRALIA, New South Wales, 11km N of Coonabarabran, *Digitaria breviglumis* (Domin) Henr., 16 Apr. 2004, M.D.E. & R.G. Shivas, BRIP 44110; ARGENTINA, Buenos Aires, 115 km NNW of Buenos Aires, *Panicum elephantipes* Nees, 29 Nov. 1999, K. & C. Vánky, BRIP 28944 = Vánky, Ust. exs. no.1117.

[3]***Anthracocystis panici-petrosi*** (Syd. & P. Syd)) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago panici-petrosi* Syd. & P. Syd., Ann. Mycol. 14: 73. 1916.
≡ *Sporisorium panici-petrosi* (Syd. & P. Syd.) M. Piepenbr., Flora Neotropica Monograph 86: 122. 2003.

[2,3]***Anthracocystis paraneurachnis*** (R.G. Shivas & Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium paraneurachnis* R.G. Shivas & Vánky, Mycol. Res. 101: 836. 1997 (as '*paraneurachnes*').

Specimen examined: AUSTRALIA, Western Australia, *Paraneurachne muelleri* (Hackel) S.T. Blake, 27 Jun. 1996, A.A. Mitchell, BRIP 26804.

[3]***Anthracocystis parodii*** (Hirschh.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago parodii* Hirschh., Darwinia 3: 404. 1939.
≡ *Sporisorium parodii* (Hirschh.) Vánky, Mycotaxon 85: 59. 2003.

Anthracocystis parodii is one of five known *Anthracocystis* species that infect a chloridoid grass. The majority of *Anthracocystis* taxa infect andropogonoid grasses.

[2,3]***Anthracocystis paspali-thunbergii*** (Henn.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago paspali-thunbergii* Henn., Hedwigia 43: 140. 1904.
≡ *Sorosporium paspali-thunbergii* (Henn.) S. Ito, Trans. Sapporo Nat. Hist. Soc. 14: 94. 1935.
≡ *Sporisorium paspali-thunbergii* (Henn.) Vánky, Publ. Herb. Ustilag. Vánky (HUV) 3: 9. 1986.
= *Sorosporium paspali* McAlpine, The smuts of Australia: 180. 1910.
= *Sorosporium paspali* McAlpine var. *verrucosum* Thirum. & M.S. Pavgi, Mycopathol. Mycol. Appl. 7: 283. 1956.

Specimen examined: CHINA, Yunnan, Mamushu, *Paspalum scrobiculatum* L., 22 Sep. 1985, L. Guo, K. Vánky, BRIP 26286 = Vánky, Ust. exs. no. 526.

[1,2]***Anthracocystis penniseti*** (Rabenh.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago penniseti* Rabenh., Hedwigia 10: 18. 1871.
≡ *Sphacelotheca penniseti* (Rabenh.) Reichert, Bot. Jahrb. Syst. 56: 679. 1921.
= *Ustilago pappiana* Baccarini, Ann. Bot. (Rome) 4: 272. 1906.
≡ *Sorosporium pappianum* (Bacc.) L. Ling, Lloydia 16: 192. 1953.
= *Sorosporium catharticum* Maire, in Recueil de travaux cryptogamiques dédiés à Louis Mangin: 359. 1931.
≡ *Sporisorium catharticum* (Maire) Vánky, Mycotaxon 35: 155. 1989.
= *Sphacelotheca panjabensis* Syd., in Sydow & Ahmad, Ann. Mycol. 37: 442. 1939.
≡ *Ustilago panjabensis* (Syd.) L. Ling, Sydowia 4: 76. 1950.
= *Sorosporium penniseti* Mundk., Trans. Brit. Mycol. Soc. 23: 116. 1939.
= *Sphacelotheca stewartii* Mundk., Mycologia 36: 290. 1944.
= *Ustilago penniseti* Rabenh. var. *verruculosa* Massenot, in Guyot, Malençon & Massenot, Rev. Mycol. (Paris) 34: 217. 1969.

Specimen examined: INDIA, Tamil Nadu, Coimbatore, Institute of Forest Genetics & Tree Breeding, Guest House, *Cenchrus ciliaris* L., 04 Jan 2010, R.G. & M.D.E. Shivas, BRIP 53217.

[2,3]***Anthracocystis penniseticola*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium penniseticola* Vánky, Mycol. Balcan. 2: 92. 2005.

Specimen examined: ETHIOPIA, Arsi, 11 km south of Asela, *Pennisetum sphacelatum* T.Durand & Schinz, 2004-11-04, T. & K. Vánky, BRIP 47137, paratype.

[3]***Anthracocystis pennisetina*** (S. Ahmad) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sphacelotheca pennisetina* S. Ahmad, Mycol. Pap. 64: 7. 1956.
≡ *Sporisorium pennisetinum* (S. Ahmad) Vánky, Mycotaxon 85: 13. 2003.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis pennisetina* (Vánky 2003b), which are most likely remnants of the non-sporogenous hyphae.

[1,2]***Anthracocystis polliniae*** (Magnus) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium polliniae* Magnus, Verh. K. K. Zool.-Bot. Ges. Wien 50: 433. 1900 (as '*Sorisporium*').
≡ *Sporisorium polliniae* (Magnus) Vánky, Mycotaxon 18: 331. 1983.

Specimen examined: GREECE, Rhodos, *Andropogon distachyos* L., 14 Apr. 1988, H.W. & I. Scholz, BRIP 19639 = Vánky, Ust. exs. no. 690.

[3]***Anthracocystis polytriadis*** (Massee) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago polytriadis* Massee, Bull. Misc. Inform. 1911: 224. 1911.
≡ *Sphacelotheca polytriadis* (Massee) L. Ling, Sydowia 3: 127. 1949.
≡ *Sporisorium polytriadis* (Massee) Vánky, Mycotaxon 62: 136. 1997.

[1,2]***Anthracocystis provinciale*** (Ellis & Galloway) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium ellisii* G. Winter var. *provinciale* Ellis & Galloway, J. Mycol. 6: 31. 1890.
≡ *Sorosporium provinciale* (Ellis & Galloway) Clinton, J. Mycol. 8: 145. 1902.
≡ *Sporisorium provinciale* (Ellis & Galloway) Vánky & Snets., in Vánky, *Mycotaxon 38*:271, 1990.

Specimen examined: USA, Iowa, Ledges State Park, Boone Co., *Andropogon gerardi* Vitman, 23 Jun. 1989, K.M. Snetselaar, BRIP 21528 = Vánky, Ust. exs. no. 759.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis*

provinciale (Vánky 1990), which are most likely remnants of the non-sporogenous hyphae.

[2,3]***Anthracocystis pseudanthistiriae*** (Syd., P. Syd & Butler) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium pseudanthistiriae* Syd., P. Syd. & Butler, Ann. Mycol. 10: 254. 1912.
≡ *Sporisorium pseudanthistiriae* (Syd., P. Syd. & Butler) Vánky, Mycotaxon 62: 145. 1997.
= *Sorosporium pseudanthistiriae-umbellatae* A.R. Patil, T.M. Patil & M.S. Patil, J. Mycol. Pl. Pathol. 34: 841. 2004.

Specimen examined: INDIA, Maharashtra, Kolhapur, Shivaji University, *Pseudanthistiria hispida* Hook., 16 Nov. 1995, Sharma, K. Vánky, BRIP 26379 = Vánky, Ust. exs. no. 969.

[2,3]***Anthracocystis pseudomaranguensis*** (Zundel) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium pseudomaranguense* Zundel, Bothalia 3: 309. 1938.
≡ *Sporisorium pseudomaranguense* (Zundel) Vánky, Mycotaxon 91: 263. 2005.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis pseudomaranguensis* (Vánky 2005), which are most likely remnants of the non-sporogenous hyphae.

[3]***Anthracocystis pseudoraphis*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium pseudoraphis* Vánky, Mycotaxon 68: 331. 1998.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis pseudoraphis* (Vánky 1998b), which are most likely remnants of the non-sporogenous hyphae.

[3]***Anthracocystis rhytachnes*** (Syd.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sphacelotheca rhytachnes* Syd., Ann. Mycol. 37: 201. 1939.
≡ *Sporisorium rhytachnes* (Syd.) Vánky, Mycotaxon 74: 171. 2000.

[2,3]***Anthracocystis rubyana*** (Vánky & N.D. Sharma) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium rubyanum* Vánky & N.D. Sharma, in Vánky, Fungal Diversity 15: 234. 2004.

Specimen examined: INDIA, Madhya Pradesh, Jabalpur, 200 km SW of Pachmarhi, *Capillipedium assimile* (Steud.) A. Camus, 31 Oct. 1992, R. Sharma, S. Raich, BRIP 51782, isoparatype.

[3]***Anthracocystis sahariana*** (Trotter) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium saharianum* Trotter, in Saccardo & Trotter, Ann. Mycol. 11: 413. 1913.
≡ *Sporisorium saharianum* (Trotter) Karatygin, in Karatygin & Azbukina, *Definitorium fungorum URSS. etc.*:78, 1989.

Anthracocystis sahariana is one of five known *Anthracocystis* species that infect a chloridoid grass. The majority of *Anthracocystis* taxa infect andropogonoid grasses.

[3]***Anthracocystis scheffleri*** (Syd. & P. Syd) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago scheffleri* Syd. & P. Syd., Bot. Jahrb. Syst. 45: 262. 1911.
≡ *Sporisorium scheffleri* (Syd. & P. Syd.) Vánky, Mycotaxon 91: 232. 2005.

[2,3]***Anthracocystis schizachyrii*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium schizachyrii* Vánky, Mycotaxon 81: 418. 2002.

Specimen examined: ZAMBIA, Southern Province, Chirundu, 75 km ESE of Kafue, *Schizachyrium exile* (Hochst.) Pilger, 28 Apr. 2001, K. & C. Vánky, BRIP 28955 = Vánky, Ust. exs. no.1128.

[3]***Anthracocystis scholzii*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium scholzii* Vánky, Mycotaxon 95: 22. 2006.

[1,2]***Anthracocystis sehimatis*** (M.S. Patil) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium sehimatis* M.S. Patil, Indian Phytopathol. 45: 181. 1992 (as '*sehimae*').
≡ *Sporisorium sehimatis* (M.S. Patil) Vánky, Mycotaxon 74: 188. 2000 (as '*sehimae*').

Specimens examined: AUSTRALIA, Western Australia, Between Halls Creek and Kununurra, *Sehima nervosum* (Rottler) Stapf, 11 Apr. 2007, A.R. McTaggart, T.S. Marney, S.M. Thompson, M.J. Ryley, A.J., G.F., M.D.E. & R.G. Shivas, BRIP 49671a.

[1,2]***Anthracocystis setariae*** (McAlpine) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium setariae* McAlpine, The smuts of Australia: 183. 1910.
≡ *Sporisorium setariae* (McAlpine) Vánky & R.G. Shivas, Fungal Diversity 14: 263. 2003.

Specimens examined: AUSTRALIA, Queensland, ca. 32 km south of Cloncurry, *Setaria pumila* (Poir.) Roem. & Schult., 10 May 1909, G.M. Robinson, BRIP 26796, isotype**;** Northern Territory, 26.2 km South of Tennant Creek, *Setaria surgens* Stapf, 26 Apr. 2007, A.R. McTaggart, R.G. Shivas, J.R. Liberato, BRIP 49637.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis setariae*

(Vánky and Shivas 2003), which are most likely remnants of the non-sporogenous hyphae.

[2,3]***Anthracocystis shivasiora*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium shivasiorum* Vánky, Mycotaxon 106: 145. 2008.

Specimen examined: THAILAND, Chiang Mai, Chiang Mai, near Mae Ngad Dam, *Eulalia trispicata* (Schult.) Henr., 28 Dec. 2005, P. Athipunyakom, S. Likhitekaraj, W. Butranu, C. & K. Vánky, A.J., M.D.E. & R.G. Shivas, BRIP 51766, holotype.

[2,3]***Anthracocystis spermoidea*** (Berk. & Broome) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago spermoidea* Berkeley & Broome, J. Linn. Soc., Bot. 14: 94. 1875.
≡ *Sphacelotheca spermoidea* (Berk. & Broome) Mundk., Trans. Brit. Mycol. Soc. 23: 96. 1939.
≡ *Sorosporium spermoideum* (Berk. & Broome) L. Ling, Sydowia 5: 48. 1951.
≡ *Sorosporium spermoideum* (Berk. & Broome) Zundel, Ustil. World: 74. 1953 (comb. superfl.).
≡ *Sporisorium spermoideum* (Berk. & Broome) Vánky, Mycotaxon 85: 31. 2003.

Specimen examined: INDIA, Tamil Nadu, ca. 65km NW of Madurai, *Cymbopogon martinii* (Roxb.) Wats., 28 Jan. 1980, K. Vánky, BRIP 45342 = Vánky, Ust. exs. no. 1245.

[2,3]***Anthracocystis sphacelata*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium sphacelatum* Vánky, Mycotaxon 85: 13. 2003.

Specimen examined: SOUTH AFRICA, Lady Gray, Eastern Cape Province, Mt. Drakensberg, *Pennisetum sphacelatum* (Nees) Dur. & Schinz, 22 Dec. 1996, C. & K. Vánky, BRIP 28972 = Vánky, Ust. exs. no. 1145.

