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Fusarium fractiflexum sp. nov. and two other species within the *Gibberella fujikuroi* species complex recently discovered in Japan that form aerial conidia in false heads

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Morphological and molecular phylogenetic analyses were conducted on 12 strains of *Fusarium*, deposited in MAFF as *F. subglutinans* (≡*F. moniliforme* var. *subglutinans*≡*F. sacchari* var. *subglutinans*) or *Fusarium* sp. because they formed aerial conidia in false heads in the dark. These strains were resolved as three distinct species within the *Gibberella fujikuroi* species complex. A new species, *F. fractiflexum*, and two species new to Japan, *F. circinatum* and *F. concentricum*, are described and illustrated and their morphological features are discussed. *Fusarium fractiflexum*, isolated from diseased yellow leaf spots of *Cymbidium* spp., is differentiated from other fusaria based on its yellowish colonies and aerial conidia formed in false heads in the dark and in zigzag-like conidial chains under black light. Japanese strains of *F. circinatum* also formed elongate, coiled sterile hyphae. Phialidic aerial conidia with a pointed apex and a wedge-shaped base were found in *F. concentricum* cultured under black light and represent a new diagnostic character of the species, in addition to colonies with alternating concentric rings when cultured on PDA. Based on DNA sequences of the β-tubulin gene and two other loci, strains of *F. fractiflexum* were resolved phylogenetically as members of the Asian clade of the *G. fujikuroi* species complex. In addition, Japanese strains of *F. circinatum* and *F. concentricum* were phylogenetically identical to the ex-type strains.

Key Words—false heads; *Fusarium circinatum*; *Fusarium concentricum*; *Fusarium fractiflexum*; *Gibberella fujikuroi* species complex.

Fusarium species that form aerial conidia only in false heads within the *Gibberella fujikuroi* (Sawada) Wollenw. species complex (the GF species complex), or section *Liseola*, have long been identified as a single taxon, *F. subglutinans* (Wollenw. & Reinking) P. E. Nelson, Toussoun & Marasas (≡*F. moniliforme* J. Sheld. var. *subglutinans* Wollenw. & Reinking), similar to the case of *F. moniliforme* (var. *moniliforme*) for species within the GF species complex that form conidial chains (Snyder and Hansen, 1945; Booth, 1971; Toussoun and Nelson, 1976). In contrast, Wollenweber and Reinking (1935), Nirenberg (1976) and Gerlach and Nirenberg (1982) recognized three to six taxa at the rank of species or variety, i.e., *F. sacchari* (E. J. Butler) W. Gams var. *sacchari* (autonym), var. *elongatum* Nirenberg, var. *subglutinans* (Wollenw. & Reinking) Nirenberg (≡*F. moniliforme* var. *subglutinans*), *F. anthophilum* (A. Braun) Wollenw., *F. succisae* (J. Schröt.) Sacc., *F. neoceras* Wollenw. & Reinking. Nelson et al. (1983), Burgess et al. (1988) and Burgess et al. (1994) recognized two species that form conidia in false heads within section *Liseola*, i.e., *F. anthophilum* and *F. subglutinans*. Species within the GF species complex, however, were reevaluated recently based on combined morphological and molecular-phylogenetic analyses (O'Donnell et al., 1998a; Nirenberg and O'Donnell, 1998). Species forming false heads within the complex were recognized as 16 different species.

Names for *Fusarium* strains stored in MAFF (Genebank, Genome and Biodiversity Research Center, National Institute of Agrobiological Sciences, Kannondai, Tsukuba, Ibaraki, Japan) have long been based on several different outdated classification systems such as Snyder and Hansen (1940, 1941, 1945), Booth (1971) and Nelson et al. (1983), depending on when each strain was first identified and accessioned. For this reason, 12 false-head-forming MAFF strains within the GF species complex were investigated morphologically and molecular-phylogenetically. This research has resulted in the discovery of a new species of *Fusarium* and the presence of two heretofore unreported species within the GF species complex from Japan. They are described and illustrated together with their phylogenetic relationships. Further, an additional diagnostic character for *F. concentricum* was discovered.

Materials and Methods

Strains examined Among *Fusarium* strains deposited in MAFF, 12 strains (Table 1) identified previously as *F. subglutinans* (≡*F. moniliforme* var. *subglutinans* ≡ *F. sacchari* var. *subglutinans*) or related unidentified *Fusarium* species within the GF species complex, forming false heads of aerial conidia, were re-evaluated morphologically and molecular-phylogenetically. The strains are stored cryogenically at −175°C or by lyophilization in MAFF and in NRRL (the Agriculture Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois, USA).

Examination of taxonomic characters Strains were grown at 20°C in 9-cm plastic Petri dishes on potato dextrose agar (PDA; Difco, Detroit, MI) in the dark to evaluate colony color, odor and growth rates. All microscopic studies and measurements were made on synthetic low nutrient agar (SNA; per liter dist. H_2O: 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g dextrose, 0.2 g sucrose, 0.6 ml 1 N NaOH, 23 g agar) with two ca. 1 × 2 cm pieces of sterile filter paper placed on the cooled agar surface (Nirenberg, 1976). Cultures were incubated for 14 d at 20°C either in complete darkness or under continuous black light (2 × fluorescent tube of National FL8BL-B 8 W/08; Nirenberg, 1990). Measurement of 0-septate aerial conidia is based on 50 conidia and that of 1-septate aerial and 3-septate sporodochial conidia on 30 conidia, in each case selected randomly from individual isolates incubated under each culture condition. Minimal and maximal sizes, arithmetic means and standard deviations (S.D.) were obtained for each character from each of the strains. For morphological comparison, *Fusarium circinatum* Nirenberg & O'Donnell NRRL 25331 (=BBA 69720, the ex-holotype strain from *Pinus radiata* D. Don), *Fusarium concentricum* Nirenberg & O'Donnell NRRL 25181 (=BBA 64354, the ex-holotype strain from *Musa sapientum* L.), NRRL 25202 (=BBA 68483), NRRL 25666 (=BBA 69855), NRRL 25667 (=BBA 69856), *Fusarium lactis* Pirotta & Riboni NRRL 25200 (=BBA 68590, the ex-neotype strain from *Ficus carica* L.), NRRL 25338 (=BBA 68591), NRRL 25339 (=BBA 69866), and *Fusarium subglutinans* MP-E, NRRL 20844 (=BBA 63621), NRRL 20981 (=BBA 65921), NRRL 22034 (=BBA 62275) (Nirenberg and O'Donnell, 1998; O'Donnell et al., 1998a) were also examined under the same culture conditions. All colors are given according to the Methuen Handbook of Colour (Kornerup and Wanscher, 1978).

Molecular biology Genomic DNA was prepared for the polymerase chain reaction (PCR) using a protocol described previously (O'Donnell and Cigelnik, 1997). PCR primers have been published for amplification and sequencing of the mitochondrial small subunit ribosomal DNA (mtSSU rDNA) (White et al., 1990; O'Donnell and Cigelnik, 1997), β-tubulin (O'Donnell and Cigelnik, 1997), and translation elongation factor (EF-1α) exons and introns (O'Donnell et al., 1998b). An Applied Biosystems 377 automated DNA sequencer (Perkin-Elmer, Foster City, California) was used to analyze all fluorescent-labeled BigDye sequencing reaction mixtures. Sequences have been deposited in GenBank under accession numbers AF333928-AF333951, or under numbers reported in O'Donnell et al. (1998a) and O'Donnell et al (2000b).

Phylogenetic analysis Results of the Wilcoxon Signed-Rank (WS-R) Templeton test implemented in PAUP*4.0b3a (Swofford, 1998), as described in O'Donnell et al. (2000a), indicated that aligned sequences from the three partitions could be analyzed as a combined dataset. Unweighted maximum parsimony analysis of the combined data was performed via a heuristic search with 1000 stepwise-addition sequences in PAUP*4.0b3a (Swofford, 1998) with MULPARS function on and with tree bisection-reconnection branch swapping.

Results

Descriptions of species Among the strains examined, the following three species of *Fusarium*, i.e., a new species and two species new to Japan, were identified based on the present morphological and molecular phylogenetic analyses.

Fusarium fractiflexum T. Aoki, O'Donnell & Ichikawa, sp. nov. Figs. 1–18.

Coloniae in PDA in quoque die ad 20°C in tenebris in proportione 2.0–3.4 mm crescentes, laxe vel dense floccosae, ad marginem integrae; reversum dilute flavum vel griseoflavum vel griseoaurantium, nonnumquam centraliter purpureogriseum. Mycelium aerium albidum vel dilute flavum, parcum, partim funiculosum. Corpora sclerotialia absentia. Odor indistinctus vel dulcis. Sporulatio in SNA in mycelio aerio et sporodochiis ad initio aetatis exordiens. Conidiophora aeria plerumque erecta, nonnumquam prostrata, primum simplicia, deinde parce, opposite vel alternatim vel sympodialiter ramosa, in phialides verticillatas exeuntia. Conidiophora nonnumquam sympodialiter proliferantia. Phialides aeriae simplices (monophialides) vel sympodialiter proliferantes (polyphialides), plerumque cylindricae, sursum attenuatae, ad 35 μm × 1.5–3.5 μm. Conidia in tenebris in mycelio aerio capitulatim massa vel in catenis brevibus irregularibus ordinata, sub illuminationem nigram saepe catenas longas fractiflexas ex conidiis usque ad 30 (raro ad 43) compositas formantia, interdum cephaloidea, raro in catenis regularibus brevibus cohaerentia. Conidia aeria 0–1(–3)–septata, ellipsoidea vel clavata, aliquando cylindrica et utrinque rotundata vel basi truncata, plerumque recta, nonnumquam leviter curvata, ubi 0-septata in tenebris 4–17 × 1.5–5.5 μm et sub illuminationem nigram 5–23 × 1.5–4.5 μm, ubi 1-septata in tenebris 10–32 × 2–4 μm et sub illuminationem nigram 8.5–37 × 2–4 μm. Conidia sporodochialia falcata vel cylindrica, cellulam apicalem acutam et cellulam basilarem pediformem praebentia, (1–)3(–5)–septata, ubi 3-septata in tenebris 20.5–63 × 2–5 μm et sub illuminationem nigram 28–76 × 2.5–4.5 μm. Chlamydosporae absentes.

Holotypus: Cultura sicca (MAFF 237529), isolata e *Cymbidio* sp. morbo affecto a K. Ichikawa, 1992, Enzan,

Table 1. Strains of examined *Fusarium* species from Japan, forming false heads of aerial conidia.

