# Validation of the description of *Gibberella circinata* and morphological differentiation of the anamorph *Fusarium circinatum*

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*Fusarium subglutinans sensu lato* is the causal agent of several diseases on a wide variety of host plants, including maize, mango, pine, pineapple and sugarcane. Pitch canker is an important disease caused by host specific *F. subglutinans* isolates from pine. Previously, *F. subglutinans* isolates occurring on different host plants could be distinguished from one another based only on pathogenicity and sexual compatibility. However, β-tubulin and histone *H3* gene sequences have recently been used to separate *F. subglutinans* isolates into distinct phylogenetic and morphological species. The pitch canker fungus was described as *F. circinatum* based on four strains. The teleomorph, *Gibberella circinata* was described based on a single cross between two of these strains. The objectives of the present study were to provide additional information and isolates to validate the description of *G. circinata* and to test the efficacy of distinguishing *F. circinatum* from *F. subglutinans sensu lato* using morphological characteristics. The single cross used in the description of *G. circinata* was repeated and vegetative compatibility tests and mating type segregation confirmed that these isolates were heterothallic. Morphological characteristics of *F. circinatum* and *G. circinata* were consistent with those given in the original description of the species with minor differences in the dimensions of the perithecia. Perithecial dimensions in this study were 332–453 µm high and 288–358 µm wide. The description of *G. circinata* was validated by providing information regarding the holotype specimen, names of collectors of isolates, date of collection and designation of the holotype specimen. Morphological criteria such as conidial morphology, type of conidiophore branching and presence of sterile coiled hyphae distinguished *F. circinatum* from *F. subglutinans sensu lato* isolates.

Keywords: *Fusarium circinatum, Fusarium subglutinans* f. sp. *pini, Gibberella fujikuroi,* mating population H.

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*Fusarium subglutinans* (Wollenw. & Reinking) Nelson & al. is a successful plant pathogen with a cosmopolitan distribution and is responsible for several important plant diseases. Pitch canker of pines is one of the most significant of these diseases and accurate identification is therefore important. Correll & al. (1992) gave isolates of *F. subglutinans* pathogenic to pines *forma specialis* status, based on host specificity and restriction fragment patterns of mtDNA indicating that pine isolates differed from non-pine isolates. *F. subglutinans* f. sp. *pini* Correll & al. could be distinguished from other host specific *F. subglutinans* isolates based only on pathogenicity and sexual compatibility (Britz & al., 1999; Viljoen & al., 1997; Correll & al., 1992; 1991). Sexual compatibility between *F. subglutinans* f. sp. *pini* isolates revealed that fertile *F. subglutinans* f. sp. *pini* were reproductively isolated and represented a distinct mating population (mating population H, MP-H) in the *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura complex (Britz & al., 1999).

O'Donnell & al. (2000; 1998) recognized 44 *Fusarium* phylogenetic lineages in the *G. fujikuroi* complex (*Liseola* and related sections) based on β-tubulin gene and translation elongation factor EF-1$\alpha$ gene sequences. Nirenberg & O'Donnell (1998) re-evaluated morphological characteristics and described morphological species that supported the phylogenetic lineages. Twelve of the 44 *Fusarium* taxa in the *G. fujikuroi* complex (residing in *Liseola* and related sections) were newly described (O'Donnell & al., 2000; 1998; Nirenberg & O'Donnell, 1998). The pitch canker fungus, *F. subglutinans* f. sp. *pini* was named *F. circinatum* Nirenberg & O'Donnell (teleomorph: *G. circinata* Nirenberg & O'Donnell). Other *Fusarium* spp. that are morphologically similar to *F. subglutinans* (= *F. subglutinans sensu lato*) described by Nirenberg & O'Donnell (1998), include *F. begoniae* Nirenberg & O'Donnell ex *Begonia elatior* hybrid, *F. bulbicola* Nirenberg & O'Donnell ex *Nerine bowdenii, Vallota* and *Haemanthus* sp., *F. concentricum* Nirenberg & O'Donnell ex *Musa sapientum* (banana), *F. guttiforme* Nirenberg & O'Donnell ex *Ananas comosum* (pineapple) and *F. pseudocircinatum* O'Donnell & Nirenberg ex *Solanum* sp., *Pinus kesiya* and *Heteropsylla incisa*. According to the Index of Fungi (1999; vol. 6: 980), the new name *G. circinata* is invalid according to Article 37.3 of the International Code of Botanical Nomenclature (ICBN, Greuter & al., 1994). Nirenberg & O'Donnell (1998) did not provide sufficient information to characterize unequivocally the type specimen in their description of *G. circinata* (John C. David, Editor of Index of Fungi, personal communication). The new names, *F. circinatum*, as well as *F. begoniae, F. concentricum F. guttiforme* and *F. pseudocircinatum* are, however, not invalid in terms of Article 37.3, because the host from

