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Fusarium kyushuense sp. nov. from Japan

Takayuki Aoki[1] and Kerry O'Donnell[2]

[1] National Institute of Agrobiological Resources, Department of Genetic Resources I, Ministry of Agriculture, Forestry and Fisheries, Kannondai, Tsukuba, Ibaraki 305–0856, Japan
[2] Microbial Properties Research, National Center for Agricultural Utilization Research, United States Department of Agriculture, Agricultural Research Service, Peoria, Illinois 61604, U.S.A.

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Four trichothecene-producing strains originally isolated from diseased wheat and a vinyl plate in Kyushu and Shikoku, Japan are described and illustrated as a new species, *Fusarium kyushuense*. This species does not produce chlamydospores, which is the key morphological character which distinguishes it from *F. sporotrichioides* with which it has been mistaken. *Fusarium kyushuense* is also differentiated from another morphologically similar species, *F. arthrosporioides*, by absence of sclerotia and by differences in conidiogenesis of obovate conidia. In *F. arthrosporioides* conidia are partly holoblastic from the aerial conidiophores and mostly phialidic from the sporodochial conidiophores, while in *F. kyushuense* conidia are mostly holoblastic and only produced from aerial conidiophores.

Key Words——*Fusarium kyushuense*; hyphomycete; Japan; mycotoxigenic; wheat scab.

Four important trichothecene-producing strains originally reported as *Fusarium nivale* Ces. ex Sacc. Fn-2, Fn-3 and Fn-2B (Tsunoda et al., 1968; Tsunoda, 1970; Ueno, 1971) and *F. episphaeria* (Tode) W. C. Snyder & H. N. Hansen Fn-M (Ueno et al., 1971) are described as a new species of *Fusarium* from Japan. It was from these strains that the trichothecenes nivalenol (Tatsuno et al., 1968), fusarenon-X (=nivalenol monoacetate; Ueno et al., 1969), and diacetylnivalenol (Tatsuno et al., 1970) were first isolated and characterized. Strains Fn-2, Fn-3 and Fn-2B were initially isolated by Dr. Hiroshi Tsunoda in 1963 from scabby grains of wheat cultivated at an agricultural research station, Kumamoto, Kyushu, Japan, while Fn-M was recovered in 1969 from a vinyl plate of a greenhouse on a farm in Nakamura-City, Kochi, Shikoku, Japan. Strains Fn-2 and Fn-3 were sent to the Agricultural Research Service Culture Collection (NRRL) for identification by Dr. Tsunoda in 1966 and Fn-2B and Fn-M by Prof. Yoshio Ueno in 1971. Subsequently these strains were identified at NRRL by John J. Ellis as *F. tricinctum* (Corda) Sacc. and at the Medical Research Council Collection (MRC; Tygerberg, South Africa) by Marasas et al. (1984) as *F. sporotrichioides* Sherb. A molecular systematic study conducted by O'Donnell (1997), however, provided evidence that these Japanese strains represent a phylogenetically distinct species, which we describe here as *F. kyushuense* after the island of Kyushu where it was first discovered close to thirty yr ago.

Materials and Methods

All strains described here are stored by lyophilization or cryogenically at ca. −175°C in the Ministry of Agriculture, Forestry and Fisheries (MAFF), National Institute of Agrobiological Resources, Tsukuba, Japan and in the Agriculture Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, IL. Strains were grown at 20°C on potato dextrose agar (PDA; Difco) in 9 cm plastic Petri dishes in the dark for the description of growth rate, colony color and odor. For morphological observations, cultures were grown on synthetic low nutrient agar (SNA), which included two pieces of ca. 1 × 2 cm sterile filter paper on the cooled agar surface (Nirenberg, 1990), for 14 d at 20°C in complete darkness or under continuous black light (Toshiba FL20S BLB 20W; Seemüller, 1968). Strains were also cultured on soil extract agar (SEA; a filtrate of 200 g dried and sieved field soil, which was boiled in 1 L of distilled water for 1 h prior to autoclaving with 15 g agar) for 30 d to test for the production of chlamydospores. All measurements were from cultures grown in complete darkness and at least 30 conidia were measured to calculate their average and the standard deviation. Colors cited are given according to the Methuen handbook of colour (Kornerup and Wanscher, 1978).

