Two new species of *Ustilaginomycetes* on *Chrysopogon fallax* from Australia

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Sporisorium fallax sp. nov. (*Ustilaginaceae*, *Ustilaginomycetes*) is described and illustrated from inflorescences of *Chrysopogon fallax* collected in the Northern Territory, Australia. Ribosomal DNA Internal Transcribed Spacer sequences confirmed *S. fallax* to be distinct from two morphologically similar species, *S. tumefaciens* and *S. tumiforme*, on *C. fallax*. *Macalpinomyces tubiformis* sp. nov. (*Ustilaginaceae*, *Ustilaginomycetes*) is described and illustrated from ovaries of *Chrysopogon fallax* collected in Queensland, Australia.

Key words: *Macalpinomyces tubiformis*, *Sporisorium fallax*, *Sporisorium tumefaciens*, *Sporisorium tumiforme*, taxonomy, *Ustilaginaceae*.

Introduction

Recent studies have lead to the discovery and classification of several new species of smut fungi in Australia (Shivas and Vánky, 2001, 2002, 2003a,b; Vánky and Shivas, 2001a,b). Vánky (2004) recognised and designed a key for eight known species (two in *Macalpinomyces* and six in *Sporisorium*) of smut fungi (*Ustilaginomycetes*) on *Chrysopogon* (tribe *Andropogoneae*, subfamily *Panicoideae*, *Poaceae*). Three of these species, *Sporisorium andropogonis-aciculati* (Petch) Vánky, *Sporisorium tumefaciens* (McAlpine) Vánky and *Sporisorium tumiforme* Vánky, occur in Australia. The latter two species produce elongated, cylindrical sori that destroy the entire inflorescence of their hosts. Examination of specimens of *Sporisorium* on *Chrysopogon* spp. with similar sori held in Herbarium BRIP revealed another species that could not be ascribed to any of the species known to occur on *Chrysopogon*.

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Ribosomal DNA Internal Transcribed Spacer (ITS) region sequences have been previously used in the taxonomy and classification of *Ustilaginomycetes* (Boyd and Carris, 1997; Boyd *et al*., 1998; Cunnington and Shivas, 2004). To confirm that this new species of *Sporisorium* was distinct from others occurring on *Chrysopogon fallax* in Australia, ITS sequences were obtained for this species and compared with those for *Sporisorium tumefaciens* and *S. tumiforme*. A new species of *Macalpinomyces* on *Chrysopogon fallax* S.T. Blake is also described and illustrated.

Materials and Methods

Pressed and dried specimens were used for studies of sorus structure and spore morphology. For light microscopy (LM) studies and spore measurements, dried spores were rehydrated in lactic acid by gently heating to boiling point. For scanning electron microscopy (SEM) studies, dried spores were dusted on double-sided adhesive tape, mounted on a specimen stub, sputter-coated with gold-palladium, *ca*. 20 nm, and examined in a SEM at 10 kV.

Six specimens of *Sporisorium*, all from *Chrysopogon fallax*, were used for molecular analysis (Table 1). DNA was extracted by grinding a small amount of spores (1 mm^3) in 50 µL of 5% Chelex-100 (Biorad). The material was spun down briefly in a microcentrifuge.

The initial PCR was performed in 25 µL containing 1 µL DNA extract, 200 µM of each dNTP, 1.5 mM $MgCl_2$, 2.5 µL 10× buffer, 4 ng each of primers ITSF1 (Gardes and Bruns, 1993) and ITSUR (TGTTCGCTATCGGTCTCTCC) (Cunnington and Shivas, 2004), and 0.5 units of Hotstar Taq (Qiagen). Reaction cycles were 15 minutes at 95°C, 35 cycles of: 30 second at 94°C, 30 seconds at 50°C, 1 minute at 72°C. A nested PCR was performed in 25 µL as outlined above, but using primers ITS5 (White *et al*., 1990) and ITSUR using 1 µL of the first round PCR product as template. PCR products were detected by running 4 µL on a 1.4% agarose gel in TBE buffer. Nested PCR products were purified using a QIAquick PCR Purification Kit (Qiagen) and sequenced directly using primers ITS5 (White *et al*., 1990) and ITS4, with an ABI PRISM® BIGDYE™ Terminator Cycle Sequencing Kit (Perkin-Elmer) according to the manufacturers instructions.