which these isolates were derived are provided (John C. David, Editor of Index of Fungi, personal communication).

The description of the pitch canker fungus, *F. circinatum* by Nirenberg & O'Donnell (1998) was based on only four isolates, while the description of *G. circinata* relied upon a single cross between two *F. circinatum* isolates (BBA 69720 and BBA 69722). Thus, virtually no consideration of variability in the teleomorph characteristics was possible for this important plant pathogen. These authors also did not consider the fertility or the thallism of the *F. circinatum* isolates. Furthermore, even though an extensive, global collection of *F. circinatum* isolates exists, variability amongst isolates of *F. circinatum* was not considered.

The objective of this study was to provide additional information as well as isolates to support the description of *G. circinata,* which is validated by fulfilling the requirements of Article 37 (Greuter & al., 1994). The previously selected mating testers for MP-H are confirmed to be the most appropriate strains to identify pitch canker isolates and the morphological differentiation of *F. circinatum* from *F. subglutinans sensu lato* isolates is reported.

## Materials and methods

### Isolates

*Fusarium circinatum* (= *F. subglutinans* f. sp. *pini*) isolates from different geographical areas, tester strains of *F. subglutinans* (MP-E) from maize (*Zea mays*) and *F. sacchari* (Butler) W. Gams (MP-B) from sugarcane (*Saccharum offinarum*) as well as ex-type *Fusarium* spp. in the *G. fujikuroi* complex that were previously identified as *F. subglutinans sensu lato* were studied (Tab. 1). All strains were single-spore isolates, preserved by lyophilization and suspensions in 15% glycerol at –70°C and are available from the culture collection of the Medical Research Council (MRC), Tygerberg, South Africa as well as from the *Fusarium* culture collection of the Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. *F. circinatum* isolates from California (FSP strain numbers) and Florida (FL strain numbers) were provided by T. R. Gordon, Department of Plant Pathology, University of California, California, USA. *F. sacchari* and *F. subglutinans* strains are also deposited in culture collections of FRC (Culture collection of the *Fusarium* Research Center, Department of Plant Pathology, Pennsylvania State University, University Park) and KSU (Collection of J. F. Leslie, Kansas State University, Department of Plant Pathology, Kansas

Tab. 1. – Origin and mating type of *Fusarium* strains in *Liseola* and related sections.