Species (deposited previously as)	MAFF[a] No.	Geographic Origin	Host/substrate	Isolator (year)	Equivalent No.[a]
Fusarium circinatum					
(*F. moniliforme* var. *subglutinans*)	236397	Japan, Kagoshima	*Pinus luchuensis* (pitch canker)	T. Kobayashi (1992)	NRRL 26431
(*F. moniliforme* var. *subglutinans*)	236399	Japan, Okinawa	*Pinus luchuensis* (pitch canker)	T. Kobayashi (1986)	NRRL 26432
(*F. subglutinans*)	237756	Japan, Amami Ohshima isl.	*Pinus luchuensis* (pitch canker)	T. Kobayashi (1991)	NRRL 29945
Fusarium concentricum					
(*Fusarium* sp.)	237649	Japan, Tsukuba, Ibaraki	*Oryza sativa* (root)	T. Aoki (1990)	NRRL 25303=BBA 69021
(*Fusarium* sp.)	237650	Japan, Ibusuki, Kagoshima	*Triticum aestivum* (seed)	T. Aoki (1992)	NRRL 25309=BBA 69012
(*Fusarium* sp.)	238110	Japan, Tsukuba, Ibaraki	soil of *Pinus densiflora* forest	T. Aoki (1990)	NRRL 29943
(*Fusarium* sp.)	238111	Japan, Tsukuba, Ibaraki	*Oryza sativa* (stem base)	T. Aoki (1990)	NRRL 26434
(*Fusarium* sp.)	238112	Japan, Tsukuba, Ibaraki	*Oryza sativa* (root)	T. Aoki (1990)	NRRL 29944
Fusarium fractiflexum					
(*F. sacchari* var. *subglutinans*)	237529[b]	Japan, Enzan, Yamanashi	*Cymbidium* sp. (leaf spot)	K. Ichikawa (1992)	NRRL 28852
(*F. sacchari* var. *subglutinans*)	237530	Japan, Hiroshima	*Cymbidium* sp. (leaf spot)	K. Ichikawa (1993)	NRRL 26794=BBA 70371
(*F. sacchari* var. *subglutinans*)	237531	Japan, Hiroshima	*Cymbidium* sp. (leaf spot)	K. Ichikawa (1993)	NRRL 28853
(*F. sacchari* var. *subglutinans*)	237532	Japan, Komoro, Nagano	*Cymbidium* sp. (leaf spot)	K. Ichikawa (1995)	NRRL 28854

[a] MAFF=National Institute of Agrobiological Resources, Tsukuba, Japan; NRRL=The Agriculture Research Service Culture Collection, National Center for Agricultural Utilization Research, USDA/ARS, Peoria, IL, USA; BBA=Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin, Germany.

[b] Ex-holotype strain.

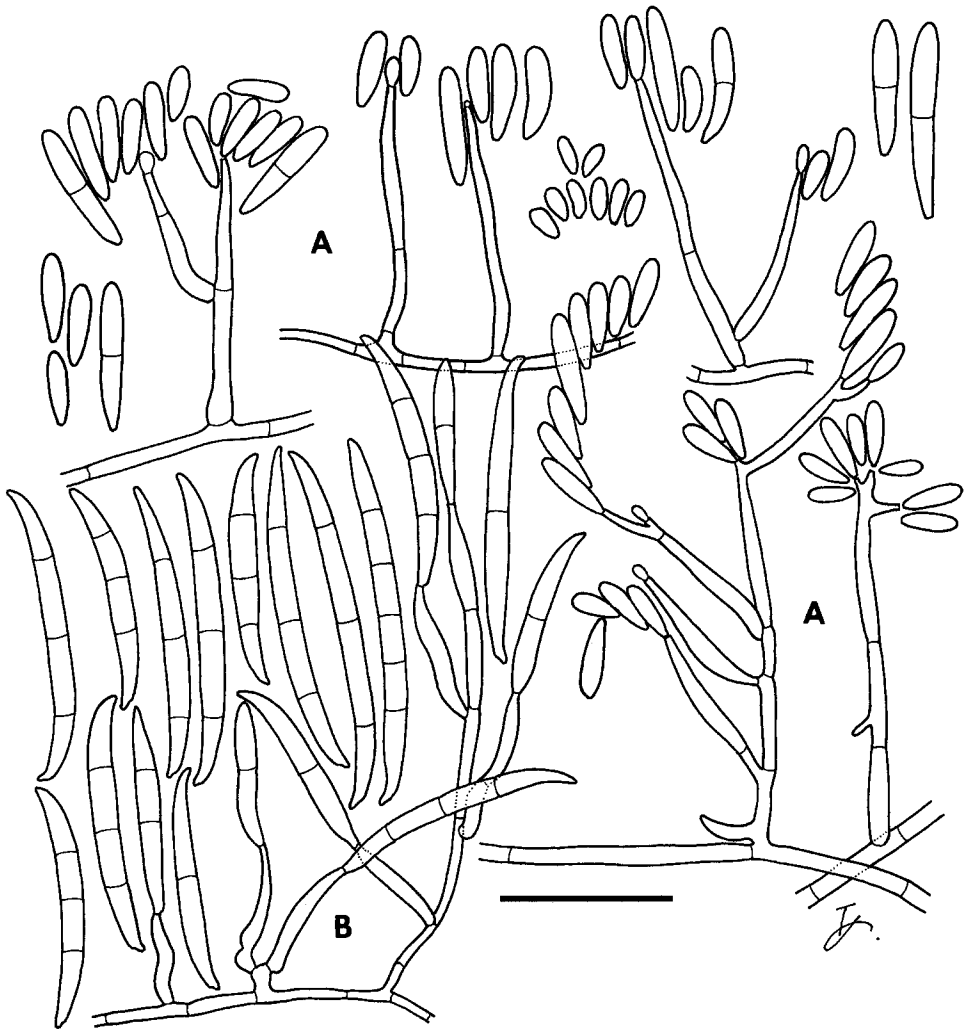


Fig. 1. *Fusarium fractiflexum* (MAFF 237529) grown on SNA in the dark. A. Aerial conidiophores with simple or proliferating conidiogenous loci, forming ellipsoidal-to-clavate conidia in false heads or adhering in side-by-side masses. Conidia possessing a rounded apex and a rounded-to-truncate base. B. Sporodochial conidia and conidiophores with phialides. Conidia consisting of an acuate apical cell and a foot-like basal cell. Sporodochial phialides produced sometimes directly on the substrate hyphae. Scale bar=25 μm.

Yamanashi Pref. in Japonia (Herb. NIAES 20515).

Colonies on PDA showing mycelial growth rates of 2.0–3.4 mm per d at 20°C in the dark. Colony margins entire. Aerial mycelia white (1A1) and partly pale yellow (4A3)-to-yellowish white (4A2), sparse, loosely-to-densely floccose, sometimes funiculose. Pigmentation in the reverse yellowish white (4A2), pale yellow (4A3), light yellow (4A4), greyish yellow (4B4)-to-greyish orange (5B5), sometimes purplish grey (13-14B2) at the center. Sclerotial bodies absent. Odor absent or, if present, sweet. Sporulation on SNA starting early in the aerial mycelium and in sporodochia. Sporodochia observed under all light conditions, on or in the agar. Aerial conidiophores mostly erect, some prostrate, at first unbranched, later branched sparsely to up to 3 times, often oppositely, alternately, or sympodially, terminating in verticillate phialides. Sympodially proliferating conidiophores sometimes present. Conidiogenous cells on the aerial conidiophores monophialidic or polyphialidic with up to 3 openings; phialides almost cylindrical, tapering toward the apex, up to 34.5 μm long and 1.5–3.5 μm wide. Conidia borne in the aerial mycelium in the dark arranged in false heads or rarely in very short irregular chains; under black light often forming long zigzag-like chains of up to 30 (rarely up to 43) conidia, some in false heads and rarely in short linear chains. Aerial conidia 0–1(–3)–septate, ellipsoidal-to-clavate, some cylindrical with rounded-to-truncate ends, mostly straight, some slightly curved, also obovate in some strains, 0-septate: measuring 4–17 × 1.5–5.5 μm in total range, 8.5–9.7 × 2.3–2.6 μm on average (ex type: 5–17 × 1.5–5.5 μm, 9.7 ± 2.9 × 2.47 ± 0.56 μm on average and S.D.) in complete darkness, 5–23 × 1.5–4.5 μm in total range, 10.5–12.4 × 2.6–2.9 μm on average (ex type: 7.5–16 × 2–3.5 μm, 10.5 ± 2.1 × 2.73 ± 0.37 μm on average and S.D.) under black light; 1-septate: 10–32 × 2–4 μm in