These sequences were aligned using ClustalX (Thompson *et al*., 1997) and the ITS sequence for *Sporisorium reilianum* (GenBank accession AF135432) was included as an outgroup. A neighbour-joining tree was created using the Kimura-2-paramater method and a complete deletion of gaps using MEGA (Kumar *et al*., 2001). 1000 bootstrap replicates were performed.

Table 1. *Sporisorium* specimens with collection details including GenBank accession numbers for ITS sequences used in this study.

Species	Herbarium accession	Location in Northern Territory, Australia	Date of collection	GenBank accession
S. fallax	BRIP 27687	268 km SE Katherine	15 Mar 2000	AY333940
S. fallax	BRIP 27690	350 km N Devil's Marbles	15 Mar 2000	AY333941
S. fallax	BRIP 27031	Todd's Monument	16 Mar 2000	AY333942
S. tumefaciens	BRIP 27688	Helen Springs	16 Mar 2000	AY333943
S. tumefaciens	BRIP 27689	Stuart Highway between Tennant Creek and Katherine	15 Mar 2000	AY333944
S. tumiforme	BRIP 26919	Newcastle Creek	16 Mar 2000	AY333945

Taxonomy

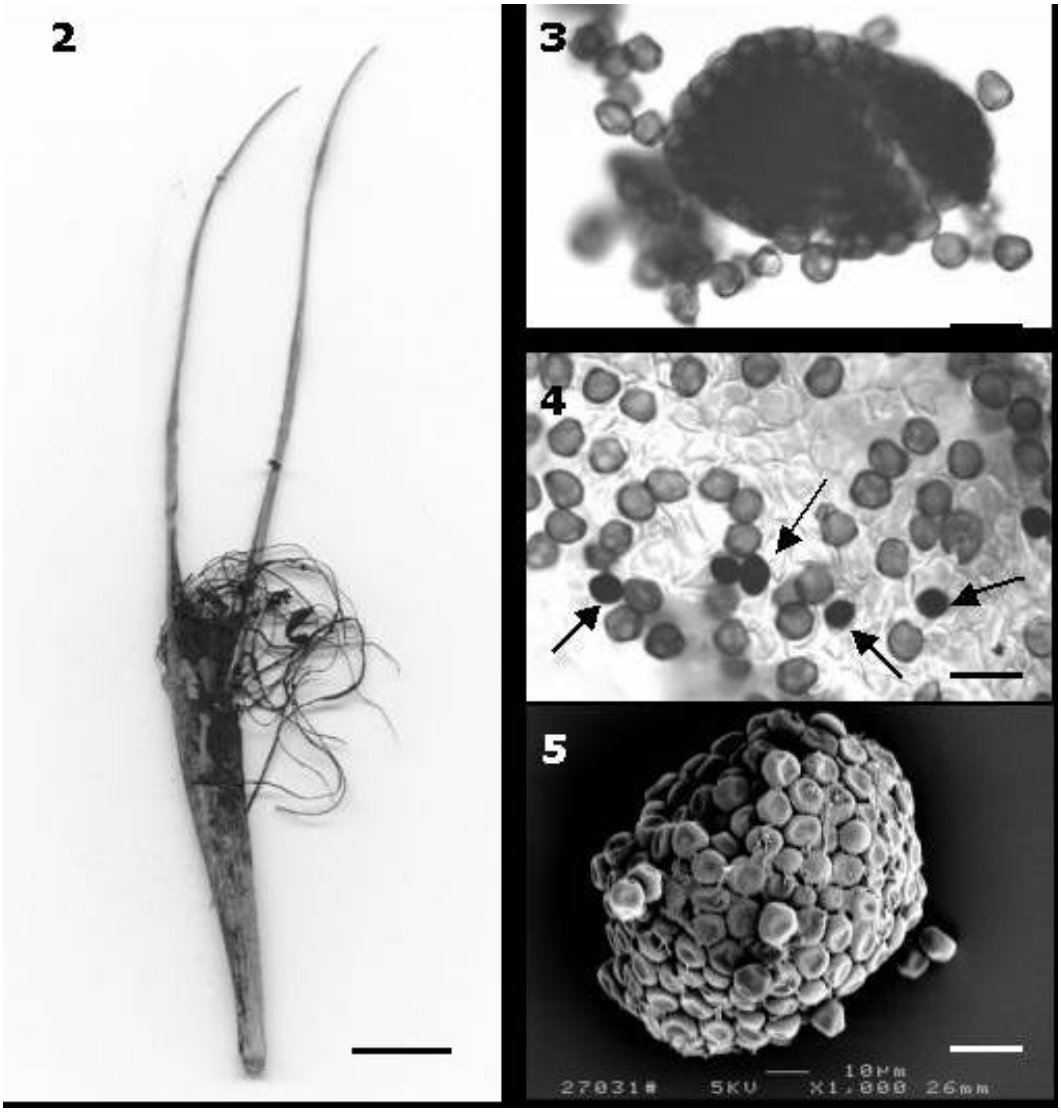
Sporisorium fallax R.G. Shivas & J.H. Cunnington, **sp. nov.** (Figs. 2-7)