| MRC no* | Other strain no§ | Fertility‡ | Mating type |
|---|---|---|---|
| *F. circinatum* South African isolates | | | |
| 6208⊕ | | FS | *MAT-2* |
| 6209 | BBA 69854, NRRL 25621 | FS | *MAT-2* |
| 6213⊕ | | H | *MAT-2* |
| 7448 | | H | *MAT-2* |
| 7452 | | H | *MAT-1* |
| 7454⊕ | BBA 69722, NRRL 25333 | H | *MAT-2* |
| 7488[e] | | H | *MAT-1* |
| *F. circinatum* California isolates | | | |
| 7869 | SL-1⊕, BBA 69720, NRRL 25331 | H | *MAT-1* |
| 7504 | FSP 14 | FS | *MAT-1* |
| 7505⊕ | FSP 48 | FS | *MAT-2* |
| 7506 | FSP 52 | H | *MAT-2* |
| 7507 | FSP 75 | H | *MAT-2* |
| 7508⊕ | FSP 90 | FS | *MAT-1* |
| *F. circinatum* Florida isolates | | | |
| 7509⊕ | FL 3 | FS | *MAT-1* |
| 7510 | FL 17 | H | *MAT-1* |
| 7511 | FL 19 | FS | *MAT-1* |
| 7512 | FL 27 | FS | *MAT-2* |
| 7513⊕ | FL 58 | FS | *MAT-1* |
| *F. circinatum* Mexican isolates | | | |
| 7568⊕ | A1 | FS | *MAT-1* |
| 7570 | A2 | FS | *MAT-1* |
| 7572 | A3 | – | – |
| 7572 | A5 | FS | *MAT-1* |
| *F. sacchari* (MP-B) from sugarcane | | | |
| 6524 | KSU 3852, FRC-M6865 | H | *MATB-1* |
| 6525 | KSU 3853, FRC-M6866 | H | *MATB-2* |
| *F. subglutinans* (MP-E) from maize | | | |
| 6483 | KSU 0990, FRC-M3696 | H | *MATE-2* |
| 6512 | KSU 2192, FRC-M3693 | H | *MATE-1* |
| *F. begoniae* | | | |
| 7542 | BBA 67781, NRRL 25300 | – | – |
| *F. bulbicola* | | | |
| 7534 | BBA 13618, NRRL 63628 | – | – |
| *F. concentricum* from banana | | | |
| 7540 | BBA 64354, NRRL 64354 | – | – |
| *F. guttiforme* from pineapple | | | |
| 7539 | BBA 69661, NRRL 25295 | – | – |
| *F. pseudocircinatum* | | | |
| 7536 | BBA 69636, NRRL 22946 | – | – |

* MRC, Medical Research Council culture collection, PROMEC, Tygerberg, South Africa

§ BBA, NRRL, FSP, FL, FRC and KSU culture collection abbreviations explained in text. A = Original number of isolates collected in Mexico deposited in TPCP culture collection, FABI, University of Pretoria, Pretoria, South Africa.

‡ H= Hermaphrodite and FS = Female sterile

⊕ Morphological characteristics examined of isolates in fertile crosses.

State University). All ex-type cultures used in this study are deposited in BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin, Germany) and NRRL (Northern Regional Research Laboratory, NCAUR, Peoria, IL, USA) culture collections.

### Sexual compatibility

The holotype herbarium specimen of *G. circinata* (BPI 74609) was examined in detail (Fig. 1a). The cross between isolates BBA 69720 and BBA 69722 (Nirenberg & O'Donnell, 1998), was repeated by inoculating these two isolates on carrot agar, 2 cm from the center of Petri dishes opposite one another, as observed on the herbarium material. The cross was incubated at the lower temperature of 17°C as suggested by Covert & al. (1999) under fluorescent and cool-white light (12 h photoperiod) until fertile perithecia were produced after 3 to 6 weeks. All crosses in this study were made on carrot agar as described by Klittich & Leslie (1988) except that we used 300 g of fresh carrots per liter of medium. Strains acting as females in the sexual cross were inoculated on carrot agar and those acting as males were inoculated on complete media (Correll & al., 1987) slants. The carrot agar plates and complete media slants were incubated for 8 days at 25°C. A spore suspension of the male parent was prepared in 2.5% (v/v) TWEEN 60 and spread over the surface of the female parent plate. Fertilized plates were incubated upright, in a single layer under fluorescent and cool-white light (12 h photoperiod) at 17°C. Female fertility of the strains was tested by reversing the roles of the two strains in the cross. Strains that were fertile only as males were designated as female-sterile, while strains serving as either the male or female parent were designated hermaphrodites. All the crosses were examined weekly for fertile crosses (perithecia with exuding ascospores). All crosses were repeated at least once and those that were fertile were repeated again. The mating type designation (*MAT*-1 or *MAT*-2) of all isolates was based on their fertility with one of the tester strains of MP-H (MRC 6213 and MRC 7488) (Steenkamp & al., 2000). Ascospores exuding from perithecia were randomly examined for viability by streaking them on 1.5% water agar and assessing germination after 24 h.