[3]***Anthracocystis stipara*** (Speg.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago stiparum* Speg., Anales Mus. Nac. Buenos Aires, Ser. 3, 12: 288. 1909.
≡ *Sorosporium stiparum* (Speg.) Zundel, Mycologia 43: 269. 1951.
≡ *Sporisorium stiparum* (Speg.) Vánky, Mycotaxon 106: 163. 2008.

[3]***Anthracocystis sulcati*** (M.S. Patil) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium sulcati* M.S. Patil, Indian Phytopathol. 45: 181. 1992.
≡ *Sporisorium sulcati* (M.S. Patil) Vánky, Mycotaxon 74: 188. 2000.

[3]***Anthracocystis tanganyikeana*** (Zundel) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium tanganyikeanum* Zundel, Mycologia 36: 408. 1944.
≡ *Sporisorium tanganyikeanum* (Zundel) Vánky, Mycotaxon 91: 235. 2005.

[2,3]***Anthracocystis tembuti*** (Henn. & Pole-Evans) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium tembuti* Henn. & Pole-Evans, in Hennings, Bot. Jahrb. Syst. 41: 270. 1908.
≡ *Sporisorium tembuti* (Henn. & Pole-Evans) Vánky, Mycotaxon 99: 63. 2007.
= *Ustilago tumefaciens* Henn., in Engler, Pflanzenwelt Ost-Afrikas, etc., C,: 48. 1895.
≡ *Sorosporium tumefaciens* (Henn.) Zundel, Mycologia 22: 149. 1930.
≡ *Sorosporium zundelianum* Cif., Nuovo Giorn. Bot. Ital., N.S., 40: 268. 1933, nom. nov.
≡ *Sporisorium leelingianum* Vánky, Fungal Diversity 12: 190, 2003.
= *Sorosporium healdii* Zundel, Mycologia 22: 147. 1930.
= *Sorosporium proliferatum* Zundel, Mycologia 22: 150. 1930.
= *Sorosporium clintonii* Zundel, Mycologia 22: 153. 1930.

Specimen examined: SOUTH AFRICA, KwaZulu-Natal, Mikes Pass, Cathedral Peak National Park, *Hyparrhenia tamba* (Steudel) Stapf, 30 Dec.1996, K. & C. Vánky, BRIP 43866 = Vánky, Ust. exs. no. 1153.

[2,3]***Anthracocystis thelepogonis*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium thelepogonis* Vánky, Mycotaxon 62: 130. 1997.

Specimen examined: EAST TIMOR, Maahui, *Thelepogon elegans* Roth ex Roem. & Schult., 06 May 2002, M.P. Weinert, A.A. Mitchell, BRIP 51275.

[1,2]***Anthracocystis themedae-arguentis*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium themedae-arguentis* Vánky, Mycotaxon 51: 154. 1994.

Specimens examined: AUSTRALIA, NT, Humpty Doo, *Themeda arguens* (L.) Hackel, 20 Mar. 1967, J.B. Heaton, BRIP 7883, paratype; INDONESIA, Bali, Denpasar, 20 km south of Mount Alas Kemayuna, *Themeda arguens* (L.) Hack., 01 Apr. 1992, Menge, C. & K. Vánky, BRIP 27277 = Vánky, Ust. exs. no. 855, isotype.

[2,3]***Anthracocystis themedae-cymbariae*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium themedae-cymbariae* Vánky, Mycotaxon 62: 141. 1997.

Specimen examined: INDIA, Karnataka, Mysore, Bandipur, *Themeda cymbaria* Hack., 05 Jun 1995, Sharma, K. Vánky, BRIP 26383 = Vánky, Ust. exs. no. 973.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis themedae-cymbariae* (Vánky 1997), which are most likely remnants of the non-sporogenous hyphae.

[3]***Anthracocystis tothii*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium tothii* Vánky, Mycotaxon 85: 14. 2003.

[1,2]***Anthracocystis trispicatae*** (R.G. Shivas, Vánky & Athipunyakom) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium trispicatae* R.G. Shivas, Vánky & Athipunyakom, Mycol. Balcan. 3: 111. 2006.

Specimen examined: THAILAND, Chiang Mai, Mae Ngad Dam, Mae Taeng District, *Eulalia trispicata* (Schult.) Henr., 28 Dec. 2005, R.G. & M.D.E. Shivas, P. Athipunyakom, W. Butranu, S. Likhitekaraj, C. & K. Vánky, BRIP 47730, isotype.

[2,3]***Anthracocystis tristachyae-hispidae*** (L. Ling) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sphacelotheca tristachyae-hispidae* L. Ling, Lloydia 16: 184. 1953.
≡ *Sporisorium tristachyae-hispidae* (L. Ling) Vánky, Mycotaxon 65: 162. 1997.

Specimen examined: SOUTH AFRICA, KwaZulu-Natal, Giants Castle Reserve, Drakensberg Mountains, *Tristachya leucothrix* Nees, 04 Jan. 1997, C. & K. Vánky, BRIP 26440 = Vánky, Ust. exs. no. 1030.

[2,3]***Anthracocystis tristachyae-nodiglumis*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium tristachyae-nodiglumis* Vánky, Mycotaxon 85: 46. 2003.

Specimen examined: ZAMBIA, Central Province, 169 km ENE of Lusaka, *Tristachya* sp., 17 Apr. 2001, C., T. & K. Vánky, BRIP 28971 = Vánky, Ust. exs. no. 1144, isotype.

[3]***Anthracocystis tristachydis*** (Syd. & P. Syd.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium tristachydis* Syd. & P. Syd., Bot. Jahrb. Syst. 45: 263. 1911.
≡ *Tolyposporium tristachydis* (Syd. & P. Syd.) Zundel, Bothalia 3: 310. 1938.
≡ *Sporisorium tristachydis* (Syd. & P. Syd.) Vánky, Mycotaxon 65: 161. 1997.

[1,2]***Anthracocystis tumefaciens*** (McAlpine) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium tumefaciens* McAlpine, The smuts of Australia: 184. 1910.
≡ *Sporisorium tumefaciens* (McAlpine) Vánky, Mycotaxon 18: 328. 1983.
= *Sphacelotheca rhaphidis* L. Ling, Sydowia 3: 128. 1949.
= *Sporisorium tumiforme* Vánky & R.G. Shivas, in Vánky, Fungal Diversity 18: 183. 2005.

Specimen examined: AUSTRALIA, Western Australia, Wyndham, 56 km SSE on Great Northern Highway, *Chrysopogon fallax* S.T. Blake, 22 Feb. 1996, A.A. Mitchell, C. & K. Vánky, BRIP 26441 = Vánky, Ust. exs. no. 1031.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells present *Anthracocystis tumefaciens*, which are remnants of the non-sporogenous hyphae.

[3]***Anthracocystis ugandensis*** (Henn.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago ugandensis* Henn., in Engler, Pflanzenwelt Ost-Afrikas, etc., C: 48. 1895.
≡ *Sporisorium ugandense* (Henn.) Vánky, Mycotaxon 91: 250. 2005.
= *Sphacelotheca dolichosora* Ainsw., Proc. Linn. Soc. London 153: 94. 1941.
≡ *Sporisorium dolichosorum* (Ainsw.) Vánky, Mycotaxon 73: 142. 1999.

Partitioning cells formed from non-sporogenous hyphae are usually absent in *Anthracocystis*. There are a few partitioning cells reported in *Anthracocystis ugandensis* (Vánky 2005), which are most likely remnants of the non-sporogenous hyphae.

[2,3]***Anthracocystis walkeri*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium walkeri* Vánky, Mycotaxon 51: 158. 1994.

Specimen examined: AUSTRALIA, Queensland, Fernvale, *Themeda triandra* Forssk., 15 Nov. 1965, R.F.N. Langdon, BRIP 7876, paratype.

[1,2]***Anthracocystis whiteochloae*** (Vánky & McKenzie) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium whiteochloae* Vánky & McKenzie, in Vánky & Shivas, Fungal Diversity 7: 160. 2001.

Specimens examined: AUSTRALIA, Western Australia, Kununurra, Fish Farm Road, *Whiteochloa cymbiformis* (Hughes) B.K. Simon, 26 Jun. 1998, R. Eichner, BRIP 26823, isotype; Northern Territory, Timber Creek, Policeman's Lookout, *Whiteochloa semitonsa* (F.Muell. ex Benth.) C.E.Hubb., 10 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51860b.

[1,2]***Anthracocystis xerofasciculata*** (R.G. Shivas, McTaggart & Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium xerofasciculatum* R.G. Shivas, McTaggart & Vánky, Mycotaxon 101: 353. 2007.

Specimen examined: AUSTRALIA, Western Australia, Between Kununurra and Halls Creek, *Xerochloa laniflora* Benth., 11 Apr. 2007, A.R. McTaggart, T.S. Marney, S.M. Thompson, M.J. Ryley, A.J., G.F., M.D.E. & R.G. Shivas, BRIP 49682, holotype**.**

[3]***Anthracocystis zambiana*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium zambianum* Vánky, Mycotaxon 85: 42. 2003.

7.3.3 *Mycosarcoma* (Clade 3)

Taxa that were included in Clade 3 all form tubular sori derived from hypertrophied host material in some ovaries of the inflorescence. All taxa in Clade 3 have partitioning cells present within the sori. Columellae were described in two of the taxa in Clade 3, but are unlikely to be homologous to the columellae that are synapomorphies of *Sporisorium* and *Anthracocystis*. The localized infection on the host forming a tubular, host derived sorus, is a synapomorphy for Clade 3.

Endosporisorium Vánky was described to accommodate smuts with long, tubular, host derived sori that contained partitioning cells and that lacked columellae (Vánky 1995a, 2002). Vánky (1995a, b) transferred four smuts into *Endosporisorium* but later synonymized it with *Macalpinomyces*, preferring to have larger, well-known genera than many smaller, unresolved genera (Vánky 1997). The type of *Endosporisorium* was based on *Macalpinomyces chrysopogonicola* (Mundk. & Thirum.) Vánky.

Endosporisorium may be an appropriate genus to accommodate species in Clade 10, although the phylogenetic position of these taxa is unresolved (Chapter 5, this thesis). Clade 10 species infect the subfamily Arundinoideae and transform the inflorescence into long tubular sori, similar to the infection described in *Endosporisorium*. *Macalpinomyces chrysopogonicola*, the type species of *Endosporisorium*, should be included in a molecular phylogenetic analysis to determine if it occurs with morphologically similar taxa in Clade 10. If this is the case, this monophyletic group could be named *Endosporisorium*. *Endosporisorium* is not a suitable genus to accommodate taxa in Clade 3 as they do not destroy the entire inflorescence, and they are phylogenetically and morphologically distinct from species similar to the type of *Endosporisorium*.

Brefeld (1912) established *Mycosarcoma* for *Ustilago maydis*, which he diagnosed as different to *Sporisorium sorghi* (as *Ustilago sorghi*) for three reasons, (i) incubation time in the host, (ii) development of the sorus at the site of penetration in the host plant, and (iii) development of aerial conidia. The peridial structure of *Ustilago maydis* was another character that Brefeld (1912) considered different to other species of *Ustilago*. Two of the characters that Brefeld (1912) described are unique characters

to Clade 3. The hypertrophied, host derived peridium and the localized infection sites on the host inflorescence are morphological synapomorphies of this monophyletic group. Furthermore, the localized, hypertrophied, often tubular sori always contain partitioning cells. Piepenbring et al. (2002) concluded from a molecular phylogenetic analysis that *Ustilago maydis* was separate to other *Ustilago* taxa, and that it could warrant placement in the genus originally assigned to it by Brefeld (1912).

Mycosarcoma Bref., Unters. Gesammtgeb. Mykol. 15: 53. 1912, **emend.** McTaggart & R.G. Shivas.

Sori in some ovaries of an inflorescence, derived from hypertrophied host material, **often tubular, splitting longitudinally to expose the spore mass mixed with partitioning cells**. Columellae usually absent. Spore balls absent. Germination of *Ustilago* type.

Type species: *Mycosarcoma maydis* (DC.) Bref.
Type on *Zea mays*.

7.3.3.1 New combinations for Mycosarcoma

The following taxa are new combinations of *Mycosarcoma* based on either results of the molecular phylogenetic analysis[1], examination of either type specimens or specimens from the exsiccata *Herbarium Ustilaginales Vánky* [2], or from type descriptions in the protologues[3].

[1,2]***Mycosarcoma arundinellae-setosae*** (R.G. Shivas & Vánky) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Macalpinomyces arundinellae-setosae* R.G. Shivas & Vánky, Mycol. Balcan. 2: 101. 2005.

Specimens examined: AUSTRALIA, Northern Territory, 13 km west of Batchelor, *Arundinella nepalensis* Trin., 06 Sep. 2006, M.J. Ryley, M.D.E. & R.G. Shivas, BRIP 47958a; Northern Territory, near Litchfield National Park, *Arundinella* sp., 12 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51868b; Queensland, 8 km south of Lakeland, *Arundinella setosa*, 04 May 2004, T.S. Marney, R.G. Shivas, BRIP 46034a, holotype.

[1]***Mycosarcoma bouriqueti*** (Maubl. & Roger) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Ustilago bouriqueti* Maubl. & Roger, in Roger, Bull. Soc. Mycol. France 50: 327, 1934.