Etymology: from Latin *fallax* (deceptive). Refers to its close resemblance to another species on the same host, as well as to the host plant species.

Typus in matrice *Chrysopogon fallax* S.T. Blake, Australia, Northern Territory, 268 km SE urbe Katherine, 16°38'29" S, 133°22'45" E, alt. 250 m.s.m., 15.III.2000, leg. R.G. Shivas, I.T. Riley, C. Vánky et K. Vánky [**Holotypus** in BRIP 27687; **isotypi** in HUV 18119 et VPRI 31661; **paratypi** in matrice *C. fallax*, Stuart Highway, prope "Todd's Monument", 16°55'23" S, 133°25'22" E, 16.III.2000, leg. R.G. Shivas, I.T. Riley, C. Vánky et K. Vánky, BRIP 27031, HUV 20374 et VPRI 31526; 349 km N urbe Alice Springs, prope "Devil's Marbles", 20°33'59" S, 134°15'34" E, alt. 460 m.s.m., 15.III.2000, leg. R.G. Shivas, I.T. Riley, C. Vánky et K. Vánky, BRIP 27690, HUV 20375; in matrice *C. latifolius* S.T. Blake, Northern Territory, Litchfield National Park, prope Lake Rum Jungle, 13°01'29" S, 130°59'04" E, alt. 140 m.s.m., 13.III.2000, leg. R.G. Shivas, I.T. Riley, C. Vánky et K. Vánky, BRIP 27685, HUV 20376].