### Thallism determination

The mating type segregation and vegetative compatibility groups (VCGs) of BBA 69720 and BBA 69722 as well as progeny from the cross were determined to establish the thallism of the cross between strains that has been used to typify *G. circinata*. The mating type segregation was determined by amplifying mating type idiomorphs using PCR primers and reaction conditions described by Steenkamp

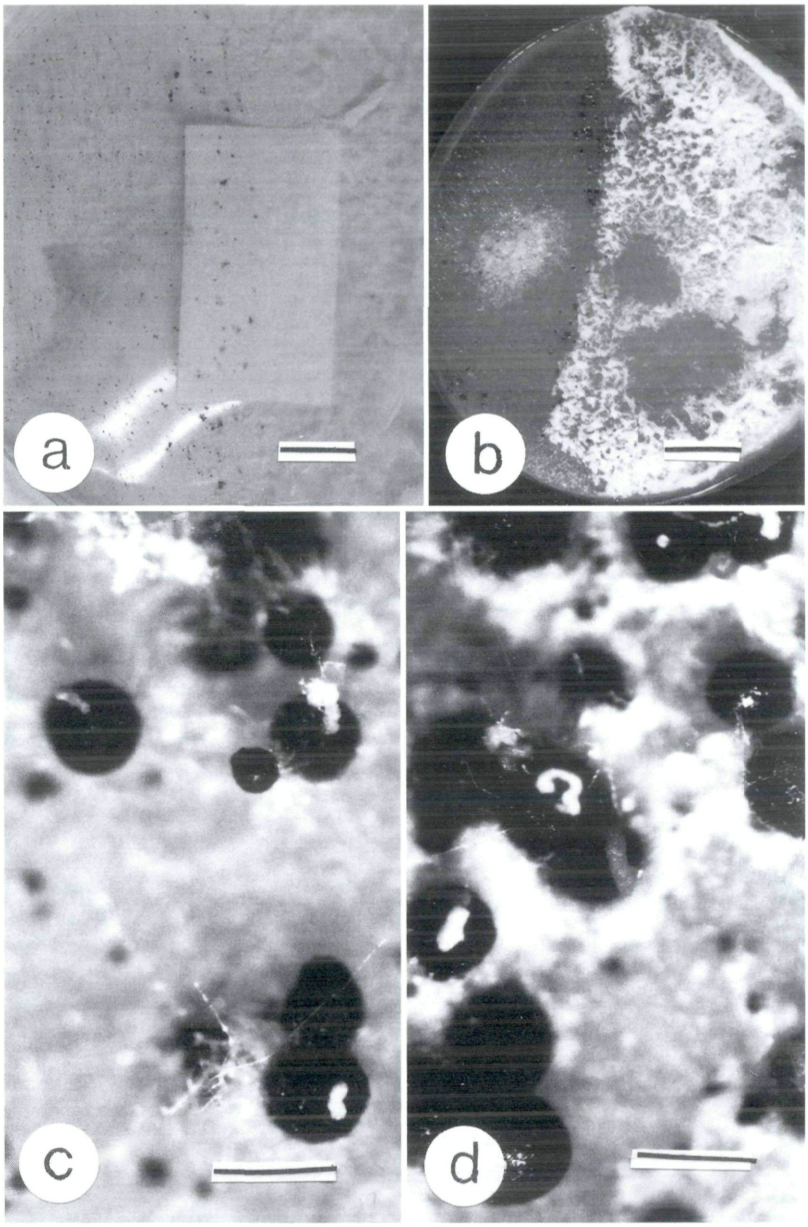


Fig. 1. – a. Holotype of *Gibberella circinata* (BPI 746094) of a cross between BBA 69720 and BBA 69722 on 5% carrot agar on a 90 mm Petri dish by Nirenberg & O'Donnell (1998). – Bar: 12 mm. – b. Cross of BBA 69720 and BBA 69722 produced on 5% carrot agar on a 65 mm Petri dish. – Bar: 12 mm. – c. Perithecia with exuding ascospores produced in a cross between BBA 69720 and BBA 69722 on carrot agar. – Bar: 200 µm. – d. Perithecia with exuding ascospores produced between mating testers, MRC 6213 and MRC 7488, on carrot agar. – Bar: 200 µm.

& al. (2000). Primers MatA and MatB amplify a product of approximately 300 base pairs (bp) in size of *MAT*-1 and primers MatC and MatD amplify a product of approximately 800 bp in size of *MAT*-2 (Steenkamp & al., 2000). The VCGs of BBA 69720, BAA 69722 and progeny were determined as previously described by Britz & al. (1999).

