# *Gibberella gaditjirrii* (*Fusarium gaditjirrii*) sp. nov., a new species from tropical grasses in Australia

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**Abstract:** A new species, *Gibberella gaditjirrii*, which was isolated from *Heteropogon triticeus* and other grasses from the tropical region of northern Australia, is described. It was differentiated from morphologically similar species that form microconidia in chains by morphological characters, sexual compatibility, Amplified Fragment Length Polymorphism (AFLP) fingerprint profiles and phylogenetic clustering based on β-tubulin, elongation factor 1-α and calmodulin gene sequences. The new species is heterothallic and abundant perithecia are formed in compatible crosses. Although female fertility is low we have isolated two reliable female fertile strains. The anamorph *Fusarium gaditjirrii* forms chlamydospores on synthetic nutrient-poor agar (SNA) and carnation leaf agar (CLA) media in the dark in fresh wild-type cultures, a feature that helps distinguish this species morphologically from other similar species which form microconidia in chains.

**Taxonomic novelties:** *Gibberella gaditjirrii* Phan, Burgess & Summerell sp. nov. (anamorph *Fusarium gaditjirrii* Phan, Burgess & Summerell sp. nov.).
**Key words:** AFLP, β-tubulin, calmodulin, DNA sequence, elongation factor 1-α, fungal endophyte, *Heteropogon,* mating population, natural ecosystems, *Themeda*.

## INTRODUCTION

The history of research on *Fusarium* Link reflects the economic importance of these fungi and has been focused largely on species associated with crop plants. However, it is reasonable to assume that many of the *Fusarium* species associated with agricultural crops have been derived from populations in natural ecosystems (Britz *et al.* 1999, Wang *et al.* 2004).

There have been several major mycogeographic studies on *Fusarium* species in natural grassland and woodland soils of Australia (e.g., Burgess *et al.* 1988, Burgess & Summerell 1992, Summerell *et al.* 1993, Sangalang *et al.* 1995a). These studies demonstrated the presence of a wide range of *Fusarium* species in such soils and led to the recognition of new taxa (Burgess & Trimboli 1986, Nelson *et al.* 1987, Sangalang *et al.* 1995b). However, there have been no studies in Australia on *Fusarium* species associated with native grasses as endophytes or latent pathogens. Zeller *et al.* (2003) recently found that *Fusarium* species are commonly associated with prairie grasses in the tallgrass prairie ecosystems in Kansas, U.S.A., as endophytes or latent pathogens of stem tissues. They described a new species, *Gibberella konza* Zeller, Summerell & Leslie (*Fusarium konzum* Zeller, Summerell & Leslie) which they commonly isolated from these grasses (Zeller *et al.* 2003). We initiated a series of community studies on *Fusarium* species associated with grasses in Australia following a personal communication from Zeller and Leslie on their initial findings.

A previously undescribed *Fusarium* population was commonly isolated from the tropical Panicoid grasses, *Heteropogon triticeus* (R. Br.) Stapf ex Craiba (giant speargrass) and *Themeda triandra* Forsk. (syn. *T. australis* (R. Br.) Stapf.; Kangaroo grass) from several geographic locations in northern Australia (Fig. 1).

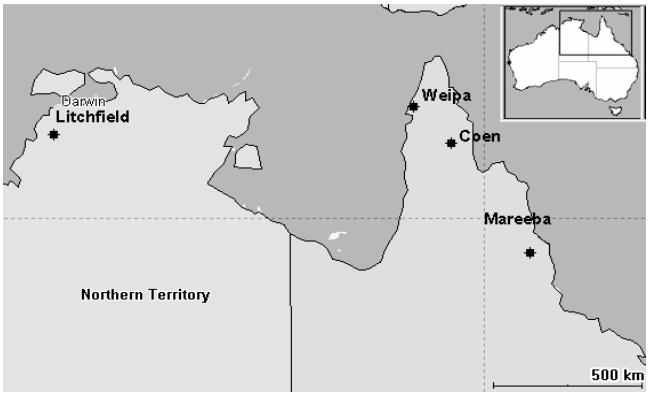


**Fig. 1.** Map of northern Australia indicating geographic origin of cultures of *G. gaditjirrii* (*F. gaditjirrii*).

**Table 1.** Geographic origin and hosts of isolation of cultures of *Gibberella gaditjirrii* (*Fusarium gaditjirrii*) in Queensland, Australia.

| Geographic origin | GPS | Host plant species |
|---|---|---|
| Davies Creek (Mareeba area) | E145°33.969' S16°59.710' | *Heteropogon triticeus, Themeda triandra* |
| Walkamin (Mareeba area) | E145°25.674' S17°07.586' | *Heteropogon triticeus, Themeda triandra* |
| Mt Molloy (Mareeba area) | E145°19.619' S16°39.986' | *Heteropogon triticeus, Themeda triandra* |
| North of Weipa (Cape York region) | E141°50.332' S12°19.699' | *Heteropogon triticeus* |
| North of Coen (Cape York region) | E143°0.183' S13°32.349' | *Heteropogon triticeus* |
| Litchfield National Park, Northern Territory | E130°42.333' S13°14.050' | *Heteropogon triticeus* |

In this paper we describe this population as the new species, *Gibberella gaditjirrii* (*Fusarium gaditjirrii*) based on morphological characters, sexual compatibility, Amplified Fragment Length Polymorphism (AFLP) and phylogenetic analysis of β-tubulin, elongation factor 1-α and calmodulin gene sequences.

## MATERIALS AND METHODS

### Morphological and cultural studies

The isolates used in this description were recovered from samples, each of 100 stems, of *H. triticeus* and *T. triandra* collected from tropical woodlands in the wet/dry tropics of northern Australia (Table 1).

The stems were washed in tap water. A nodal section, 0.5–1 cm in length, was removed from each stem approximately 10–15 cm above the crown, washed in sterile water and surface-sterilized with 70 % ethanol. Each section was then cut longitudinally and one subsection was plated cut-side down on Peptone PCNB Agar (PPA) (Nash & Snyder 1962). The plates were incubated at 25 °C day/ 20 °C night with a 12 h photoperiod.

Colonies of *Fusarium* which developed from the stem sections were subcultured onto carnation leaf agar (CLA) (Fisher *et al*. 1982) and incubated as described above for 10 d. Pure cultures were established using the single-spore method on three different media, CLA, potato-dextrose agar (PDA) and synthetic nutrient-poor agar (SNA) (Nirenberg 1976) for morphological examination.

Cultures on CLA and PDA were incubated at 25 °C day/ 20 °C night with a 12 h photoperiod (Burgess *et al.* 1994) while cultures on SNA were incubated in the dark (Nirenberg 1976) at 25 °C. After 12 d of incubation, the cultures were examined for the following morphological features: shape, size and mode of formation of microconidia, morphology of conidiogenous cells, size and shape of macroconidia and presence of chlamydospores (Burgess *et al.* 1994, Nirenberg & O'Donnell 1998). Measurements were according to Zeller *et al.* (2003). Colony type and pigmentation in the agar were assessed on PDA after 10 d, the latter by means of the Methuen handbook of colour (Kornerup & Wanscher 1963). Colony diameters were measured at 25 °C and 30 °C on PDA using three replicate cultures of each isolate at each temperature.