= *Sphacelotheca mauritiana* Zundel, Mycologia 36: 405. 1944. (Syn. by Vánky 1996:107).

= *Sorosporium stenotaphri* Vien.-Bourg., Ann. Inst. Natl. Agron. 47: 23. 1963.

Specimen examined: GALLIA, St. Paul, Ins. Reunion, pr. urbem St. Paul, *Stenotaphrum dimidiatum* (L.) Brongn., 18 Dec. 1994, K. Vánky, BRIP 26403a: Vánky, Ust. exs. no. 993.

[1]***Mycosarcoma dietelianum*** (Henn.) McTaggart & R.G. Shivas **comb. nov.** MycoBank

Basionym: *Ustilago dieteliana* Henn., Hedwigia 37: 268. 1898.

≡ *Lundquistia dieteliana* (Henn.) Vánky, Fungal Diversity 17: 163. 2004.

≡ *Sporisorium dietelianum* (Henn.) Vánky, Mycotaxon 91: 266. 2005.

= *Ustilago kellermanii* Clinton, North American Flora 7: 15. 1906.

[3]***Mycosarcoma elionuri-tripsacoidis*** (Vánky) McTaggart & R.G. Shivas **comb. nov.** MycoBank

Basionym: *Macalpinomyces elionuri-tripsacoidis* Vánky, Mycotaxon 85: 43. 2003.

[3]***Mycosarcoma flaccidum*** (S.H. He & L. Guo) McTaggart & R.G. Shivas **comb. nov.** MycoBank

Basionym: *Macalpinomyces flaccidus* S.H. He & L. Guo, Mycotaxon 101: 99. 2007.

[1,2]***Mycosarcoma mackinlayi*** (McTaggart & R.G. Shivas) McTaggart & R.G. Shivas **comb. nov.** MycoBank

Basionym: *Macalpinomyces mackinlayi* McTaggart & R.G. Shivas, Persoonia 23: 187. 2009.

Specimens examined: AUSTRALIA, Western Australia, Kalumburu Rd. creek crossing north of Drysdale River, *Eulalia mackinlayi* (Benth.) Kuntze, 10 May 2009, A.R. McTaggart, V.L. Challinor, M.J. Ryley, C.F. Gambley, T. Scharaschkin, M.D.E. & R.G. Shivas, BRIP 52549a, holotype; Western Australia, between King Edward River Crossing and Mitchell Falls' Campsite, Mitchell Plateau, *Eulalia mackinlayi* (Benth.) Kuntze, 10 May 2009, A.R. McTaggart, V.L. Challinor, M.J. Ryley, C.F. Gambley, T. Scharaschkin, M.D.E. & R.G. Shivas, BRIP 52546a, paratype.

[1,2]***Mycosarcoma maydis*** (DC.) Bref. Unters. Gesammtgeb. Mykol. 15: 53. 1912.

Basionym: *Uredo maydis* DC., Fl. franç., ed. 3, 6: 77. 1815.

≡ *Ustilago maydis* (DC.) Corda, Icones Fungorum Hucusque Cognitorum 5: 3. 1842.

= *Lycoperdon zeae* Beckm., Hannover. Mag. 6: 1330. 1768.

= *Uredo segetum* Pers. δ *mays-zeae* DC., Fl. franç. 2: 596. 1805.

≡ *Ustilago zeae-maydis* (DC.) G. Winter, in Rabenhorst's Kryptogamen-Flora, Ed. 2, 1: 97, 1881.

≡ *Ustilago mays-zeae* (DC.) Magnus, Verh. Bot. Vereins Prov. Brandenburg 37: 72. 1895 (1896).

= *Uredo zeae* Schwein., Schriften Naturf. Ges. Leipzig 1: 71. 1822.

= *Caeoma zeae* Link, in Linné's Species Plantarum, Ed. 4, 6(2): 2. 1825.

≡ *Ustilago zeae* (Link) Unger, Ueber den Einfluß des Bodens: 211. 1836.

= *Ustilago euchlaenae* Archang., Erbario Crittogamico Italiano, Ser. 2, no. 1152, 1882.

Specimens examined: HUNGARY, Zala, comit. Zala, pr. urbem Keszthely, *Zea mays* L., 15 Jul. 1986, K. Vánky, BRIP 27365a: Vánky, Ust. exs. no. 593; BOLIVIA, Potosi, Toro Toro, Toro Toro National Park, *Zea mays* L., 21 Apr. 2009, R.G. & M.D.E. Shivas, A.R. McTaggart, W.A. Arce, C. & K. Vánky, BRIP 52746a.

[3]***Mycosarcoma nodiglume*** (Vánky) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Macalpinomyces nodiglumis* Vánky, Mycotaxon 81: 414. 2002.

[2,3]***Mycosarcoma siamense*** (R.G. Shivas, Vánky & Athipunyakom) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Macalpinomyces siamensis* R.G. Shivas, Vánky & Athipunyakom, in Vánky, Shivas & Athipunyakom, Mycol. Balcan. 3: 108. 2006.

Specimen examined: THAILAND, Chiang Mai, Chiang Mai, 57 km NNE of Phrao District, *Coelorachis striata* (Nees ex Steud.) A. Camus, 28 Dec. 2005, R.G. & M.D.E. Shivas, BRIP 47765a, isotype.

[3]***Mycosarcoma trachypogone*** (Zundel) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Sphacelotheca trachypogonis* Zundel, Mycologia 25: 353, 1933.
≡ *Sporisorium trachypogonis* (Zundel) Vánky, Mycotaxon 56: 206. 1995.

[1,2]***Mycosarcoma tubiforme*** (R.G. Shivas & Vánky) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Macalpinomyces tubiformis* R.G. Shivas & Vánky, in Shivas, Cunnington & Vánky, Fungal Diversity 16: 152. 2004.

Specimens examined: AUSTRALIA, Northern Territory, Mary River, east of Mary River Crossing, *Chrysopogon* sp. 12 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51865a; Queensland, 20 km north of Corner of Monduran and Gympie Highways, Gin Gin, *Chrysopogon fallax*, S.T. Blake, 25 Apr. 2003, M.D.E. & R.G. Shivas, BRIP 39858a, HUV 20303, holotype.

[1]***Mycosarcoma vetiveriae*** (Padwick) McTaggart & R.G. Shivas **comb. nov.** MycoBank

Basionym: *Ustilago vetiveriae* Padwick, Mycol. Pap. 17: 5. 1946.

[3]***Mycosarcoma zonotriches*** (Vánky) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Macalpinomyces zonotriches* Vánky, Mycotaxon 59: 122. 1996.

7.3.4 *Stollia* (Clade 4)

The new genus *Stollia* is proposed to accommodate smut fungi that occur on grasses in the tribe Andropogoneae as localized galls in the ovaries of an inflorescence. The sori are enclosed by a peridium of host tissue. The swollen ovaries consist of spores mixed with partitioning cells. Columellae and spore balls are absent.

Stollia McTaggart & R.G. Shivas ***gen. nov.***
MycoBank ; Fig. 23a-f.
Etymology: Named after the German mycologist Mattias Stoll in recognition of his substantial contribution towards resolving the *Ustilago-Sporisorium-Macalpinomyces* complex.

Sori in ovariis Andropogonearum tumidis, locati in inflorescentia, globosi ad obovoideos, tecti peridio crasso facto ex hospite contextu, initio viridi, fusciore ex maturitate, qui adultus rumpitur et massam sporarum pulverulentam exponit cellulis dividentibus mixtam. Sporae singulae, globosae, subglobosae ad ellipsoideas, saepe echinulatae. Cellulae dividentes in gregibus laxis, irregularibus, globosis, hyalinis. Columellae et sporae conglobatae absunt. Germinatio typi Ustilaginis.

Sori in swollen ovaries of Andropogoneae, localized in the inflorescence, globose to obovoid, covered by a thick peridium derived from host tissue, initially green, darker with age, which ruptures at maturity to expose the pulverulent spore mass mixed with partitioning cells. Spores single, globose, subglobose to ellipsoidal, often echinulate. Partitioning cells in loose irregular groups, globose, hyaline. Columellae and spore balls are lacking. Germination of *Ustilago* type.

Type species: *Stollia ewartii* (McAlpine) McTaggart & R.G. Shivas
Type on *Sorghum stipoideum*, Australia, Western Australia, Napier, Broome Bay, 22 May 1910, A.J. Ewart, MEL 1055129.

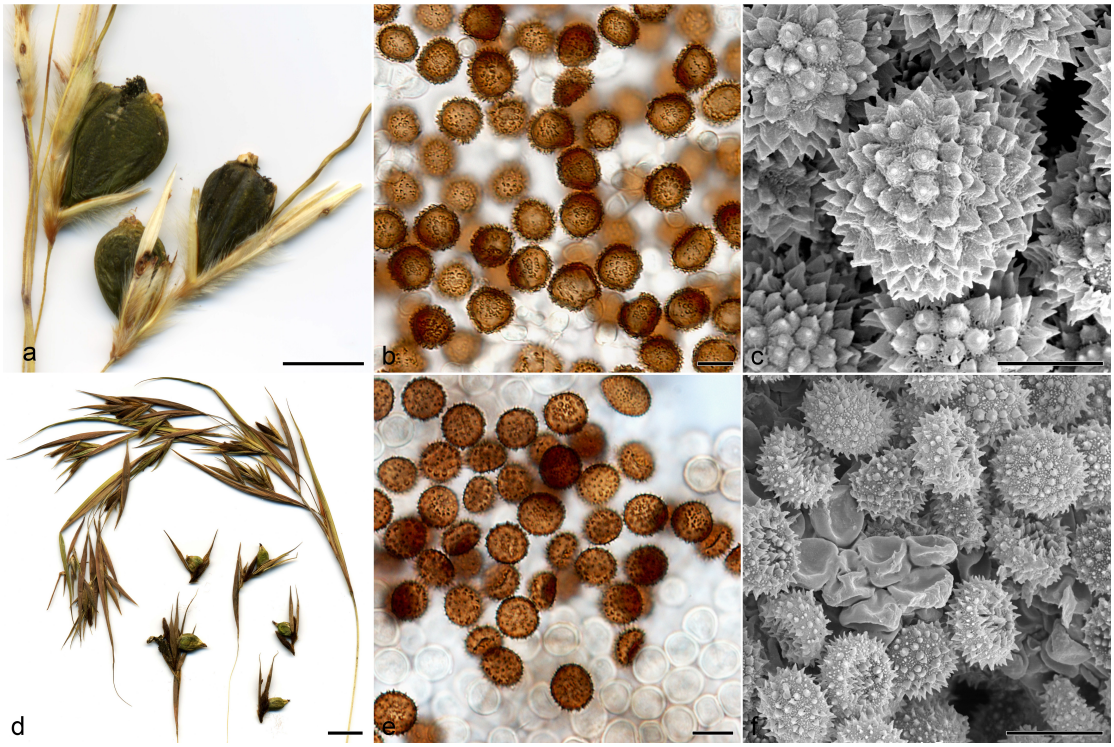

Fig. 22. *Stollia ewartii* and *S. bursa*. a. *S. ewartii* sori. b. *S. ewartii* spores. c. *S. ewartii* spores under SEM. d. *S. bursa* sori. e. *S. bursa* spores. f. *S. bursa* spores under SEM. Scale a, d = 5 mm; b-c, e-f = 10 µm.

7.3.4.1 New combinations for Stollia

The following taxa are new combinations of *Stollia* based on either results of the molecular phylogenetic analysis[1], examination of either type specimens or specimens from the exsiccata *Herbarium Ustilaginales Vánky* [2], or from the type descriptions in protologues[3].

[2,3]***Stollia bothriochloae*** (L. Ling) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Ustilago bothriochloae* L. Ling, Mycological Papers 11: 4. 1945.
≡ *Macalpinomyces bothriochloae* (L. Ling) Vánky, Fungal Diversity 15: 225. 2004.

Specimens examined: BOLIVIA, La Paz, Sud Yungas, Between Chulumani and Inquisivi, *Bothriochloa bladhii* (Retz.) S.T. Blake, 18 Apr. 2009, R.G. & M.D.E. Shivas, A.R. McTaggart, W.A. Arce, C. & K. Vánky, BRIP 52756a; THAILAND, *Bothriochloa bladhii* (Retz.) S.T. Blake, 27 Dec. 2005, R.G. Shivas, P. Athipunyakom, BRIP 47762a.

[1,2]***Stollia bursa*** (Berk.) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Ustilago bursa* Berk., Hookers's J. Bot. Kew Gard. Misc. 6: 206. 1854.
≡ *Tolyposporium bursum* (Berk.) McAlpine, The smuts of Australia: 186. 1910.
≡ *Sphacelotheca bursa* (Berk.) Mundk. & Thirum., Mycol. Pap. 16: 6. 1946.
≡ *Sporisorium bursum* (Berk.) Vánky, Mycotaxon 31: 403. 1988.

≡ *Macalpinomyces bursus* (Berk.) Vánky, Mycotaxon 81: 427. 2002.

Specimen examined: THAILAND, Chiang Rai, 42 km east of Chiang Saen, *Themeda villosa* Hack., 16th Dec. 2007, P. Athipunyakom, S. Likhitekaraj, V.L. Challinor, T.S. Marney, A.R. McTaggart, M.D.E. & R.G. Shivas, BRIP 51544a.