### Morphological description

Morphological characteristics such as perithecial dimensions and ascospore characteristics of *G. circinata* obtained from ten fertile crosses between *F. circinatum* isolates (BBA 69720 × BBA 69722, MRC 6213 × MRC 7488, BBA 69722 × MRC 7488, MRC 6208 × MRC 7488, MRC 7452 × MRC 6213, MRC 7505 × MRC 6213, MRC 7508 × MRC 7488, MRC 7509 × MRC 6213, MRC 7513 × MRC 6213 and MRC 7568 × MRC 6213) on carrot agar were compared with characteristics of *G. circinata* described by Nirenberg & O'Donnell (1998). The morphological characteristics, including: the shape of the conidia, type of conidiophore branching, origin of the conidiophore from the substrate, presence of chlamydospores and presence of sterile coiled hyphae (Nirenberg & O'Donnell, 1998), of *F. subglutinans sensu lato* isolates were also considered in detail (Tab. 1).

To stimulate culture and conidial development, isolates were transferred to carnation leaf agar (CLA, Fisher & al., 1982) and potassium chloride (KCl) agar (Nelson & al., 1983). Cultures were incubated at 25°C under fluorescent and cool-white light with a 12 h photoperiod and examined after 10 to 14 days, using a Zeiss Axioskop microscope. Secondary characteristics such as growth rate and colony colour were determined on potato dextrose agar (PDA) after incubation at 25°C in the dark (Nelson & al., 1983). Photomicrographs were taken of cultures grown on CLA. Fifty micro- and macroconidia, perithecia and ascospores were measured for each isolate and results represent minimum, mean and maximum values with the standard deviations in parentheses.

## Results

### Sexual compatibility

Nirenberg & O'Donnell (1998) did not describe details of how they made the cross between BBA 69720 and BBA 69722, but it was clear from herbarium material (Fig. 1a) that isolates had been paired alongside each other. We repeated the cross in the same way and, although this is not an ideal technique, perithecia were produced (Fig. 1b). Mature perithecia exuding ascospores (Fig. 1c) also were produced when this cross was repeated using the standard method

(Klittich & Leslie, 1988). The standard method was considerably more effective than when isolates were placed alongside each other. BBA 69720 and BBA 69722 were shown to be hermaphrodites and can, therefore, serve as effective tester strains. The fertility of the cross between BBA 69720 and BBA 69722 was compared with the fertility between the MP-H tester strains, MRC 6312 and MRC 7488. The fertile cross between these tester strains produced approximately three times more perithecia exuding ascospores (Fig. 1c) than the cross between BBA 69720 and BBA 69722 (Fig. 1d). All the fertile crosses recorded in this study were repeated successfully in at least two different tests. The percentage germination of random ascospores was higher than 80% in all cases.

The hermaphroditic strains MRC 6213 and MRC 7488 (Britz & al., 1999) were identified as *MAT*-2 and *MAT*-1, respectively as has also been shown by Steenkamp & al. (2000). The mating type designation of all the other *F. circinatum* isolates was of the opposite mating type to the tester strains (MRC 6213 or MRC 7488) with which a fertile cross was produced (Tab. 1).

### Thallism determination

Strain BBA 69720 amplified a PCR product of approximately 300 bp in size and BBA 69722 amplified a PCR product of approximately 800 bp in size. Five of the ascospore progeny produced a PCR product of approximately 800 bp in size and the other five produced PCR products of approximately 300 bp in size. Vegetative compatibility tests determined that the parent strains and 10 ascospore progeny all belonged to different VCGs.

## Morphological description and validation

***Gibberella circinata*** Nirenberg & O'Donnell Mycologia 90: 440. 1998.

Immersed and superficial perithecia formed on carrot agar. Ovoidal to obpyriform, dark purple to black perithecia (329–)332–396–453(–463) µm high and 288–337–358(–386) µm wide (Fig. 2a). Within 3–6 weeks after fertilization the perithecia exude pale brown, ellipsoidal 1–septate ascospores in cirrhi from perithecia (Fig. 1c; 2b). – Anamorphic state, *F. circinatum*, characterized by sterile coiled hyphae (Fig. 2d), sympodially branched conidiophores bearing polyphialides (Fig. 2c) and conidiophores that originate directly from the substrate hyphae (erect). – Macroconidia 3-septate, slender and cylindrical (lunate) (Fig. 2e). – Microconidia non-septate, obovoid (Fig. 2f), occasionally oval to allantoid.