All isolates were preserved by lyophilisation and also maintained as conidial suspensions in 15 % glycerol at –70 °C. A dried culture of isolate F15048 was deposited as the holotype culture of *F. gaditjirrii* at New South Wales Plant Pathology Herbarium, Orange, NSW, Australia as DAR 76663. A preserved heterothallic cross between isolates F15017 and F15048 was deposited as holotype culture of *Gibberella gaditjirrii* as DAR 76662. Isolate F15048 (= DAR 76663, FRC M-8754, MRC 8568, CBS 116011), *MAT-1* and F15017 (= DAR 76664, FRC M-8755, MRC 8569, CBS 116010), *MAT-2* were deposited at *Fusarium* Research Center, Department of Plant Pathology, Pennsylvania State University, University Park, Pennsylvania, U.S.A. (FRC numbers), PROMEC Unit, Medical Research Council, Tygerberg, South Africa (MRC numbers), Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS numbers).

Standard mating population tester strains were used in this study: FGSC 7600 (*Gibberella moniliformis*, *MAT-1*), FGSC 7603 (*G. moniliformis, MAT-2*), FGSC 7611 (*G. sacchari*, *MAT-1*), FGSC 7610 (*G. sacchari, MAT-2*), FGSC 8931 (*G. fujikuroi, MAT-1*), FGSC 8932 (*G. fujikuroi, MAT-2*), FGSC 7615 (*G. intermedia, MAT-1*), FGSC 7614 (*G. intermedia, MAT-2*), FGSC 7616 (*G. subglutinans*, *MAT-1*), FGSC 7617 (*G. subglutinans, MAT-2*), FGSC 7057 (*G. thapsina*, *MAT-1*), FGSC 7056 (*G. thapsina, MAT-2*), FGSC 8934 (*G. nygamai, MAT-1*), FGSC 8933 (*G. nygamai, MAT-2*), FGSC 8910 (*G. konza*, *MAT-1*), FGSC 8911 (*G. konza, MAT-2*).

Sixteen isolates of 14 previously described species were used together with isolates of *F. gaditjirrii* to estimate genetic similarity of the species with potentially related species. These isolates were: *Fusarium andiyazi* Marasas, Rheeder, Lamprecht, Zeller & Leslie (KSU 4647, FRC 6905), *F. brevicatenulatum* Nirenberg, O'Donnell, Kroschel & Andrianaivo (KSU 10756, MRC-7531, BBA 69197), *F. fujikuroi* Nirenberg (KSU 01993, FRC 1148; KSU 01995, FRC 1150), *F. globosum* Rheeder, Marasas & Nelson (KSU 11554), *F. lactis* (Pirotta & Riboni) Nirenberg & O'Donnell (KSU 10757, MRC-7532, BBA 68590), *F. napiforme* Marasas, Nelson & Rabie (F 9794, MRC 4144), *F. nygamai* Burgess & Trimboli (KSU 05111), *F. phyllophilum* Nirenberg & O'Donnell (KSU 10768, MRC-7543, BBA 63625), *F. proliferatum* (Matsushima) Nirenberg (KSU 04854, *MAT-1*; KSU 04853,

*MAT-2*), *F. pseudoanthophilum* Nirenberg, O'Donnell & Mubatanhema (KSU 10755, MRC-7530, BBA 69002), *F. pseudocircinatum* Nirenberg & O'Donnell (KSU 10761, MRC-7536, BBA 69636), *F. pseudonygamai* Nirenberg & O'Donnell (KSU 10762, MRC-7537, BBA 69552), *F. thapsinum* Klittich, Leslie, Nelson & Marasas (KSU 04093, FRC-6563, MRC-6537, *MAT-2*; KSU 04094, FRC-6564, MRC-6288, *MAT-1*), and *F. verticillioides* (Sacc.) Nirenberg (KSU 00149, *MAT-1*; KSU 00999, *MAT-2*).

**DNA isolation**

Fungal mycelium was harvested from liquid SNA (Schilling 1996) cultures grown in the dark for 6 d for DNA extraction, which was conducted using the FastDNA® Kit (Qbiogene, Inc., Irvine, CA, U.S.A.) according to the manufacturer's instructions. Genomic DNA was assessed using gel electrophoresis for the estimation of concentration and integrity. DNA samples were stored at -20 °C.

**Amplified Fragment Length Polymorphism (AFLP) analysis**

The AFLP similarity of five isolates of *F. gaditjirrii* (F15006, F15012, F15048, F15066, F15071) and 16 isolates of 14 morphologically related species was analyzed. The protocol for AFLP was based on the methods described by Vos *et al.* (1995) and followed Zeller *et al.* (2000). Selective amplification was conducted with selective nucleotide extension primers EcoR1+GG and Mse1+CT with the EcoR1+GG primer labeled with fluorescent marker hexachloro-6-carboxy-fluorescine (HEX). Final products of the selective PCR were fractionated through a denaturing polyacrylamide gel using the ABI PRISM® 3700 DNA Analyzer (Applied Biosystems Inc., Foster City, CA, U.S.A.). AFLP fragment analysis was performed with ABI PRISM® GeneScan® Analysis Software version 3.7 (Applied Biosystems), which was optimized to identify and measure bands ranging in size from 100 to 400 base pairs. The bands (alleles) were scored as present or absent in the form of a binary matrix. AFLP patterns were analyzed qualitatively using NTSYSpc version 2.10q (Applied Biostatistics, Inc., New York, U.S.A.). Genetic similarities among isolates were calculated using the DICE coefficient (Nei & Li 1979) and clustered using Unweighted Pair Group Method of Analysis (UPGMA).

**DNA sequencing and phylogenetic analysis**

PCR amplifications of the elongation factor 1-α, β-tubulin and calmodulin gene regions from five strains of *F. gaditjirrii* (F15006, F15012, F15048, F15066, F15071) were performed using the primers listed in Table 2. Approximately 50 ng of template DNA was used in each reaction. The protocol was based on the method described by O'Donnell & Cigelnik (1997). PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Australia). DNA sequences were obtained using an ABI PRISM® 3700 DNA Analyzer at the Sydney University Prince Alfred Macromolecular Analysis Centre (SUPAMAC, The University of Sydney). Sequences were deposited into GenBank (Accession number: AY639624–AY639638).

The sequences were then aligned with sequences obtained from GenBank (Table 3) using ClustalX (V1.81) (Thompson *et al.* 1997). Alignment data sets were deposited in TreeBASE (Accession number: SN1956). Phylogenetic analyses were conducted based on the aligned sequences for each of the data sets using PAUP v. 4.0b10 (Swofford 1998). All alignment gaps were treated as missing data. Each data set was initially analyzed based on different optimality criteria: maximum parsimony (MP), weighted parsimony (WP), maximum likelihood (ML) and neighbour-joining (NJ). Maximum parsimony trees were obtained using heuristic searches with random sequence addition of 1000 replicates and tree-bisection-reconnection branch-swapping algorithm. The transition : transversion ratios for each of the data set estimated from ML (2.5 : 1 for β-tubulin, 3 : 1 for elongation factor 1-α and 2.2 : 1 for calmodulin) were used in the WP analysis. The NJ tree was generated based on the HKY85 model (Hasegawa *et al.* 1985). For ML and NJ analyses, base frequencies were estimated with among-site ratios assumed to be equal and the gamma distribution was also estimated. Clade stability was assessed by 1000 bootstrap replicates with random sequence addition. Consistency index (CI) and retention index (RI) were calculated for the indication of the amount of homoplasy present.