[1,2]***Stollia ewartii*** (McAlpine) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Ustilago ewartii* McAlpine, Proc. Linn. Soc. New South Wales 36: 45. 1912('1911'; as '*ewarti*').
≡ *Macalpinomyces ewartii* (McAlpine) Vánky & R.G. Shivas, Mycotaxon 80: 346. 2001.
= *Ustilago sorghi-stipoidei* L. Ling, Sydowia 7: 154. 1953.

Specimens examined: AUSTRALIA, Western Australia, between Wyndham and Kununurra, *Sarga timorense* (Kunth) Spangler, 04 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51814a; Western Australia, between Wyndham and Kununurra, *Sarga timorense* (Kunth) Spangler, 08 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. & R.G. Shivas, BRIP 51818a; Northern Territory, Katherine, *Sarga timorense* (Kunth) Spangler, 24 Apr. 1947, S.T. Blake, BRIP 7791a, isotype.

[3]***Stollia ovariicolopsis*** (Vánky) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Sporisorium ovariicolopsis* Vánky, Mycotaxon 74: 203. 2000.
≡ *Macalpinomyces ovariicolopsis* (Vánky) Vánky, Mycotaxon 81: 427. 2002.

[3]***Stollia pseudanthistiriae*** (A.R. Patil, T.M. Patil & M.S. Patil) McTaggart & R.G. Shivas **comb. nov.** MycoBank
Basionym: *Macalpinomyces pseudanthistiriae* A.R. Patil, T.M. Patil & M.S. Patil, J. Mycol. Pl. Pathol. 34: 839. 2004.

7.3.5 *Langdonia* (Clade 9)

The new genus *Langdonia* is proposed to accommodate the monophyletic group of smut fungi that infect the ovaries of *Aristida*, have coiled sporogenous hyphae and lack columellae.

Langdonia McTaggart & R.G. Shivas ***gen. nov.***
MycoBank ; Fig. 24a-c
Etymology: Named after the Australian mycologist Raymond F. N. Langdon who first described the mode of soral development in the *Ustilago-Sporisorium-Macalpinomyces* complex.

Inficientes hospites Aristidorum. Sori in nonnullis vel omnibus ovariis paniculae. Columella abest. Sporae plerumque compactae in sporas conglobatas. Cellulae dividentes factae ex hyphis non-sporogenis absunt. Germinatio typi Ustilaginis.

Infecting hosts of *Aristida*. Sori in some or all ovaries of a panicle. Columella absent. Spores usually compacted into spore balls. Partitioning cells formed from non-sporogenous hyphae absent. Germination of *Ustilago* type.

Type species: *Langdonia consanguinea* (Ellis & Everhart) McTaggart & R.G. Shivas
Type on *Aristida adscensionis*, India, Uttar Pradesh, Varanasi, Banaras Hindu University, 02 Nov 1948, M.S. Pavgi, HCIO 20048.

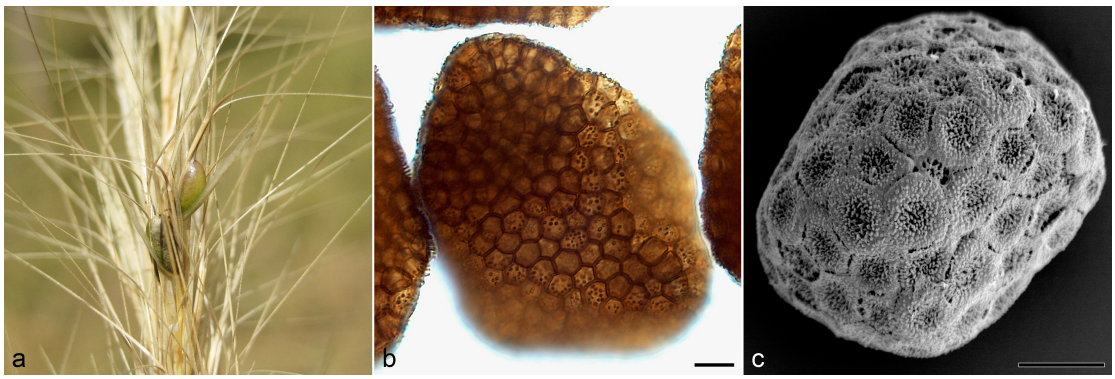

Fig. 23. *Langdonia*. a. *L. aristidicola* sori. b. *L. aristidicola* spores in spore ball. c. *L. aristidicola* spore ball under SEM. Scale b-c = 10 µm.

7.3.5.1 New combinations for Langdonia

The following taxa are new combinations of *Langdonia* based on either results of the molecular phylogenetic analysis[1], examination of either type specimens or specimens from the exsiccata *Herbarium Ustilaginales Vánky* [2], or from the type descriptions in the protologues[3].

[3]***Langdonia aristidaria*** (Durán) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago aristidarius* Durán, Ustil. Mexico: 222. 1987.

[1,2]***Langdonia aristidicola*** (Speg.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Urocystis aristidicola* Speg., Anales Mus. Nac. Buenos Aires, Ser. 3, 12: 294. 1909.

≡ *Tuburcinia aristidicola* (Speg.) Liro, Ann. Univ. Fenn. Abo., Ser. A, 1: 26. 1922.

≡ *Sporisorium aristidicola* (Speg.) Vánky, Mycotaxon 78: 305. 2001.

= *Sorosporium consanguineum* Ellis & Everh. var. *bullatum* Pavgi & Thirumalachar, Sydowia 5: 10. 1951.

≡ *Sorosporium bullatum* (Pavgi & Thirum.) Pavgi & Thirum., in Thirumalachar & Pavgi, Mycopathol. Mycol. Appl. 7: 284. 1956 (later homonym, not J. Schröter 1869). (Syn. by Vánky 2001:305).

= *Sorosporium penuriasorus* Durán, Ustil. Mexico: 67. 1987.

Specimens examined: AUSTRALIA, Northern Territory, Victoria River, 15 km east of Victoria Highway, *Aristida* sp., 12 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E & R.G. Shivas, BRIP 51871a; Northern Territory, 72 km NNW of Alice Springs, *Aristida jerichoensis* (Domin) Henr., 26 Mar. 2000, C. & K. Vánky, BRIP 26930a: Vánky, Ust. exs. no. 1119.

[2,3]***Langdonia clandestina*** (R.G. Shivas, Vánky & Athipunyakom) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium clandestinum* R.G. Shivas, Vánky & Athipunyakom, in Vánky, Shivas & Athipunyakom, Mycol. Balcan. 3: 108. 2006.

Specimens examined: THAILAND, Nakhon Phanom, 31 km west of Sri Songkram, *Aristida balansae* Henrard, 12 Dec. 2007, P. Athipunyakom, S. Likhitekaraj, V.L. Challinor, T.S. Marney, A.R. McTaggart, M.D.E. & R.G. Shivas, BRIP 51520a; Kalasin Province, 10 km NW of Na Khu, *Aristida setacea* Retz., 20 Dec. 2005, R.G. Shivas, P. Athipunyakom, BRIP 47754a, isotype.

[1,2]***Langdonia confusa*** (H.S. Jackson) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium confusum* H.S. Jackson, Bull. Torrey Bot. Club 35: 148. 1908.

≡ *Sporisorium confusum* (H.S. Jackson) Vánky, Mycotaxon 78: 306. 2001.

Specimens examined: AUSTRALIA, Northern Territory, 63.4 km north of Alice Springs, *Aristida inaequiglumis* Domin, 23 Apr. 2007, A.R. McTaggart, J.R.Liberato, R.G. Shivas, BRIP 49660a; Queensland, 1 km south of Mount Morgan, *Aristida queenslandica*, 24 Mar. 2003, R.G. & M.D.E. Shivas, BRIP 42670a; BOLIVIA, Potosi, Charcas, between Toro Toro and Punata, *Aristida* sp., 22 Apr. 2009, R.G. & M.D.E. Shivas, A.R. McTaggart, W.A. Arce, C. & K. Vánky, BRIP 52755a.

[1,2]***Langdonia consanguinea s. lat.*** (Ellis & Everhart) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium consanguineum* Ellis & Everhart, J. Mycol. 3:56, 1887.
≡ *Sporisorium consanguineum* (Ellis & Everhart) Vánky, Mycotaxon 31: 402. 1988, *s. lat.*
= *Ustilago aristidae* Peck, Bull. Torrey Bot. Club 12: 35. 1885 (not *Sporisorium aristidae* (S. Ahmad) Vánky).
= *Sorosporium aristidae* Neger, Ann. Univ. Chile 95: 789. 1896 (as '*Sorisporium*').
= *Sorosporium bornmuelleri* Magnus, Verh. K. K. Zool.-Bot. Ges. Wien 50: 434. 1900 (as '*Sorisporium*').
= *Sorosporium concelatum* L. Ling, Lloydia 14: 106. 1951.
≡ *Sporisorium arundinellae-nepalensis* Vánky, Mycotaxon 89: 91. 2004 (not *Sporisorium concelatum* (Zundel) M. Piepenbr.

Specimens examined: AUSTRALIA, Western Australia, Kununurra, Ivanhoe Crossing, *Aristida hygrometrica* R.Br., 09 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E & R.G. Shivas, BRIP 51839a; Western Australia, Gingin, Cemetery, *Aristida* sp., 14 Sep. 1999, C. & K. Vánky, BRIP 27723a.

[1,3]***Langdonia fraseriana*** (Syd.) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sorosporium fraserianum* Syd., Ann. Mycol. 35: 25. 1937.
≡ *Sporisorium fraserianum* (Syd.) Vánky, Mycotaxon 78: 308. 2001.

Specimen examined: AUSTRALIA, Northern Territory, Alice Springs, near Standley's Gap, *Aristida nitidula* (Henr.) S.T.Blake ex J.M.Black, 22 Apr. 2007, A.R. McTaggart, J.R. Liberato, R.G. Shivas, BRIP 49668a.

[3]***Langdonia goniospora*** (Massee) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Ustilago goniospora* Massee, Bull. Misc. Inform. 1899: 183. 1899.
≡ *Sorosporium goniosporum* (Massee) L. Ling, Lloydia 16: 189. 1953.
≡ *Sporisorium goniosporum* (Massee) Vánky, Mycotaxon 78: 308. 2001.

[2,3]***Langdonia inopinata*** (Vánky) McTaggart & R.G. Shivas, **comb. nov.** MycoBank
Basionym: *Sporisorium inopinatum* Vánky, Mycotaxon 81: 384. 2002.

Specimen examined: THAILAND, *Aristida* sp., 20 Dec. 2005, R.G. Shivas, P. Athipunyakom, BRIP 47757a; ZIMBABWE, Matabeleland North, 30 km SE of Bulawayo, *Aristida scabrivalvis* Hack., 02 Mar. 1999, C. & K. Vánky, BRIP 28935a: Vánky, Ust. exs. no. 1108, isotype.

7.3.6 Taxa of uncertainty

[1,3]***Sporisorium modestum*** (Syd.) H. Scholz, in Fraiture, Bull. Jard. Bot. Nat. Belg. 66: 171. 1997.
Basionym: *Ustilago modesta* (Syd.), Ann. Mycol. 33: 231. 1935.
≡ *Sphacelotheca modesta* (Syd.) Zundel, Bothalia 3: 301. 1938.

Sporisorium modestum sat in the *Ustilago s. lat*. clade in the molecular phylogenetic analyses by McTaggart et al. (submitted 2010a) and Stoll et al. (2005). It was described with a stout columella and partitioning cells, which indicated that it belongs to *Sporisorium*. It infects *Enneapogon*, which is in the subfamily Chloridoideae. Few species of *Sporisorium* infect chloridoid hosts. The structure of the columella should be examined to determine if it is homologous to the columellae of *Anthracocystis* and *Sporisorium*.

[1,2]***Sporisorium panici*** (E. Mackinnon) Vánky, Mycotaxon 78: 295. 2001.
Basionym: *Sorosporium panici* E. Mackinnon, J. & Proc. Roy. Soc. New South Wales 46: 201. 1912.
= *Ustilago panici-gracilis* E. Mackinnon, J. & Proc. Roy. Soc. New South Wales 46: 202. 1912. (Syn. by Vánky & Shivas, 2008: 126).
= *Ustilago clelandii* H. Sydow, Ann. Mycol. 35: 24. 1937. (Syn. by Ling as U. panici-gracilis, 1953:181).

Specimen examined: AUSTRALIA, New South Wales, Nyngan, Nyngan Experiment Farm, *Paspalidium aversum* Vickery, Feb. 1911, E. Mackinnon, BRIP 39182, isotype,

Sporisorium panici is not a true *Sporisorium*. It sat in Clade 8 of the molecular phylogenetic analysis by McTaggart et al. (2011b). It does not possess true columella according to our definition.

[1,3]***Sporisorium veracruzianum*** (Zundel & Dunlap) M. Piepenbr., Mycol. Res. 99: 787. 1995.
Basionym: *Sphacelotheca veracruziana* Zundel & Dunlap, in Zundel, North American Flora 7: 994. 1939.

Sporisorium veracruzianum is not a true *Sporisorium*. It occurred in Clade 7 of the molecular phylogenetic analysis by McTaggart et al. (submitted 2010a). It is unlikely that the columella described in *S. veracruzianum* is homologous to the synapomorphic columellae of *Sporisorium* and *Anthracocystis*.