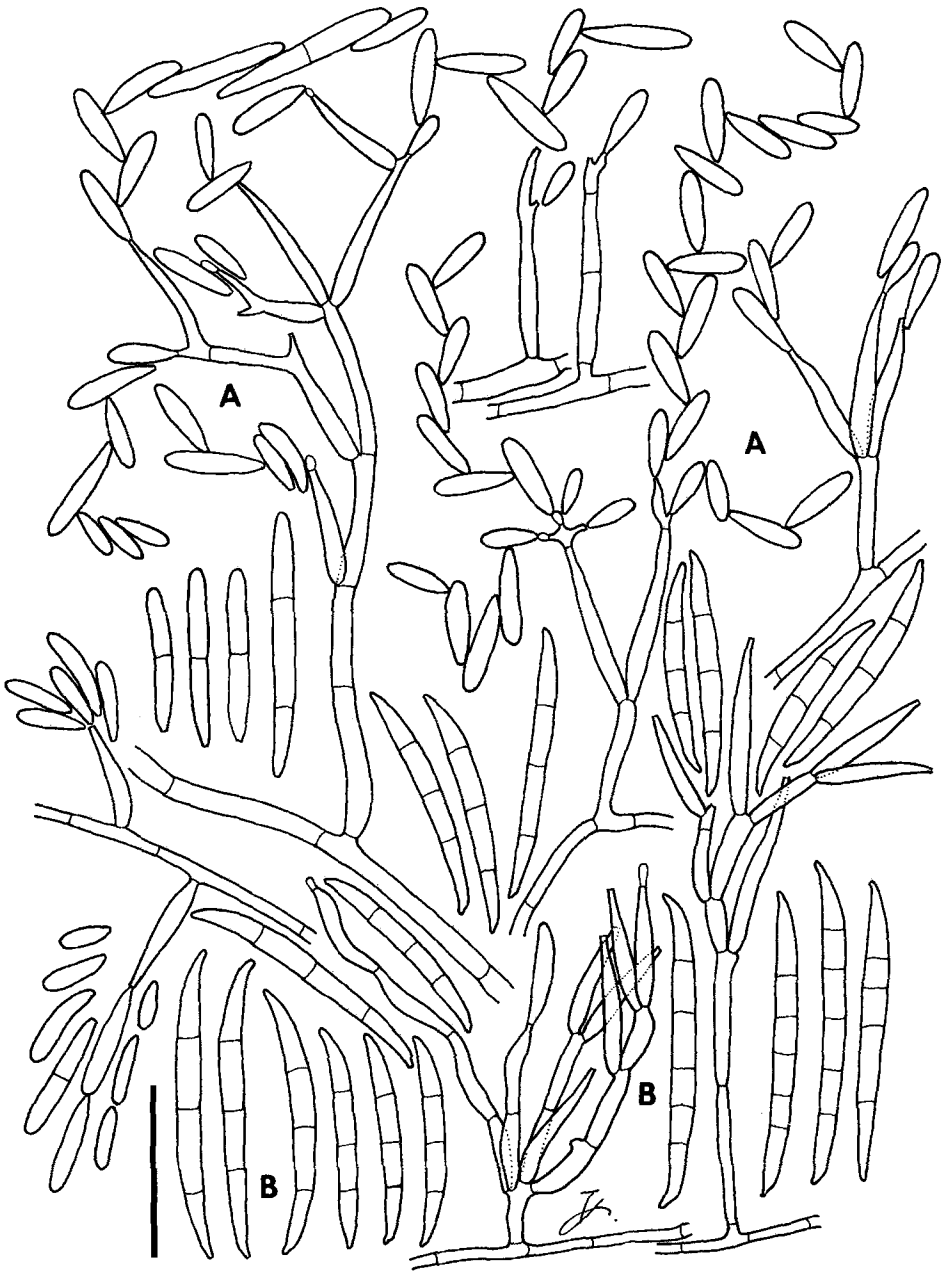
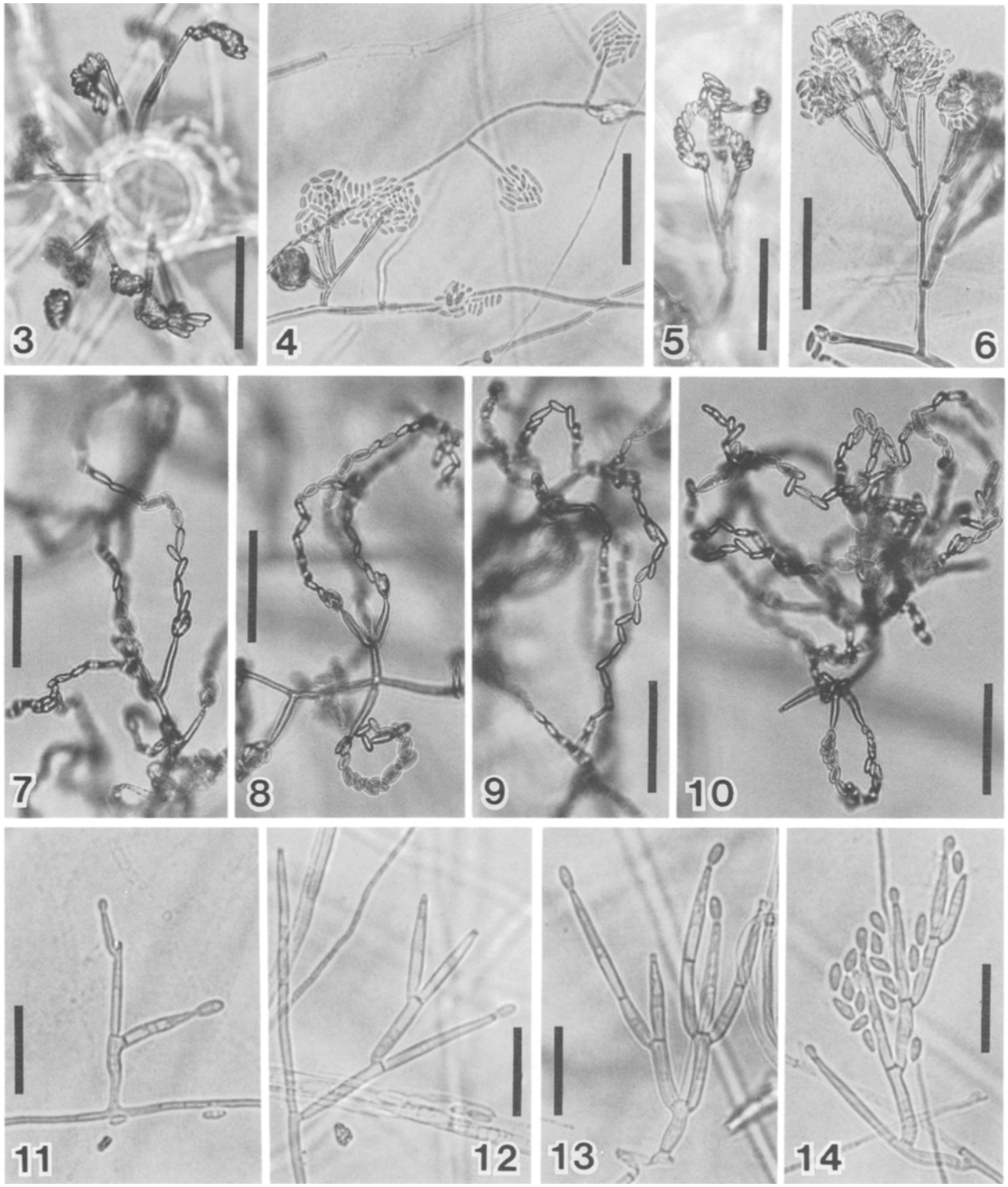


Fig. 2. *Fusarium fractiflexum* (MAFF 237529) grown on SNA under black light. A. Aerial conidiophores with simple or proliferating conidiogenous loci, forming zigzag-like chains or false heads of ellipsoidal-to-clavate conidia together with some cylindrical conidia with a rounded-to-truncate base. B. Sporodochial conidia and branched conidiophores with phialides. Conidia consisting of an acuate apical cell and a foot-like basal cell. Scale bar = 25 μm.

total range, 14.6–20.9 × 2.9–3.1 μm on average (ex type: 11.5–32 × 2–4 μm, 20.9 ± 5.6 × 2.93 ± 0.40 μm on average and S.D.) in complete darkness, 8.5–37 × 2–4 μm in total range, 16.6–25.5 × 3.1–3.2 μm on average (ex type: 11–25.5 × 2.5–4 μm, 16.6 ± 3.1 × 3.05 ± 0.31 μm on average and S.D.) under black light. Sporodochial conidia (1–)3(–5)-septate, falcate-to-cylindrical, with an acuate apical cell and a distinct foot-like basal cell, mostly formed in sporodochia on or in the agar, sometimes also observed on conidiophores arising from funiculose aerial mycelia in the dark; 3-septate: 20.5–63 × 2–5 μm in total range, 37.8–43.2 × 3.0–3.6 μm on average (ex type: 20.5–63 × 2–4.5 μm, 43.2 ± 8.6 × 3.23 ± 0.43 μm on average and S.D.) in complete darkness, 28–76 × 2.5–4.5 μm in total range, 42.7–48.9 × 3.1–3.7 μm on average (ex type: 28–61.5 × 2.5–4 μm, 42.7 ± 6.7 × 3.18 ± 0.26 μm on average and S.D.) under black light. Some 0-septate, curved cylindrical-to-naviculate conidia produced on short monophialides on and in the agar: measuring 7–22.5 × 1.5–3 μm in total range, 10.5–11.7 × 1.8–2.2 μm on average in complete darkness, 7–17.5 × 1.5–2.5 μm in total range, 10.6–13.0 × 2.2–2.3 μm on



Figs. 3–14. *Fusarium fractiflexum* (MAFF 237529) grown on SNA (3–6, and 11–14 in the dark, 7–10 under black light). 3–5. Aerial conidiophores arising from the substrate hyphae on the agar surface, forming false heads of ellipsoidal-to-clavate and some cylindrical conidia (aerial view). 6. Long, branched aerial conidiophores forming false heads of conidia (aerial view). 7–10. Conidiophores arising from the aerial mycelium forming long zigzag-like chains of conidia (aerial view). 11. Conidiophore formed on the substrate hyphae with a proliferating conidiogenous locus. 12–14. Aerial conidiophores branched sympodially or oppositely. Scale bars: 3–10=50 μm; 11–14=20 μm.

average under black light. Chlamydospores absent.

Etymology: *fractiflexus* (Lat. zigzag), referring to arrangement of aerial conidia under continuous black light.

Holotype: NIAES 20515, a dried culture, isolated from a diseased leaf of *Cymbidium* sp. (yellow spot), Enzan, Yamanashi Pref., 1992, K. Ichikawa, deposited in the herbarium of NIAES (National Institute of Agro-Environmental Sciences, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki), Japan.

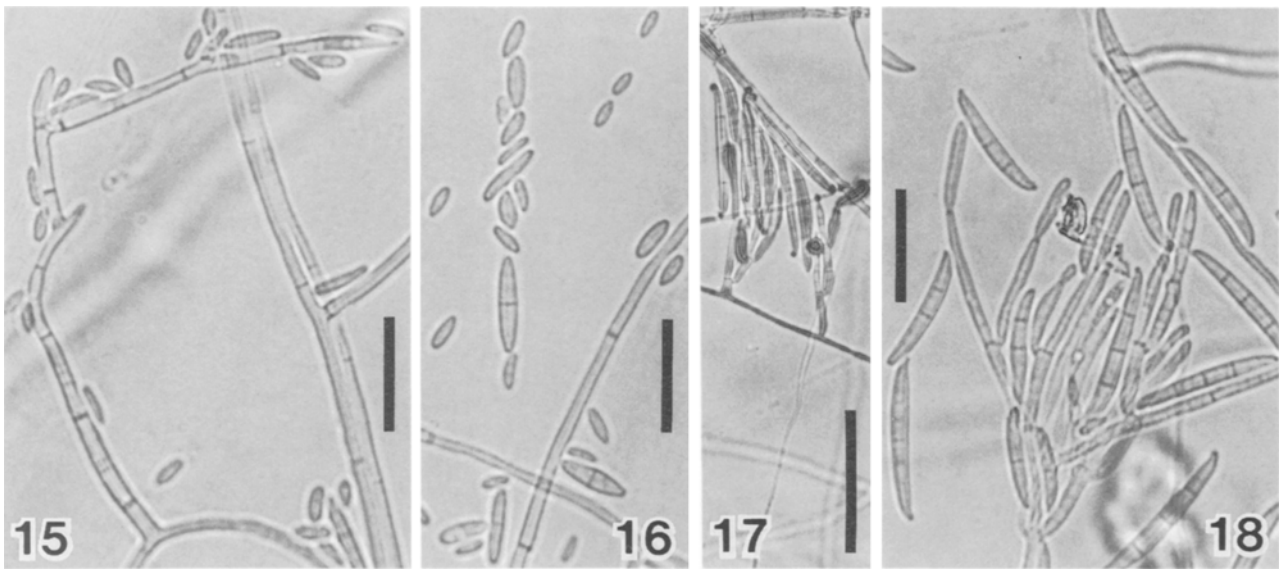
Isotype: BPI 747913, dried culture, deposited in the herbarium of BPI (US National Fungus Collection, Beltsville, MD), USA.