**Mating type and sexual compatibility tests**

The mating type of an isolate was determined using specific PCR amplification of two mating type genes as described by Kerényi *et al.* (1999) and Steenkamp *et al.* (2000). Both *MAT-1* and *MAT-2* gene amplifications were done to ensure that either *MAT-1* or *MAT-2* of each putative strain was amplified. Isolates of opposite mating types were crossed using the protocol described by Klittich & Leslie (1988). Each negative cross was repeated twice and each positive cross was repeated at least once.

Crosses were maintained for up to 6 wk after fertilization. The viability of ascospores was assessed after 4 wk following the procedure of Britz *et al.* (1999).

**Table 2.** Primers used to amplify the gene regions of elongation factor 1-α, β-tubulin and calmodulin.

| Primer | Sequence (5' → 3') |
|---|---|
| Elongation factor 1-α | (O'Donnell *et al.* 1998) |
| EF1 | ATGGGTAAGGA(AG)GACAAGAC |
| EF2 | GGA(GA)GTACCAGT(GC)ATCATGTT |
| β-tubulin | (O'Donnell & Cigelnik 1997) |
| T1 | AACATGCGTGAGATTGTAAGT |
| T22 | TCTGGATGTTGTTGGGAATCC |
| Calmodulin | (O'Donnell *et al.* 2000) |
| CL1 | GA(GA)T(AT)CAAGGAGGCCTTCTC |
| CL2A | TTTTTGCATCATGAGTTGGAC |

**Table 3.** Elongation factor 1-α, β-tubulin and calmodulin gene sequences of *Fusarium* spp. obtained from GenBank and used in the present study.

| | GenBank Accession Number | | |
|---|---|---|---|
| **Species** | **Elongation factor 1-α** | **β-tubulin** | **Calmodulin** |
| *Fusarium acutatum* | AF160276 | U34431 | AF158329 |
| *F. anthophilum* | AF160292 | U61541 | AF158345 |
| *F. begoniae* | AF160293 | U61543 | AF158346 |
| *F. circinatum* | AF160295 | U61547 | AF158348 |
| *F. concentricum* | AF160282 | AF333951 | AF158335 |
| *F. dlamini* | AF160277 | U34430 | AF158330 |
| *F. fujikuroi* | AF160279 | U34415 | AF158332 |
| *F. globosum* | AF160285 | U61557 | AF158338 |
| *F. guttiforme* | AF160297 | U34420 | AF158350 |
| *F. hostae* | AF331812 | AF324327 | NS* |
| *F. inflexum* | AF008479 | U34435 | AF158366 |
| *F. miscanthi* | AF060385 | AF324332 | NS* |
| *F. napiforme* | AF160266 | U34428 | AF158319 |
| *F. nisikadoi* | U61555 | AF324330 | NS* |
| *F. nygamai* | AF160273 | U34426 | AF158326 |
| *F. oxysporum* | AF160312 | U34424 | AF158365 |
| *F. phyllophilum* | AF160274 | U34432 | AF158327 |
| *F. proliferatum* | AF291058 | AF060389 | AF158333 |
| *F. pseudonygamai* | AF160263 | U34421 | AF158316 |
| *F. redolens* | AF331806 | AF324321 | NS* |
| *F. sacchari* | AF160278 | U61552 | AF158331 |
| *F. subglutinans* | AF160289 | U34417 | NS* |
| *F. thapsinum* | AF160270 | U34418 | AF158323 |
| *F. verticillioides* | AF273318 | U34413 | AF158315 |

*NS: no sequence availabile in GenBank.

Sexual compatibility tests were conducted using isolates of *F. gaditjirrii* and tester strains of morphologically related mating populations to examine if the new species belonged to any of the described mating populations

One hundred and thirty two putative isolates of *F. gaditjirrii* were first treated as male parents in the crosses with the female fertile testers of *G. moniliformis, G. sacchari, G. intermedia, G. subglutinans, G. thapsina* and *G. konza*. Crosses were not made with *G. fujikuroi* or *G. nygamai* because the tester strains of these species did not produce fertile crosses with each other. The isolates of *F. gaditjirrii* of opposite mating types were then intercrossed to test for female fertility, in which each field isolate was treated as both male and female parent. Effective population size ($N_e$) was estimated based on mating type ratio and the frequency of female fertile isolates (Leslie & Klein 1996).

## RESULTS

### Morphological and cultural studies

***Gibberella gaditjirrii*** Phan, Burgess & Summerell, **sp. nov.** MycoBank MB500072. Fig. 2A–E.

*Anamorph*: *Fusarium gaditjirrii* Phan, Burgess & Summerell, sp. nov.

*Etymology*: *Gaditjirri*, in the Rirratjinu language, Yirritja moiety, Australian Aboriginal, refers to *Heteropogon triticeus*, the grass species from which this fungus was first isolated (Yunupingu *et al.* 1995).

Perithecia superficilia, livida, 210–294 µm alta, 210–350 µm diam. Asci clavati, dehiscentes, octospori. Ascosporae exudatae in cirrhis, laeves, hyalinae, ellipsoidae vel

obovoideae, 1–3-septatae et ad septum paulo constrictae, 4–6 × 12–16 µm. Anamorphosis: *Fusarium gaditjirrii* Phan, Burgess et Summerell.

*Holotypus*: Cultura exsiccata in agaro ex DAR 76664 (= CBS 116010) × DAR 76663 (= CBS 116011) (DAR 76662). Holotypus in DAR.

*Perithecia* superficial, solitary to aggregated in groups of a few and seated on a minute basal stroma, broadly pyriform, and warty; 210–294 (mean = 263) µm high, 210–350 (mean = 274) µm diam; blue-black, colour not changing in 3 % KOH, turning red in 100 % lactic acid (Fig. 2A, B). Perithecial wall 22.8–35.6 (mean = 27.3) µm thick, consisting of two regions. Outer region, including cells making up the warts, 14.4–29.4 (mean = 19.6) µm thick; cells angular to elliptic, 4.4–11.8 (mean = 8.7) µm long × 2.6–5.8 (mean = 4.2) µm, largest at the exterior and smallest toward the interior of the perithecial wall. Walls of cells 1–2 (mean = 1.4) µm thick and pigmented. Inner region 7.6–11.8 (mean = 9.4) µm thick, cells ellipsoid, 4.8–14.2 (mean = 9.1) × 0.4–2.6 (mean = 1.7) µm, increasingly more compacted and thin-walled toward the centrum, cell walls 0.4–1.2 (mean = 0.9) µm thick and pigmented. Perithecial apex continuous with the inner and the outer wall (Fig. 2C). *Asci* clavate, dehiscent, 80–120 (mean = 96) × 8–14 (mean = 10.2) µm, eight-spored (Fig. 2D). *Ascospores* ellipsoidal to obovoid, 12–16 (mean = 14.4) × 4–6 (mean = 5.3) µm, 1–3-septate, mostly 1-septate, slightly constricted at the septum (Fig. 2E).