7.3.7 Identification of genera

A key to the genera of the *Ustilago-Sporisorium-Macalpinomyces* complex is presented. The genera can be identified by soral characteristics and host plant.

1a	Columella, partitioning cells or spore balls present	2
b	Columella, partitioning cells and spore balls absent	***Ustilago***
2a	Columella present	3
b	Columella absent	4
3a	Columella filiform, flattened, flexuous. Spore balls formed from coiled sporogenous hyphae. Partitioning cells usually absent	***Anthracocystis***
b	Columella stout, cylindrical, woody. Spore balls usually absent. Partitioning cells present	***Sporisorium***
4a	Sori cylindrical or tubular, derived from host tissue	5
b	Sori globose or ovoid in hypertrophied host ovaries	6
5a	Sori localized on host inflorescence	***Mycosarcoma***
b	Sori destroying entire inflorescence	***Macalpinomyces s. lat.***
6a	Spore balls formed from coiled sporogenous hyphae	7
b	Spore balls absent	8
7a	Sori on *Aristida*	***Langdonia***
b	Sori on *Panicum trachyrachis*, partitioning cells present	***Anomalomyces***
8a	Sori on *Eriachne*	***Macalpinomyces s. str.***
b	Sori on grasses in the subfamilies Paniceae or Chloridoideae	9
9a	Sori on grasses in the tribe Andropogoneae	***Stollia***
b	Sori on grasses in Panicoideae or Chloridoideae	***Macalpinomyces s. lat.***

7.4 Discussion

Six of the ten monophyletic groups within the *Ustilago-Sporisorium-Macalpinomyces* complex are well supported and can be defined by morphology or host classification. Relationships among these groups are however, still ambiguous. Character evolution within the complex cannot be determined confidently while there is doubt about the evolutionary relationships among the clades. For example, because the relationship of *Sporisorium* to *Anthracocystis* is not fully resolved, it is unknown whether columellae arose once in a shared common ancestor, or whether columellae were derived in two separate clades. The results of our previous studies indicate that the two structural types of columellae arose separately in smut fungi that infect the Andropogoneae.

To resolve relationships among the clades and character evolution, it will be necessary to add more taxa to future phylogenetic analyses. The taxa should be

chosen from *Mycosarcoma*, *Stollia*, *Ustilago s. lat*., Clade 8 and the group of smut fungi similar to the genus *Endosporisorium*. It is not necessary to add *Sporisorium* or *Anthracocystis* species as these clades are already well represented. Sequences should be obtained from the nuclear rDNA loci ITS and LSU. The addition of more nuclear sequence data would be another approach if nuclear rDNA loci do not resolve the relationships.

Clades 7 and 8 and the monophyletic group of smut fungi that included *Macalpinomyces loudetiae*, remain unresolved here. Morphological synapomorphies could not be determined for these groups. It is essential to resolve these clades to accurately define *Ustilago* and *Macalpinomyces*, which are both currently polyphyletic groups. *Macalpinomyces* occurs in three unrelated clades, (i) the monotypic type species *Macalpinomyces eriachnes*, (ii) Clade 10, which has smut fungi that destroy the host inflorescence with tubular sori and are similar to the description of *Endosporisorium*, and (iii) the species in Clade 8 that form galls on panicoid and chloridoid hosts and that have partitioning cells. Future phylogenetic analyses must include more taxa that occur on hosts in the subfamilies Chloridoideae and Arundinoideae, and the tribe Paniceae. Inclusion of taxa will help to determine synapomorphies and resolve clades and subclades of these challenging groups.

References

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8.0 Conclusions

8.1 Cumulative impact of publications

The genera *Ustilago*, *Sporisorium* and *Macalpinomyces* represented an unresolved complex at the generic level. The original generic concepts did not accommodate the diversity of species assigned to these three genera. Previous studies indicated morphology alone was inadequate to differentiate genera within the complex.

The purpose of the current study was to resolve the systematics of the *Ustilago-Sporisorium-Macalpinomyces* complex through five approaches, namely to (i) define the complex, (ii) examine the diversity of the complex, (iii) comprehensively reconstruct the evolutionary history of the complex using combined molecular and morphological phylogenetic analyses, (iv) identify characters that could delimit monophyletic groups within the complex, and (v) incorporate these monophyletic groups into a new taxonomic framework. This was accomplished in five publications in progress, culminating in the emendation of *Sporisorium*, together with the establishment of two new genera, *Langdonia* and *Stollia*, and resurrection of two unused genera *Anthracocystis* and *Mycosarcoma*, to accommodate newly recognised monophyletic groups within the *Ustilago-Sporisorium-Macalpinomyces* complex.

8.2 Significance of the thesis

The phylogenetic inferences, re-evaluation of character homology and resulting new classification of the *Ustilago-Sporisorium-Macalpinomyces* complex presented here make a new contribution to plant pathology and fungal taxonomy, in particular towards a more comprehensive understanding of the systematics of the Basidiomycotina. The findings have enabled the reclassification of a taxonomically challenging and agriculturally important group of fungi. The key findings are summarized below.

8.2.1 Key findings in Chapter 3: Toward a resolution of the *Ustilago-Sporisorium-Macalpinomyces* complex

The concepts of *Ustilago* and *Sporisorium* have changed considerably since their original descriptions approximately 200 years ago. *Ustilago* was originally a broad genus that accommodated a wide diversity of smut fungi. It was later subdivided and refined to contain only smut fungi that occurred on grasses. *Sporisorium* was neglected for over 150 years; mycologists instead used the genera *Sphacelotheca* and *Sorosporium* to classify species. The Australian mycologists Ray Langdon and Robert Fullerton resurrected *Sporisorium* and described its defining morphological characteristics, namely a columella, partitioning cells and a peridium. These characters were not present in *Ustilago*. Problems of classification were created by the subsequent discovery of a diversity of new taxa that exhibited a combination of soral characters that could not be strictly accommodated within *Sporisorium* or *Ustilago*. These taxa, lacking an appropriate genus, were often placed into *Macalpinomyces*, which became polyphyletic.

Systematic problems with the *Ustilago-Sporisorium-Macalpinomyces* complex were not resolved using traditional morphological characters, including spore ornamentation or soral structure. Furthermore, clades derived from molecular phylogenetic analyses did not correlate with morphology. One approach to resolving the complex is to group recognised species into a single genus, *Ustilago*. This approach neglects the great morphological diversity within the complex and the relationships among taxa. The genera need further subdivision to reflect and encompass this diversity. This requires a thorough examination of soral morphology to determine morphological synapomorphies within the monophyletic groups derived from combined morphological and molecular phylogenetic analyses.

Clearly defining the *Ustilago-Sporisorium-Macalpinomyces* complex was an important step towards its resolution. Although it was widely accepted that a complex existed, there was ambiguity in the nature of the complex and how it had arisen. Understanding the complex, and the diversity within the complex, strengthened the case for its resolution and removed any argument to regard all of the species within the complex as *Ustilago*, which was taxonomically unacceptable.

8.2.2 Key findings in Chapter 4: *Macalpinomyces mackinlayi*

Macalpinomyces mackinlayi was a novel species found on a collecting trip to a remote region in Western Australia during the current study. It was placed in *Macalpinomyces* because of the combination of characters typical of both *Ustilago* and *Sporisorium*. Molecular phylogenetic analysis including nine similar taxa and assessed with parsimony in an exhaustive search, demonstrated *Macalpinomyces mackinlayi* was no more closely related to other *Macalpinomyces* species than it was to species in *Ustilago*. This study highlighted that *Macalpinomyces* constitutes a polyphyletic genus.

This project helped to discover previously unknown fungal biodiversity within Australia. Five new smut taxa were described and a new disease report was recorded for Australia in the course of this work, as is evident in the publications presented in the appendix. Determining the species of fungi present within Australia is crucial for biosecurity, as this enables the distinction between endemic and invasive or non-native species. Documenting the biodiversity of Australia's fungi is also important for their conservation.

8.2.3 Key findings in Chapter 5: Phylogenetic utility of molecular and morphological data for determining monophyletic groups in a complex of smut fungi

There were several outcomes that arose from the molecular phylogenetic analyses of molecular and morphological data in the *Ustilago-Sporisorium-Macalpinomyces* complex. Molecular phylogenetic analyses demonstrated ITS2 secondary structure could not resolve higher taxonomic levels of closely related smut fungi. Relationships among taxa generated with a mitochondrial locus, COX3, were shown to be incongruent with that from nuclear rDNA and nuclear protein-coding loci.

Phylogenetic analyses provided insight into the use of morphological data for reconstructing fungal evolutionary trees. Smut fungi have few morphological characters available for incorporation into phylogenetic datasets. The characters that were scored were considered putatively homologous until they had been tested in a phylogenetic reconstruction. Results from this study showed that some of the scored

characters in the complex were not homologous. A re-evaluation of the homology of the characters was undertaken from these data, however, the inclusion of morphological characters in a phylogenetic analysis did not help to resolve the *Ustilago-Sporisorium-Macalpinomyces* complex.

In terms of the debate for adding more molecular loci or including data for more taxa, this study conclusively supported the addition of taxa. Inclusion of additional species in the dataset effectively resolved phylogenetic relationships and led to identification of synapomorphies within the recovered clades. Additional taxa strengthened support for several clades and recovered clades that were not resolved in previous phylogenetic studies.

Ten clades were established here using both Bayesian inference and maximum likelihood optimality criteria. Nuclear rDNA and nuclear protein-coding loci resolved the same clades. Relationships among clades varied with use of different optimality criteria and different loci.

Insights were gained into the use of morphological data in reconstruction of fungal phylogenies. Morphology is rarely included in systematic studies of fungi and this is due to the paucity of available characters. For example, many anamorphic fungi within the Ascomycetes have only two structures that can be assessed, the conidia and conidiophores, and these characters are often considered homoplasious. Smut fungi within the *Ustilago-Sporisorium-Macalpinomyces* complex offered a unique opportunity for using morphological data in phylogenetic analyses, because of their diverse soral characters and host specificities.

8.2.4 Key findings in Chapter 6: A reassessment of character homology in the *Ustilago-Sporisorium-Macalpinomyces* complex (Ustilaginaceae).

Resolution of the *Ustilago-Sporisorium-Macalpinomyces* complex depended on identifying morphological synapomorphies that could define monophyletic groups. Morphological synapomorphies determined here had previously been disregarded as a useful way to delimit the complex. For example, columellae occurred in several groups and were thought to be convergent; this study demonstrated that there was a

difference in the formation of the columella among certain clades. The different formation and structure of columellae in *Sporisorium* and *Anthracocystis* were synapomorphic for the two groups. Spore balls and partitioning cells were also both considered to be convergent characters across the complex. This study determined that spore balls produced by coiled sporogenous hyphae and partitioning cells formed from non-sporogenous hyphae were synapomorphic characters that could be used in combination with other soral characters to define the ten clades.

Two of the groups within the *Ustilago-Sporisorium-Macalpinomyces* complex can be defined by their host classification. Delimiting genera by the host subfamily or tribe is warranted when morphological characters prove inadequate to define monophyletic groups within the complex. The smut fungi that occur on the arid grass *Triodia* possibly warrant a genus separate from *Ustilago*. This clade was well supported, but its relationship to *Ustilago s. lat.* was unknown. A lack of other morphological characters, at this stage, best places these taxa within *Ustilago*.

The determination of character evolution and host coevolution in the *Ustilago-Sporisorium-Macalpinomyces* complex may have wide applications across other fungal systematic studies. Host family classification has been used previously to delimit genera of smut fungi, but lower levels of classification, namely sub-family and tribe, have not been considered. Other groups of smut fungi, such as *Tilletia*, or complexes within rust fungi, such as *Uromyces* and *Puccinia*, could benefit from delimiting groups by host classification. Rigorous examination of seemingly convergent characters in other fungal groups could provide a means to reassess these characters and improve the taxonomy. For example, analysing conidial secession in the Mycosphaerellaceae could identify another character to define the many morphologically similar genera.

8.2.5 Key findings in Chapter 7: A taxonomic revision of *Ustilago*, *Sporisorium* and *Macalpinomyces*.

Reconciliation of the homologous characters within the *Ustilago-Sporisorium-Macalpinomyces* complex enabled the clades to be defined by combing morphology and host classification. *Sporisorium* was emended to contain smuts that are

morphologically similar to the type species *S. sorghi*. *Anthracocystis* was re-established to accommodate taxa previously regarded as *Sporisorium* possessing filiform columellae, spore balls, a fungal derived peridium, and no partitioning cells. Over 150 species were reassigned to *Anthracocystis*.

Ustilago maydis is a widely studied plant pathogen and a model organism. *Mycosarcoma* was described for the clade that contained *Ustilago maydis* and other smut fungi with tubular, host derived sori and partitioning cells. This genus now contains 13 taxa.

Langdonia and *Stollia* were described for smut fungi that occurred in clades with a common host. *Langdonia*, named after Australian mycologist Raymond F. N. Langdon, accommodates smut fungi infecting the host *Aristida*. These smut fungi form a gall in the ovaries and typically have spore balls formed from coiled sporogenous hyphae. *Stollia*, named after German mycologist Matthias Stoll, accommodates smut fungi that form a gall in some ovaries of hosts in the tribe Andropogoneae.