Ex holotype culture: MAFF 237529 (=NRRL 28852).

Isolates studied: MAFF 237529 (=NRRL 28852) from a diseased leaf of *Cymbidium* sp. (yellow spot), Enzan, Yamanashi Pref., 1992, K. Ichikawa, MAFF 237530 (=NRRL 26794) and MAFF 237531 (=NRRL 28853) from diseased leaves of *Cymbidium* sp. (yellow spot), Hiroshima Pref., 1993, K. Ichikawa, MAFF 237532 (=NRRL 28854) from a diseased leaf of *Cymbidium* sp. (yellow spot), Komoro, Nagano Pref., 1995, K. Ichikawa.

Specimens prepared: dried cultures, NIAES 20515 and BPI 747913 from MAFF 237529; NIAES 20516 from MAFF 237530; NIAES 20517 from MAFF 237531; NIAES 20518 from MAFF 237532.

Notes *Fusarium fractiflexum* is characterized by its white-to-yellowish, floccose colonies on PDA in the dark, its zigzag-like arrangement of aerial conidia formed on SNA under continuous black light (Table 2, Figs. 2A, 7–10), and production of sporodochia under all light conditions (Figs. 1B, 2B, 17, 18). This species is morphologically most similar to *F. lactis* (Wollenweber and Reinking, 1935; Nirenberg and O'Donnell, 1998), because of the zigzag-like arrangement of aerial conidia. Formation of long zigzag-like conidial chains in the former species was, however, observed only under continuous black light. In complete darkness, aerial conidia were mostly formed in false heads as amorphous or side-by-side masses (Figs. 1A, 3–6), similar to those observed in *Clonostachys* (Schroers et al., 1999). In general, conidia of *F. fractiflexum* are longer than those of *F. lactis*, especially the 0-septate aerial conidia and 3-septate sporodochial conidia. *Fusarium lactis* was reported to form obovoid aerial conidia (Nirenberg and O'Donnell, 1998), but those formed in *F. fractiflexum* are mostly ellipsoidal-to-clavate (Figs. 1A, 2A, 3–10, 14–16). Colony color of *F. fractiflexum* on PDA in the dark was, without exception, yellowish with occasionally a purplish tint at the center, but that of *F. lactis* was reported as rose-to-red, or violet (Wollenweber and Reinking, 1935). No yellowish coloration was observed when strains of *F. lactis* (NRRL 25200=BBA 68590 ex neotype, NRRL 25338, NRRL 25339) were examined under the same culture conditions. Host/substrate ranges reported for *F. lactis* are *Ficus carica* L., *Malus domestica* Borkh. (=*Pyrus malus* L.), *Pyrus communis* L., *Cydonia vulgaris* Pers., milk and soil (Wollenweber and Reinking, 1935; Nirenberg and O'Donnell, 1998), but *F. fractiflexum* has been isolated only from *Cymbidium* spp. *Fusarium lactis* and *F. fractiflexum* (MAFF 237529=NRRL 28852 ex holotype, MAFF 237530=NRRL 26794) are nested, respectively, within the African and Asian clades of the GF species complex (O'Donnell et al., 2000b). *Fusarium fractiflexum* is pathogenic to *Cymbidium* spp. and caused yellow spots on the leaves. Detailed descriptions of the symptoms caused by an isolate (MAFF 237530), previously identified as *F. subglutinans*, were given by Ichikawa and Aoki (2000).



Figs. 15–18. *Fusarium fractiflexum* (MAFF 237529) grown on SNA in the dark. 15. Sympodially proliferating conidiophore in the aerial mycelium. 16. Elliptical-to-clavate, plus some cylindrical aerial aseptate and 1-septate conidia. 17 & 18. Sporodochial conidiophores producing falcate conidia from phialides. Scale bars: 15, 16, 18=20 μm; 17=50 μm.

Fusarium circinatum Nirenberg & O'Donnell, Mycologia **90**: 442, 1998. Figs. 19–34.

Colonies on PDA showing mycelial growth rates of 4.0–4.6 mm per d at 20°C in the dark. Colony margins entire. Aerial mycelia with zonate coloration, white (1A1), purplish white (14A2), purplish grey (14B2), or reddish lilac (14B3) at the center, violet-brown (11E5), greyish red (11C-D4), greyish ruby (12D4), greyish magenta (14E5) or purplish grey (14B2) towards the margin, pale yellow (3A3)-to-yellowish white (3A2) at the marginal zone, veltinous, centrally floccose. Pigmentation in the reverse greyish ruby (12D3)-to-dark ruby (12F4-5) at the center, greyish red (10D4)-to-violet-brown (10E4-6) towards margins, yellow (4A5-6), pastel yellow (3A4), pale yellow (3A3)-to-yellowish white (3A2) at the margin. Sclerotia absent. Odor absent. Sporulation on SNA starting early in the aerial mycelium. Sporodochia formed later on or in the agar under any light condition, generally more frequent under black light, with conidiophores branched verticillately. Aerial conidiophores mostly erect, sometimes prostrate, formed directly from the substrate hyphae on the agar or in the aerial

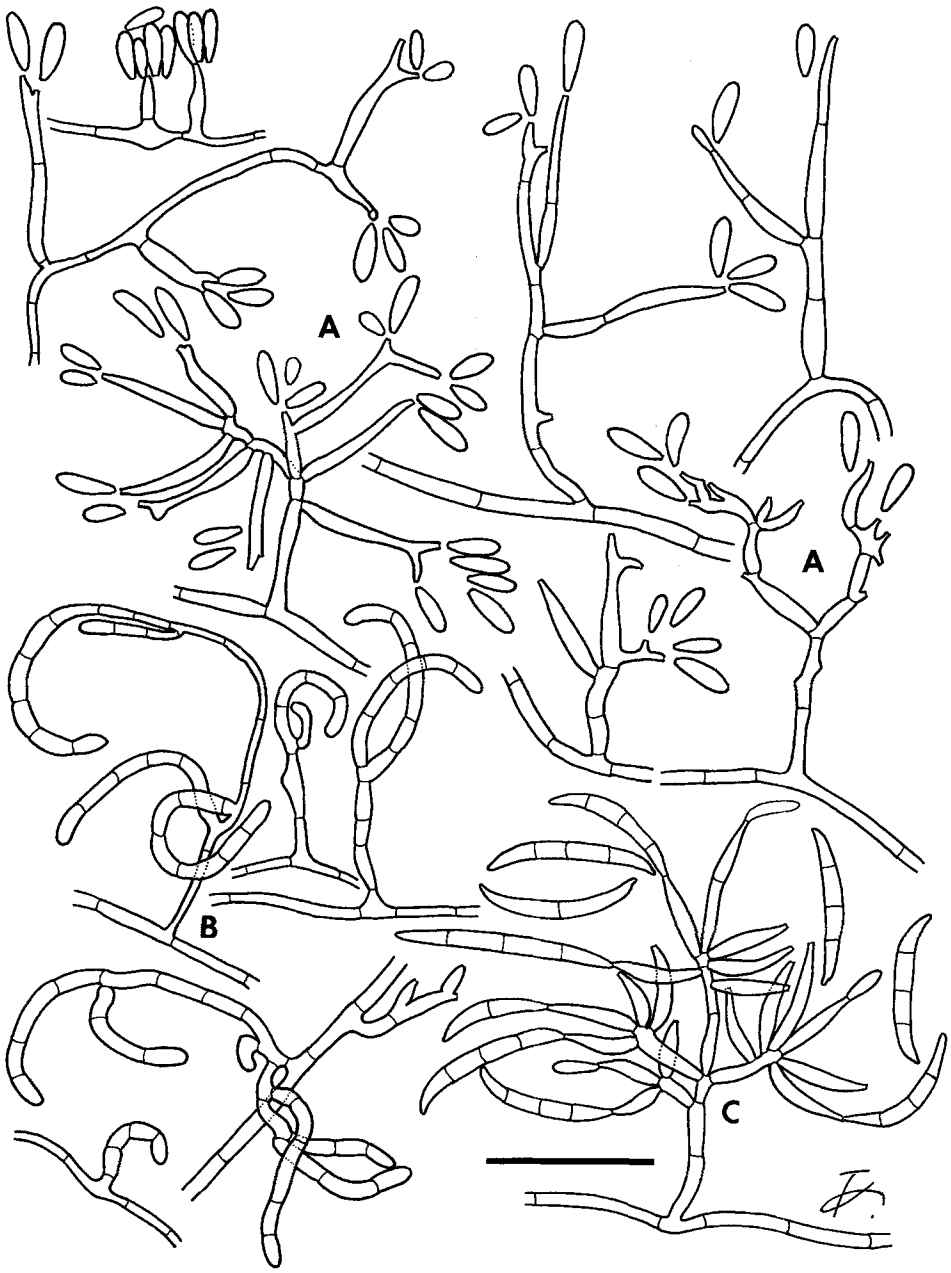
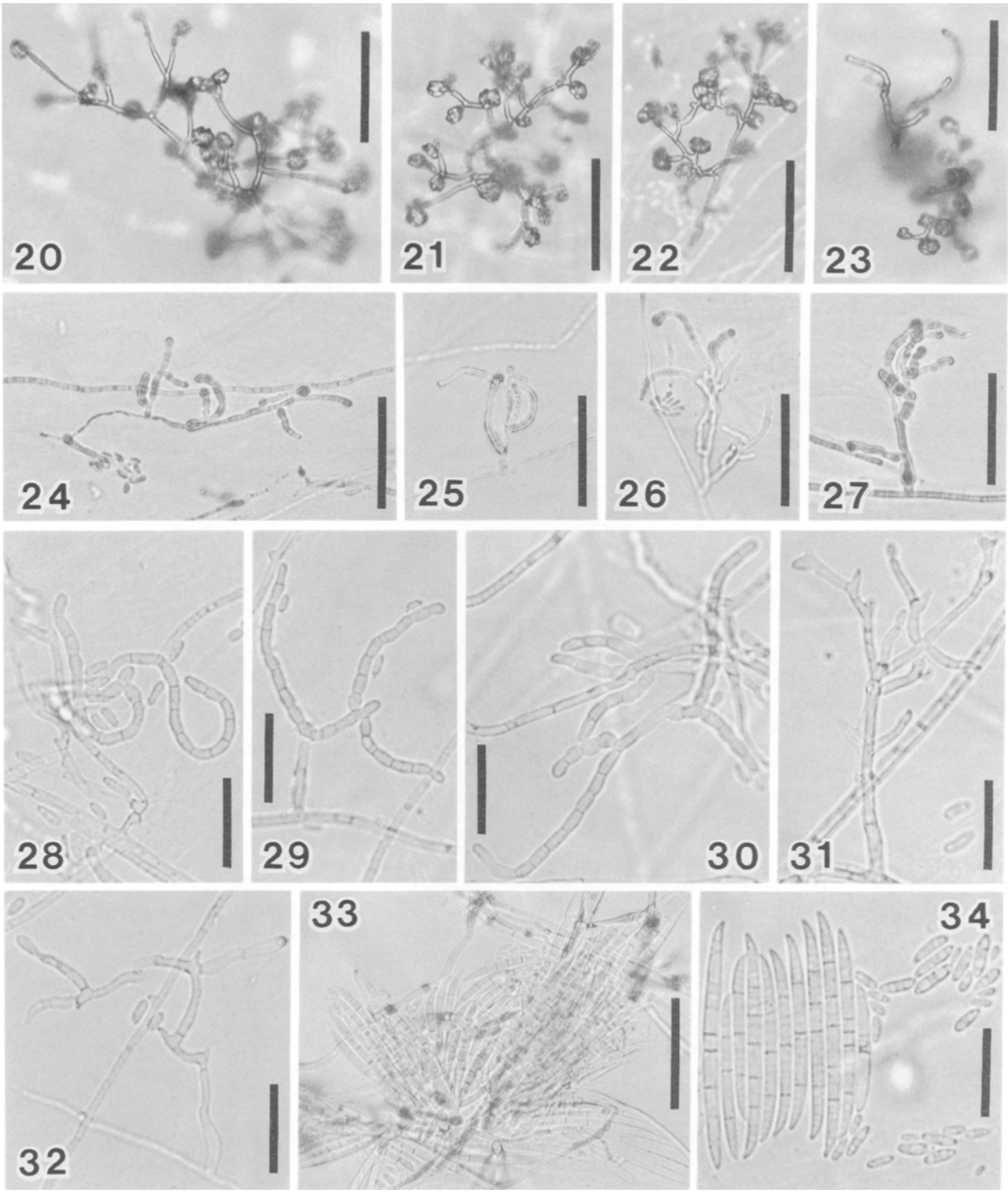