## ***Fusarium gaditjirrii*** Phan, Burgess & Summerell **sp. nov.** MycoBank MB500073. Fig. 2F–M.

Coloniae in agaro PDA post 72 horas 25 °C 28 mm diam, 30 °C 39 mm diam, floccosae, primum candidae, deinde violaceae. Coloniae reversum cinerascens, violaceum ad atroviolaceum. Conidiophora aeria 3–4 phialides verticillatas proferentia. Sporodochia in agaro CLA numerosa, pallide aurantiaca. Microconidia longa, gracilia, hyalina, 3–5-septata, cellula basali vix indentata, cellula apicali paulo curvata, 40–70 × 3–5 µm. Microconidia in catenis longitudine media et capitulis falsis formata, hyalina, obovoidea, 0–1-septata, 8 × 2 µm, in monophialidibus, raro polyphalidibus, formata. Chlamydosporae catenatae, terminales vel intercalares.
*Holotypus*: Cultura exsiccata in agaro ex F15048 (= CBS 116011), sejuncta a ramentis plantarum (*Heteropogon triticeus*) in humo, Australia (DAR 76663). Holotypus in DAR.

*Sporodochia* pale orange, forming within 8 d on carnation leaf pieces in colonies on CLA; sporodochial phialides cylindrical and short. *Phialides* 5.5–34.2 (mean = 18.8) µm long × 2–3.2 (mean = 2.6) µm wide. Primary conidiophores terminating in verticils of 3–4 phialides. Polyphialides, if present, producing not more than 2 openings. *Macroconidia* long, slender, usually 3–5-septate, forming abundantly in sporodochia from short, cylindrical phialides, thin-walled with slightly curved apical cells and barely notched basal cells (Fig. 2H, I), 40–70 (mean = 59.3) µm long × 3–5 (mean = 3.8) µm wide. *Microconidia* forming abundantly in false heads and in medium (>15 microconidia) to long chains (>30 microconidia), arising from monophialides (Fig. 2L, M), or rarely from polyphialides. Microconidia 5–11 (mean = 8.1) µm × 1.5–3.5 (mean = 2.6) µm, obovoid, non-septate, occasionally 1-septate (Fig. 2J, K). *Chlamydospores* formed within 14 d on SNA or CLA only in darkness in most isolates, varying in abundance in different isolates, typically borne in clusters on aerial hyphae, occasionally in chains, terminal or intercalary, subglobose to cylindrical, 6.7–13.7 (mean = 9.4) × 3.5–12.7 (mean = 7.1) µm, smooth or slightly rough-walled, and hyaline (Fig. 2F, G).

*Cultural characteristics*: Colonies on PDA with floccose, white aerial mycelium, becoming greyish violet in older cultures. Pale orange conidial masses present in most isolates. Pigment on reverse side of culture greyish violet to bluish violet. Colony diameters on PDA 2.9 cm at 25 °C and 3.9 cm at 30 °C.

*Isolates examined*: Over 200 isolates of *F. gaditjirrii* have been examined. Representative isolates used for morphological description, sexual compatibility tests and molecular characterization are listed below under the accession numbers in the Fusarium Research Laboratory, The University of Sydney, Australia. **Australia**, QLD, Mareeba region, Davies Creek, cultures isolated from *Heteropogon triticeus*, collected Sep. 2001 by L.W. Burgess: F14978, *MAT-1*; F14984, *MAT-1*; F14986, *MAT-1*; F14990, *MAT-1*; F14999, *MAT-1*; F15002, *MAT-1*; F15005, *MAT-1*; F15006, *MAT-2*; F15012, *MAT-1*; F15014, *MAT-1*; F15015, *MAT-1*; F15019, *MAT-2*; F15026, *MAT-1*; F15030, *MAT-2*; F15032, *MAT-1*; collected Sep. 2002 by L.W. Burgess: F15106, *MAT-2*; culture isolated from *Themeda triandra*, collected Sep. 2001 by L.W. Burgess: F15071, *MAT-1*; Mareeba region, Walkamin Research Station, cultures isolated from *H. triticeus*, collected Sep. 2001 by L.W. Burgess: F15036, *MAT-1*; F15037, *MAT-1*; F15038, *MAT-1*; F15044, *MAT-2*; F15048, *MAT-1*; F15056, *MAT-1*; F15066, *MAT-2*; F15096, *MAT-1*; Mareeba region, Mt. Molloy, culture isolated from *H. triticeus*, collected Sep. 2001 by L.W. Burgess: F15083, *MAT-1*; North of Weipa, culture isolated from *H. triticeus*, collected Nov. 2001 by J.R. Clarkson: F15164, *MAT-2.*

### AFLP analysis

AFLP divergence between five isolates of *F. gaditjirrii* and 14 morphologically related species was estimated. A total of 235 bands was amplified and analysed.