For comparison of micromorphology, the following strains were also incubated under the same conditions prior to microscopic observation: *F. sporotrichioides* ATCC 48018 (=MAFF 236626), ATCC 56095 (=MAFF 236627), ATCC 60310 (=MAFF 236629), ATCC 62360 (=MAFF 236628), MAFF 236638, MAFF 236639 and MAFF 236650; *F. arthrosporioides* NRRL 13995 (=CBS 829.85=BBA 64134) and NRRL 20899 (=CBS 173.32=BBA 4128).

Specimens, i.e., dried cultures, prepared in this study were deposited in the herbaria of National Institute of Agro-Environmental Sciences (NIAES), Ministry of

Agriculture, Forestry and Fisheries, Tsukuba, Japan and in the US National Fungus Collection (BPI), USDA/ARS, Beltsville, MD, USA, including the holo- and the isotypes.

Species description

Fusarium kyushuense O'Donnell & T. Aoki, sp. nov. Figs. 1–12

Coloniae in PDA ca. 1.7–6.2 mm in dies ad 20°C crescentes, margine integro vel undulato; mycelium aerium rubrum, pallide rubrum, rubescenti-albidum, griseo-aurantiacum, pallide luteum vel albidum, plerumque abundans, interdum sparse evolvens, laxe vel dense floccosum; reversum rubrum, atrorubrum, vinos-rubrum, rubescenti-albidum vel albidum; hyphae 1–5(–7.5)μm latae; sclerotia absentia; odor nullus. Initium sporulationis in SNA praecox, ad mycelium aerium vel ad conidiophora ex superficie agari stantibus exoriens. Conidia plerumque ad apicem conidiophori proliferantis singulatim formata, itaque culturae aspectus pulveraceus videtur sunt. Sporodochia plerumque sparse sed interdum copiose formata. Conidiophora aeria initio non ramosa, deinde laxe vel dense ramosa, interdum verticillata sed plerumque sympodialiter prolificantia. Cellulae conidiogenae conidiophororum aeriorum typice holoblasticae, interdum phialidicae, plerumque sympodialiter prolificantes, usque ad 50 μm longae, 2.5–4.5 μm latae; cellulae conidiogenae sporodochii monophialidicae, interdum sympodialiter prolificantes. Conidia ad mycelia aeria elliptica vel clavata, interdum obovata vel subglobosa, 0–3(–5)-septata, aliquando fusiformia vel falcata, ad extrema utrinque attenuato-angustata; ea 0-septata 4–18.7 × 2.2–4.5 μm (obovata 4–9.3 × 2.7–4.5 μm); ea 1-septata 10.5–22.3 × 2.2–4.5 μm; ea 3-septata 20–38.3 × 2.7–4.7 μm. Conidia sporodochialia ex monophialidibus subulatis vel ampulliformibus formata, typice falcata vel fusiformia, sed interdum clavata, plerumque cellula apicali acuta et cellula basali pediformi distincta vel indistincta praedita, (1–)3–5(–7)-septata; ea 3-septata 27.7–44 × 2.7–4 μm; ea 5-septata 41.7–60.8 × 3.2–4.8 μm. Chlamydosporae absentes.

Holotypus: NIAES 99701, colonia exsiccata, in cultura ex semine mucido *Tritici aestivi* L., Kumamoto, Kyushu, Japonia, 1963, H. Tsunoda, in Herbario NIAES (MAFF), Japonia deposita.

Isotypus: BPI 806248, colonia exsiccata in Herbario BPI, USA deposita.

Culturae ex holotypo: NRRL 3509 (=NRRL A-14732 =Fn-2)=MAFF 237645.

Etymology: *Kyushu*+*-ensis*; indicating the collecting place of the type material.