Fig. 19. *Fusarium circinatum* (MAFF 236399) grown on SNA in the dark. A. Aerial conidiophores with simple or proliferating conidiogenous loci, forming false heads of clavate, ellipsoidal conidia with a rounded-to-truncate base. B. Coiled and septate sterile hyphae formed on the substrate hyphae. C. Sporodochial conidia and branched conidiophores with phialides. Conidia possessing an acuate apical cell and a foot-like basal cell. Scale bar = 25 μm.



Figs. 20–34. *Fusarium circinatum* grown on SNA in the dark. (20–26 and 28–34 from MAFF 237756, and 27 from MAFF 236399). 20–22. Erect aerial conidiophores arising from the substrate hyphae on the agar surface, forming false heads of conidia (aerial view). 23. Aerial conidiophore adjacent to coiled sterile hyphae. 24–27. Coiled sterile hyphae formed on the substrate hyphae. 28–30. Enlargement of the coiled septate sterile hyphae. 31 & 32. Branched aerial conidiophores showing proliferating conidiogenous loci. 33. Sporodochia formed on the agar surface. 34. Sporodochial conidia consisting of an acuate apical cell and a foot-like basal cell. Scale bars: 20–27, 33=50 μm; 28–32, 34=20 μm.

mycelia, often branched several times at mostly right angles, or proliferating to form a dendroid morphology, but less frequently under black light than in the dark. Coiled sterile hyphae formed in the dark, arising singly from the substrate hyphae or associated with aerial conidiophores, not observed under continuous black light. Conidiogenous cells on the aerial conidiophores monophialidic or polyphialidic, often with more than 3 openings, bearing conidia in false heads. Phialides almost cylindrical, tapering toward the apex, up to 29 μm long and 2–4 μm wide. Aerial conidia 0–1(–2)–septate, clavate, ellipsoidal-to-allantoid, sometimes obovate, with a rounded-to-truncate base and a rounded apex; 0-septate: measuring 3.5–14 × 1.5–3 μm in total range, 6.4–8.4 × 2.4–2.6 μm on average in complete darkness, 4.5–21.5 × 2–3.5 μm in total range, 8.2–10.9 × 2.6–2.8 μm on average under black light; 1-septate: 6–22 × 2.5–3.5 μm in total range, 11.3–14.5 × 2.7–3.0 μm on average in complete darkness, 10–22.5 × 2.5–4 μm in total range, 14.5–16.6 × 2.9–3.2 μm on average under black light. Sporodochial conidia typically 1–3–septate, curved cylindrical, slender, tapering towards both ends, with an acuate apical cell and a distinct foot-like basal cell; 1-septate: 14.5–29 × 2.5–3 μm in total range, 21.5–25.0 × 2.7–2.9 μm on average in complete darkness, 16–32.5 × 2.5–3.5 μm in total range, 22.8–26.1 × 2.8–2.9 μm on average under black light; 3-septate: 26.5–47.5 × 2.5–3.5 μm in total range, 29.8–37.3 × 2.9–3.1 μm on average in complete darkness, 28–55 × 2.5–4.5 μm in total range, 30.7–37.8 × 3.1–3.2 μm on average under black light. Clavate-to-elliptical, sometimes allantoid conidia without a basal foot often formed from phialides on the substrate hyphae in the agar. Chlamydospores absent.

Isolates studied: MAFF 236397 (=NRRL 26431) from diseased *Pinus luchuensis* Mayr (pitch canker), Kagoshima Pref., 1992, T. Kobayashi, MAFF 236399 (=NRRL 26432) from diseased *P. luchuensis* (pitch canker), Okinawa Pref., 1986, T. Kobayashi, MAFF 237756 (=NRRL 29945) from diseased *P. luchuensis* (pitch canker), Amami Ohshima isl., Kagoshima Pref., 1991, T. Kobayashi.

Notes *Fusarium circinatum* was characterized as forming coils of elongate sterile hyphae (Nirenberg and O'Donnell, 1998; Table 2). An authentic strain of the species (NRRL 25331=BBA 69720 ex holotype) produced masses of sterile coiled hyphae often associated with aerial conidiophores on SNA under all light conditions. Among the Japanese strains, however, the sterile coils were not always associated with conidiophores (Fig. 23) but often formed directly from the substrate hyphae on the agar (Figs. 19B & 24–30). This structure was not observed under continuous black light. Except for the slight differences noted above, morphological characteristics of the strains examined agreed with the given description (Nirenberg and O'Donnell, 1998) and those of an authentic strain of *F. circinatum* (NRRL 25331=BBA 69720). Aerial conidiophores formed by the Japanese isolates were branched extensively, yielding a dendroid morphology (Figs. 20–22 & 31), especially in darkness, as described and observed in an authentic strain. *Fusarium circinatum* causes pitch canker disease of various species of *Pinus* (Hepting and Roth, 1946; Correll et al., 1991). Kobayashi and Muramoto (1989) reported pathogenicity of the fungus to *P. luchuensis* by artificial inoculation, as well as two additional common Japanese pines, *P. densiflora* Siebold & Zucc. and *P. thunbergii* Parl., but natural occurrence of the disease in Japan is only known on *P. luchuensis*.

Fusarium concentricum Nirenberg & O'Donnell, Mycologia **90**: 442–445, 1998. Figs. 35–58.

Colonies on PDA showing mycelial growth rates of 3.8–4.7 mm per d at 20°C in the dark. Colony margins entire, sometimes jagged. Aerial mycelia white (1A1), sometimes pale yellow (2A3), pastel yellow (2A4)-to-light yellow (2A5), often with pinkish or purplish coloration at the center, then, dull red (11C3), greyish brown (11D3), brownish grey (11C-D2), lilac-grey (16B2), sparse, loosely to densely floccose, sometimes velutinous. Pigmentation in the reverse yellowish white (4A2), pale yellow (4A3), pastel yellow (3A4), dull yellow (3B3), yellowish grey (4B3)-to-greyish yellow (3-4B4), sometimes brownish grey (11C-E2)-to-greyish brown (11E-F3), often centrally purplish grey (14D2)-to-greyish magenta (14D3-14E5), sometimes with alternating yellowish-to-greyish concentric rings. Sclerotia absent. Odor absent or, if present, somewhat sweet. Sporulation on SNA starting early in the aerial mycelium and in the sporodochia. Sporodochia observed under all light conditions, on or in the agar. Aerial conidiophores in the dark mostly prostrate, formed as side-branches of aerial hyphae or the substrate hyphae, generally short, unbranched or with a few lateral branches; under continuous black light, erect, formed directly from substrate hyphae on the agar or sometimes from aerial hyphae, branched verticillately several times or proliferating to form a dendroid morphology. Conidiogenous cells on the aerial conidiophores monophialidic or polyphialidic, bearing conidia in false heads, sometimes holoblastic under black light, bearing conidia solitarily or in pairs. Phialides of aerial conidiophores almost cylindrical, tapering toward the apex, up to 30 μm long and 1.5–4 μm wide. Aerial conidia of two types: (1) conidia with a rounded apex and a rounded-to-truncate base formed in the dark and under black light, clavate-to-ellipsoidal, or rarely obovate, mostly straight, rarely slightly curved, 0–1(–3)–septate; 0-septate: measuring 5–17.5 × 1.5–4 μm in total range, 7.9–10.4 × 2.5–2.8 μm on average in complete darkness, 5.5–20.5 × 1.5–4 μm in total range, 10.5–12.2 × 2.7–3.0 μm on average under black light; 1-septate: 9.5–28 × 2.5–4 μm in total range, 14.4–19.2 × 3.0–3.2 μm on average in complete darkness, 8.5–35.5 × 2.5–4 μm in total range, 16.9–22.3 × 2.9–3.2 μm on average under black light. Under black light, (2) aerial conidia with a hooked or pointed apex and a wedge-shaped basal cell formed additionally and constantly, naviculate-to-fusiform, straight or often falcate, (0–)3(–5)–septate, often adhering side-by-side; 3-septate: 26.5–58 × 2.5–4.5 μm in total range, 37.3–39.9 × 3.0–3.4 μm on