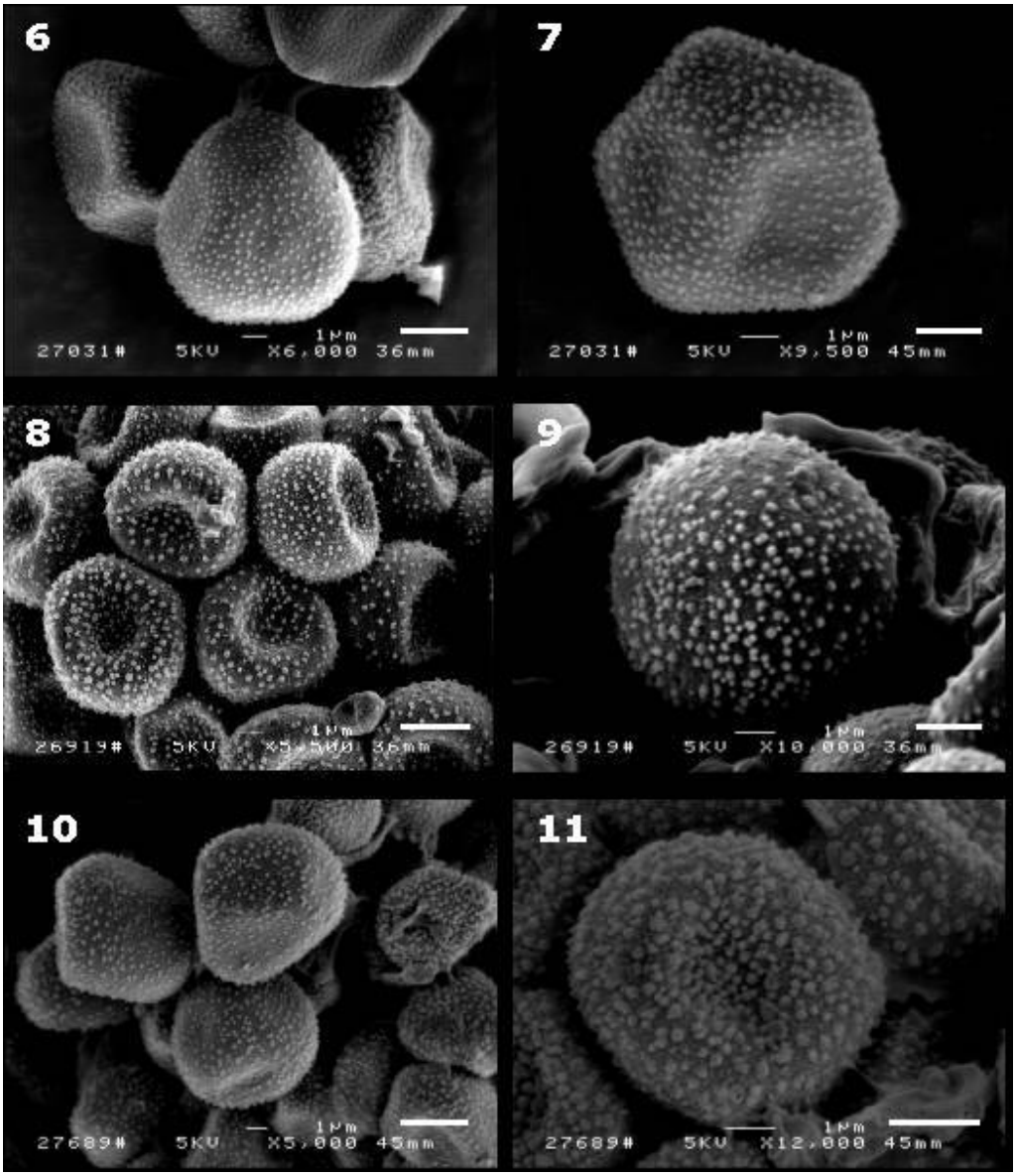
Sori flores recentes omnes destruentes, vagina foliorum primo celati, elongati cylindrati, 50 × 5 mm, peridio denso, cinerascenti tecti, postea protrudenti. Peridium, ubi maturavit, paulatim decidit, patefaciens massam laxarum sporarum glomerum atram, pulveream et columellas plurimas, longas, simplices, flagelliformes. *Glomera sporarum* globosa, subglobosa vel ovata, atrobrunnea, 30-65 × 30-85 µm diam., semi-permanentia, secedentia in doe genera *sporarum* (exteriores et interiores). Exteriores sporae globosae, subglobosae usque ad subpolyedriciter inaequales, atrorubrobrunneae, 6-8 × 6-10 µm, leves usque ad dense verruculosae, pariete 1-3 µm crasso. Interiores sporae globosae, subglobosae, usque ad subpolyedriciter inaequales, saepe angulares, pallide flavidobrunneae usque mediocriter rubrobrunneae, 7-10 × 8-12 µm, dense punctatae-verruculosae, paries 0,5-1,0 µm. *Cellulae steriles* non conspectae.

