Mycoscience (2003) 44:227–237
DOI 10.1007/s10267-003-0103-6

FULL PAPER

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Conidium morphology of *Curvularia geniculata* and allied species

Received: September 26, 2002 / Accepted: March 12, 2003

Abstract The species delimitation of some members of *Curvularia* with 4-septate conidia was studied. Conidial shapes and sizes were variable to some extent depending on isolates and herbarium materials examined. By crossing experiments, *C. affinis*, *C. fallax*, and *C. senegalensis* showed no reproductive isolation from *C. geniculata*. Some isolates obtained from culture collections and from fields were not fertile with tester isolates of *C. geniculata*. Their conidial morphology were somewhat different from *C. geniculata*. SEM observations also revealed their morphological difference from *C. geniculata*. Restriction fragment length polymorphism (RFLP) of total DNA was analyzed with several isolates of species belonging to the "geniculata" group of genus *Curvularia*. DNA fingerprinting with probes prepared from GPD-1 and tublin coding regions gave clear differences among them, resulting in different banding positions. The results of analyses also confirmed that *C. senegalensis*, *C. affinis*, and *C. fallax* are synonymous to *Curvularia geniculata*. Two populations having 4-septate conidia with warping hilum and one population with rough surface conidia were clearly different from *C. geniculata*.

Key words "Geniculata" group · New combination · Reproductive isolation · RFLP analyses · Type studies

Introduction

Members of the genus *Curvularia* have been separated into three groups, "geniculata," "lunata," and "maculans," on the basis of relative position and numbers of septa in their conidia (Boedijn 1933). These characteristics are easily recognizable under light microscopic observation. However, species identification of the members in each group is difficult because of the vague descriptions without illustrations in the early-described species and the marked variation in their conidial morphology, depending on the environmental conditions under which they were produced (Groves and Skolko 1945; Upsher 1975; Tsuda 1992; Tsuda and Ueyama 1982, 1983).

Several species of *Curvularia*, such as *C. affinis* Boedijn, *C. fallax* Boedijn, *C. senegalensis* (Speg.) C.V. Subramanian, and *C. geniculata* (Tracy et Earle) Boedijn in the "geniculata" group, had been widely accepted as independent species (Groves and Skolko 1945; Subramanian 1953, 1956; Ellis 1966, 1971; Sivanesan 1987). However, the differences of these species are difficult to recognize because of inconsistency of morphological characteristics used for species delimitation among them. In such cases, the concept of sexual reproductive isolation is useful for the delimitation of the species because interspecific hybridization is rare or difficult in these group members (Tsuda et al. 1985; Tsuda and Ueyama 1987; Shimizu et al. 1998). Fortunately, the teleomorph of *C. geniculata* has been reported by Nelson (Nelson 1964). However, crossing experiments have not been conducted for the aforementioned species except for our preliminary results using new field isolates and some cultural collections (Tsuda 1988).

Comparisons of conidial morphology of the isolates among reproductively compatible populations would be the best approach to determine the species to which they belong. Comparison of herbarium materials, as well as cultural studies on conidial morphology of field isolates, is also useful for this purpose. Further, scanning electron microscopy (SEM) observation of surface ornamentation of the conidium is useful for elucidation of critical characteristics for separation of species, as has been shown for *C. lunata* (Wakker) Boedijn and *C. aeria* (Batista, Lima et Vasconcelos) Tsuda et Ueyama (Tsuda et al. 1985).

In this article, we report the species delimitation of some members in the "geniculata" group of the genus *Curvularia* based on reproductive isolation and conidial morphology, including surface ornamentation of the conidia under SEM observation. We also present the results of restriction frag-

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ment length polymorphism (RFLP) analyses for several isolates in the "geniculata" group of the genus. Next, we discuss the relations between conidial morphology and the results of RFLP analyses for populations that are difficult to allocate to already known species in the "geniculata" group. Based on the results of the present study, the synonyms of *C. geniculata* and two new combinations for erroneously described species are presented.

Materials and methods

Isolates and specimens used in this study

Four-septate *Curvularia* species without a protuberant hilum were selected for this study. Field isolates and cultural collections used in this study are listed in Table 1. They were grown on modified V-8 juice agar medium and soil decoction agar medium (Tsuda and Ueyama 1987) or complete agar medium (CM) (Shimizu et al. 1997) in slant cultures maintained at 25°C. The specimens deposited at IMI, BPI, UT, TNS, and other herbaria examined are listed in Table 2.

