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FULVISPORIUM, A NEW GENUS OF USTILAGINALES *

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ABSTRACT

A new, monotypic genus, *Fulvisporium* K. Vánky is described, based on *Tolyposporium restifaciens* D. E. Shaw (type on *Stipa aristiglumis* F. Mueller, Australia). Its sorus- and spore morphology, ultrastructure of the septal pore and host-parasite relationship, as well as molecular biological characters are described. *Fulvisporium* is compared with *Tolyposporium*, *Sorosporium*, *Thecaphora*, *Aurantiosporium* and *Tolyposporella*.

Key words: Ustilaginales, *Fulvisporium*, *Fulvisporium restifaciens*, morphology, ultrastructure, molecular phylogeny, rDNA.

INTRODUCTION

A peculiar smut fungus, "ropy smut", was described from Australia, on *Stipa aristiglumis* F. Mueller, under the name of *Tolyposporium restifaciens* D. E. Shaw (1952:145), producing light coloured, permanent spore balls in the culms of the host plants. Mature sori resemble twisted, teased-out ropes. Discussing the generic affinity of this smut fungus, Shaw (1952:144) compared it with *Tolyposporium* and *Thecaphora* and remarked that "The organism does not conform entirely to either genus". Indeed, most of the characters of this fungus do not fit either with those of *Tolyposporium* or with any other known genus (comp. Vánky, 1987). The ultrastructural and molecular biological results rather show a relationship to the *Microbotryum* group than to the *Tolyposporium* or *Thecaphora* species.

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MATERIALS AND METHODS

Smatted plants of *Stipa stiposa* Hughes were collected in Australia (Tasmania, Hobart, Queen's Domain, 5.III.1996, C. & K. Vánky), pressed and dried. Material used for studies of the sorus structure, spore morphology, spore germination and molecular biological analysis was taken from these specimens, preserved in the senior author's herbarium (Herb. Ustil. Vánky, HUV 17637) and in the Tasmanian Herbarium, Hobart (HO). Further specimens, used for molecular analysis are: *Aurantiosporium subnitens* (Schröter & P. Henn.) Piep., K. Vánky & Oberw. on *Scleria pratensis* Lindley ex Nees (Herb. M. Piepenbring 1173, = HUV 17125), *Thecaphora seminis-convolvuli* (Desm.) Ito on *Convolvulus arvensis* L. (Herb. A. Nagler 775), *Tolyposporella brunckii* (Ell. & Gall.) G. P. Clinton on *Andropogon saccharoides* Swartz (HUV 17816), and *Tolyposporium junci* (Schröter) Wor. on *Juncus bufonius* L. (HUV 17169).

For light microscopical (LM) studies, dried spores were rehydrated in lactophenol by gently heating to the boiling point. For scanning electron microscopical (SEM) studies, dried spores were dusted on double-sided adhesive tape, mounted on specimen stub, sputter-coated with gold-palladium, ca. 20 nm, and examined in a SEM at 10 kV.

For the study of germination, spores were spread thinly on water agar (WA) and on malt yeast peptone agar (MYP), in Petri dishes, kept at room temperature (ca. 22 °C). As soon as basidiospores were produced, a suitable piece of medium (about 10 mm square) was cut out, transferred to a slide, and covered with a coverglass. A small droplet of lactophenol with cotton blue, added to the side of the square of medium, fixed and stained the basidia and basidiospores.

For studies of the soral and ultrastructural characters, fresh material was fixed with 2% glutaraldehyde in 0.1 M Na-cacodylate buffer at pH 7.2 for several days. After six transfers in 0.1 M Na-cacodylate buffer, the material was postfixed in 1% osmiumtetroxide in the same buffer for 1 h in the dark, washed in distilled water, and stained in 1% aqueous uranyl acetate for 1 h in the dark. After five washes in distilled water, the material was dehydrated in acetone series, embedded in Spurr's plastic and sectioned with a diamond knife. Semi-thin sections were stained with new fuchsin and crystal violet, mounted in "Entellan" and studied in a light microscope (LM). Ultra-thin sections were mounted on copper-slot-grids, post-stained with lead citrate for 5 minutes, and examined in a transmission electron microscope (TEM) at 80 kV.