Sori (Fig. 2) destroying the entire inflorescence, at first concealed by the leaf sheath, elongated cylindrical, 50 × 5 mm, covered by a thick, greyish peridium, later protruding, at maturity the perdium flakes away exposing the black, powdery mass of loose spore balls and several, long, simple, flagelliform columella. *Spore balls* (Figs. 3, 5) globose, subglobose to ovoid, dark brown, 30-65 × 30-85 µm diam., semi-permanent, separating into two



Figs. 2-5. *Sporisorium fallax* (from BRIP 27031, holotype). **2.** Sorus in an infected inflorescence of *Chrysopogon fallax* showing flagelliform columellae. **3.** Spore ball and loose spores. **4.** Spores in LM (some dark outer spores arrowed). **5.** Spore ball in SEM. Bars: 2 = 1 cm; 3-5 = 20 µm.

types of *spores* (outer and inner) (Figs. 4, 6-7). Outer spores globose, subglobose to subpolyhedrally irregular, dark reddish-brown, 6-8 × 6-10 µm, smooth to densely verruculose, wall 1-3 µm thick. Inner spores globose, subglobose to subpolyhedrally irregular, often angular, pale yellowish-brown to medium reddish-brown, 7-10 × 8-12 µm, densely punctate-verruculose, wall 0.5-1.0 µm. *Sterile cells* not seen.



Figs. 6-11. Spores. **6-7.** *Sporisorium fallax* (from BRIP 27031, holotype). **8-9.** *Sporisorium tumiforme* (from BRIP 26919). **10-11.** *Sporisorium tumefaciens* (from BRIP 27689). Bars: 6, 8, 10 = 3 μm; 7, 9, 11 = 2 μm.

On *Poaceae*: *Chrysopogon fallax* S.T. Blake, *C. latifolius* S.T. Blake, Australia.

Sporisorium fallax is macroscopically similar to *S. tumefaciens* and *S. tumiforme*, in that each of these smut fungi produces long (5 cm), cylindrical sori that are partly hidden by the uppermost leaf sheath. Furthermore, the sori of these three species destroy the entire inflorescence and have thick peridia

and numerous filiform columellae. Microscopically the three species can be separated on the morphology of spores and spore balls. *Sporisorium fallax* has rather permanent spore balls and two types (dimorphic) of spores that are often angular. *Sporisorium tumefaciens* (Figs. 10-11) and *S. tumiforme* (Figs. 8-9) have spore balls that easily separate by pressure and spores of one type that are mostly rounded rather than angular. Vánky (2004) discusses the differences between *S. tumefaciens* and *S. tumiforme.*

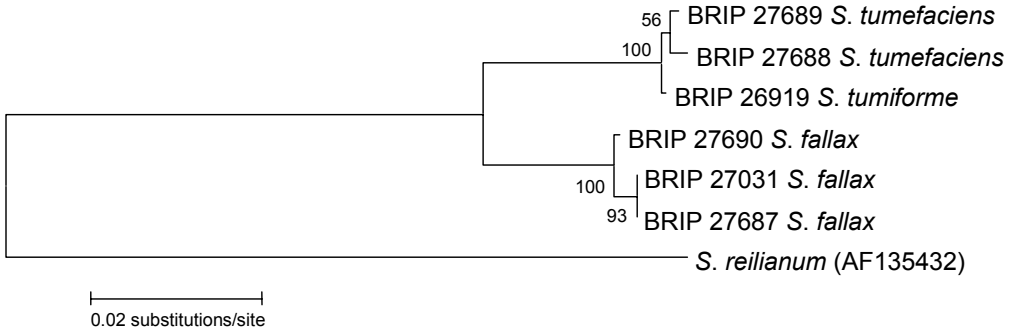


Fig. 1. Phylogenetic relationships of *Sporisorium fallax, S. tumefaciens* and *S. tumiforme* based on ITS sequences.