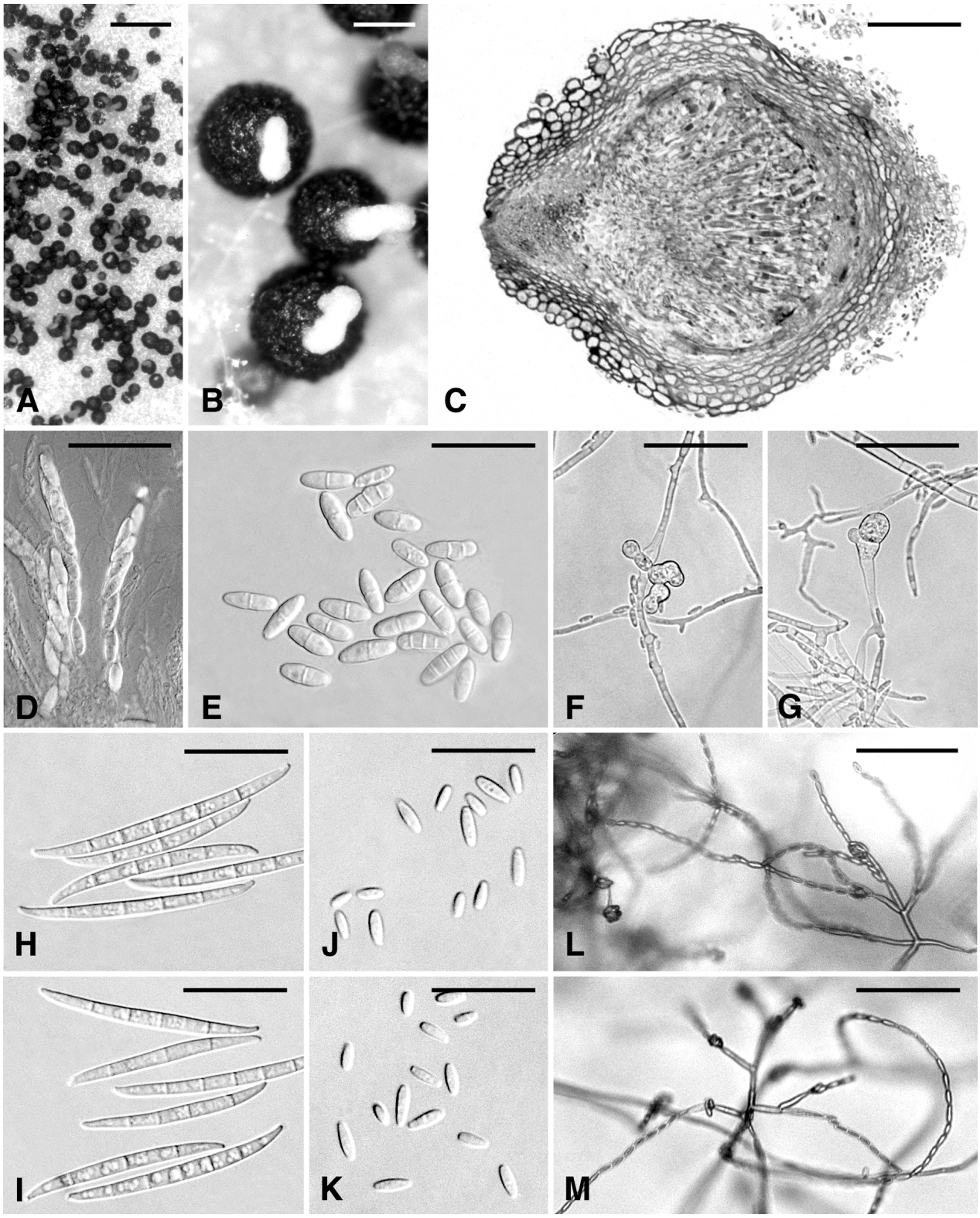


**Fig. 2.** Morphological characters of *Gibberella gaditjirriii* and *Fusarium gaditjirrii*. A, B. Perithecia. Scale bars = 1.2 mm (A), 125 µm (B). C. Longitudinal section of a perithecium stained with toluidine blue. Scale bar = 50 µm. D–E. Asci and ascospores. Scale bars = 40 µm (D), 25 µm (E). F–G. Chlamydospores produced in SNA cultures. Scale bars = 50 µm. H–I. Macroconidia. Scale bars = 25 µm. J–K. Microconidia. Scale bars = 25 µm. L–M. Microconidia produced in chains and false heads in CLA cultures. Scale bars = 50 µm.

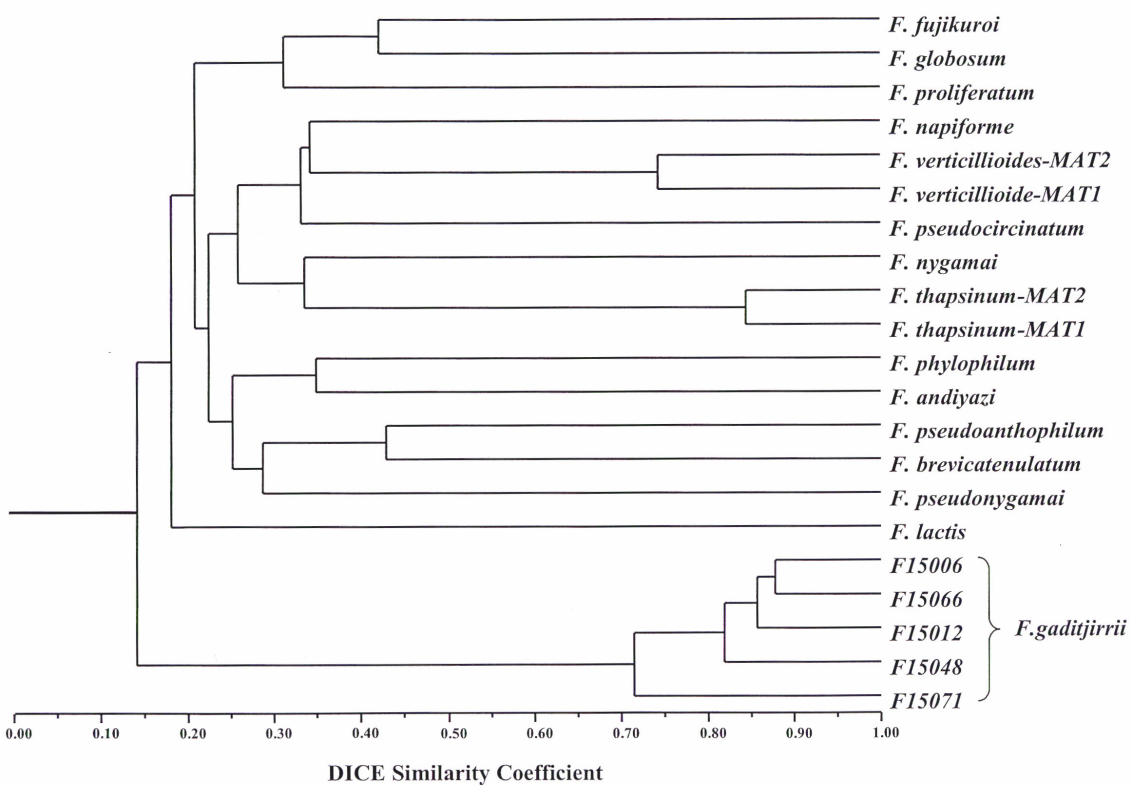


**Fig. 3.** Dendrogram derived from UPGMA cluster analysis of AFLP similarities of *Fusarium gaditjirrii* and morphologically related species.

Based on this analysis, the genetic relationships among all isolates studied are presented in the form of a dendrogram (Fig. 3).

All *F. gaditjirrii* isolates clustered together with more than 70 % similarity and were distinct from the isolates of the other species analysed. No other species had more than 20 % similarity with the new species. We also compared AFLP profiles of 60 isolates of *F. gaditjirrii* and all isolates were shown to be within 70 % similarity (results not given).

**Phylogenetics - DNA sequence analysis**

The β-tubulin gene data set consisted of 526 nucleotide characters, of which 65 were parsimony-informative. WP analysis yielded 54 equally most-parsimonious trees of 158 steps (CI = 0.848, RI = 0.911) (Fig. 4). The 678 bp elongation factor 1-α gene data set, which included 152 parsimony-informative characters, yielded 3 equally most-parsimonious trees of 418 steps (CI = 0.737, RI = 0.784) (Fig. 5). The calmodulin gene data set consisted of 672 nucleotide characters, of which 68 were parsimony-informative. Weighted parsimony analysis yielded 36 equally most-parsimonious trees of 160 steps (CI = 0.869, RI = 0.917) (Fig. 6).

For each gene, no major topological variations were detected among trees obtained using the different optimality criteria (MP, WP, ML and NJ) (results not shown). However, slight incongruities were observed among the different gene trees. Nevertheless, in all trees isolates of *F. gaditjirrii* formed a strongly supported clade with bootstrap confidence of 100 %.