Colony on PDA shows an average growth rate of 1.7–6.2 mm per d at 20°C. Colony margin entire to undulate. Aerial mycelium red, pale red, reddish-white, grayish-orange, light yellow to white; generally abundant, some sparsely developed, loosely to dense floccose. Reverse pigmentation red, deep red, wine red, reddish-white to white. Hyphae 1–5(–7.5)μm wide. Sclerotia absent. Odor absent. Sporulation on SNA starting within a few days in the aerial mycelium or on conidiophores arising directly from the agar surface; conidia mostly formed singly on the tips of proliferating conidiophores giving the cultures a powdery appearance; sporodochia normally formed sparsely, but abundantly in some strains. Aerial conidiophores at first unbranched, becoming loosely to densely branched, sometimes verticillate often proliferating sympodially. Conidiogenous cells of aerial conidiophores typically holoblastic, some phialidic, often proliferating sympodially, up to 50 μm long, 2.5–4.5 μm wide; sporodochial conidiogenous cells monophialidic, some proliferating sympodially. Aerial conidia ellipsoidal to clavate, some obovate to subglobose, 0–3(–5)-septate, occasionally fusiform to falcate, tapering towards both ends; 0-septate: 4–18.7 × 2.2–4.5 μm, 9.6 ± 3.20 × 3.1 ± 0.53 μm on average and S.D. (obovate: 4–9.3 × 2.7–4.5 μm, 7.1 ± 1.22 × 3.6 ± 0.53 μm on average and S.D.); 1-septate: 10.5–22.3 × 2.2–4.5 μm, 16.0 ± 3.02 × 3.5 ± 0.54 μm on average and S.D.; 3-septate: 20–38.3 × 2.7–4.7 μm, 30.0 ± 4.89 × 3.5 ± 0.45 μm on average and S.D. Sporodochial conidia formed from subulate to ampuliform monophialides, typically falcate to fusiform, but some clavate mostly with an acuate apical cell and a distinct or indistinct basal foot cell, (1–)3–5(–7)-septate; 3-septate: 27.7–44 × 2.7–4 μm, 37.9 ± 4.00 × 3.3 ± 0.32 μm on average and S.D.; 5-septate: 41.7–60.8 × 3.2–4.8 μm, 50.1 ± 4.78 × 3.9 ± 0.40 μm on average and S.D. Chlamydospores absent.

Holotype: NIAES 99701, a dried culture, isolated from a scabby seed of *Triticum aestivum* L., Kumamoto, Kyushu, Japan, 1963, H. Tsunoda, deposited in the herbarium of NIAES (National Institute of Agro-Environmental Sciences, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki), Japan.

Isotype: BPI 806248, dried culture, deposited in the herbarium of BPI (US National Fungus Collection, Beltsville, MD), USA.

Ex holotype cultures: NRRL 3509 (=NRRL A-14732 =Fn-2)=MAFF 237645.

Isolates studied: NRRL 3509 (=NRRL A-14732= Fn-2)=MAFF 237645, NRRL 6490 (=NRRL A-18476= Fn-2B)=MAFF 237646, and NRRL 26204 (=NRRL A-14731=Fn-3)=MAFF 237648 from a scabby wheat seed, collected by H. Tsunoda in 1963, Kumamoto, Kyushu, Japan; NRRL 6491 (=NRRL A-18478=Fn-M) =MAFF 237647 from a vinyl plate of a greenhouse on a farm, collected by H. Tsunoda in 1969, Nakamura City, Kochi, Shikoku, Japan.

Specimens prepared: dried cultures, NIAES 99701 and BPI 806248 from NRRL 3509; NIAES 99702 from NRRL 6490; NIAES 99703 from NRRL 26204; NIAES 99704 from NRRL 6491.

Notes The strains available for study, including the ex-type strain NRRL 3509 (=Fn-2), are somewhat degenerated. Degeneration of mycelial pigmentation in NRRL 6490 (=Fn-2B) is reflected in the production of sparse aerial mycelium together with the completely white appearance of cultures; however, this strain is not pionnotal. Reddish, floccose sectors are occasionally formed at the colony margin which may represent the wild-type phenotype. Colonies produced by strains

