Mycoscience 38: 329–333, 1997

Fusarium nisikadoi, a new species from Japan

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Accepted for publication 29 August 1997

A new species of *Fusarium*, *F. nisikadoi*, isolated from *Phyllostachys nigra* var. *henonis* (bamboo) and *Triticum aestivum* (wheat) in Japan, is described, illustrated and discussed. This species is differentiated from other known species of the genus by the following characteristics: whitish colony color, long zigzag-like chains of 0–3(–5)-septate clavate conidia, intermixed with pyriform conidia, produced mostly from monophialides and rarely from polyphialides in the aerial mycelium, very long and slender sporodochial conidia, and no chlamydospores. The long chains of septate conidia are known only in this species of the genus *Fusarium*. The conidiophores on the aerial mycelium sometimes proliferate sympodially. The species is tentatively placed in the form-section *Liseola*.

Key Words——conidial morphology; form-section *Liseola*; *Fusarium nisikadoi*; *Phyllostachys nigra* var. *henonis*; *Triticum aestivum*.

Fusarium anamorphs of the section *Liseola* and their allies have recently undergone a series of taxonomical re-explorations, such as discoveries of new species (Marasas et al., 1985; Burgess and Trimboli, 1986; Marasas et al., 1987; Nelson et al., 1987; Marasas et al., 1988; Summerell et al., 1995; Rheeder et al., 1996), proposal of a new section *Dlaminia* (Kwasna et al., 1991), and phylogenetic consideration based on molecular analyses (Peterson and Logrieco, 1991; Voigt et al., 1995; Waalwijk et al., 1996a, b; O'Donnell and Cigelnik, 1997; O'Donnell et al., 1998). These new discoveries have been triggered mainly by rejection of the lumping classification system (Nelson et al., 1983) adopted by Snyder and Hansen (1940, 1941, 1945) and re-appreciation of the splitting species concept of the German school (Wollenweber and Reinking, 1935; Nirenberg, 1976; Gerlach and Nirenberg, 1982; Nirenberg, 1989). The discovery of increasing numbers of mating populations, i.e., biological species, identified within the section *Liseola* or the *Gibberella fujikuroi* species complex, as some American mycologists prefer to call it (Kuhlman, 1982; Leslie, 1991, 1995), might also have stimulated the reassessment.

During a survey on the occurrence of *Fusarium* species from gramineaceous substrates in Japan, *Fusarium* strains of unusual morphology were isolated from three different locations. The combination of morphological characters observed in the isolates has not yet been reported among the known species of the genus. In this report, we formally describe and illustrate the fungus as a new species of *Fusarium*, and discuss the morphological features related to the systematic positioning of the species.

Materials and Methods

Origins of the strains examined *Fusarium* strains were isolated on three separate occasions from the following substrates and regions within Japan: slime flux in clumps of the bamboo *Phyllostachys nigra* Munro var. *henonis* Stapf on the campus of the National Institute of Agrobiological Resources (NIAR), Ministry of Agriculture, Forestry and Fisheries (MAFF), Tsukuba, Ibaraki Prefecture, June 1991; wheat seed (*Triticum aestivum* L.) collected in a farmer's field in Hita, Oita Prefecture, April 1992; and a mixed culture sent to the Centraalbureau voor Schimmelcultures (CBS), The Netherlands (W. Gams, pers. comm.), originating from a nut in Japan.

Examination of taxonomic characters Strains were grown at 20°C in plastic Petri dishes on potato dextrose agar (PDA; Difco, Detroit) in the dark to diagnose colony color, odor and growth rate. All microscopic studies and measurements were conducted on synthetic low nutrient agar (SNA; per L 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 a ca. 1 × 2 cm piece of sterile filter paper placed on the cooled agar surface (Nirenberg, 1976). Cultures were incubated for 10 to 14 d at 20°C either in complete darkness or under permanent black light (Philips TLD 18 W/08; Nirenberg, 1990). These light regimes and the medium are essential for the production and comparison of certain morphological characters of *Fusarium* strains. A Zeiss Axiomat photomicroscope was used for all measurements and to record diagnostic morphological features photographically. Unless otherwise stated, all micropictures were taken of colonies grown on SNA in complete darkness,

and at least 30 conidia were measured to record the minimum and maximum size with standard deviation (S.D.) as well as mean value. All colors are given according to the Methuen Handbook of Colour (Kornerup and Wanscher, 1978).