The conidiophores of *F. circinatum* examined in this study had 2–5 openings and hyphal swellings occurred in some isolates.

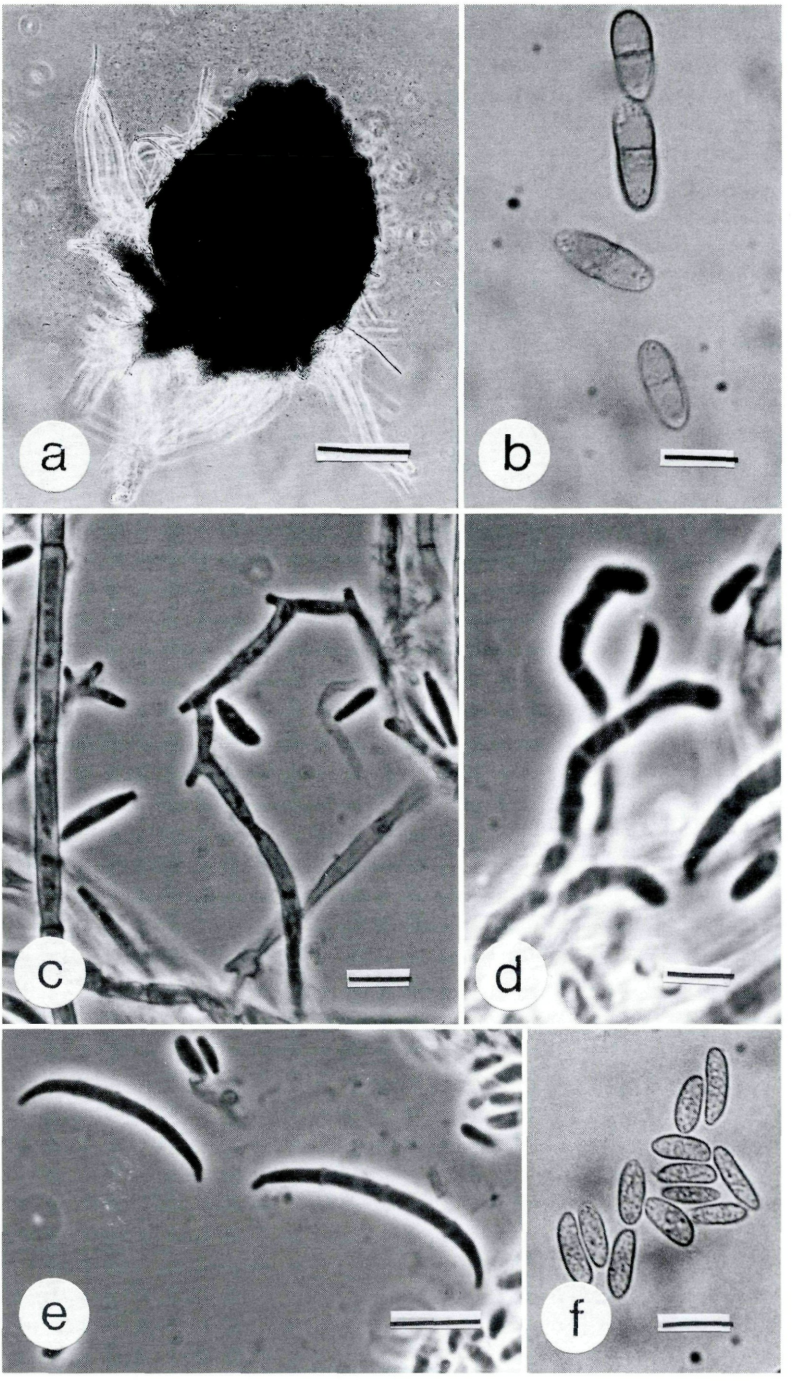


Fig. 2. – Morphological characteristics of *G. circinata* and *F. circinatum* (MRC 6213). – a. Mature perithecia. – Bar: 100 µm. – b. Septate ascospores. – Bar: 10 µm. – c. Branched conidiophores. – Bar: 10 µm. – d. Sterile coiled hyphae. – Bar: 10 µm. – e. Slender and cylindrical macroconidia. – Bar: 10 µm. – f. Obovoid microconidia. – Bar: 10 µm.