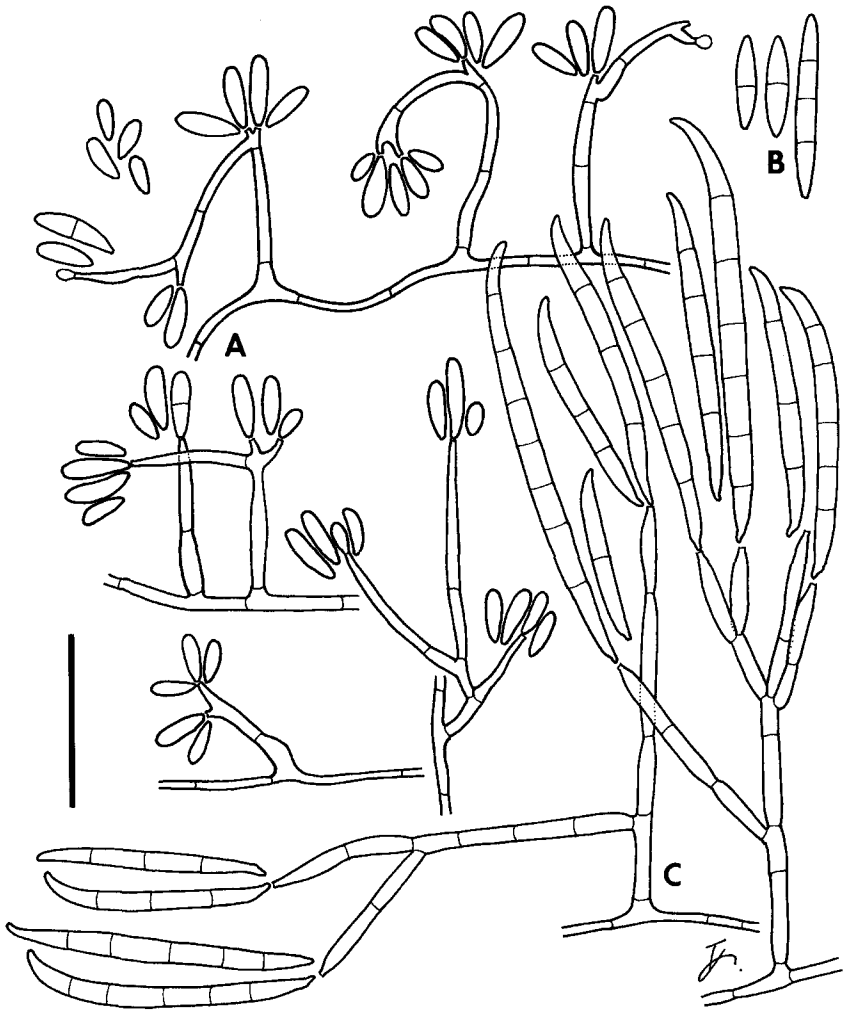


Fig. 35. *Fusarium concentricum* (MAFF 237649) grown on SNA in the dark. A. Aerial conidiophores with simple or proliferating conidiogenous loci, forming false heads of clavate, ellipsoidal and obovate conidia with a rounded-to-truncate base. B. Septate aerial conidia with a rounded-to-truncate base. C. Sporodochial conidia and branched conidiophores with phialides. Conidia possessing an acuate apical cell and a foot-like basal cell. Scale bar=25 μm.

average, 5-septate: 45.5–70.5×2.5–4.5 μm in total range, 53.1–63.4×3.1–3.5 μm on average. Sporodochial phialides flask-to-bottle-shaped. Sporodochial conidia with an acuate apical cell and a distinct foot-like basal cell, (1–)3–5-septate, falcate-to-cylindrical, slender, tapering towards both ends; 3-septate: 26.5–57.5 ×2.5–4 μm in total range, 34.9–45.2×3.0–3.2 μm on average in complete darkness, 27.5–67.5×2.5–4 μm in total range, 44.3–51.2×3.1–3.3 μm on average under black light; 5-septate: 31–76.5×2.5–4 μm in total range, 44.1–59.7×3.2–3.4 μm on average in complete darkness, 40–87×2.5–5 μm in total range, 57.8–64.0×3.2–3.7 μm on average under black light. Chlamydospores absent.

Isolates studied: MAFF 237649 (=NRRL 25303=BBA 69021) and MAFF 238112 (=NRRL 29944) from roots of rice (*Oryza sativa* L.), Tsukuba, Ibaraki Pref., 1990, T. Aoki, MAFF 238111 (=NRRL 26434) from stem base of rice, Tsukuba, Ibaraki Pref., 1990, T. Aoki, MAFF 237650 (=NRRL 25309=BBA 69012) from a seed of *Triticum aestivum* (L.) Thell., Ibusuki, Kagoshima Pref., 1992, T. Aoki, MAFF 238110 (=NRRL 29943) from soil of a pine (*Pinus densiflora* Siebold & Zucc.) forest, Tsukuba, Ibaraki Pref., 1990, T. Aoki.

Notes Based on the original description given by Nirenberg and O'Donnell (1998), *F. concentricum* was characterized as producing alternating pale orange and reddish-grey concentric color rings when cultured on PDA at 20°C. Two strains (MAFF 238111=NRRL 26434 and MAFF 238110=NRRL 29943) examined in this study produced similar yellowish and greyish concentric rings when cultured on PDA in the dark, but this character was not exhibited uniformly by all strains. Some strains showed irregular discoloration of the colony suggestive of degeneration. However, a stable feature of all strains of *F. concentricum* examined was found, i.e., formation of dendroid aerial conidiophores under continuous black light (Figs. 36A, 43, 46, 47). More-

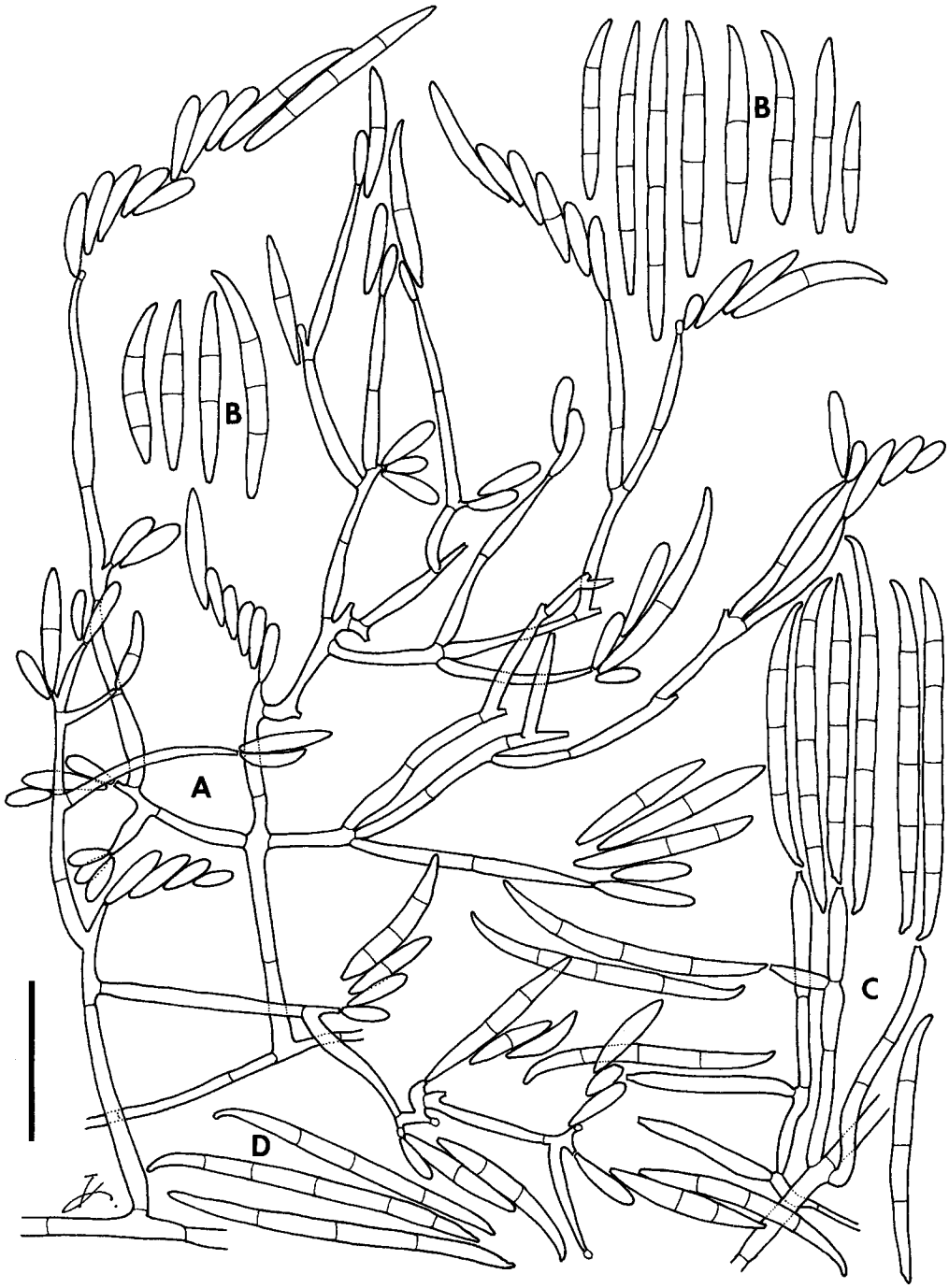
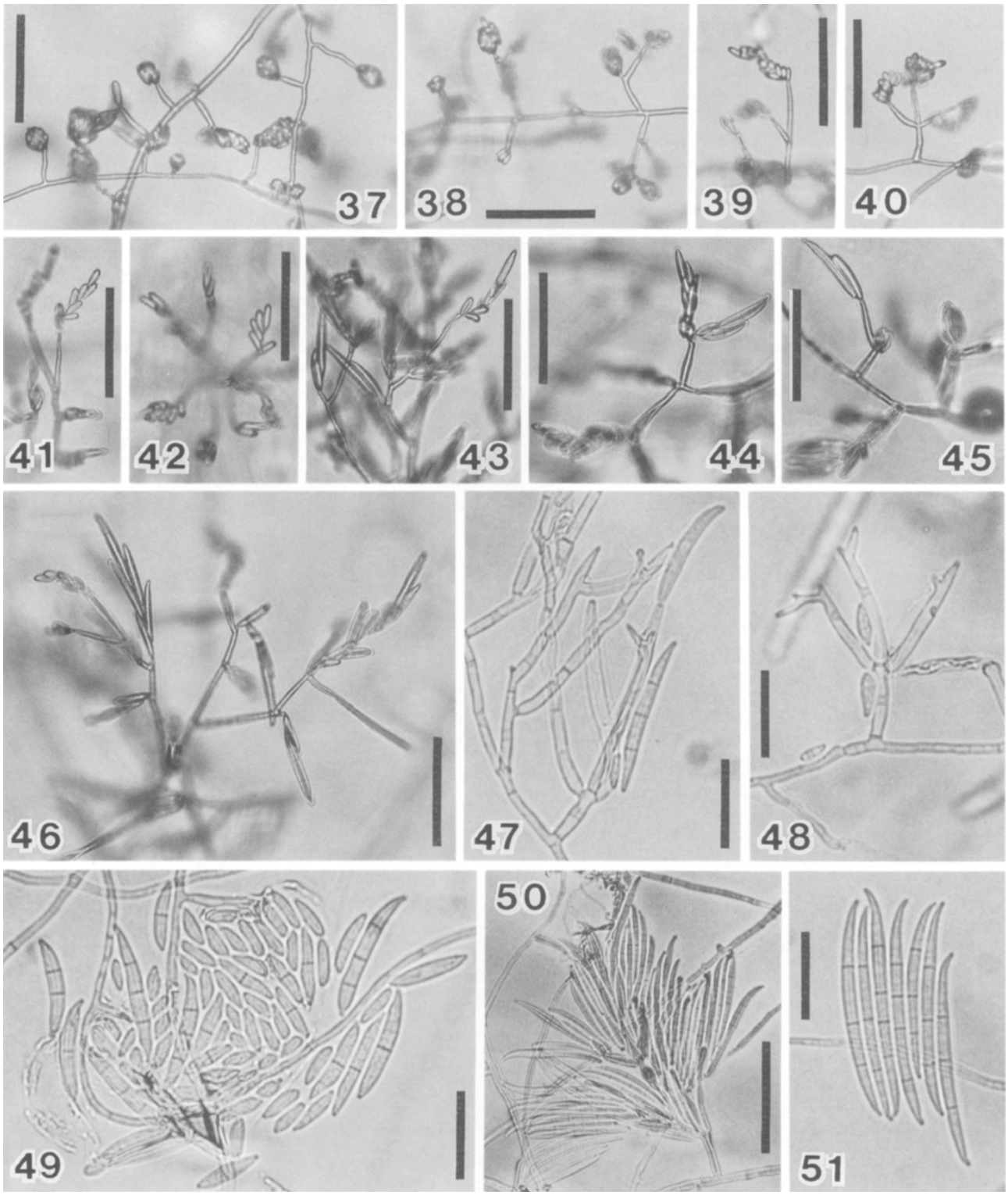