Strains examined were deposited in BBA (Institut für Mikrobiologie, Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany), NRRL (National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois, USA), and MAFF (Genetic Resources Center, National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries, Kannondai, Tsukuba, Ibaraki, Japan).

Results and Discussion

Fusarium nisikadoi T. Aoki & Nirenberg, sp. nov.

Figs. 1–7

Coloniae in PDA ad 20°C 3.9 mm in dies crescentes, margine integra, ex mycelio aerio albido, farinaceo usque lanoso constantes; reversum griseo-aurantium vel aurantium; sclerotia nulla; odor nullus; temperatura crescentes minima 2.5°C, optima 25–27.5°C, maxima 32.5–35°C. Sporulatio in mycelio aerio cito oriens. Sporodochia parva, indistincta. Conidiophora ad mycelium aereum, sparce ramosa, nonnumquam sympodialiter proliferantia, plerumque monophialidica, interdum polyphialidica; phialides cylindricae, ad 40 μm longae, 3.5 μm latae. Conidia mycelii aerii longe catenata vel in capitulis adhaerentia, clavata, 0–3(–5)-septata, saepe pyriformia ubi 0-septata; conidia clavata et 0-septata 5.8–21.4 × 2.6–3.9 μm; conidia 3-septata 32–44.5 × 4.0–5.2 μm; conidia pyriformia 7.4–11.6(–16) × 5.2–9.5 μm. Conidia sporodochialia in agaro formata, multo longiora, recta vel falcata, (1–)3–7(–9)-septata, cellula apicali acutata et basilari pediformi; conidia 5-septata 56.0–92.0 × 3.5–4.0 μm. Chlamydosporae nullae.

Holotypus: Cultura exsiccata, ex semine *Tritici aestivi* L., Hita, Oita Pref., Japonia, Apr. 1992, T. Aoki, in Herbario B depositus.

Culturae ex holotypo: BBA 69015=NRRL 25308=MAFF 237506.

Colonies on PDA showing average mycelial growth rate of 3.9 mm per day at 20°C. Colony margin entire. Aerial mycelium whitish; loosely mealy to lanose. Pigmentation in the reverse greyish orange to orange. Sclerotial bodies absent. Odor absent. Cardinal temperatures: minimum 2.5°C, optimum 25–27.5°C, maximum 32.5–35°C. Sporulation starting early in the aerial mycelium; true sporodochia not observed. Conidiophores arising from the aerial mycelium, sparsely branched, sometimes proliferating sympodially, mostly monophialidic, sometimes polyphialidic; phialides cylindrical, up to 40 μm long and 3.5 μm wide. Conidia borne in the aerial mycelium arranged in long chains of often zigzag-like and in false heads, clavate 0–3(–5)-septate, occasionally pyriform and mostly 0-septate; clavate conidia, 0-septate: 5.8–21.4 × 2.6–3.9 μm, 13.3 ± 4.0 × 3.1 ± 0.6 μm on average and S.D.; 3-septate: 32–44.5 × 4.0–5.2 μm, 35.6 ± 3.8 × 4.2 ± 0.4 μm on average and S.D.; pyriform conidia: 7.4–11.6(–16) × 5.2–9.5 μm, 9.4 ± 1.0 × 6.0 ± 0.7 μm on average and S.D. Sporodochial conidia mostly in the agar, very long, straight to falcate, (1–)3–7(–9)-septate, with an acute apical cell and a foot-like basal cell; 5-septate: 56.0–92.0 × 3.5–4.0 μm, 75.6 ± 10.4 × 3.9 ± 0.2 μm on average and S.D. Chlamydospores absent.

Holotype: A dried culture, isolated from a seed of *Triticum aestivum* L., Hita, Oita Prefecture, Japan, Apr. 1992, T. Aoki, deposited in B (the herbarium, Botanisches Museum, Berlin-Dahlem, Germany).