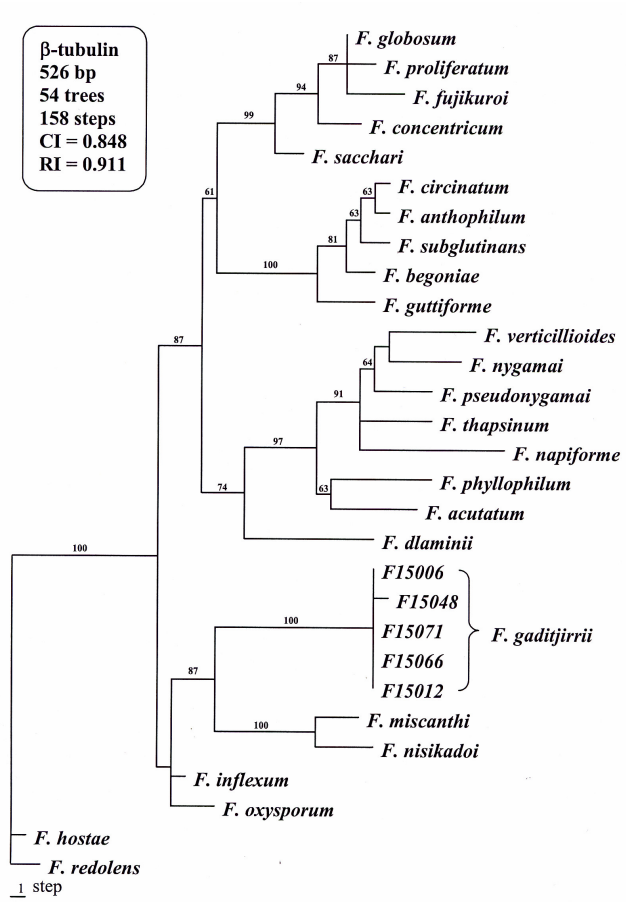


**Fig. 4.** One of 54 most-parsimonious trees inferred from β-tubulin gene sequences of *F. gaditjirrii* and closely related species. Bootstrap values > 50 % from 1000 maximum parsimony replications are indicated above the nodes.

Genealogical analysis of the three genes clearly distinguished *F. gaditjirrii* isolates as a separate clade from all other related groups.

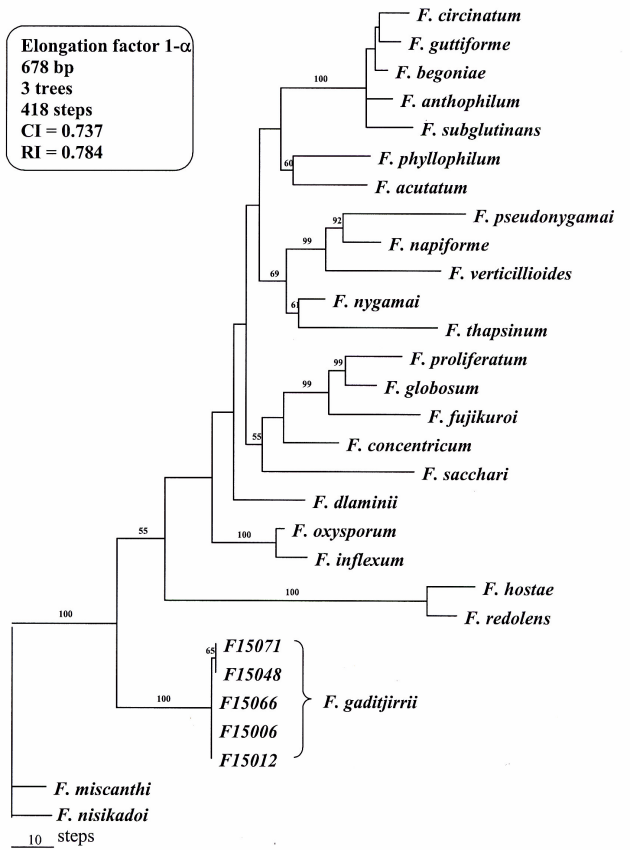


**Fig. 5.** One of the 3 most-parsimonious trees inferred from elongation factor 1-α gene sequences of *F. gaditjirrii* and closely related species. Bootstrap values > 50 % from 1000 maximum parsimony replications are indicated above the nodes.

However, the position of *F. gaditjirrii* among these groups is not clearly resolved due to conflicts among the gene trees.

**Sexual compatibility**

Seventy-six *MAT-1* and 56 *MAT-2* isolates were determined with the PCR-based assays. None of the isolates of *F. gaditjirrii* produced fertile crosses with the tester strains of *G. moniliformis, G. sacchari, G. intermedia, G. subglutinans, G. thapsina* or *G. konza*. Two female fertile isolates, F15017 (*MAT-2*) and F15019 (*MAT-2*) produced fertile crosses with 51 *MAT-1* isolates when all the putative isolates of opposite mating types were intercrossed. No fertile crosses were obtained from any self-cross tests. Twenty-five isolates were infertile as male (male-sterile).

No *MAT-1* female fertile isolate was obtained from any of the intra-specific crosses. These results suggest that the number of female fertile strains of this population is probably very low in nature.

Among 132 isolates studied, *MAT-1* and *MAT-2* segregated 76 : 56, giving an $N_e$ of approximately 97.7 % of the count based on mating type ratio. Based on female fertility, the $N_e$ is 7.2 % of the count excluding the male-sterile isolates and 5.9 % of the count if the male-sterile isolates are included.

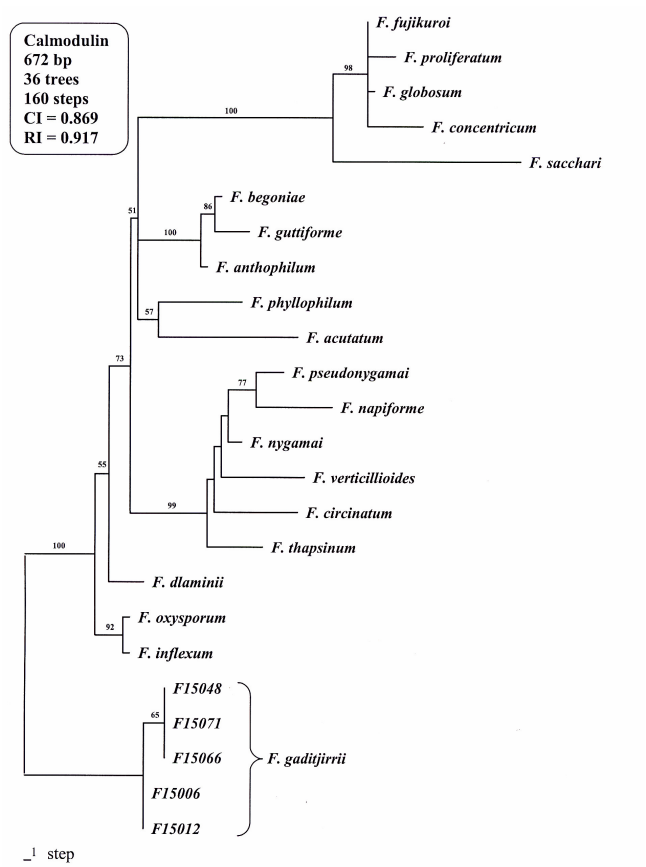


**Fig. 6.** One of 36 most-parsimonious trees inferred from calmodulin gene sequences of *F. gaditjirrii* and closely related species. Bootstrap values > 50 % from 1000 maximum parsimony replications are indicated above the nodes.

These values were lower than those reported for *G. moniliformis, G. thapsina, G. intermedia*, *G. circinata* and *G. konza* (Leslie & Klein 1996, Britz *et al.* 1998, Chulze *et al.* 2000, Zeller *et al.* 2003). The viability of ascospores was greater than 90 %.