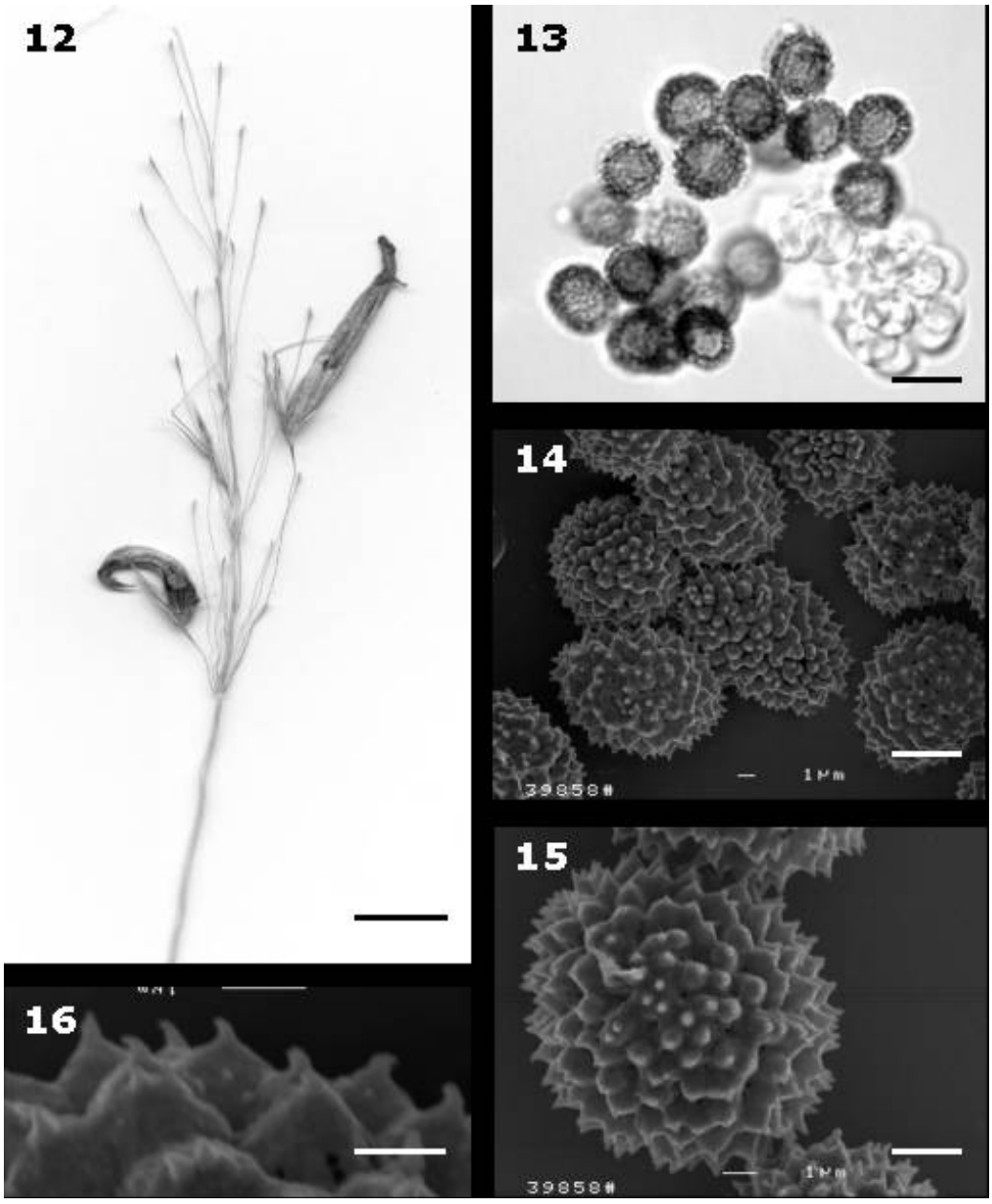
The neighbour-joining tree based on ITS sequences (Fig. 1) clearly showed *S. fallax* to be distinct from both *S. tumiforme* and *S. tumefaciens*. The latter two species were found to have very similar ITS sequences. Although only three specimens were examined, the sequence differences between *S. tumiforme* and *S. tumefaciens* were comparable to the difference found between different specimens of *S. fallax*. This agrees with the large number of morphological similarities shared by the two species and indicates that they have only recently diverged from a common ancestor.