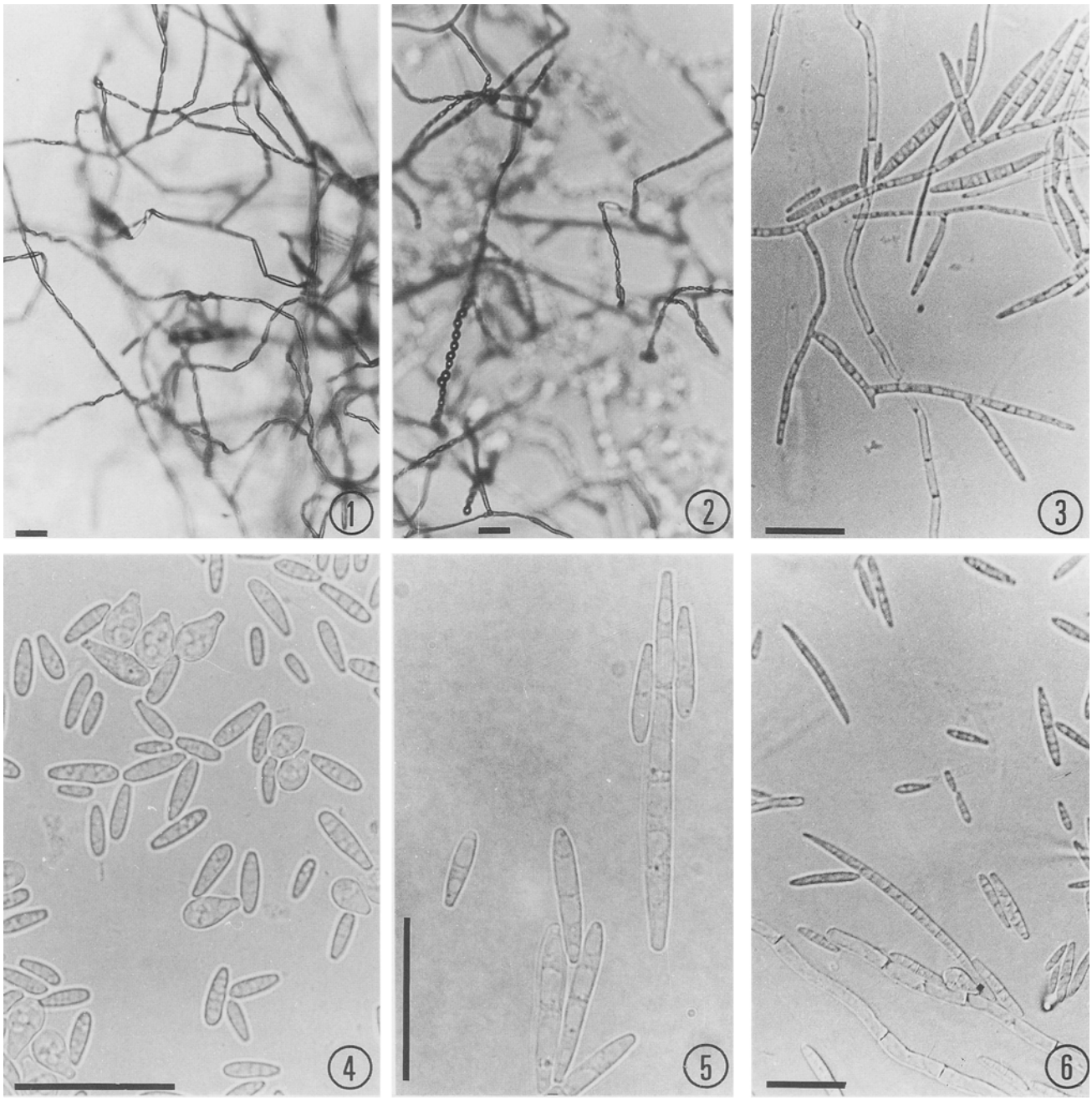
Ex holotype cultures: BBA 69015=NRRL 25308=MAFF 237506.