Crossing experiments

Crossing experiments were made on Sach's rice-straw agar medium as described previously (Tsuda and Ueyama 1975) with tester isolates that retain maternal factor(s). They were arbitrarily selected from fresh field isolates or ascospore progenies (Tsuda and Ueyama 1987).

RFLP analyses

RFLP analyses were performed according with the methods presented by Nakada et al. (1994). In this case, the length of DNA fragments produced by digestion with specific

Table 1. Fungal isolates used in this study and their typing by restriction fragment length polymorphism (RFLP) analyses

Isolates	Host plants	Locality	RFLP type
Curvularia geniculata population			
I54 cu1-1	*Oryza sativa*	Indonesia	I
Clover 84-3	*Trifolium repens*	Kumamoto, Japan	I
Myoga cu4-2	*Zingber mioga*	Kumamoto, Japan	I
19A4-1	*Oryza sativa*	Burma	I
NC29-1-1	Graminea plant[a]	New Caledonia	I
NC29-1-2	Graminea plant[a]	New Caledonia	I
19A5	*Oryza sativa*	Burma	I
Niigata 5	*Oryza sativa*	Niigata, Japan	I
Blue Stem 3	*Andropogon furcatus*	Kumamoto, Japan	I
Ascos. g-4	Ascospore isolate		I
Yaseiine g	*Oryza sativa*	Indonesia	I
Rough surface conidia-producing population			
B71	*Oryza sativa*	Burma	II
B77-1	*Oryza sativa*	Burma	II
B110-7-1	*Oryza sativa*	Burma	II
B110-7-2	*Oryza sativa*	Burma	II
Awa-Kenjyo	*Setaria italica*	Kagoshima, Japan	II
Hilum warping conidia-producing population			
Cyp ina-g 2-1	*Cyperus iria*	Kyoto, Japan	V
Cyp ina-g 2-2	*Cyperus iria*	Kyoto, Japan	V
Weeping 1-1	*Eragrostis curvula*	Kumamoto, Japan	V
Host-Kuma 3	Graminea plant[a]	Kagoshima, Japan	V
Kobayashi-1	Graminea plant[b]		V
Kobayashi-4	Graminea plant[b]		V
Lemon g-1	*Cymbopogon citratus*	Kyoto, Japan	V
Lemon g-2	*Cymbopogon citratus*	Kyoto, Japan	V
Tojin 1-1	*Pennisetum glaucum*	Kyoto, Japan	VI
Tojin 1-2	*Pennisetum glaucum*	Kyoto, Japan	VI
Ohi-Akatorii	*Pennisetum alopecuroides*	Tokushima, Japan	VI
Isolates from culture collections			
Curvularia genuiculata IMI 69539			I
Curvularia genuiculata ICMP 6140-78			I
Curvularia genuiculata ATCC 6671			IV
Curvularia affinis IMI 38975			I
Curvularia fallax IMI 79732			I
Curvularia senegalensis IMI 80285b			I
Curvularia senegalensis ATCC 24154			I
Curvularia inaequalis IMI 12950			III

[a] Genus or species was not identified
[b] Isolated from unidentified turf grass by Dr. T. Kobayashi

Table 2. Conidial measurements of *Curvularia* "gegiculata" group from herbarium specimens