Holotype. – BPI 74609 = Dried preserved culture on 5% carrot agar of cross between isolates BBA 69720 and BBA 69722. *F. circinatum* BBA 69720 (ex-holotype culture) is *MAT*-1 hermaphrodite collected by T. R. Gordon from a symptomatic *Pinus radiata* branch in San Lorenzo, Almeda County, California, USA in 1988. Holotype deposited as a dried specimen in herbarium of Botanischer Garten und Botanisches Museum, Berlin-Dahlem, Germany (B), no accession number available (B. Hein, Curator of fungi, personal communications). Strain BBA 69722 is *MAT*-2 hermaphrodite collected by A. Viljoen from *P. patula* seedlings in the Ngodwana nursery, Mpumalanga, South Africa during July 1990 (Viljoen & al., 1994). The isotype of *G. circinata* is deposited in herbarium B with no accession number.

The morphological characteristics of *G. circinata* produced in ten crosses on carrot agar between members of MP-H of *G. fujikuroi* were compared with those in the original description of *G. circinata*. In general, the perithecia and ascospore characteristics were consistent with those described by Nirenberg & O'Donnell (1998). However, perithecia in this study were 332–396–453 µm high and 288–337–358 µm wide, in contrast with the smaller perithecia (ca 325 µm high and 230 µm wide) reported by Nirenberg & O'Donnell (1998).

Isolates examined. – Twenty-two *F. circinatum* isolates and ten laboratory crosses between *F. circinatum* were examined (Tab. 1).

## Morphological differentiation

Morphological characteristics of ex-type *Fusarium* spp. previously identified as *F. subglutinans* (Tab. 1) were examined to establish whether *F. circinatum* isolates could be distinguished from *F. subglutinans sensu lato*. All the *F. subglutinans sensu lato* isolates had obovoid microconidia. Oval to allantoid or fusoid microconidia are present in all *F. subglutinans sensu lato* isolates except in *F. guttiforme*, which only produces obovoid microconidia. Sterile coiled hyphae are only produced by *F. circinatum* and *F. pseudocircinatum* isolates. *F. circinatum* (MP-H), *F. pseudocircinatum* and *F. sacchari* (MP-B) produce sympodial conidiophores (defined by Nirenberg & O'Donnell (1998: 455) as "proliferating conidiophores – conidiophores with intercalary phialides often created by sympodially proliferating growth of the conidiophores"). Most of the *F. subglutinans sensu lato* isolates produce phialides with 3 and more conidiogenous openings, except for *F. begoniae, F. bulbicola* and *F. subglutinans* that produce 3 and fewer conidiogenous openings on the phialides. *F. guttiforme* (BBA 69661) produced sporodochia and 3-septate, falcate macroconidia with basal cell. Macroconidia with 3–5 septa are produced by *F. bulbicola, F. concentricum* and *F. subglutinans* whereas *F. begoniae, F. circinatum, F. guttiforme, F. pseudocircinatum* and *F. sacchari* produce 3-septate macroconidia. No short false chains

were observed in *F. pseudocircinatum* (BBA 69636) (Nirenberg & O'Donnell, 1998) on CLA incubated under continuous black light.

## Discussion

Our study has provided sufficient information regarding the collector, date of collection and direct designation of the holotype specimen as specified by Article 37.3 of the ICBN (Greuter & al., 1994) to validate the description of *G. circinata*. Results showed that the single cross used for the description for *G. circinata* is heterothallic, which is important because sexual compatibility tests are used to distinguish different mating populations in the *G. fujikuroi* complex. The variability of the morphological characteristics amongst a large collection of *F. circinatum* and *G. circinata* was consistent with those given in the original description of the species, except for minor differences in the perithecial dimensions. Morphological characteristics such as conidial morphology, type of conidiophore branching and presence of sterile coiled hyphae were used to differentiate *F. circinatum* from *F. subglutinans sensu lato* isolates.