Isolates studied: BBA 69009=NRRL 25179=MAFF 237509, BBA 69010=NRRL 25183=MAFF 237510, BBA 69016=NRRL 25191=MAFF 237508, BBA 69014=NRRL 25203=MAFF 237507, MAFF 237566, MAFF 237567, Tsukuba, Ibaraki, Japan, slime flux of *Phyllostachys nigra* var. *henonis*; BBA 69015=NRRL 25308=MAFF 237506, Hita, Oita, Japan, a *Triticum aestivum* seed; BBA 69597=NRRL 25327, isolated at the CBS, The Netherlands, from a mixed culture, which originated from a nut of *Bertholletia excelsa* Humb. & Bonpl., Japan.

Notes The species epithet refers to the late Dr. Yosikazu Nisikado, a Japanese phytopathologist, who conducted research on *Fusarium* head blight of wheat.

Fusarium nisikadoi is characterized by 0-3(-5)-septate, long clavate conidia, which are produced in long, sometimes zigzag-like chains on often sympodially proliferating conidiophores on the aerial mycelium. This feature, 3- to 5-septate clavate conidia in chains, has not been observed in other *Fusarium* species yet. Sometimes pyriform conidia can also be seen in the chains. Production ratio of the clavate and the pyriform conidia varies with strains and cultural conditions. Degenerated strains that have lost these characters through extended cultivation on high nutritional media, resemble *Fusarium verticillioides* (Sacc.) Nirenberg. Some cultures of *F. nisikadoi* also resemble *Fusarium proliferatum* (Matsushima) Nirenberg in producing pyriform conidia in chains, sympodially proliferating conidiophores and occasional polyphialides. But still the 0-septate clavate conidia and the sporodochial conidia of *F. nisikadoi* are much longer than those of the other two species (Nirenberg, 1976).

The collective morphological features, including the absence of chlamydospores, suggest that *F. nisikadoi* is a member of the morphologically-defined infrageneric species grouping called section *Liseola*. This section and the related section *Elegans* are now under critical evaluation by molecular phylogenetic analyses based on DNA sequence data (O'Donnell and Cigelnik, 1997; O'Donnell et al., 1998). Heterogeneity and artificial features of the form-sections *Liseola* and *Elegans* have gradually been elucidated. The molecular evidence indicates that section *Liseola* is paraphyletic by definition, because chlamydospore-forming species such as *Fusarium dlaminii* Marasas, Nelson & Toussoun (Marasas et al., 1985), *Fusarium napiforme* Marasas, Nelson & Rabie



Figs. 1–6. *Fusarium nisikadoi* on SNA in the dark.
1. Aerial mycelium with conidiophores producing long clavate conidia adhering in long, zigzag-like chains (BBA 69014). 2. Aerial mycelium with conidiophores producing clavate and pyriform conidia adhering in long, linear chains (BBA 69010). 3. Branched conidiophore on the aerial mycelium with sympodial proliferation (BBA 69015). 4. Clavate and pyriform conidia borne in the aerial mycelium (BBA 69015). 5. Short and long clavate conidia borne in the aerial mycelium. 6. Sporodochial conidia (BBA 69014). All scale bars = 25 μm.

(Marasas et al., 1987), and *Fusarium nygamai* Burgess & Trimboli (Burgess and Trimboli, 1986; Marasas et al., 1988) are artificially excluded from this section. *Fusarium nisikadoi*, producing no chlamydospore, is then nested within a lineage of the section *Elegans* and does not share a common ancestor with the *Liseola* fusaria (O'Donnell et al., 1998). The sections *Liseola* and *Elegans*, therefore, have to be emended to cover the species with natural relationships, regardless of their chlamydospore production. The sections of the form-genus *Fusarium* are not natural but artificial by definition, based mainly on morphology and created for an easier way of keying. We, thus, tentatively place *F. nisikadoi* into the form-section *Liseola* of the current definition based on its