For molecular biological studies, DNA was isolated from herbarium material or from culture obtained from germinated spores, using the SDS method of Edwards et al. (1991) modified by Henrion et al. (1992). The 5' region of the nuclear large subunit ribosomal RNA gene was amplified using the polymerase chain reaction and the primers NL1 and NL4 (Boekhout et al., 1995). The PCR product was purified using the QIAquick™ protocol (QIAGEN). This dsDNA was sequenced directly using the ABI PRISM™ Dye-Termination Cycle Sequencing Kit (Perkin Elmer) on an automated sequencer (ABI 373, Perkin Elmer). An alignment of 551 bp was obtained using ClustalW program (Thompson et al., 1994). The sequence data were analysed by PHYLIP, version 3.5c (Felsenstein, 1993).

RESULTS

The study of the sori, spore balls, spores and spore germination of *Tolyposporium restifaciens*, complemented by ultrastructural and molecular biological characters, revealed that this smut fungus cannot be placed in any earlier known genus. Therefore a new genus is proposed for it:

Fulvisporium K. Vánky, **gen. nov.**

Sori in telis vegetativis Graminearum glomerulos intercellulares sporarum formantes. Peridium nullum, columella nulla, greges sporarum sterilium inter glomerulos sporarum nulli. Glomeruli sporarum permanentes, tenuiter colorati, e sporis tantum compositi. Germinatio sporarum secundum typum *Ustilago*.

Sori in vegetative tissues of Gramineae in which intercellularly spore balls are produced. No peridium, no columella, no groups of sterile cells between the spore balls. Spore balls permanent, poorly pigmented, composed only of spores. Spore germination of *Ustilago*-type. Typus generis:

Fulvisporium restifaciens (D. E. Shaw) K. Vánky, **comb. nov.**

Basionym: *Tolyposporium restifaciens* D. E. Shaw, Proc. Linn. Soc. New South Wales 77:145, 1952. – Type on *Stipa aristiglumis* F. Mueller, Australia, New South Wales, Breeza Plains, 10.XII.1951, G. Dickson. Holotype in SYD (693), isotype in IMI (47686).

Sori (Figs. 1, 2) in the stems, in the distal internode(s), when mature resembling twisted, teased-out ropes, 0.5–1 cm wide, 20–50(–90) cm long, consisting of vascular bundles, whereas the intervascular parenchymatous tissue is destroyed and replaced by a yellowish-brown, when older light cinnamon-brown, granular powdery mass of spore balls. Spore balls (Figs. 3, 4, 6) subglobose, broadly ellipsoidal, ovoid, often elongated or slightly irregular, (10–)13–32 x (12–)16–40(–55) μm , golden-yellow to pale yellowish-brown, translucent, composed of (1–)5–25 (or more?) firmly united spores. Spores (Figs. 3, 4, 6) variable in shape and size, rounded with flattened contact sides, from ovoid, ellipsoidal, elongated, hemiglobose to subpolyhedrally irregular, 6–12(–13) x 8–14(–16) μm , pale golden-yellow; wall smooth, 1–2(–3) μm thick, thinner on the contact sides, thicker at the angles. In the centre of the free surface the exospore is thin forming a ca. 2 μm wide, round germ pore which in rehydrated spores slightly protrude forming a small papilla (Fig. 3, arrows), in dehydrated spores is depressed (Fig. 6, arrow). In young stages, sporogenous hyphae are growing intercellularly in the parenchyma tissue of the stem. Later, between the parenchyma cells, thin-walled, hyaline, spore ball initials are formed which increase in size, become thick-walled and slightly pigmented (Figs. 2, 5). At maturity, the epidermis ruptures releasing the spore masses. The remaining, naked vascular bundles give to the old sori the appearance of a teased-out rope. Spore germination (Fig. 8; of 6 weeks old spores, on water agar (WA) or on malt yeast peptone agar (MYP), at room temperature, after 1 day) results in 4-celled basidia measuring 2–3(–4) x 15–25(–28) μm . On the basidia, laterally and terminally, ovoid or long-ellipsoidal basidiospores of 1.5–2 x 4–8 μm are produced directly, without sterigmata. The septa separating two fungal cells (Fig. 7) show typical basidiomycetous substructure: two outer electron-opaque