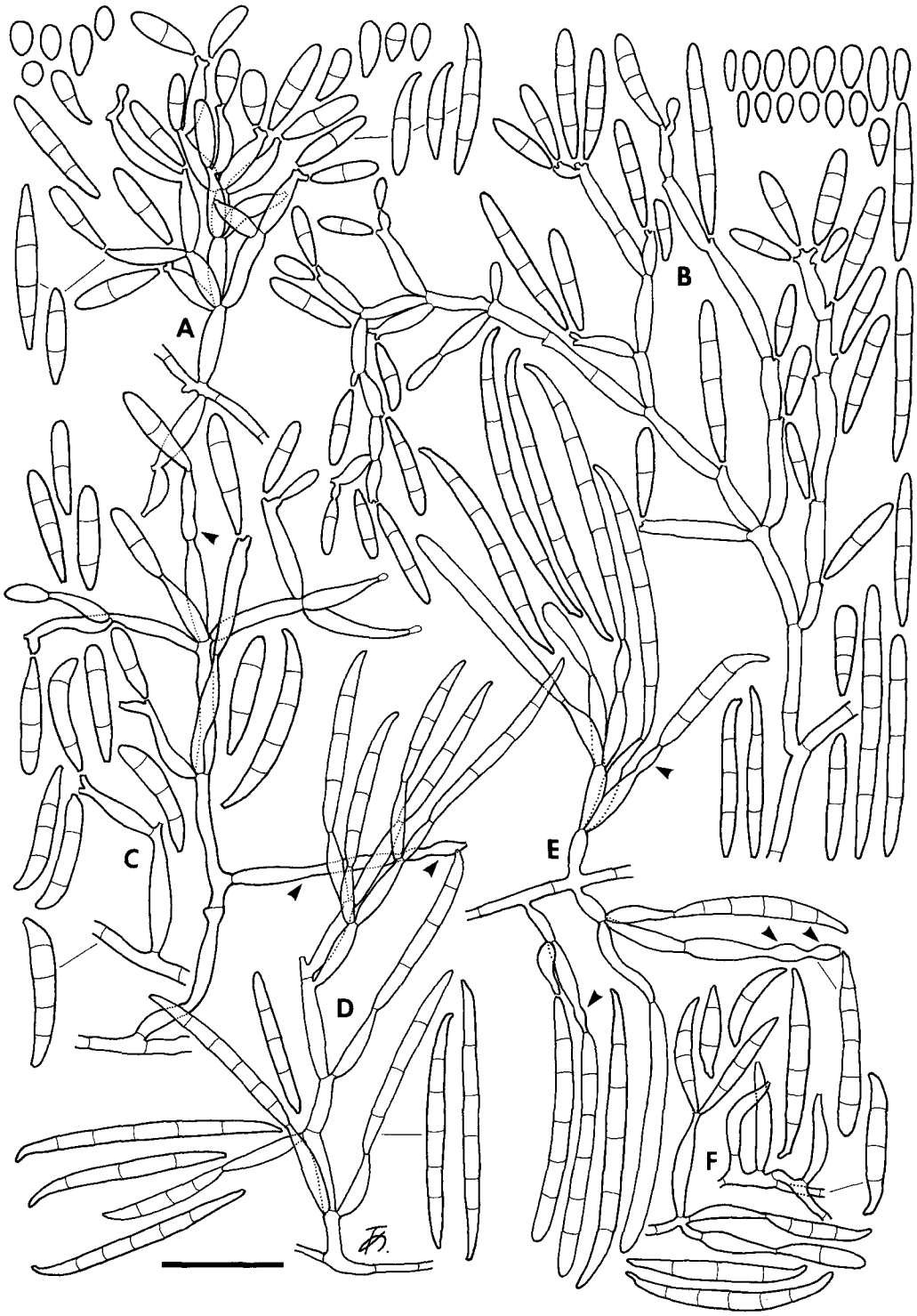
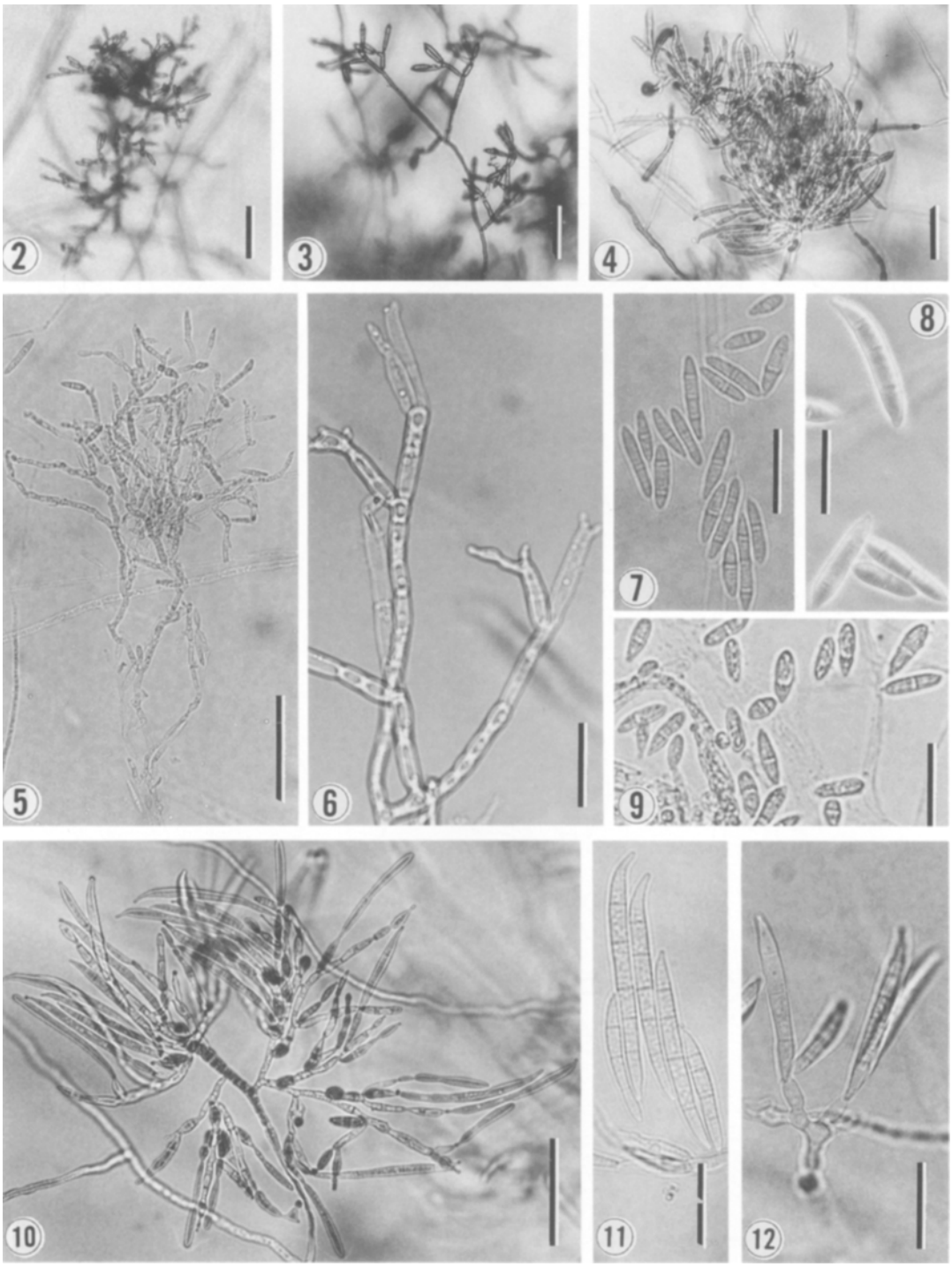