The cross between *F. circinatum* strains BBA 69720 (ex-type) and BBA 69722 used to describe *G. circinata* (Nirenberg & O'Donnell, 1998) was shown to be heterothallic. This was indicated by the 1:1 (*MAT*-1:*MAT*-2) mating type segregation of the ascospore progeny as well as the recombination indicated by vegetative compatibility tests, where progeny belonged to different VCGs than parent strains (BBA 67920 and BBA 69722). This removed any concern that the single cross might have been homothallic, an event previously observed in the related species, *F. sacchari* (= *F. subglutinans,* MP-B) (Britz & al., 1999; Leslie & al., 1986).

*Fusarium circinatum* is reproductively isolated from other species in the *G. fujikuroi* complex and reside in MP-H of the *G. fujikuroi* complex (Britz & al., 1999). Sexual compatibility can be used as an alternative method to differentiate *Fusarium* spp. residing in different mating populations of the *G. fujikuroi* complex (Leslie, 1995). To accomplish the definition of mating populations, suitable tester strains are required. Suitable tester strains are hermaphrodites of opposite mating type that produces a fertile cross (Leslie, 1995). The strains BBA 69720 and BBA 69722 were identified as *MAT*-1 and *MAT*-2, respectively and were hermaphrodites. However, MRC 6213 and MRC 7488 have previously been identified as mating tester strains of MP-H (Britz & al., 1999). MRC 6213 and MRC 7488 should preferably be used as the standard tester strains of MP-H of *G. fujikuroi,* because they produce more perithecia exuding ascospores than crosses between BBA 69720 and BBA 69722.

Hyphal swellings were observed in some *F. circinatum* isolates. These swellings should, however, not be confused with chlamydospores, which could result in incorrect identification of this fungus. Nirenberg & O'Donnell (1998) based the description of the teleomorph on a single cross and consequently could not consider variability in the morphological characteristics. The observation of 3-septate, falcate macroconidia produced by *F. guttiforme* isolate (BBA 69661) facilitated the differentiation of *F. circinatum* from *F. subglutinans sensu lato*. In the original description by Nirenberg & O'Donnell (1998) only 'conidia borne in aerial mycelium, obovoid, mostly 0-septate, occasionally 1-septate' were described for *F. guttiforme*. This illustrates the need to include a relatively large set of isolates displaying relevant characteristics, when describing new species of *Fusarium* (Burgess & al., 1982).

Newly described *Fusarium* spp. that are morphologically similar to *F. subglutinans* were examined to identify differentiating morphological characteristics. *F. subglutinans sensu lato* isolates can be differentiated using conidial morphology, type of conidiophore branching, origin of the conidiophore from the substrate and presence of sterile coiled hyphae. In this study, we referred to "conidiophores formed by sympodial branching" (van Wyk & al., 1991) as sympodial conidiophores, rather than proliferating conidiophores (Nirenberg & O'Donnell, 1998). Only *F. circinatum* and *F. pseudocircinatum* produce sterile coiled hyphae, but *F. pseudocircinatum* produces short false chains under continuous black light (Nirenberg & O'Donnell, 1998). This characteristic was not observed in our study in the ex-type culture of *F. pseudocircinatum*, under the same conditions. However, *F. circinatum* has erect conidiophores, whereas *F. pseudocircinatum* has prostrate conidiophores.

The use of morphological characteristics to identify fungi that are very similar to each other, such as *Fusarium* spp., is time-consuming and difficult. This is particularly true for researchers not familiar with the taxonomy of this group of fungi. Sexual compatibility tests have been used to distinguish morphologically similar *Fusarium* spp., in the *G. fujikuroi* complex in the past. However, O'Donnell & al. (2000; 1998) and Steenkamp & al. (1999) have identified a number of conserved genes that can be used to distinguish *Fusarium* spp. in the *G. fujikuroi* complex. Rapid identification of closely related *Fusarium* spp. in the *G. fujikuroi* complex is possible with a PCR-RFLP technique based on histone *H3* gene sequences (Steenkamp & al., 1999). This technique is reliable and provides the non-taxonomist with a valuable test for identification. *F. circinatum* can be differentiated from *F. subglutinans sensu lato* using morphological, genetic and molecular characteristics.

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