Fig. 1. Sori of *Fulvisporium restifaciens* (D. E. Shaw) K. Vánky on the culms of *Stipa stiposa* Hughes (Vánky, Ust. exs. No. 414). Bar = 1 cm

layers are separated by a thin electron-transparent middle lamella (Fig. 7, arrow). The septal wall tapers towards the pore lip, without flaring. The septal pore is simple (Fig. 7, arrow head). The pore canal is small, occasionally occluded by electron-opaque material.

On Gramineae: *Stipa aristiglumis* F. Mueller, *S. blackii* C. E. Hubb., *S. stuposa* Hughes, and *Stipa* sp., Australia [N.S.W., Tas.].

DISCUSSIONS

Tolyposporium species [type *Tol. junci* (Schröter) Woronin ex Schröter on *Juncus bufonius* L.] are parasitising members of the Cyperaceae and Juncaceae families. The spore balls are produced on the surface of the host tissue, most commonly on the internal floral organs (gynoeceum, filaments), rarely on the floral envelopes and pedicels. The parasitic hyphae are intracellular. The spore balls are differentiating within a mass of sporogenous hyphae, closest to the host tissues and the ripening is centrifugal. The spore mass is black, the spore balls are blackish-brown to opaque.

In contrast, *Fulvisporium restifaciens* is parasitising members of the Gramineae family. The spore balls are produced within the culm tissues and are liberated after the covering host tissues are ruptured. The parasitic hyphae are intercellular. There is no mass of sporogenous hyphae in which the spore balls are differentiating. The spore mass is yellowish-brown, the spore balls are golden-yellow to pale yellowish-brown, translucent. The phragmobasidial type of spore germination has *Fulvisporium* in common with *Tolyposporium* species.

Species of *Sorosporium* [type: *S. saponariae* Rudolphi] and *Thecaphora* [type: *Thec. hyalina* Fingerh. = *Thec. seminis-convolvuli* (Desm.) Ito] are parasitising members of a great number of dicots families. They are producing more or less permanent, pigmented spore balls in various organs of the host plants. The parasitic hyphae are intracellular. The spore balls are differentiating from grouped, coiled sporogenous hyphae. The spore mass is light yellowish- to dark reddish-brown. The type of germination (where it is known) is different from that of *Fulvisporium restifaciens*, and usually results in production of one to several, ovoid or long ellipsoidal, aerial "conidia" on the top of shorter or longer hyphae or hyphal branches (comp. Ingold, 1987:473 + Fig. 2; Nagler, 1986:56-60 + Figs 26-27).

Aurantiosporium [type: *A. subnitens* (Schröter & Hennings) Piepenbring, K. Vánky & Oberwinkler], on a member of the Cyperaceae family (*Scleria melaleuca* Reichb. ex Schldl. & Cham.), is producing parenchymatous galls in some of both male and female spikelets of an inflorescence. The sori are filled with orange to cinnamon coloured spore masses composed of light- to orange-yellow spores, single or aggregated into irregular groups of easily separating "pseudo spore balls" held together by a thin, gelatinous spore sheath. The parasitic hyphae are intercellular. The septal pore is simple. Spore germination results in phragmobasidia. These characters bring *Aurantiosporium* and *Fulvisporium* close to each other. The main differentiating character between these two genera is the presence of firm, permanent spore balls in *Fulvisporium* in contrast to single spores or spores adhering in loose groups in *Aurantiosporium*. The host plants also belong to different families.