Fig. 1. *Fusarium kyushuense* grown on SNA in the dark.
(A) Densely branched aerial conidiophore with proliferating conidiogenous loci, producing septate and aseptate, ellipsoidal to clavate and some obovate and fusiform conidia (NRRL 6490=Fn-2B). (B) Loosely branched aerial conidiophore with sympodially-proliferating conidiogenous cells, producing septate and aseptate, clavate, ellipsoidal to obovate, some fusiform conidia (NRRL 3509=Fn-2). (C) Branched aerial conidiophores with proliferating conidiogenous cells producing septate conida (NRRL 26204=Fn-3). Arrowheads indicate unusual percurrent proliferation which gives the conidiogenous cells a torulose or nodulated appearance. (D) Sporodochial conidia and a branched conidiophore with phialides. Phialides sometimes proliferate from the apex of a phialide formed previously (NRRL 3509=Fn-2). (E) Sporodochial conidia and conidiophores produced as short lateral branches of a hypha (NRRL 26204=Fn-3). Arrowheads indicate percurrent proliferation of conidiogenous cells. (F) Sporodochial conidia and phialides produced directly on hyphae and on a short branch (NRRL 6490=Fn-2B). Scale bar=25 μm.



Figs. 2–12. *Fusarium kyushuense* grown on SNA in the dark.