Fig. 36. *Fusarium concentricum* (MAFF 237649) grown on SNA under black light. A. Aerial dendroid conidiophores consisting of proliferating conidiogenous loci, forming false heads and masses of conidia adhering side-by-side. Two types of aerial conidia are formed: (1) clavate-to-ellipsoidal conidia with a rounded apex and a rounded-to-truncate base, and (2) naviculate-to-fusiform conidia with a hooked or pointed apex and a wedge-shaped base. B. Naviculate-to-fusiform, septate aerial conidia with a hooked or pointed apex and a wedge-shaped base. C. Sporodochial conidia and branched conidiophores with phialides. D. Sporodochial conidia with an acuate apical cell and a foot-like basal cell. Scale bar = 25 μm.

over, (0–)3(–5)–septate aerial conidia with a hooked apex and a wedge-shaped basal cell, were constantly observed among the isolates within 2 wk under black light (Table 2, Figs. 36A, B, 43–47, 49, 52–58). They were mostly phialidic and straight, but often even falcate, and exhibited a morphology intermediate between typical aerial and sporodochial conidia. In complete darkness, aerial conidiophores formed by the strains were mostly short and prostrate, and aerial conidia were phialidic, consistently clavate-to-ellipsoidal or obovate with a



Figs. 37–51. *Fusarium concentricum* grown on SNA (37 and 40 from MAFF 237650, 38, 44, 45 and 50 from MAFF 238112, 39, 47–49, 51 from MAFF 237649, 41–43, 46 from MAFF 238110; 37–40 in the dark, 41–51 under black light). 37–40. Aerial conidiophores in the dark arising from the aerial mycelium, producing false heads of conidia with a rounded apex (aerial view). 41–45. Aerial conidiophores formed under black light, forming false heads of conidia with a rounded apex but often with a hooked or pointed apex (in Figs. 43–45) (aerial view). 46. Dendroid aerial conidiophores forming fusiform conidia with a hooked or pointed apex. 47. Part of a branched aerial conidiophore forming naviculate-to-fusiform conidia with a hooked or pointed apex and a wedge-shaped base. 48. Aerial conidiophore forming clavate-to-ellipsoidal conidia with a rounded apex and a truncate base. 49. Variously shaped aerial conidia. 50. Sporodochial conidiophores producing falcate conidia from phialides. 51. Sporodochial conidia consisting of an acuate apical cell and a foot-like basal cell. Scale bars: 37–46, 50=50 μm; 47–49, 51=20 μm.

rounded apex and a rounded-to-truncate base (Figs. 35A, 37–40). This was also observed in authentic strains of the species (NRRL 25181=BBA 64354 ex holotype, NRRL 25666=BBA 69855 and NRRL 25667=BBA 69856, all isolated from *Musa sapientum*, and NRRL 25202=BBA 68483 from *Nilaparvata lugens* Stål), showing the same reaction to the different light conditions. Authentic strains also formed aerial conidia with a hooked apex and a wedge-shaped basal cell under black light, (in NRRL 25181=BBA 64354 ex holotype) 3-septate: 26–55.5×2.5–4 μm in total range, 39.8±7.9×3.2±0.21 μm on average and S.D.; 5-septate: 50.5–66×3–4 μm in total range, 59.5×3.4 μm on average. Only typical aerial and sporodochial conidia were formed in the dark. Production of dendroid aerial conidiophores and aerial conidia with a hooked apex and a wedge-shaped basal cell under black light represents an additional morphological character to diagnose *F. concentricum*. Strains of *F. concentricum* have been reported to produce a mycotoxin, moniliformin or an insect toxin, beauvericin (Gupta et al., 1991; Vesonder et al., 1995; Nirenberg & O'Donnell, 1998).

Molecular phylogenetic relationship of *F. fractiflexum*, *F. circinatum* and *F. concentricum* The combined dataset consisted of partial sequences from three independent loci: mtSSU rDNA (701 bp), β-tubulin (588 bp), and EF-1α (646 bp, excluding 25 ambiguously aligned positions). The taxon matrix consisted of 21 and 16 ingroup strains, respectively, from the American and Asian clades of the GF species complex. Gene trees inferred from the individual and combined data were rooted by the outgroup method using sequences of two members of the *Fusarium oxysporum* Schltdl. species complex (Baayen et al., 2000). Maximum parsimony trees inferred from the individual partitions (not shown) yielded the following: mtSSU rDNA, 60 trees of 93 steps with 7 nodes with≥50% bootstrap support; β-tubulin, 9 trees of 83 steps with 19 nodes with≥50% bootstrap support; and EF-1α, 5520 trees of 209 steps with 19 nodes with≥50% bootstrap support. Compared to the β-tubulin and EF-1α partitions, there was a slight increase in bootstrap support with the combined dataset (22 nodes≥50% bootstrap intervals). Phylograms based on the combined dataset, which were represented by 387 trees of 412 steps (Fig. 59), resolved two distinct lineages corresponding to the American and Asian clades

Figs. 52–58. Conidia of *Fusarium concentricum* formed on SNA under black light (52, 53 and 55 from MAFF 237649, 54 from MAFF 238112, 56–58 from MAFF 237650). 52–54. Naviculate-to-fusiform aerial conidia with a hooked or pointed apex and a wedge-shaped base. 55–58. Comparison of three different types of conidia: (1) clavate, ellipsoidal or obovate aerial conidia with a rounded apex and a truncate base; (2) naviculate-to-fusiform aerial conidia with a hooked or pointed apex and a wedge-shaped base; and (3) falcate-to-cylindrical sporodochial conidia with an acuate apical cell and a foot-like basal cell. 57. Enlargement of Fig. 56. Scale bars: 52–56=20 μm; 57, 58=10 μm.

of the GF species complex (O'Donnell et al., 1998a; O'Donnell et al., 2000b). Japanese strains causing pitch canker of pine, *F. circinatum* (teleomorph: *G. circinata* Nirenberg & O'Donnell), formed an exclusive group with the type strain NRRL 25331 (97% bootstrap) within the American clade of the GF species complex. This species is strongly supported as a sister to *Fusarium* sp. NRRL 29123 and 29124 (92% bootstrap) isolated from the noxious weed, *Bidens pilosa* L., in Florida (O'Donnell et al., 2000b). All of the remaining Japanese strains included in this study were resolved phylogenetically as *F. concentricum* or as a newly described species causing yellow spot of *Cymbidium* sp., *F. fractiflexum*. Both of these species are strongly supported as exclusive groups (100% bootstrap) within the Asian clade of the GF species complex (98% bootstrap). *Fusarium concentricum* appears to form a moderately supported sister group (74% bootstrap) to two unnamed species: 1) *Fusarium* sp. NRRL 25226 responsible for the mango malformation disease in India (Kumar et al., 1993) and Israel (Freeman et al., 1999), and 2) *Fusarium* sp. NRRL 26427 from tropical forest soil in Papua-New Guinea. Although *F. fractiflexum* is strongly supported as a member of the Asian clade, phyletic relationships of this species within this clade vary depending on the partition(s) analyzed (O'Donnell et al., 2000b). Strains of *F. fractiflexum* were previously identified as *F. subglutinans* (≡*F. sacchari* var. *subglutinans*) (Table 1) based on older criteria by Gerlach and Nirenberg (1982) and Nelson et al. (1983). Molecular phylogenetic analyses, however, revealed that *F. fractiflexum* and *F. subglutinans* (MP-E) belonged, respectively, to the Asian clade and the American clade of the GF species complex, demonstrating that both species are only distantly related.

Discussion

A new species of *Fusarium* and two species never before reported from Japan were investigated in the present study: *F. fractiflexum*, *F. circinatum* and *F. concentricum*. Strains of the three species examined have been identified previously as *F. subglutinans* (≡*F. moniliforme* var. *subglutinans* ≡*F. sacchari* var. *subglutinans*) or a related unidentified species, because they formed false