Tolyposporella is an insufficiently known, small, apparently heterogeneous genus. Its type [*T. chrysopogonis* Atkinson on *Sorghastrum nutans* (L.) Nash., Gramineae] is characterised by sori on the inner surface of the leaf sheaths forming more or less confluent, at first subepidermal, later bursting striae filled with a black, granular-agglutinated mass of spore balls composed of a great number of apparently loose but firmly agglutinated spores (illustrated by Vánky, 1987:115). The exospore is irregularly thickened (2.5–8, rarely up to 14 μm), laminated, smooth. Spore germination (Atkinson, 1897:16) "by a delicate promycelium which becomes branched, septate. Sporidia borne laterally, single, subclavate or fusoid, 2–2.5 x 9–12 μm ". *Tolyposporella chrysopogonis* has intercellular hyphae and no simple septal pore. The pigmented spores with a peculiar morphology (due to the irregularly thickened exospore), the type of spore germination (illustrated by Thirumalachar, Whitehead & O'Brien, 1967:392), the lack of simple septal pore differentiate *Tolyposporella* from *Fulvisporium restifaciens*.

Among the smuts, *Fulvisporium restifaciens* shares the combination of presence of phragmobasidia and absence of intracellular hyphae only with *Microbotryum* (sensu Bauer & Oberwinkler), *Liroa*, *Sphacelotheca*, *Ustilentyloma* and *Aurantiosporium*. As in *F. restifaciens*, the septa in *Ustilentyloma* and *Aurantiosporium* possess simple pores, whereas they are poreless in *Microbotryum*, *Liroa* and *Sphacelotheca*. Thus, the ultrastructural characteristics indicate that *Fulvisporium restifaciens* is closely related to *Ustilentyloma* and *Aurantiosporium*. Of these genera *Ustilentyloma* has single spores, *Aurantiosporium* also single spores but often aggregated into irregular, easily separating groups (pseudo spore balls), whereas *Fulvisporium* has firmly united, permanent spore balls.

Figs. 2–7. *Fulvisporium restifaciens* (D. E. Shaw) K. Vánky on *Stipa stiposa* Hughes (HUV 17637).