Specimens		Length × width (μm)	Mean value (μm)	L/W
Curvularia geniculata (Tracy et Earle) Boedijn				
IMI	69539	20.0–30.0 × 9.3–14.3	26.1 × 11.3	2.29
IMI	108765	22.5–33.7 × 7.5–15.0	28.0 × 10.6	2.67
IMI	103899	No measurement (conidia few)		
IMI	117731(a)	No conidia		
UT	176605[a]	21.2–33.7 × 8.1–11.2	28.8 × 9.8	2.94
UT	180648[a]	No measurement (conidia few)		
Cochliobolus geniculatus Nelson (type)				
BPI	212BH85	22.5–34.3 × 7.5–11.2	29.1 × 9.8	2.90
Helminthosporium geniculatum Tracy et Earle (type)				
BPI	2485	25.0–43.7 × 10.6–13.7	33.4 × 12.0	2.78
Brachysporium sesami Sawada (type)				
IMI	31926	24.3–34.3 × 9.3–13.1	29.4 × 10.8	2.70
TNS	F-220576	25.0–32.5 × 8.7–12.5	29.1 × 9.8	2.90
Curvularia affinis Boedijn				
IMI	38975[a]	26.2–36.2 × 8.1–13.4	31.5 × 11.2	2.88
IMI	85415[c]	No measurement (conidia broken)		
UT	176589[a]	27.5–45.0 × 8.7–13.1	33.8 × 10.3	3.28
Curvularia fallax Boedijn				
IMI	79732	17.5–25.0 × 7.5–11.2	21.4 × 9.4	2.26
IMI	110075	26.2–38.7 × 8.7–12.5	32.1 × 10.3	3.09
IMI	112670	18.7–23.7 × 8.1–11.2	21.2 × 9.8	2.14
Curvularia senegalensis (Spegazzini) Boedijn				
IMI	86098	No measurement (no 4-septate conidia)		
IMI	80285(b)	18.7–32.5 × 8.7–12.5	23.0 × 11.0	2.08
IMI	91989	20.0–30.0 × 9.3–13.7	23.3 × 11.3	2.06
UT	176605	No measurement (conidia few)		
Brachysporium senegalense Spegazzini (type)				
Spegazzini herbarium, LaPlata Univ. 27233		No measurement (no conidia)		
Acrothecium falcatum Tehon (type)				
Univ. Illinois 14509		22.5–30.6 × 9.3–13.1	26.6 × 11.9	2.23
Curvularia inaequalis (Shear) Boedijn				
IMI	12950[b]	18.9–30.0 × 7.9–11.0	24.5 × 9.1	2.60
IMI	53747[c]	22.1–31.6 × 7.9–10.0	26.6 × 9.9	2.69
IMI	106078	23.7–39.5 × 10.2–16.6	32.5 × 13.5	2.39
Helminthosporium inaequale Shear (type)				
BPI	450	No measurement (conidia few)		

L/W, length to width ratio
[a] Authorized by Boedijn
[b] Authenticated for *Acrothecium arenariae*
[c] Authenticated for *Helminthosporium inaequale*

enzymes was identified by Southern hybridization. As probes, the genomic DNA fragments of *Bipolaris maydis* (Nisikado) Shoemaker (GPD-1) and *C. lunata* (tubulin) were employed, and as the digestion enzymes, four types of 6-base recognizing cutters, *Hin*dIII, *Eco*RI, *Bam*HI, and *Pst*I were used.

SEM observations

The conidia produced on soil decoction agar slant after 2–3 weeks of culture were collected and mounted on aluminum stubs with double-sided electro-conducting adhesive tape. Samples were coated with platinum-palladium using a sputtering device (E120; Hitachi) for about 120 s. The conidia were observed immediately after coating under a Hitachi S-800 scanning electron microscope at 10 kV.

Results

Conidial morphology of field isolates and cultural collections

The conidial morphology of the isolates typical for *C. geniculata* and its allied species is shown in Fig. 1, and the conidia measurements of representative isolates are given in Table 3.

The conidia are geniculate to almost straight and boat shaped owing to the dispropotional enlargement at the third cell from the base. They are straw colored to dark brown and sometimes paler at both ends, depending on the isolates or cultural conditions in which they were produced. This conidial morphology concurs with that depicted by Ellis (1966) for *C. geniculata*, *C. fallax*, *C. affinis*, and *C. senegalensis*. However, identification of these isolates to species level is difficult, as pointed out in many previous reports (Groves and Skolko 1945; Somal 1976; Tsuda 1992). The conidial morphology of these isolates was sometimes