2. Aerial conidiophore arising from the agar surface exhibiting a complex dendroid morphology, producing obovate, ellipsoidal to clavate conidia at the tips (NRRL 3509=Fn-2; an aerial view). 3. Conidiophores on the aerial mycelium, producing clavate conidia (NRRL 26204=Fn-3; an aerial view). 4. Sporodochia produced on the agar surface (NRRL 26204=Fn-3; an aerial view). 5. Aerial branched conidiophore, showing a complex dendroid morphology (NRRL 3509=Fn-2). 6. Irregularly branched conidiophores in the aerial mycelium, showing sympodially proliferating conidiogenous cells. (NRRL 6490=Fn-3). 7. Ellipsoidal to clavate aerial conidia with 0–3 septa (NRRL 3509=Fn-2). 8. Falcate to ellipsoidal aerial conidia with 1–3 septa (NRRL 26204=Fn-3; phase contrast microscopy). 9. Ellipsoidal to clavate, 0–1-septate obovate aerial conidia (NRRL 6490=Fn-2B). 10. Sporodochial conidiophores producing falcate to fusiform conidia (NRRL 26204=Fn-3). 11. Falcate to fusiform sporodochial conidia with 3–5 septa (NRRL 26204=Fn-3). 12. Sporodochial conidia formed from simple phialides (NRRL 6490=Fn-2B). Scale bars=50 μm in Figs. 2–5, 10, and 20 μm in Figs. 6–9, 11, 12.

NRRL 3509 (=Fn-2), NRRL 26204 (=Fn-3) and NRRL 6491 (=Fn-M) appear to exhibit the variable wild-type pigmentation, though the growth rate of NRRL 26204 (=Fn-3) is restricted: 1.7 mm/d at 20°C. Degeneration of microscopic morphology was observed in NRRL 26204 and NRRL 6491. These strains frequently show unusual percurrent proliferation of conidiogenous cells in both aerial and sporodochial conidiophores which gives these cells a torulose or nodulated appearance (arrowheads, Figs. 1C, E; Fig. 10). In addition, sporodochial conidia of NRRL 26204 and NRRL 6491 occasionally have rounded apices (Fig. 1E) which resemble those of *Cylindrocarpon*. Production of sporodochial conidia in NRRL 26204 and NRRL 6491 starts rapidly. Micromorphology of NRRL 3509 (=Fn-2) and NRRL 6490 (=Fn-2B) is quite stable and the much healthier appearance of the conidial structures of NRRL 3509 (Figs. 1B, D; Figs. 2, 5, 7) is accepted as representative of the wild-type. Therefore, the description of colony characteristics represents a synthesis of all of the strains, while microscopic morphology was based exclusively on the ex-type strain, NRRL 3509. Conidia of NRRL 26204 (Figs. 1C, E; Figs. 8, 10, 11) and NRRL 6491 were somewhat longer and wider than those of NRRL 3509 (Figs. 1B, D; Fig. 7) and NRRL 6490 (Figs. 1A, F; Figs. 9, 12).

Fusarium kyushuense is characterized by floccose colonies with reddish pigmentation on PDA, aseptate or septate conidia produced singly and holoblastically on the tips of sympodially-proliferating aerial conidiophores, falcate to fusiform septate sporodochial conidia, and the absence of chlamydospores and sclerotia. Except for the absence of chlamydospores, *F. kyushuense* closely resembles *F. sporotrichioides* and *F. chlamydosporum* Wollenw. & Reinking in the production of similar aerial conidia which give the colonies a powdery appearance. Although *F. kyushuense* has been mistaken for *F. sporotrichioides* (Marasas et al., 1984), *F. kyushuense* produces obovate conidia which are much smaller than the so-called 'pyriform' conidia produced by *F. sporotrichioides* (Seemüller, 1968; Gerlach and Nirenberg, 1982). Conidia produced on aerial conidiophores in *F. kyushuense* represent a continuous range of size and shapes (Figs. 1A, B; Figs. 7, 9). In contrast to *F. sporotrichioides* and *F. chlamydosporum*, which produce abundant chlamydospores singly or in chains when cultured for 2 wk on either SNA or SEA in complete darkness and under continuous black light (Seemüller, 1968; Gerlach and Nirenberg, 1982), chlamydospores were not produced by strains of *F. kyushiuense* when cultured up to 30 d under the same conditions.