Macalpinomyces tubiformis R.G. Shivas & Vánky, **sp. nov.** (Figs. 12-17)

Etymology: from Latin *tubus* (pipe) and *-formis* (forming). Refers to the shape of the sorus.

Typus in matrice *Chrysopogon fallax* S.T. Blake, Australia, Queensland, cca. 20 km N oppid. Gingin, 24°54'13" S, 151°54'12" E, alt. cca. 108 m.s.m., 25.IV.2003, leg. M.D.E. Shivas & R.G. Shivas [**Holotypus** in BRIP 39858; **isotypus** in HUV 20303].

Sori in spiculis et sessilibus et pedicellati nonnullis inflorescentiae eiusdem, organa floralia intima destruentes, longe tubiformes, saepe inclinati vel torti, 2-3 × 25-50 mm, peridio cinereo origine plantae nutrientis et fungali cooperti, quo longitudinaliter rupto massam atrobrunneam, pulveream sporarum, catervis cellularum sterilium intermixtam ostendentes. *Sporae* globosae, subglobosae, plerumque ellipsoidales, 8-11 × 9-13,5 µm, olivaceobrunneae; pariete aequali, simul cum verrucis dense dispositis, acutis, pyramidalibus inclusis 1,5-2,5 µm ceasso. *Cellulae steriles* in catervis irregularibus, cellulae singulae globosae, subglobosae, ellipsoidales, 5-8(-10) × 5-9,5(-12) µm, hyalinae; pariete tenui, cca. 0,5 µm, levi.



Figs. 12-16. *Macalpinomyces tubiformis* (from BRIP 39858, holotype). **12.** Sori in ovaries of *Chrysopogon fallax*. **13.** Spores and sterile cells in LM. **14-15.** Spores in SEM. **16.** Surface warts on spore. Bars: 12 = 1 cm; 13 = 10 µm; 14 = 5 µm; 15 = 2 µm; 16 = 1 µm.

Fig. 17. *Macalpinomyces tubiformis* (from BRIP 39858, holotype). Sori in some sessile and pedicelled spikelets of an inflorescence of *Chrysopogon fallax* (left). A triplet of spikelets with a sorus protruding from the sessile, hermaphrodite spikelet and a sorus from one of the pedicelled, male spikelets (right). Bars: (left) = 1 cm; (right) = 2.5 mm.

Sori (Figs. 12, 17) in some sessile and pedicelled spikelets of an inflorescence, destroying the innermost floral organs, long tubiform, often bent or twisted, 2-3 × 25-50 mm, covered by a grey peridium of host and fungal origin which ruptures longitudinally disclosing the dark brown, powdery mass of spores intermixed with groups of sterile cells. *Spores* (Figs. 13-15) globose, subglobose, usually ellipsoidal, 8-11 × 9-13.5 µm, olivaceous-brown; wall even, 1.5-2.5 µm thick including the densely situated, acute, pyramidal warts (Fig. 16). Sterile cells (Fig. 13) in irregular groups, single cells globose, subglobose, ellipsoidal, 5-8(-10) × 5-9.5(-12) µm, hyaline; wall thin, *ca*. 0.5 µm, smooth.

On *Poaceae*: *Chrysopogon fallax* S.T. Blake, Australia. Known only from the type collection.

Macalpinomyces tubiformis has spores ornamented with acute, pyramidal warts and is thereby distinct from all known smut fungi on *Chrysopogon*. SEM showed that the acute tips of the spore ornamentations were hooked (Fig. 16).

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