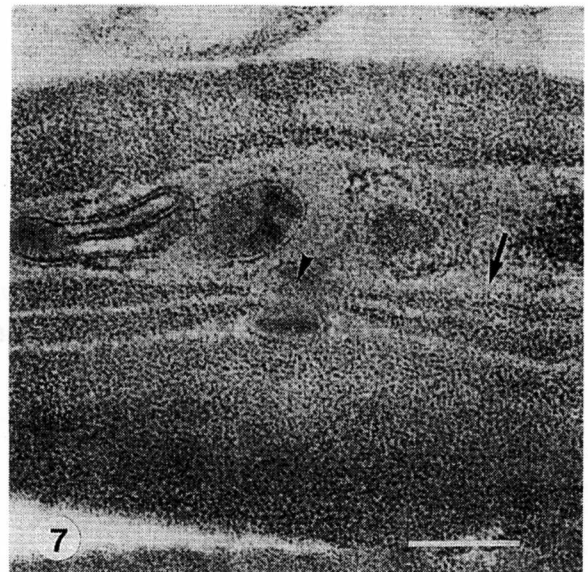
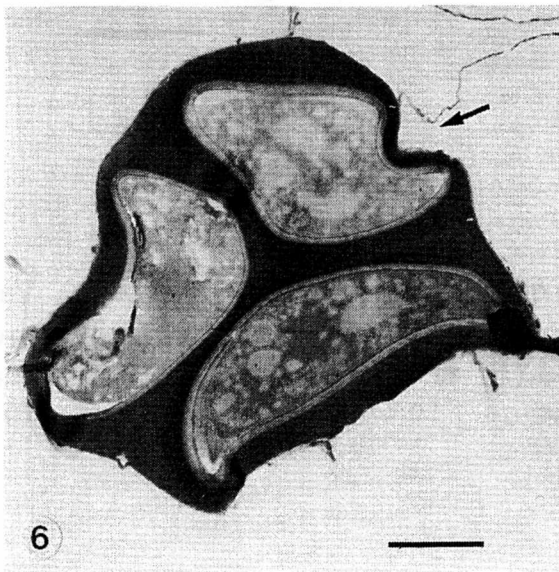
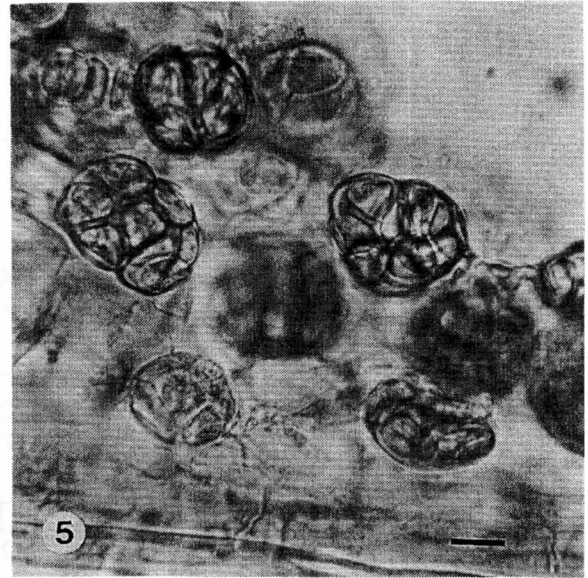
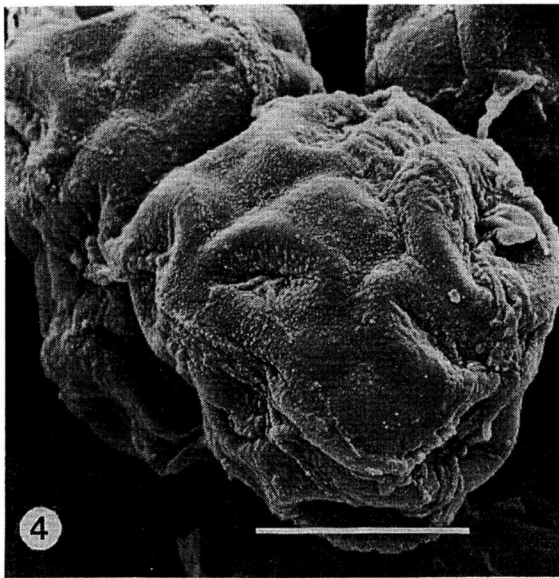
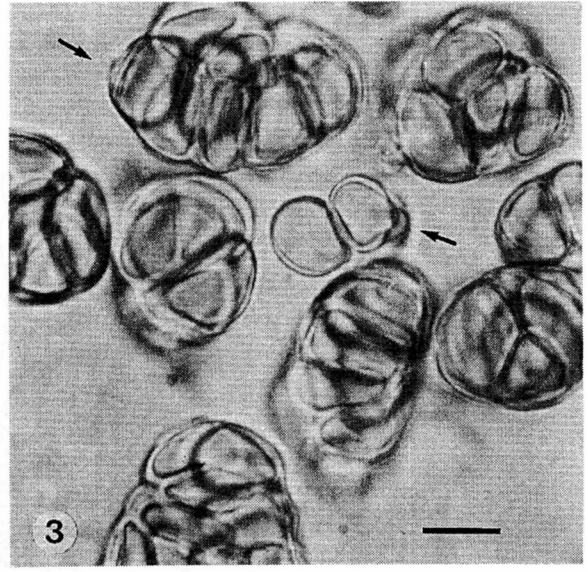
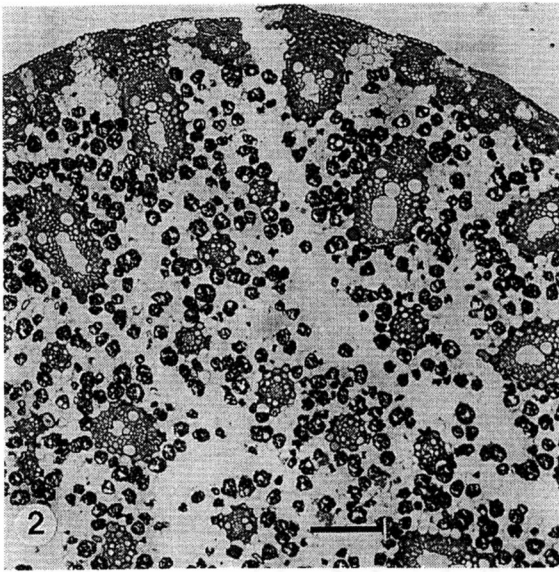
Fig. 2. TS of a young sorus, still protected by the epidermis and subepidermal host tissues. Between the intact vascular bundles the parenchyma tissue is more or less destroyed and replaced by spore balls. Bar = 100 μm