As a result of this study, the taxonomy of *Sporisorium* has been resolved, however, *Ustilago* and *Macalpinomyces* are still polyphyletic. No morphological synapomorphies were identified for the clade containing species described as *Ustilago*, *Sporisorium* and *Macalpinomyces* (Clade 8). Until this clade is resolved, *Macalpinomyces* will be a polyphyletic genus. The *Ustilago* clade (Clade 7) could not be resolved. It is possible that this group could be divided into smaller groups delimited by host, such as the smut fungi that occur on *Triodia*.

Resolving the majority of the *Ustilago-Sporisorium-Macalpinomyces* complex is a significant contribution to fungal taxonomy. The complex contains many important fungal pathogens including *Sporisorium sorghi*, *S. scitamineum*, *S. cruentum*, *Ustilago hordei*, *U. tritici* and *U. maydis* (now *Mycosarcoma maydis*). These taxa can now be named with certainty and stability, owing to the new robust taxonomy. Description of newly discovered species will be less ambiguous for taxa within the resolved clades. The genera can be defined based on morphological synapomorphies,

making the taxonomy more accessible to mycologists that are unable to use molecular techniques for identification.

8.3 Future work

8.3.1 Resolving the relationship among the clades

Monophyletic groups within the complex were well supported and can be defined by morphology. Relationships among these groups are, however, still ambiguous. Character evolution within the complex cannot be determined confidently while there is doubt about the evolutionary relationships of the clades. For example, the relationship of *Sporisorium* to *Anthracocystis* was not fully resolved, which means it cannot be determined whether columellae arose once in a shared common ancestor or whether columellae were derived multiple times.

To resolve the relationships among the clades and to assess character evolution, addition of taxa to future phylogenetic analyses will be required. The additional taxa should be chosen from *Mycosarcoma*, *Stollia*, *Ustilago s. lat.*, Clade 8 and the group of smut fungi similar to the genus *Endosporisorium* in Clade 10. It will not be necessary to add *Sporisorium* or *Anthracocystis* species as these clades are already well represented. Sequences should be obtained from the nuclear rDNA loci ITS and LSU. The addition of more nuclear protein-coding sequence data would be another approach if the nuclear rDNA loci were unable to resolve all relationships.

8.3.2 Resolution of *Ustilago* and *Macalpinomyces*

Clades 7, 8 and 10 remained unresolved here. Morphological synapomorphies were unable to be determined confidently for these groups. It will be essential to resolve these clades to accurately define *Ustilago* and *Macalpinomyces*. Future phylogenetic analyses must include more taxa that occur on hosts in the subfamilies Chloridoideae and Arundinoideae, and the tribe Paniceae. Inclusion of taxa will help to determine synapomorphies and resolve clades and subclades of these challenging groups.

Appendices

Appendix 1. Taxa used in this study with host and GenBank details. GenBank numbers in bold were obtained in this investigation. *Mac* = *Macalpinomyces*; *Spo* = *Sporisorium*; *Ust* = *Ustilago*.

Taxon	Host	Source/Accession number	Locus and GenBank details				
			ITS	LSU	COX3	EF1a	GAPDH
Anomalomyces panici Vánky, R.G. Shivas & M. Lutz	*Panicum trachyrachis*	Vanky et al. 2006	DQ459348	DQ459347			
Mac arundinellae-setosae R.G. Shivas & Vánky	*Arundinella nepalensis*	BRIP 47958	**HQ013086**				
		BRIP 51868			**HQ012972**		**HQ013055**
Mac bursus (Berk.) Vánky	*Themeda quadrivalvis*	Stoll et al. 2005	AY740154 (as *Spo bursum*)				
	Themeda villosa	BRIP 51544			**HQ012973**		
Mac eragrostiellae Vánky & C. Vánky	*Eragrostiella bifaria*	Stoll et al. 2005	AY740036	AY740089			
Mac eriachnes (Thüm.) Langdon & Full.	*Eriachne aristidea*	Stoll et al. 2005	AY740037	AY740090			
	Eriachne ciliata	BRIP 51816			**HQ012974**		
Mac ewartii (McAlpine) Vánky & R.G. Shivas	*Sarga timorense*	BRIP 51814			**HQ012975**		
		BRIP 51818	**HQ013087**	**HQ013127**		**HQ013026**	**HQ013056**
Mac loudetiae (Vienn.-Bourg.) Vánky	*Loudetia flavida*	Stoll et al. 2005	AY740151				
Mac mackinlayi McTaggart & R.G. Shivas	*Eulalia mackinlayi*	BRIP 52549	**GU014817**	**HQ013131**	**HQ012976**	**HQ013027**	**HQ013057**
Mac neglectus (Niessl) Vánky	*Setaria pumila*	Stoll et al. 2005	AY740056 (as *Spo neglectum*)	AY740109 (as *Spo neglectum*)			
	Setaria viridis	BRIP 47112			**HQ012977**		
Mac simplex Vánky	*Loudetia simplex*	Stoll et al. 2005	AY740152				
Mac spermophorus (Berk. & M.A. Curtis ex de Toni) Vánky	*Eragrostis ferruginea*	Stoll et al. 2005	AY740171 (as *Ust spermophora*)				
	Sporobolus australasicus	BRIP 51810	**COX3**		**HQ012978**		
		BRIP 51858		**FQ013130**		**HQ013028**	**HQ013058**
Mac trichopterygis Vánky & C. Vánky	*Trichopteryx dregeana*	Stoll et al. 2005	AY740039	AY740092			
Mac tristachyae Vánky & C. Vánky	*Loudetiopsis chrysothrix*	Stoll et al. 2005	AY740164				

Taxon	**Host**	**Source/Accession number**	ITS	LSU	COX3	EF1a	GAPDH
Mac tubiformis R.G. Shivas & Vánky	*Chrysopogon fallax*	BRIP 51865	**HQ013088**		**HQ012979**	**HQ013029**	**HQ013059**
Mac viridans R.G. Shivas, McTaggart & Vánky	*Sporobolus actinocladus*	BRIP 49133	**HQ013089**	**HQ013125**	**HQ012980**	**HQ013030**	**HQ013060**
Mel pennsylvanicum Hirschh.	*Polygonum glabrum*	Stoll et al. 2005	AY740040	AY740093			
Moe bullatus (J. Schröt.) Vánky	*Paspalum distichum*	Stoll et al. 2005	AY740153	AY740153			
Spo absconditum Vánky	*Schizachyrium fragile*	BRIP 49648	**HQ013090**				
Spo aegyptiacum (A.A. Fisch. Waldh.) Vánky	*Schismus arabicus*	Stoll et al. 2005	AY344970	AY740129			
Spo andropogonis (Opiz) Vánky	*Bothriochloa saccharoides*	Stoll et al. 2005	AY740042	AY740095			
Spo anthistiriae (Cobb) Vánky	*Themeda triandra*	BRIP 49775			**HQ012981**	**HQ013031**	**HQ013061**
Spo anthracoideisporum Vánky & R.G. Shivas	*Pseudoraphis spinescens*	Stoll et al. 2005	AY740044	AY740097			
Spo apludae-aristatae (B.V. Patil & Thirum.) Vánky	*Apluda mutica*	Stoll et al. 2005	AY740045	AY740098			
Spo aristidicola (Speg.) Vánky	*Aristida jerichoensis*	BRIP 26930	**HQ013091**		**HQ012982**	**HQ013032**	
	Aristida sp.	BRIP 51871					**HQ013062**
Spo arthraxonis (Pat.) L. Guo	*Arthraxon lanceolatus*	Stoll et al. 2005	AY740046	AY740099			
Spo bothriochloae (L. Ling) Vánky	*Dichanthium sericeum*	BRIP 51819	**HQ013092**				**HQ013063**
Spo caledonicum (Pat.) Vánky	*Heteropogon contortus*	BRIP 51854	**HQ013093**		**HQ012984**		**HQ013064**
		BRIP 28043				**HQ013033**	
Spo cenchri (Lagerh.) Vánky	*Cenchrus pilosus*	Stoll et al. 2005	AY344972	AF453943			
Spo cenchri-elymoidis Vánky & R.G. Shivas	*Cenchrus elymoides*	BRIP 26491	**HQ013094**	**HQ013122**	**HQ012985**	**HQ013034**	**HQ013065**
Spo chrysopogonis Vánky	*Chrysopogon fulvus*	Stoll et al. 2005	AY344973	AY740131			
Spo confusum (H.S. Jacks.) Vánky	*Aristida inaequiglumis*	BRIP 49660			**HQ012986**		
	Aristida queenslandica	BRIP 42670	**HQ013095**	**HQ013132**			**HQ013066**
	Aristida sp.	BRIP 52755	**HQ013096**		**HQ012987**		
Spo consanguineum (Ellis & Everh.) Vánky	*Aristida hygrometrica*	BRIP 51839	**HQ013096**		**HQ012983**		

Taxon	**Host**	**Source/Accession number**	ITS	LSU	COX3	EF1a	GAPDH
Spo consanguineum (Ellis & Everh.) Vánky	*Aristida hygrometrica*	BRIP 27723	**HQ013098**		**HQ012988**		**HQ013067**
Spo cruentum (J.G. Kühn) Vánky	*Sorghum halepense*	Stoll et al. 2005	AY344974	AF453939			
Spo culmiperdum (J. Schröt.) Vánky	*Andropogon gerardii*	Stoll et al. 2005	AY344975	AF133580			
Spo cymbopogonis-bombycini R.G. Shivas & Vánky	*Cymbopogon bombycinus*	BRIP 52511	**HQ013099**		**HQ012989**	**HQ013035**	
Spo destruens (Schltdl.) Vánky	*Panicum miliaceum*	Stoll et al. 2005	AY344976	AY747077			
Spo dietelianum (Henn.) Vánky	*Tripsacum* sp.	Cunnington et al. 2005	AY998100				
Spo dimeriae-ornithopodae Vánky & C. Menge	*Dimeria ornithopoda*	Stoll et al. 2005	AY344977	AY740132			
Spo doidgeae (Zundel) Langdon & Full.	*Bothriochloa ewartiana*	BRIP 49669		**HQ013126**	**HQ012990**	**HQ013036**	**HQ013068**
		Stoll et al. 2005	AY740047 (as *S. andropogonis-micranthi*)				
S. elionuri (Henn. & Pole-Evans) Vánky	*Elionurus muticus*	Stoll et al. 2005	AY740157				
S. enteromorphum (McAlpine) Vánky	*Themeda triandra*	Stoll et al. 2005	AY740158				
S. erythraeense (Syd. & P. Syd.) Vánky	*Hackelochloa granularis*	Stoll et al. 2005	AY740049	AY740102			
Spo everhartii (Ellis & Galloway) M. Piepenbr.	*Andropogon virginicus*	Stoll et al. 2005	AY740159				
Spo exsertum (McAlpine) L. Guo	*Themeda triandra*	BRIP 52545			**HQ012991**		
Spo fallax R.G. Shivas & Cunningt.	*Chrysopogon fallax*	Shivas et al. 2004	AY333940				
Spo fastigiatum Vánky	*Andropogon angustatus*	Stoll et al. 2005	AY344978	AY740133			
Spo formosanum (Sawada) Vánky	*Panicum repens*	Stoll et al. 2005	AY344979	AY740134			
Spo foveolati (Maire) Vánky	*Eremopogon foveolatus*	Stoll et al. 2005	AY740050	AY740103			
Spo fraserianum (Syd.) Vánky	*Aristida nitidula*	BRIP 49668	**HQ013100**		**HQ012992**		

Taxon	**Host**	**Source/Accession number**	ITS	LSU	COX3	EF1a	GAPDH
Spo heteropogonicola (Mundk. & Thirum.) Vánky	*Heteropogon contortus*	BRIP 51822	**HQ013101**	**HQ013135**	**HQ012993**	**HQ013037**	**HQ013069**
		BRIP 52496				**HQ013038**	
Spo holwayi (G.P. Clinton & Zundel) Vánky	*Andropogon bicornis*	Stoll et al. 2005	AY344980	AF453941			
Spo hwangense Vánky & C. Vánky	*Sporobolus panicoides*	Stoll et al. 2005	AY740051	AY740104			
Spo iseilematis-ciliati Vánky	*Iseilema* sp.	BRIP 51870	**HQ013102**		**HQ012994**	**HQ013039**	**HQ013070**
		BRIP 52517			**HQ012995**	**HQ013040**	
Spo lacrymae-jobi (Mundk.) Vánky	*Coix lacryma-jobi*	Stoll et al. 2005	AY740052	AY740105			
Spo lanigeri (Magnus) Ershad	*Cymbopogon ambiguus*	BRIP 46819	**HQ013103**				
Spo lepturi (Thüm.) Vánky	*Hemarthria uncinata*	Stoll et al. 2005	AY344981	AY740135			
Spo loudetiae-pedicellatae Vánky & C. Vánky	*Loudetia pedicellata*	Stoll et al. 2005	AY740053	AY740106			
Spo manilense (Syd. & P. Syd.) Vánky	*Sacciolepis indica*	Stoll et al. 2005	AY740059	AY740112			
		BRIP 51516			**HQ012996**		
Spo mexicanum (Vánky) Vánky & Cunningt.	*Andropogon* sp.	Cunnington et al. 2005	AY998101				
Spo mishrae Vánky	*Apluda mutica*	Stoll et al. 2005	AY344983	AY740136			
Spo mitchellii (Syd. & P. Syd.) Vánky	*Iseilema dolichotrichum*	BRIP 49696			**HQ012997**		
	Iseilema sp.	BRIP 52538				**HQ013041**	
Spo modestum (Syd.) H. Scholz	*Enneapogon avenaceus*	Stoll et al. 2005	AY740054	AY740107			
Spo monakai (Mishra) Vánky	*Isachne globosa*	Stoll et al. 2005	AY740161				
Spo moniliferum (Ellis & Everh.) L. Guo	*Heteropogon contortus*	Stoll et al. 2005		AF453940			
		BRIP 52504	**HQ013104**		**HQ012998**	**HQ013042**	**HQ013071**
Spo mutabile (Syd.) Vánky	*Cymbopogon refractus*	BRIP 44111	**HQ013105**		**HQ012999**		
Spo nealii (Ellis & F.W. Anderson) Vánky	*Heteropogon melanocarpus*	Stoll et al. 2005	AY740055	AY740108			