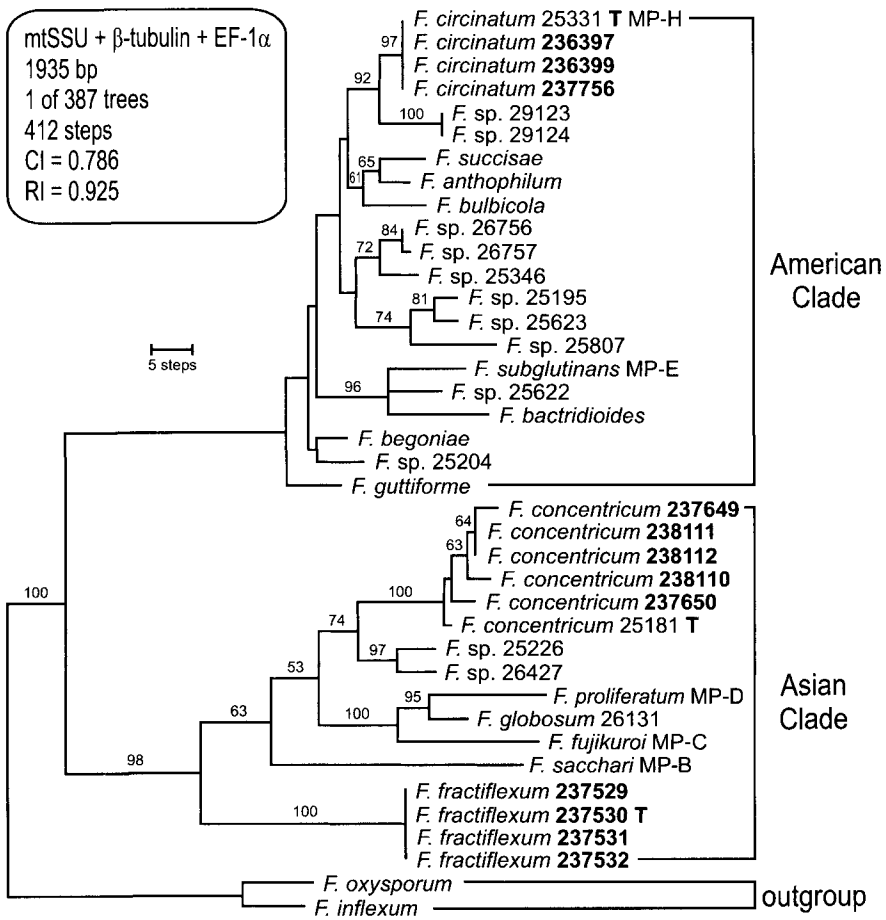


Fig. 59. One of 387 most-parsimonious trees of 412 steps inferred from the combined mtSSU rDNA, β-tubulin and EF-1α gene sequences rooted with two sequences from the *Fusarium oxysporum* species complex. Numbers above nodes represent bootstrap intervals from 1000 replications. Strains in bold were isolated in Japan and represent MAFF accession numbers. All other strain numbers are those of the ARS Culture Collection (NRRL). T=type strain; MP=biological species in the *Gibberella fujikuroi* species complex.

heads of aerial conidia under ordinary cultural conditions. They were, however, resolved as distinct species based on detailed morphological study using black light illumination, as well as by molecular phylogenetic analyses of DNA sequences from three independent loci. A morphological comparison of the three species described is given in Table 2, together with three morphologically similar species.

Results of the present study indicate that combined morphological (phenotypic) and molecular phylogenetic (genotypic) analyses are often necessary for the systematic study of fusaria. Secondly, morphological characteristics of strains should be recorded on SNA in complete darkness and under continuous black light. Morphological differences between strains grown in the dark and under black light were clearly revealed for *F. fractiflexum* and *F. concentricum* in the present study. Aerial conidia of *F. fractiflexum* were formed in long zigzag-like chains under continuous black light (Table 2, Figs. 2A, 7–10), while only false heads were formed in darkness (Figs. 1A, 3–6). Further, *F. concentricum* formed (0–)3(–5)-septate and phialidic aerial conidia with a hooked apex and a wedge-shaped basal cell under continuous black light (Table 2, Figs. 36A, B, 43–47, 49, 52–58), while in the dark aerial conidia were only clavate-to-ellipsoidal with rounded-to-truncate ends (Figs. 35A, 37–42). In addition, a new character found in *F. concentricum* represents an additional morphological feature that can be used to diagnose the species more accurately. Aoki and Nirenberg (1999) suggested application of a mixed light regime, i.e., an alternating illumination program of 12 h darkness/12 h black light, for examination of *F. globosum* Rheeder, Marasas & P. E. Nelson. To determine whether such morphological differences are related to light, however, cultures should be grown under two separate light conditions as shown in *F. fractiflexum* and *F. concentricum*. By effective control of the different light conditions, additional morphological features to diagnose species of *Fusarium* might be found, even in species that form only false heads of aerial conidia in the dark. Such information is essential to properly diagnose fusarial diseases such as yellow spot of *Cymbidium* spp. induced by *F. fractiflexum*.

Ichikawa and Aoki (2000) reported on a strain examined in the present study (MAFF 237530=K. Ichikawa 93–1–1) as *F. subglutinans* as the cause of yellow spot of *Cymbidium*, based on the identification at the time it was deposited in MAFF (as *F. sacchari* var. *subglutinans*) using the taxonomy of Nelson et al. (1983). Ichikawa and Aoki (2000) mentioned that the fungus produced aerial conidia only in false heads. This was an oversight, because we have determined that it produces zigzag-like conidial chains on SNA under continuous black light. In a key and a synopsis, Nirenberg and O'Donnell (1998) indicated that *F. subglutinans* (≡*F. sacchari* var. *subglutinans*) and *F. sacchari* (var. *sacchari*) do not form conidial chains when cultured under either complete darkness or continuous black light. Pascoe (1990a, b) has reported that *F. subglutinans* forms mostly holoblastic or sometimes phialidic, straight fusiform and septate (meso-) conidia from the aerial conidiophores, and their production was markedly accelerated under continuous black light. These reported features were also reconfirmed when authentic strains of *F. subglutinans* were examined in the present study (Table 2). It has also been reported that sporodochia of *F. sacchari* were absent in wild-type strains or not formed without black light (Nirenberg, 1976; Gerlach and Nirenberg, 1982; Nirenberg and O'Donnell, 1998), and therefore the species was originally described as *Cephalosporium sacchari* E. J. Butler (Gams, 1971). All strains of *F. fractiflexum* cultured on SNA, however, formed sporodochia under any light condition and zigzag-like conidial chains under continuous black light. They were all isolated from the similar disease symptoms of *Cymbidium* spp. Here we formally report that yellow spot of *Cymbidium* spp. (Ichikawa and Aoki, 2000) is caused by *F. fractiflexum*, not *F. subglutinans*. Therefore, the present study has documented a new pathogen of *Cymbidium*. Six species of *Fusarium* within the GF species complex have been reported as pathogenic to *Cymbidium* spp.: *F. anthophilum*, *F. bulbicola* Nirenberg & O'Donnell (≡*F. sacchari* var. *elongatum*), *F. phyllophilum* Nirenberg & O'Donnell (≡*F. proliferatum* (Matsush.) Nirenberg ex Gerlach &

Table 2. Comparison of major diagnostic morphological features of *Fusarium circinatum*, *F. concentricum* and *F. fractiflexum* with three morphologically similar species grown on SNA under different light conditions.

Species	Coiled elongation of sterile hyphae	Naviculate to fusiform, phialidic aerial conidia	Fusiform and solitary, holoblastic aerial conidia	Zigzag-like chains of aerial conidia	Sporodochia and sporodochial conidia
Fusarium circinatum	D(+B)[a]	—	—	—	D+B
Fusarium concentricum	—	B	[B][a]	—	D+B
Fusarium fractiflexum	—	—	—	B	D+B
Fusarium lactis[b]	—	—	—	D+B	D(+B)
Fusarium sacchari	—	—	—	—	(B)[c]
Fusarium subglutinans	—	[B]	D+B	—	D+B

[a] D: present in complete darkness, or B: under continuous black light, —: absent, ( ): occasionally absent, [ ]: occasionally present.
[b] based on *F. lactis* NRRL 25200 (=BBA 68590, ex neotype), NRRL 25338 and NRRL 25339.
[c] Nirenberg (1976) and Nirenberg and O'Donnell (1998) stated that sporodochia were absent in wild-type cultures or rarely formed even under black light.

Nirenberg var. *minus* Nirenberg) and *F. proliferatum* (Nirenberg, 1976; Ichikawa and Aoki, 2000), causing leaf spots or lesions; *F. moniliforme* (Gleason et al., 1966) causing seedling wilt; and *F. subglutinans* (D'Agliano and Carrai, 1994; Honda et al., 1995; Broadhurst and Hartill, 1996) causing necrotic lesions on leaves and bulbs. Species concepts of *F. moniliforme*, a later synonym of *F. verticillioides* (Sacc.) Nirenberg, and *F. subglutinans* (≡*F. moniliforme* var. *subglutinans*) within the GF species complex have long been applied differently, depending on the taxonomic system used. Therefore, these reports, at least as they relate to the latter two species, should be reevaluated based on current taxonomic knowledge (Nirenberg and O'Donnell, 1998; O'Donnell et al., 2000b), as in the present study.

Pitch canker of *Pinus* species was originally reported in the United States (Hepting and Roth, 1946), and the pathogen was first identified as *F. lateritium* Nees: Fr. f. sp. *pini* (Snyder et al., 1949). Later the fungus was reidentified as *F. moniliforme* var. *subglutinans* (Kuhlman et al., 1978), and then as *F. subglutinans* f. sp. *pini* (Correll et al., 1991). In Japan, pitch canker of *P. luchuensis* was originally reported by Kobayashi and Muramoto (1989), and the causal fungus was reported as *F. moniliforme* var. *subglutinans* (≡*F. subglutinans*). Three reference strains deposited in MAFF were morphologically identical to *F. circinatum* in that they formed slender falcate sporodochial conidia, and false heads of aerial conidia together with coiled sterile hyphae (Figs. 19–34; Nirenberg and O'Donnell, 1998). Based on DNA sequences of β-tubulin and other genes, the three Japanese strains were phylogenetically identical to the ex-type strain of *F. circinatum* (Fig. 59). These results indicate that the etiological agent of pitch canker of *P. luchuensis* in Japan is *F. circinatum*.

Fusarium concentricum was observed to form septate aerial conidia with a hooked apex and a wedge-shaped basal cell under continuous black light. Pascoe (1990a, b) re-characterized conidial types formed by *Fusarium* species, and proposed a new conidial type "mesoconidium (pl. -a)" in addition to the "micro-" and "macroconidium (pl. -a)". The septate aerial conidia with a hooked apex and a wedge-shaped basal cell of *F. concentricum* (Figs. 36A, B, 43–47, 49, 52–58) agree morphologically with mesoconidia as defined by Pascoe (1990a, b), but aerial conidia formed by *F. concentricum* appeared to be phialidic (Figs. 36A, 43–46), as indicated by the successive production of plural conidia from the same loci. However, some aerial conidia formed singly could be considered as holoblastic. Nirenberg and O'Donnell (1998) and Aoki and Nirenberg (1999) recently applied alternative terminology for conidial types, e.g., aerial conidia or sporodochial conidia, in order to describe morphological features more precisely. It is also apparent that the globose aerial conidia of *F. globosum* (mostly holoblastic but round-shaped) and septate catenate aerial conidia of *F. nisikadoi* T. Aoki & Nirenberg, as well as the septate aerial conidia of *F. concentricum*, cannot be classified as either micro-, macro- or mesoconidia, according to Pascoe (1990a, b). Anatomical descriptions of conidial types and morphology would be more precise if they followed the cultural methods presented in this and the previous studies (Nirenberg and O'Donnell, 1998; Aoki and Nirenberg, 1999).

Fusarium species within the GF species complex and related species are presently under critical evaluation by molecular phylogenetic analyses based on DNA sequence data of multiple loci (O'Donnell and Cigelnik, 1997; O'Donnell et al., 1998a; O'Donnell et al., 2000b). Precise morphological analyses of the newly discovered phylogenetic species are required to advance the systematics of the fusaria by linking the morphological phenotypes with the multilocus genotypes.

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