Figs. 3, 4. Spore balls in LM and in SEM. The germ pores, possessing thinner wall, are slightly protruding in the rehydrated spores (Fig. 3, arrows), but depressed in dried spores (Fig. 6, arrow). Bars = 10 μm

Fig. 5. Squashed young sorus with spore balls in various developmental stages (in lactophenol with cotton blue). Bar = 10 μm

Fig. 6. TS of a spore ball between empty cells of the host plant, seen in TEM. The spores have a thin endospore and an irregularly thick, electron-dense exospore. Bar = 2 μm

Fig. 7. Septal wall and porus in TEM. The wall (arrow) has a thin, electron-transparent middle lamella and two outer, electron-opaque layers. The porus (arrow head) is simple, the pore canal is small, occluded by electron-opaque material. Bar = 0.1 μm



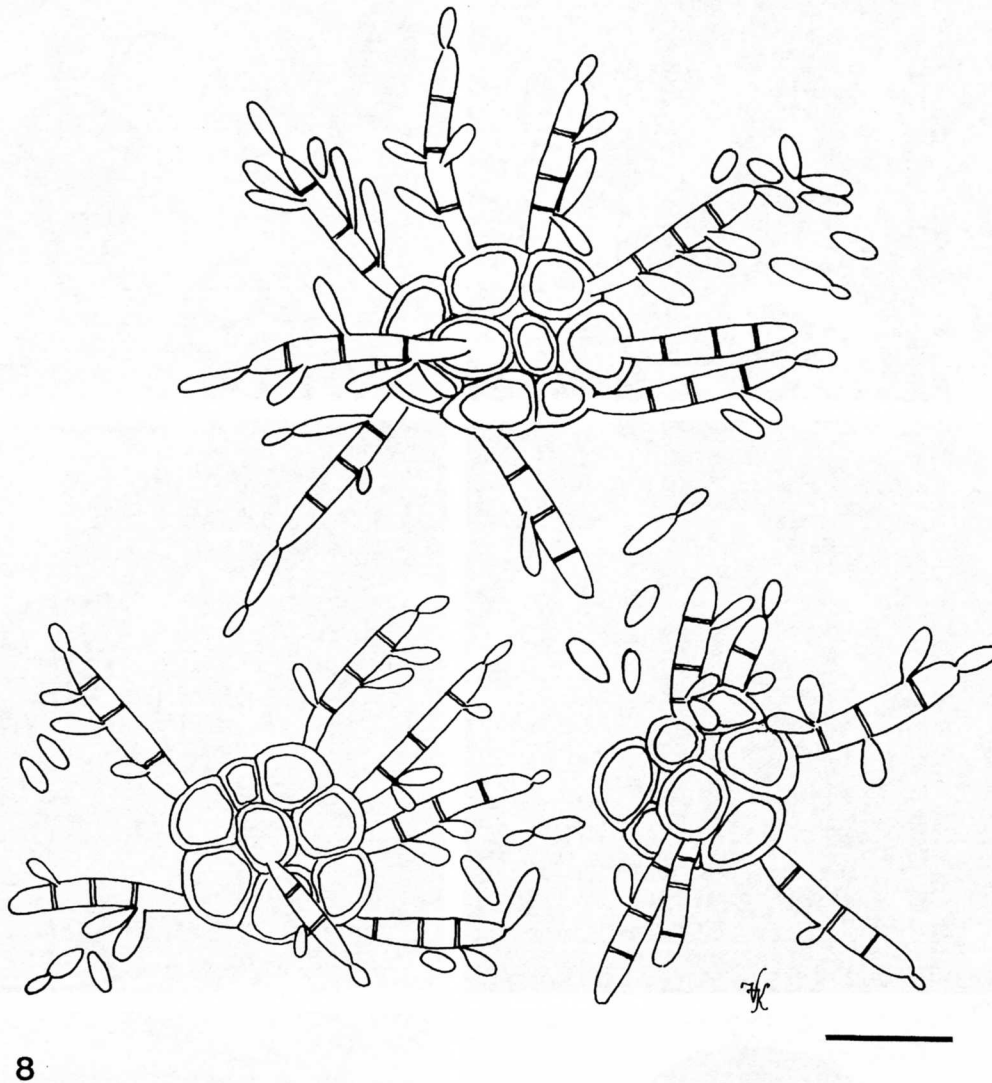


Fig. 8. Germinating spores of *Fulvisporium restifaciens* (D. E. Shaw) K. Vánky with septate, 4-celled basidia on which laterally and terminally ovoid basidiospores are produced (on MYP, at room temp., after 1–2 days; HUV 17637). Bar = 10 μ m