Taxon	**Host**	**Source/Accession number**	ITS	LSU	COX3	EF1a	GAPDH
Spo nervosum Vánky, C. Vánky & R.G. Shivas	*Sehima nervosum*	Stoll et al. 2005		AY740110			
		BRIP 27019	**HQ013106**				
Spo occidentale (Seym. ex G.P. Clinton) Vánky & Snets.	*Andropogon gerardii*	Stoll et al. 2005	AY344985	AY740137			
Spo ophiuri (Henn.) Vánky	*Rottboellia cochinchinensis*	Stoll et al. 2005	AY740019	AJ236136			
		BRIP 25772			**HQ01300**		
Spo ovarium (Griffiths) Vánky	*Urochloa fasciculata*	Stoll et al. 2005	AY740020	AJ236137			
Spo panici (E. Mackinnon) Vánky	*Paspalidium caespitosum*	BRIP 43942	**HQ170519**		**HQ013001**		
Spo panici-leucophaei (Bref.) M. Piepenbr.	*Digitaria brownii*	Stoll et al. 2005	AY740035 (as *Spo fascicularis*)	AY740088 (as *Spo fascicularis*)			
Spo paspali (Speg.) Vánky	*Paspalum notatum*	Stoll et al. 2005	AY344982 (as *Spo paspali-notati*)	AF453944 (as *Spo paspali-notati*)			
Spo penniseti (Rabenh.) Ershad	*Pennisetum setaceum*	Stoll et al. 2005	AY344971	AY740130			
Spo polliniae (Magnus) Vánky	*Andropogon distachyos*	Stoll et al. 2005	AY344987	AY740138			
Spo provinciale (Ellis & Galloway) Vánky & Snets.	*Andoropogon gerardii*	Stoll et al. 2005	AY344988	AY747076			
Spo pseudechinolaenae Vánky & C. Menge	*Pseudechinolaena polystachya*	Stoll et al. 2005	AY344989	AY740139			
Spo pulverulentum (Cooke & Massee) Vánky	*Saccharum strictum*	Stoll et al. 2005	AY740162				
Spo queenslandicum Vánky, C. Vánky & R.G. Shivas	*Sehima nervosum*	BRIP 49706	**HQ013107**				
Spo rarum R.G. Shivas, McTaggart & Vánky	*Eulalia aurea*	BRIP 49134	**HQ013108**		**HQ013002**	**HQ013043**	**HQ013072**
Spo reilianum (J.G. Kühn) Langdon & Full.	*Zea mays*	Zhang and Gao unpublished	FJ167357				
	Not provided	Matheny et al. 2006		DQ832228			
	Sorghum sp.	Munkacsi et al. 2007			DQ327814	DQ352827	DQ352815
Spo ryleyi Vánky & R.G. Shivas	*Sarga leiocladum*	BRIP 51726			**HQ013003**		**HQ013074**
	Sarga timorense	BRIP 49713	**HQ013109**			**HQ013044**	**HQ013073**

Taxon	**Host**	**Source/Accession number**	ITS	LSU	COX3	EF1a	GAPDH
Spo sehimatis (M.S. Patil) Vánky	*Sehima nervosum*	BRIP 49671	**HQ013110**		**HQ013004**		
Spo setariae (McAlpine) Vánky & R.G. Shivas	*Setaria surgens*	BRIP 49636	**HQ013111**		**HQ013005**	**HQ013045**	
		BRIP 26910					**HQ013075**
Spo sorghi Ehrenb. ex Link	*Sorgum bicolor*	Roux et al. 1998	AF038828				
Spo sorghi Ehrenb. ex Link	*Sorgum bicolor*	Begerow et al. 1997		AF009872			
	Sorghum sp.	Munkacsi et al. 2007			DQ327815	DQ352828	DQ352816
Spo spinulosum S.H. He & L. Guo	*Capillipedium parviflorum*	Vanky and Lutz 2009	GU139172	GU139171			
Spo tenue (Syd. & P. Syd.) Vánky	*Bothriochloa decipiens*	BRIP 48629	**HQ013112**		**HQ013006**	**HQ013046**	**HQ013076**
Spo themedae-arguentis Vánky	*Themeda arguens*	Stoll et al. 2005	AY344991	AY740140			
Spo trachypogonicola Vánky & C. Vánky	*Trachypogon plumosus*	Stoll et al. 2005	AY344992	AY740141			
Spo trachypogonis-plumosi Vánky	*Trachypogon plumosus*	Stoll et al. 2005	AY740060	AY740113			
Spo trispicatae R.G. Shivas, Vánky & Athip.	*Eulalia trispicata*	BRIP 47730	**HQ13113**		**HQ013007**		
Spo tumefaciens (McAlpine) Vánky	*Chrysopogon aciculatus*	Stoll et al. 2005	AY344969	AY740128			
Spo vanderystii (Henn.) Langdon & Full.	*Hyparrhenia hirta*	Stoll et al. 2005	AY740058 (as *Spo puellare*)	AY740111 (as *Spo puellare*)			
Spo veracruzianum (Zundel & Dunlap) M. Piepenbr.	*Panicum viscidellum*	Stoll et al. 2005	AY344993	AY740114			
Spo vermiculum R.G. Shivas, McTaggart & Vánky	*Sarga plumosum*	BRIP 49748	**HQ013114**	**HQ013134**	**HQ013008**	**HQ013047**	**HQ013077**
Spo whiteochloae Vánky & McKenzie	*Whiteochloa semitonsa*	BRIP 51860	**HQ013115**		**HQ013009**	**HQ013048**	**HQ013078**
Spo wynaadense (Sundaram) Vánky & R.G. Shivas	*Sarga leiocladum*	BRIP 27640	**HQ013116**	**HQ013124**	**HQ013010**	**HQ013049**	**HQ013079**
Spo xerofasciculatum R.G. Shivas, McTaggart & Vánky	*Xerochloa laniflora*	BRIP 49682	**HQ013117**				
Ust affinis Ellis & Everh.	*Stenotaphrum secundatum*	Stoll et al. 2005	AY344995	AF133581			

Taxon	**Host**	**Source/Accession number**	ITS	LSU	COX3	EF1a	GAPDH
Ust altilis Syd.	*Triodia pungens*	Stoll et al. 2005	AY740166				
	Triodia sp.	BRIP 52543		**HQ013136**			
Ust austro-africana Vánky & C. Vánky	*Enneapogon cenchroides*	Stoll et al. 2005	AY740061	AY740115			
Ust avenae (Pers.) Rostr.	*Avena barbata*	Stoll et al. 2005	AY344997	AF453933			
Ust bouriqueti Maubl. & Roger	*Stenotaphrum dimidiatum*	Stoll et al. 2005	AY740167				
Ust bromivora (Tul. & C. Tul.)	*Bromus catharticus*	Stoll et al. 2005	AY740064	AY740118			
Ust bromivora (Tul. & C. Tul.)	*Bromus* sp.	BRIP 52238			**HQ013012**		
Ust bullata J. Schröt.	*Bromus diandrus*	Stoll et al. 2005	AY344998	AF453935			
Ust calamagrostidis (Fuckel) G.P. Clinton	*Calamagrostis epigeios*	Stoll et al. 2005	AY740065	AY740119			
Ust crameri Körn.	*Setaria italica*	Stoll et al. 2005	AY344999	AY740143			
Ust curta Syd.	*Tripogon loliiformis*	Stoll et al. 2005	AY740165 (as *Ust alcornii*)				
		BRIP 26929		**HQ013123**	**HQ013013**		**HQ013080**
Ust cynodontis (Pass.) Henn.	*Cynodon dactylon*	Stoll et al. 2005	AY345000	AF009881			
		BRIP 51207			**HQ013014**	**HQ013050**	**HQ013081**
Ust davisii Liro	*Glyceria multiflora*	Stoll et al. 2005	AY740169				
Ust drakensbergiana Vánky	*Digitaria tricholaenoides*	Stoll et al. 2005	AY740170				
Ust echinata J. Schröt.	*Phalaris arundinacea*	Stoll et al. 2005	AY345001	AY740144			
Ust esculenta Henn.	*Zizania latifolia*	Stoll et al. 2005	AY345002	AF453937			
Ust filiformis (Schrank) Rostr.	*Glyceria fluitans*	Stoll et al. 2005	AY740066	AY740120			
Ust hordei Bref.	*Hordeum vulgare*	Stoll et al. 2005	AY345003	AF453943			
	Hordeum sp.	Munkacsi et al. 2007			DQ327819	DQ352832	DQ352820
Ust inaltilis Vánky & A.A. Mitch.	*Triodia longiloba*	BRIP 49123	**HQ013118**		**HQ013015**		
Ust ixophori Durán	*Ixophorus unisetus*	Stoll et al. 2005	AY740067	AY740121			
Ust lituana R.G. Shivas, Vánky & Cunningt.	*Triodia epactia*	BRIP 46795	**HQ013119**		**HQ013016**		
Ust maydis (DC.) Corda	*Zea mays*	Stoll et al. 2003	AY345004				
		Piepenbring et al. 2002		AF453938			
		Munkacsi et al. 2007			DQ327817	DQ352830	DQ352818

Taxon	**Host**	**Source/Accession number**	ITS	LSU	COX3	EF1a	GAPDH
Ust nuda (C.N. Jensen) Rostr.	*Hordeum leporinum*	Stoll et al. 2005	AY740069	AJ236139			
	Hordeum sp.	BRIP 52237			**HQ013017**		
Ust pamirica Golovin	*Bromus gracillimus*	Stoll et al. 2005	AY345005	AY740145			
Ust phrygica Magnus		Berner et al. 2007	DQ139961				
Ust porosa Langdon	*Sarga timorense*	BRIP 51811			**HQ013018**		
		BRIP 51842	**HQ13120**	**HQ013128**	**HQ013019**	**HQ013051**	**HQ013082**
Ust schmidtiae Vánky	*Enneapogon polyphyllus*	BRIP 26906			**HQ013020**		
	Enneapogon sp.	BRIP 51848	**HQ013121**	**HQ013129**	**HQ013021**		**HQ013083**
Ust schroeteriana Henn.	*Paspalum paniculatum*	Stoll et al. 2005	AY345006	AY740146			
Ust scitaminea Syd.	*Saccharum* sp.	Stoll et al. 2005	AY740070 (as *Spo scitamineum*)	AY740147 (as *Spo scitamineum*)			
		Munkacsi et al. 2007			DQ327816 (as *Spo scitamineum*)	DQ352829 (as *Spo scitamineum*)	DQ352817 (as *Spo scitamineum*)
Ust sparsa Underw.	*Dactyloctenium radulans*	Stoll et al. 2003	AY345008				
		BRIP 51829			**HQ013022**		
Ust sporoboli-indici L. Ling	*Sporobolous pyramidalis*	Cunnington and Shivas 2006	AY772736				
Ust striiformis (Westend.) Niessl	*Alopecurus pratensis*	Stoll et al. 2005	AY740172				
		Begerow et al. 2006		DQ875375			
Ust syntherismae (Schwein.) Peck	*Digitaria ternata*	Stoll et al. 2005	AY740071	AY740123			
Ust tragana Zundel	*Tragus berteronianus*	Stoll et al. 2005	AY740072	AY740124			
Ust trichophora (Link) Kunze	*Echinochloa colona*	Stoll et al. 2005	AY345009	AY740148			
	Echinolchloa utilis	BRIP 51725			**HQ013023**		
		BRIP 49159				**HQ013052**	**HQ013084**
Ust triodiae Vánky	*Triodia microstachya*	Stoll et al. 2005	AY740074	AY740126			
		BRIP 26907			**HQ013024**		
		BRIP 49124				**HQ013053**	**HQ013085**
Ust tritici (Bjerk.) E. Rostrup	*Triticum aestivum*	Bakkeren et al. 2000	AF135424				
Ust turcomanica Tranzschel	*Eremopyrum distans*	Stoll et al. 2005	AY345011	AF453936			

Taxon	**Host**	**Source/Accession number**	ITS	LSU	COX3	EF1a	GAPDH
Ust vetiveriae Padwick	*Vetiveria zizanioides*	Stoll et al. 2005	AY345011	AY740149			
Ust xerochloae Vánky & R.G. Shivas	*Xerochloa imberbis*	Stoll et al. 2005	AY345012	AY740150			
	Xerochloa barbata	BRIP 51826			**HQ013025**		
		BRIP 49820				**HQ013054**	

Appendix 2. Character and character state selection for scoring of morphological characters.