## DISCUSSION

*Gibberella gaditjirrii* (*Fusarium gaditjirrii*) is described as a new species based on morphological characters, sexual compatibility, AFLP and phylogenetic analysis of DNA sequences. DNA fragment analysis on the basis of overall genome scan (AFLP) and sequence analysis of three gene regions (β-tubulin, elongation factor 1-α and calmodulin) strongly indicate that *F. gaditjirrii* is distinct from all other species of this genus included in the study. In the AFLP analysis isolates of *F. gaditjirrii* clustered together with more than 70 % similarity while having less than 20 % similarity with all of the other species.

**Table 4.** Comparison of key morphological characters of *Fusarium* species that form chains of microconidia in aerial mycelia on SNA.[a]

| Species | Shape of microconidia | | | | Chains of microconidia | | Phialide | | Chlamydospores |
|---|---|---|---|---|---|---|---|---|---|
| | Clavate | Obovoid | Pyriform | Globose | Short (<15 conidia) | Medium to long (>15 conidia) | Polyphialidic | Monophialidic | |
| *Fusarium andiyazi*[b] | +[c] | – | – | – | – | + | – | + | +[h] |
| *F. annulatum* | + | – | – | – | – | + | + | + | – |
| *F. brevicatenulatum* | – | + | – | – | +[f] | – | +[g] | + | – |
| *F. fractiflexum* | + | – | – | – | + | +[f] | + | + | – |
| *F. fujikuroi* | + | – | – | – | – | + | + | + | – |
| *F. gaditjirriii* | + | + | – | – | – | + | (+) | + | + |
| *F. globosum* | (+)[d] | + | – | + | + | (+) | + | + | – |
| *F. lactis* | – | + | – | – | + | (+) | + | + | – |
| *F. miscanthi* | + | – | + | – | – | + | +[g] | + | – |
| *F. napiforme* | + | (+) | + | – | – | + | – | + | + |
| *F. nisikadoi* | + | – | – | – | – | + | +[g] | + | – |
| *F. nygamai* | (+) | + | – | – | + | – | + | + | + |
| *F. phyllophilum* | + | – | – | – | + | – | + | + | – |
| *F. proliferatum* | + | – | (+) | – | – | + | + | + | – |
| *F. pseudoanthophilum* | + | + | (+) | – | (+) | – | + | + | + |
| *F. pseudocircinatum* | – | + | – | – | + | – | +[g] | + | – |
| *F. pseudonygamai* | (+) | + | – | – | + | (+) | +[g] | + | –[i] |
| *F. thapsinum* | + | + | +[e] | – | – | + | – | + | – |
| *F. verticillioides* | + | – | – | – | – | + | – | + | +[j] |

[a]*F. decemcellulare* forms chains of microconidia but is not included as it can be differentiated from the other species on morphological criteria (Burgess *et al.* 1994).
[b]The characters of *F. andiyazi* on SNA were observed in isolates KSU 04647 and KSU 11155 by us, original description was based on CLA (Marasas *et al.* 2001).
[c] + / – Indicates the presence / absence of the character.
[d](+) Indicates that the character is not present in all isolates of this species.
[e]The formation of pyriform conidia in the reference cultures KSU 01087, KSU 00966, KSU 04093 was confirmed by Tijana Petrovic (pers. comm.).
[f]Indicates the presence of this character under black light condition.
[g]Indicates that the character is rarely, occasionally or sometimes present according to original description (Nirenberg & Aoki 1997; Nirenberg & O'Donnell 1998; Gams *et al.* 1999).
[h]Indicates the presence of pseudochlamydospores (Marasas *et al.* 2001).
[i]Indicates the absence of true chlamydospores, but a few swollen hyphal cells are present in chains (Nirenberg & O'Donnell 1998).
[j]Indicates the presence of thickened hyphae (Gerlach & Nirenberg 1982).

On the basis of genealogical concordance of gene trees obtained from the three different gene regions, *F. gaditjirrii* isolates are shown to represent a distinct clade from other sister groups. Although these phylogenetically distinct groups are closely related to each other, the precise inter-relationships among them cannot be conclusively resolved due to incongruity among gene trees.

*Fusarium gaditjirrii* may not be reliably differentiated from other species using morphological criteria (Table 4). The majority of wild-type cultures of this species produced chlamydospores which differentiate it from most other described species that form microconidia in chains. However, cultures of *F. gaditjirrii* that have been subcultured repeatedly can lose the ability to form chlamydospores. Furthermore, there may be other undescribed populations in nature that are morphologically similar to, but differ genetically from, *F. gaditjirrii*. The issues related to cryptic species have been discussed in detail by Steenkamp *et al.* (2002). Consequently, we recommend that identification based on morphological markers be confirmed using sexual compatibility studies and DNA analysis.

Although only two female fertile isolates were found in 132 isolates of *F. gaditjirrii,* abundant perithecia were produced from successful crosses with these two female fertile isolates. The low effective population number based on hermaphrodite frequency suggests that sexual reproduction in *G. gaditjirrii* is probably not common in nature. This situation is similar to that found in *G. konza* (Zeller *et al.* 2003). *Fusarium gaditjirrii* was commonly isolated from the lower stem region of *H. triticeus* from the Mareeba and Cape York areas of northern Australia (Fig. 1), accounting for 30 % of the total *Fusarium* isolates recovered in a community study (authors' unpubl. data). It was isolated less commonly from *T. triandra* in a parallel community study in the Mareeba area. However *F. gaditjirrii* was not isolated from *Coix gasteenii* B.K. Simon (Johansen pers. comm.) or *Sorghum plumosum* (R. Br.) P. Beauv. var. *plumosum* from Lakefield National Park in Cape York (Walsh pers. comm.) from this northern region. It was isolated infrequently from *Sorghum interjectum* Lazarides from Litchfield National Park, Northern Territory, Australia in recent community studies (Walsh pers. comm.).

*Fusarium gaditjirrii* has not been isolated from the following hosts during intensive community studies in the sub-tropical/warm temperate region of northern-western New South Wales, Australia: *Sorghum bicolor* (L.) Moench. (Petrovic pers. comm.), *Sorghum halepense* (L.) Pers. (Lee *et al.* pers. comm.) and *Austrostipa aristiglumis* (F. Muell.) S.W.L. Jacobs & J. Everett. (Bentley pers. comm.).

These data indicate that *F. gaditjirrii* is likely to be relatively common in the tropical tall grass regions of northern Australia where *H. triticeus* is a common component of the grass flora. Furthermore, it indicates that *F. gaditjirrii* is less abundant or does not occur in the cooler sub-tropical/warm temperate region of New South Wales. Tropical tall grasses such as *H. triticeus* do not occur in New South Wales.

We note that *F. konzum* was isolated from surface-sterilised prairie grass stems from Kansas, U.S.A. (Zeller *et al.* 2003) and the authors concluded that it was existing as an endophyte or latent plant pathogen (Sinclair & Cerkauskas 1996, Stone *et al.* 2000) in these grasses. *Fusarium gaditjirrii* was also isolated from surface-sterilised stems of *H. triticeus* indicating that it may also colonise this and other grasses as an endophyte or a latent plant pathogen. Further studies are needed to clarify the relationship of *F. gaditjirrii* with *H. triticeus* and other grasses.