Sequence data of 5 smut fungi were completed with the known sequences of *Microbotryum violaceum* (Pers. : Pers.) G. Deml & Oberw., *Tilletia caries* (DC.) L.-R. & C. Tul., and *Ustilago hordei* (Pers.) Lagerh. (Boekhout et al., 1995; Berres et al., 1995). An alignment of 551 bp was created using the multiple alignment from ClustralW.

Neighbour joining analysis of the alignment results in the topology shown in Fig. 9. Other analysis methods like maximum parsimony or maximum likelihood are leading largely to the same topology. Rooting the tree in the longest internal branch yields two different lineages of evolution. One including *Aurantiosporium subnitens*, *Fulvisporium restifaciens* and *Microbotryum violaceum* represents a group of smut fungi having phragmobasidia and intercellular hyphae. The second lineage including *Thecaphora seminis-convolvuli*, *Tilletia caries*, *Tolyposporella brunckii*, *Tolyposporium junci* and *Ustilago hordei* has either taxa with phragmobasidium and intracellular hyphae or taxa with holobasidium and intercellular hyphae.

These data also support the separation of *Fulvisporium restifaciens* from the genus *Tolyposporium*. Molecular sequence data show on the one hand that there is no close relationship between *Fulvisporium restifaciens* and the analysed members of the genera *Thecaphora*, *Tolyposporella* and *Tolyposporium* and no close relationship between these three last-mentioned genera. On the other hand the molecular sequence data reveal a close relationship between *Fulvisporium restifaciens* and *Aurantiosporium subnitens*, discussed above.

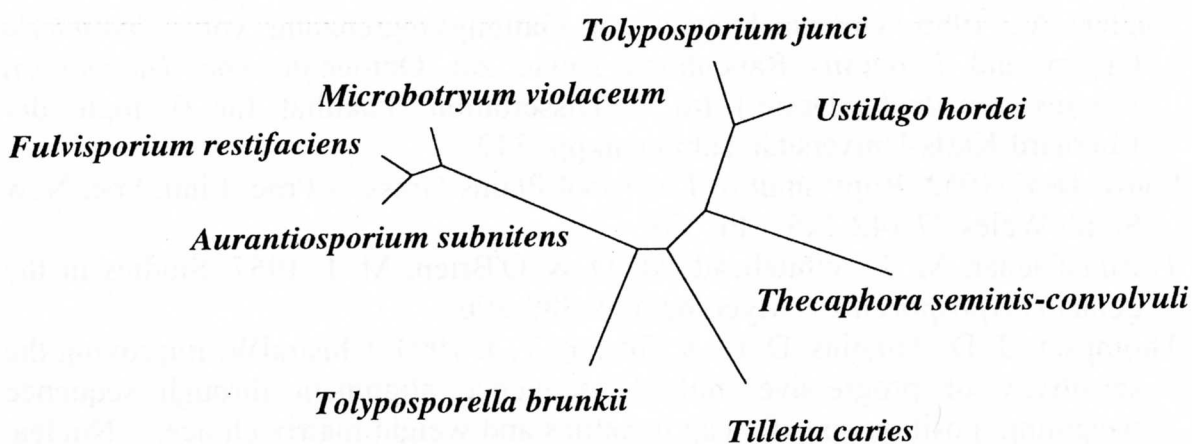


Fig. 9. Topology resulting from neighbour joining analysis of partial LSU rDNA gene sequences from 8 smut fungi. Branch lengths represent the distances of the sequences.

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NOTE

The alignment of rDNA sequences is available from the third author upon request.