Taxon	Position on host	Host Subfamily	Panicoideae	Chloridoideae	Appearance on Host	Peridium	Columella	Partitioning cells	Spore balls	Spore mean size
Anomalomyces panici	0	0	2	?	0	1	0	1	1	2
Mac arundinellae-setosae	0	7	?	?	1	1	0	1	0	1
Mac bursus	0	0	1	?	0	1	0	1	0	3
Mac eragrostiellae	0	1	?	1	0	1	0	1	0	3
Mac eriachnes	0	5	?	?	3	1	0	1	1	5
Mac ewartii	0	0	1	?	0	1	0	1	0	4
Mac loudetiae	0	7	?	?	?	?	0	1	0	2
Mac mackinlayi	0	0	1	?	1	1	0	1	0	3
Mac neglectus	0	0	2	?	3	1	1	1	0	3
Mac simplex	0	7	?	?	1	1	0	1	0	3
Mac spermophorus	0	1	?	1	0	1	0	1	0	3
Mac spinulosus	0	1	?	1	0	1	0	1	0	2
Mac trichopterygis	0	7	?	?	2	1	0	1	0	3
Mac tristachyae	0	7	?	?	2	1	0	1	0	1
Mac tubiformis	0	0	1	?	1	1	0	1	0	4
Mac viridans	0	1	?	1	0	1	0	1	0	3
Mel pennsylvanicum	0,1,2	?	?	?	0,4	1	0	0	0	3
Moe bullatus	0	0	2	?	0	1	0	1	1	3
Spo absconditum	0	0	1	?	2	2	2	0	1	4
Spo aegyptiacum	0	4	?	?	3	1	1	1	0	4
Spo andropogonis	0	0	1	?	2	1	1	1	2	2
Spo anthistiriae	0	0	1	?	1	2	2	2	1	4
Spo anthracoideisporum	0	0	1	?	1,3	2	2	1	2	6
Spo apludae-aristatae	0	0	1	?	1,3	2	2	2	1	3
Spo aristidicola	0	4	?	?	0	1	0	2	1	2
Spo arthraxonis	0	0	1	?	1,3	?	1,2	1	0	4
Spo bothriochloae	0	0	1	?	1	2	2	0	1	4
Spo caledonicum	0	0	1	?	2	2	2	0	2	3
Spo cenchri	0	0	2	?	2	2	2	1,2	2	3
Spo cenchri elymoidis	0	0	2	?	1	?	1	0	2	3
Spo chrysopogonis	0	0	1	?	2	2	2	2	1	4
Spo confusum	0	4	?	?	0	1	0	0	2	4
Spo consanguineum	0	4	?	?	0	1	0	0,2	2	2
Spo cruentum	0	0	1	?	3	1	1	1	0	2
Spo cryptum	0	0	2	?	3	1	1	0,1	2	3
Spo culmiperdum	0	0	1	?	2	1	1	0	0	6
Spo cymbopogonis-bombycini	0	0	1	?	1	2	2	2	2	4
Spo destruens	0	0	2	?	2	2	2	1	2	3
Spo dietelianum	0	?	?	?	2	?	2	1	0	3
Spo dimeriae-ornithopodae	0	0	1	?	2	2	2	1	0	4
Spo doidgeae	0	0	1	?	2	1	1	1	2	3
Spo elionuri	0	0	1	?	1,3	2	2	0	0	2
Spo enteromorphum	0	0	1	?	2	?	2	1	2	1
Spo erythraeense	0	0	1	?	2	1	1	1	0	3

Taxon	Position on host	Host Subfamily	Panicoideae	Chloridoideae	Appearance on Host	Peridium	Columella	Partitioning cells	Spore balls	Spore mean size
Spo everhartii	0	0	1	?	1	2	2	2	1	3
Spo exsertiformum	0	0	1	?	3	1	1	1	2	2
Spo exsertum	0	0	1	?	3	1	1	1	2	2
Spo fallax	0	0	1	?	2	2	2	2	2	2
Spo fastigiatum	0	0	1	?	3	1	1	1	0,2	3
Spo flagellatum	0	0	1	?	2	1	1	1	2	6
Spo formosanum	0	0	2	?	2	2	2	1	2	1
Spo foveolati	0	0	1	?	2	1	1	1	0	3
Spo fraserianum	0	4	?	?	0	1	0	0	2	1
Spo hainanae	0	0	1	?	3	1	1	1	0	2
Spo heteropogonicola	0	0	1	?	1	2	2	1,2	1	4
Spo holwayi	0	0	1	?	2	1	1	1	0	5
Spo hwangense	0	1	?	1	3	1	1	1	1	3
Spo ischaemicola	0	0	1	?	3	1	1	1	0	4
Spo iseilematis ciliati	0	0	1	?	3	1	1	1	2	3
Spo lacrymae-jobi	0	0	1	?	3	1	1	1	2	3
Spo lanigeri	0	0	1	?	3	1	1	1	1	2
Spo lepturi	0	1	?	4	2	1	1	1	0	2
Spo loudetiae pedicellatae	0	7	?	?	2	2	2	1	2	3
Spo magnusianum	0	0	2	?	3	1	1	1	2	3
Spo manilense	0	0	2	?	3	1	1	1	2	4
Spo mexicanum	0	0	1	?	2	2	2	1	0	3
Spo mishrae	0	0	1	?	1,2,3	1	1	0,2	1	3
Spo mitchellii	0	0	1	?	3	1	1	1	2	4
Spo modestum	0	1	?	3	3	1	1	1	0	4
Spo monakai	0	0	2	?	3	1	1	1	2	3
Spo moniliferum	0	0	1	?	3	1	1	1	2	3
Spo mutabile	0	0	1	?	1,2,3	2	2	?	2	6
Spo nealii	0	0	1	?	1	2	2	1	0	2
Spo nervosum	0	0	1	?	3	?	?	0	1	4
Spo occidentale	0	0	1	?	3	1	1	1	0	5
Spo ophiuri	0	0	1	?	2	1	1	1	0	4
Spo ovarium	0	0	2	?	3	?	1	1	2	3
Spo panici	0,1	0	2	?	2,4	1	1	0	0,2	4
Spo panici-leucophaei	0,2	0	2	?	2,4	1	1,2	0	2	1
Spo paspali	0	0	2	?	3	1	1	1	0	2
Spo penniseti	0	0	2	?	3	1	1	1,2	2	4
Spo polliniae	0	0	1	?	3	2	2	0	1,2	3
Spo provinciale	0	0	1	?	2	2	2	1	2	6
Spo pseudechinolaenae	0	0	2	?	2	1	1	1	2	4
Spo pulverulentum	0	0	1	?	3	1	1	1	2	4
Spo queenslandicum	0	0	1	?	2	1	1	1	2	2
Spo rarum	0	0	1	?	3	1	1	1	2	2
Spo reilianum	0,1,2	0	2	?	2,4	2	2	1	2	4
Spo ryleyi	0	0	1	?	3	1	1	1	0	1
Spo sehimatis	0	0	1	?	1	2	2	0	1	2

Taxon	Position on host	Host Subfamily	Panicoideae	Chloridoideae	Appearance on Host	Peridium	Columella	Partitioning cells	Spore balls	Spore mean size
Spo setariae	0	0	2	?	1,3	2	1,2	1	1	3
Spo sorghi	0	0	1	?	3	1	1	1	0	1
Spo tenue	0	0	1	?	2	1	1	1	2	2
Spo themedae-arguentis	0	0	1	?	1,2,3	2	2	0,2	1	5
Spo trachypogonicola	0	0	1	?	2	1	1	1	0	3
Spo trachypogonis	0	0	1	?	0,1	1	1	1	2	4
Spo trispicatae	0	0	1	?	1	2	2	2	2	4
Spo tumefaciens	0	0	1	?	2	2	2	1	2	2
Spo vanderystii	0	0	1	?	2	1	1	1	0	3
Spo veracruzianum	0	0	2	?	0	1	?	1	0	3
Spo vermiculum	0	0	1	?	2	1	1	1	0	0
Spo whiteochloae	0	0	2	?	3	1	1	0	1	3
Spo wynaadense	0	0	1	?	3	1	1	1	0	2
Spo xerofasciculatum	0	0	2	?	?	2	2	1	0,2	2
Ust affinis	0	0	2	?	2,3	1	0	1	0	2
Ust agropyri	0	2	?	?	2,3,4	0,1	0	0	0	4
Ust altilis	1	1	?	1	4	1	0	0	0	3
Ust austroafricana	0	1	?	3	0	1	0	0	0	2
Ust avenae	0	2	?	?	2,3	0	0	0	0	1
Ust bouriquetii	0	0	2	?	1	1	0	1	0	2
Ust bromivora	0	2	?	?	2,3	0	0	0	0	3
Ust bullata	0	2	?	?	2,4	0,1	0	0	0	2
Ust calamagrostidis	2	2	?	?	4	0	0	0	0	5
Ust comburens	0	2	?	?	2	0	0	0	0	0
Ust crameri	0	0	2	?	3	1	0	0	0	3
Ust curta	0	1	?	1	0	0,1	0	0	0	6
Ust cynodontis	0	1	?	2	2	0,1	0	0	0	1
Ust davisii	2	2	?	?	4	1	0	0	0	3
Ust drakensbergiana	0	0	2	?	2,3,4	1	0,2	0	0	1
Ust echinata	2	2	?	?	4	1	0	0	0	5
Ust egenula	0	1	?	1	0	1	0	1	0	4
Ust esculenta	1	3	?	?	4	0,1	0	0	0	3
Ust filiformis	2	2	?	?	4	1	0	0	0	1
Ust hordei	0	2	?	?	2,3	1	0	0	0	2
Ust inaltilis	1	1	?	1	4	1	0	0	0	2
Ust ixophori	0	0	2	?	0	1	0	0	0	3
Ust lituana	0	1	?	1	2	1	0	0	0	1
Ust maydis	0	0	1	?	0,4	1	0	1	0	3
Ust nuda	0	2	?	?	2,4	0	0	0	0	2
Ust pamirica	0	2	?	?	2,3,4	1	0	0	0	5
Ust phrygica	0	2	?	?	2,3	1	0	0	0	2
Ust porosa	0	0	1	?	2	0,1	0,1	0	0	1
Ust schmidtiae	0	1	?	3	0	1	0	0	0	3
Ust schroeteriana	0	0	2	?	2,3	0	0	0	0	6
Ust scitaminea	0	0	1	?	2	1	1	1	0	2
Ust sparsa	0	1	?	1	0	1	0	0	0	2
Ust spinificis	0	0	2	?	0,3	0,1	0	0	0	0

Taxon	Position on host	Host Subfamily	Panicoideae	Chloridoideae	Appearance on Host	Peridium	Columella	Partitioning cells	Spore balls	Spore mean size
Ust sporoboli indici	2	1	?	1	4	1	0	0	0	2
Ust striiformis	2	2	?	?	4	1	0	0	0	4
Ust syntherismae	0	0	2	?	2	0	0	0	0	4
Ust tragana	0	1	?	2	0	1	0	0	0	2
Ust trichophora	0	0	2	?	0	1	0	0	0	3
Ust triodiae	0	1	?	1	2	1	0	0	0	3
Ust tritici	0	2	?	?	2,4	0	0	0	0	2
Ust turcomanica	0	2	?	?	2,3	1	0	0	0	4
Ust vetiveriae	0	?	?	?	0	1	0	0	0	2
Ust xerochloae	0	0	2	?	0,4	1	0	0	0	3

Character states

1. *Position on host*: (0) Inflorescence (1) Culms/stems (2) Leaves
2a. *Host Subfamily*: (0) Panicoideae (1) Chloridoideae (2) Pooideae (3) Oryzoideae (4) Aristoideae (5) Eriachne (6) Danthonioideae (7) Arundineae
2b. *Panicoideae*: (0) Andropogoneae (1) Paniceae
2c. *Chloridoideae*: (0) Chlorideae (1) Eragrostideae (2) Cynodonteae (3) Pappophoreae (4) Lepturus
3. *Appearance on Host*: (0) Forming galls in hypertrophied host ovaries (1) Sorus localized in host inflorescence (2) Sorus destroying whole inflorescence (3) Sori destroying all ovaries in inflorescence (4) Galls in stems or culms
4. *Peridium*: (0) Absent (1) Derived from host material (2) Derived from fungal material
5. *Columella*: (0) Absent (1) Stout/woody (2) Filiform
6. *Partitioning cells*: (0) Absent (1) Present (2) Dimorphic spores
7. *Spore balls*: (0) Absent (1) Persistent (2) Ephemeral
8. *Spore mean size*: (0) Less than 5.0 microns (1) Between 5.5 and 7.0 microns (2) Between 7.5 and 9.0 microns (3) Between 9.5 and 11.0 microns (4) Between 11.5 and 13.0 microns (5) Between 13.5 and 15.0 microns (6) Between 15.5 and 20.0 microns (7) Greater than 20.0 microns

Appendix 3. *Tilletia challinorae*

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Appendix 4. Three new species of *Tilletia* on native grasses from northern Australia

Appendix 5. *Tilletia micrairae*

Appendix 6. First report of the smut fungus *Ustanciosporium appendiculatum* in Australia

Appendix 7. Additions to the smut fungi (Ustilaginomycetes) of Bolivia