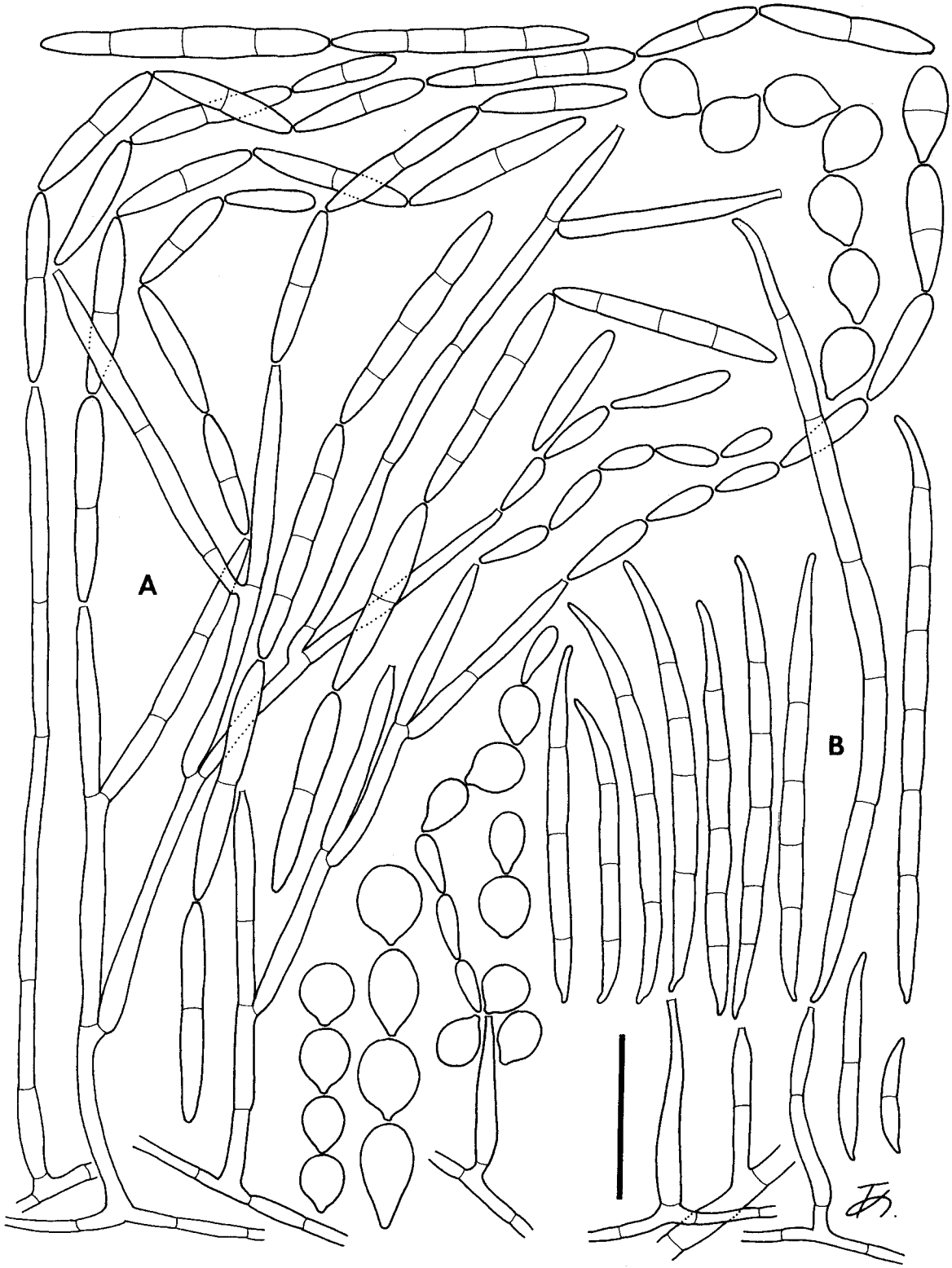


Fig. 7. *Fusarium nisikadoi* (BBA 69015) on SNA in the dark.
A: Conidiophores with septate and 0-septate, clavate and pyriform conidia in the aerial mycelium. B: Sporodochial conidia and conidiophores. Scale bar = 25 μm.

morphology. Additional genetic evidence is expected to contribute toward the systematic assessment of the sections in the genus.

Acknowledgements——The authors would like to thank Prof. Walter Gams for the Latin diagnosis, and Ms. Heidrum Anders for her excellent technical assistance. We are also grateful for the financial support from the 1994 OECD Fellowship, Cooperative Research Project on Biological Resources Management from OECD Directorate for Food, Agriculture and Fisheries, Paris, France.

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