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## REFERENCES

Britz H, Coutinho TA, Wingfield MJ, Marasas WFO, Gordon TR, Leslie JF (1999). *Fusarium subglutinans* f. sp. *pini* represents a distinct mating population in the *Gibberella fujikuroi* species complex. *Applied and Environmental Microbiology* **65**: 1198–1201.

Britz H, Wingfield MJ, Coutinho TA, Marasas WFO, Leslie JF (1998). Female fertility and mating type distribution in a South African population of *Fusarium subglutinans* f. sp. *pini*. *Applied and Environmental Microbiology* **64**: 2094–2095.

Burgess LW, Nelson PE, Toussoun TA, Forbes GA (1988). Distribution of *Fusarium* species in sections *Roseum, Arthrosporiella, Gibbosum* and *Discolor* recovered from grassland, pasture and pine nursery soils of eastern Australia. *Mycologia* **80**: 815–824.

Burgess LW, Summerell BA (1992). Mycogeography of *Fusarium*: survey of *Fusarium* species in subtropical and semi-arid grassland soils from Queensland, Australia. *Mycological Research* **96**: 780–784.

Burgess LW, Summerell BA, Bullock S, Gott KP, Backhouse D (1994). *Laboratory manual for Fusarium research.* Fusarium Research Laboratory, The University of Sydney, Australia.

Burgess LW, Trimboli D (1986). Characterization and distribution of *Fusarium nygamai* sp. nov. *Mycologia* **78**: 223–229.

Chulze SN, Ramírez ML, Torres A, Leslie JF (2000). Genetic variation in *Fusarium* section *Liseola* from no-

till maize in Argentina. *Applied and Environmental Microbiology* **66**: 5312–5315.

Fisher NL, Burgess LW, Toussoun TA, Nelson PE (1982). Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* **72**: 151–153.

Gams W, Klamer M, O'Donnell K (1999). *Fusarium miscanthi* sp. nov. from *Miscanthus* litter. *Mycologia* **91**: 263–268.

Gerlach W, Nirenberg H (1982). The genus *Fusarium* - a pictorial atlas. *Mitteilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft*, Berlin-Dahlem **209**: 1–405.

Hasegawa M, Kishino K, Yano TA (1985). Dating of human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **21**: 160–174.

Kerényi Z, Zeller KA, Hornok L, Leslie JF (1999). Molecular standardization of mating terminology in the *Gibberella fujikuroi* species complex. *Applied and Environmental Microbiology* **65**: 4171–4076.

Klittich CJR, Leslie JF (1988). Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics* **118**: 417–423.

Kornerup A, Wanscher JH (1963). *Methuen handbook of colour*. Methuen & Co Ltd, London, UK.

Leslie JF, Klein KK (1996). Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics* **144**: 557–567.

Marasas WFO, Rheeder JP, Lamprecht SC, Zeller KA, Leslie JF (2001). *Fusarium andiyazi* sp. nov., a new species from sorghum. *Mycologia* **93**: 1203–1210

Nash SM, Snyder WC (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology* **52**: 567–572.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences USA* **76**: 5269–5273.

Nelson PE, Toussoun TA, Burgess LW (1987). Characterization of *Fusarium beomiforme* sp. nov. *Mycologia* **79**: 884–889.

Nirenberg H (1976). Untersuchungen über die morphologische und biologische differenzierung in der *Fusarium*-Sektion *Liseola*. *Mitteilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft*, Berlin-Dahlem **169**: 1–111.

Nirenberg HI, Aoki T (1997). *Fusarium nisikadoi*, a new species from Japan. *Mycoscience* **38**: 329–333.

Nirenberg H, O'Donnell K (1998). New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* **90**: 434–458.

O'Donnell K, Cigelnik E (1997). Two devergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**: 103–116.

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences, USA* **95**: 2044–2049.

O'Donnell K, Nirenberg HI, Aoki T, Cigelnik E (2000). A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. *Mycoscience* **41**: 61–78.

Sangalang AE, Burgess LW, Backhouse D, Duff J, Wurst M (1995a). Mycogeography of *Fusarium* species in soils from tropical, arid and mediterranean regions of Australia. *Mycological Research* **99**: 523–528.

Sangalang AE, Summerell BA, Burgess LW, Backhouse D (1995b). Taxonomy of *Fusarium*: characterisation of *Fusarium avenaceum* subsp. *aywerte* and *Fusarium avenaceum* subsp. *nurragi*. *Mycological Research* **99**: 287–290.

Schilling AG (1996). *Characterization and differentiation of the cereal pathogens Fusarium culmorum and F. graminearum by PCR-based molecular markers*. Ph.D. thesis. Stuttgart, Germany.

Sinclair JB, Cerkauskas RF (1996). Latent infection vs. endophytic colonization by fungi. In: *Endophytic fungi and grasses and woody plants: systematics, ecology and evolution* (Redlin SC, Carris LM, eds). APS Press: St. Paul, MN, U.S.A.: 3–29.

Steenkamp ET, Britz H, Coutinho TA, Wingfield BD, Marasas WFO, Wingfield MJ (2000). Molecular characterization of *Fusarium subglutinans* associated with mango malformation. *Molecular Plant Pathology* **1**: 187–193.

Steenkamp ET, Wingfield BD, Desjardins AE, Marasas WFO, Wingfield MJ (2002). Cryptic speciation in *Fusarium subglutinans*. *Mycologia* **94**: 1032–1043.

Stone JK, Bacon CW, White JF (2000). An overview of endophytic microbes: Endophytism defined. In: *Microbial endophytes* (Bacon CW, White JF, eds). Marcel Dekker, Inc.: New York, U.S.A.: 3–29.

Summerell BA, Rugg CA, Burgess LW (1993). Mycogeography of *Fusarium*: survey of *Fusarium* species associated with forest and woodland communities in north Queensland, Australia. *Mycological Research* **97**: 1015–1019.

Swofford DL (1998*). PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods).* Version 4. Sinauer Associates: Sunderland, Massachusetts.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876–4882.

Vos P, Hogers R, Bleeker M, Reijans M, Lee Tvd, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407–4414.

Wang B, Brubaker CL, Burdon JJ (2004). *Fusarium* species and *Fusarium* wilt pathogens associated with native *Gossypium* populations in Australia. *Mycological Research* **108**: 35–44.

Yunupingu B, Yunupingu-Marika L, Marika D, Marika B, Marika R, Wightman G (1995). Rirratjinu ethnobotany: Aboriginal plant use from Yirrkala, Arnhem Land, Australia. *Northern Territory Botanical Bulletin* **21**: 1–112.

Zeller KA, Jurgenson JE, El-Assiuty EM, Leslie JF (2000). Isozyme and Amplified Fragment Length Polymorphisms from *Cephalosporium maydis* in Egypt. *Phytoparasitica* **28**: 121–130.

Zeller KA, Summerell BA, Bullock S, Leslie JF (2003). *Gibberella konza* (*Fusarium konzum*) sp. nov. from

prairie grasses, a new species in the *Gibberella fujikuroi* species complex. *Mycologia* **95**: 943–954.