Fusarium kyushuense resembles *F. arthrosporioides* Sherb. (Sherbakoff, 1915; Gerlach and Nirenberg, 1982) in that both species produce holoblastic septate conidia, i.e., arthrosporial conidia (Sherbakoff, 1915), and obovate or pyriform conidia, i.e., sporotrichial conidia of *F. arthrosporioides* (Sherbakoff, 1915), and neither produce chlamydospores. Examination of authentic strains of *F. arthrosporioides* (NRRL 13995=CBS 829.85 and NRRL 20899=CBS 173.32) revealed that pyriform conidia of *F. arthrosporioides* were produced primarily from phialides in sporodochia or phialides formed directly on hyphae on the agar surface. Phiaides in both the strains were mostly monophialidic and ampuliform as seen in *F. poae* (Peck) Wollenw. Obovate conidia, whose morphology represented a continuous range of size and shapes to other conidia, were occasionally observed on the aerial conidiophores of *F. arthrosporioides*, as observed by Sherbakoff (1915) and by Gerlach and Nirenberg (1982). They were produced holoblastically on sympodially-proliferating conidiogenous loci. In contrast, obovate conidia in *F. kyushuense* were formed only on aerial conidiophores, and these were mostly holoblastic on sympodially-proliferating conidiogenous cells (Figs. 1A, B); however, those on phialidic sporodochial conidiophores were never observed in this species. Another morphological feature that appears to distinguish these species is that sclerotia are produced by *F. arthrosporioides* (observed only in NRRL 13995=BBA 64134; Gerlach and Nirenberg, 1982) but not by *F. kyushuense*. Sclerotia formed by the former species were round to oval shaped, white to red, ca. 50–500 μm in diam on PDA, consisted of plectenchymatic somewhat swollen hyphal masses without pigmented outer wall layers.

Sherbakoff (1915) recognized *F. arthrosporioides* var. *asporotrichius* Sherb., which was differentiated from *F. arthrosporioides* (var. *arthrosporioides*) by the absence of "sporotrichial" sporodochia (i.e., sporodochia do not produce pyriform conidia). If strains of *F. arthrosporioides* var. *asporotrichius* were to lose their ability to produce pyriform conidia from sporodochia, then their morphology would converge on that of *F. kyushuense*. Sherbakoff (1915) also illustrated pyriform to obovate aerial conidia in *F. arthrosporioides* var. *asporotrichius*, and included measurements of these conidia observed under various culture conditions, although these conidia were proportionally very low in number. Booth (1971) followed Wollenweber and Reinking (1935), who synonymized this variety with *F. arthrosporioides*. Presumably Gerlach and Nirenberg (1982) also followed this synonymy because they did not mention var. *asporotrichius* in their treatment of the genus. Because Sherbakoff's (1915) diagnosis of *F. arthrosporioides* var. *asporotrichius* is so brief, and type material and ex-type cultures are not available for study, there is no way to ascertain whether this variety and *F. kyushuense* are conspecific. We, therefore, tentatively consider the variety *asporotrichius* also as distinct from *F. kyushuense*, although we are able to suggest a close morphological relationship between both taxa. Even if both taxa turn out to be conspecific, priority still exists for recognizing *F. kyushuense* at the rank species. Cladistic analyses of DNA sequences from multiple loci (O'Donnell and Cigelnik, unpubl.) clearly indicate *F. kyushuense* and *F. arthrosporioides* (as var. *arthrosporioides*) are phylogenetically distinct. Included in these analyses was NRRL 25793 received as *F. arthrosporioides* (=CBS 314.73=ATCC 24361=IMI 125834b ex *Azalea* sp. from New Zealand determined by C. Booth). Although this strain now is morphologically degenerate and without aerial conidia, phylogenetic ana-

lyses indicate that it is *F. cerealis* (Cooke) Sacc. (synonym=*F. crookwellense* Burgess et al. (1982)), not *F. arthrosporioides*.

Lastly, we have refrained from placing *F. kyushuense* in a section within *Fusarium*, because phylogenetic studies indicate that many of these infrageneric groupings are nonmonophyletic (Mulé et al., 1997; Nirenberg and Aoki, 1997; O'Donnell, 1997; O'Donnell and Cigelnik, 1997).

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