Table 3. Conidial measurements of representative isolates used in this study

Isolates	Length × width (μm)	Mean value (μm)	L/W
Field isolate			
I54 cu1-1	20.0–31.2 × 7.5–11.2	24.2 × 9.5	2.55
Clover 84-3	20.0–31.2 × 8.1–11.2	24.4 × 9.7	2.40
19A4-1	17.5–28.7 × 9.3–13.7	24.6 × 10.9	2.25
19A4-1[a]	21.8–38.7 × 6.2–11.2	27.0 × 9.9	2.70
NC29-Cu1-1	25.0–37.5 × 8.7–13.1	30.4 × 11.1	2.72
NC29-Cu1-2	25.0–30.0 × 8.1–12.5	27.9 × 10.1	2.76
NC29[a]	25.0–38.7 × 10.0–15.2	31.0 × 12.7	2.42
19A5	21.8–32.3 × 8.1–13.1	25.7 × 10.0	2.57
Niigata 5	22.5–28.7 × 8.8–11.8	25.9 × 10.5	2.46
Blue Stem 3	25.0–35.0 × 8.7–13.1	28.5 × 10.2	2.78
Ascos. g-4	21.8–26.2 × 6.2–9.3	24.2 × 8.0	3.01
Yaseiine g	25.0–35.0 × 6.2–12.5	30.5 × 9.8	3.11
B71	26.8–35.0 × 7.5–11.2	30.9 × 9.8	3.13
B77-1	20.0–38.7 × 7.5–13.1	29.4 × 10.0	2.94
B110-7-1	26.2–32.5 × 8.1–10.6	29.0 × 9.4	3.09
B110-7-2	23.7–35.0 × 8.1–11.2	26.7 × 9.5	2.79
Awa-Kenjyo	21.2–37.5 × 6.2–11.2	28.2 × 8.4	3.33
Cyp ina-g 2-1	28.7–43.7 × 8.7–12.5	37.6 × 9.8	3.78
Cyp ina-g 2-2	23.8–34.9 × 7.9–10.3	28.5 × 9.0	3.16
Cyp ina-g 2-2[b]	26.2–45.0 × 6.2–11.2	34.8 × 8.5	4.07
Weeping 1-1	26.2–36.2 × 7.5–11.2	29.9 × 9.0	3.30
Host-Kuma 3	27.5–41.2 × 6.2–13.7	34.3 × 10.2	3.36
Kobayashi-1	28.1–45.0 × 6.2–12.5	34.7 × 9.5	3.62
Kobayashi-4	20.0–33.7 × 6.2–9.3	28.8 × 7.6	3.75
Lemon g-1	26.2–41.2 × 6.2–10.0	31.9 × 8.4	3.78
Lemon g-2	32.5–46.2 × 8.1–11.2	38.7 × 9.3	4.13
Tojin 1-1	30.0–43.7 × 8.7–11.2	36.8 × 10.2	3.61
Tojin 1-2	31.2–40.0 × 8.7–13.7	35.2 × 10.2	3.43
Ohi-Akatorii	27.5–40.0 × 8.7–13.1	33.5 × 10.6	3.15
Curvularia geniculata			
IMI 69539	21.2–26.8 × 8.7–11.2	24.5 × 10.0	2.45
ATCC 6671	31.2–42.5 × 8.7–11.8	37.9 × 10.4	3.63
Curvularia affinis			
IMI 38975	27.5–40.0 × 8.1–12.5	33.0 × 9.8	3.34
Curvularia fallax			
IMI 79732	21.2–30.0 × 8.1–11.8	26.0 × 9.9	2.61
Curvularia senegalensis			
IMI 80285(b)	21.8–27.5 × 8.7–11.2	24.5 × 9.6	2.55
ATCC 24154	21.2–30.0 × 8.1–13.1	25.2 × 10.2	2.53
Curvularia inaequalis			
IMI 12950	18.9–30.0 × 7.9–11.0	24.5 × 9.1	2.60

[a] Conidia produced on host plant
[b] Conidia produced on different medium

different when they were produced in different conditions. Typical examples are shown in Fig. 2.

In addition, we recognized some field isolates whose conidial morphology was clearly different from that of the *C. geniculata* population (including *C. fallax*, *C. affinis*, and *C. senegalensis*). These were divided into three populations based on their conidial morphology. The rough surface conidia-producing population had pale-colored, slender, geniculate, or boat-shaped conidia (Fig. 1J, isolates B77-1, Awa-Kenjyo). The second population was composed of isolates having almost straight conidia with a warped hilum; these were further divided into two subpopulations (Fig. 1G, isolates Cyp ina-g 2-1, Lemon g-1, and others; Fig. 1H, J, isolates Tojin 1-1, Tojin 1-2, and Ohi-Akatorii). *C. geniculata* ATCC 6671 were also different in having dark-colored, relatively large-sized, almost straight to boat-shaped conidia (Fig. 1F). The conidia of *C. inaequalis* IMI 12950 resembled those of the warped hilum type population, although differing slightly (Fig. 1E).

Conidial morphology of herbarium materials

We have examined herbarium specimens of the "geniculata" group including type specimens deposited at IMI, BPI, UT, TNS, and other herbaria. The conidial measurements are given in Table 2; the typical conidia, if any, are shown in Fig. 3. Some specimens had no conidia.

The conidial morphology of the type specimen of *C. geniculata* (=*Helminthosporium geniculatum* Tracy et Earle) (BPI 3485) is shown in Fig. 4B. Conidia were 4-septate, brown to dark brown, almost straight to boat shaped, swollen at the third cell, and measured 25–44 × 11–14 μm (mean, 33.4 × 12.0 μm) with a length/width ratio of 2.78.

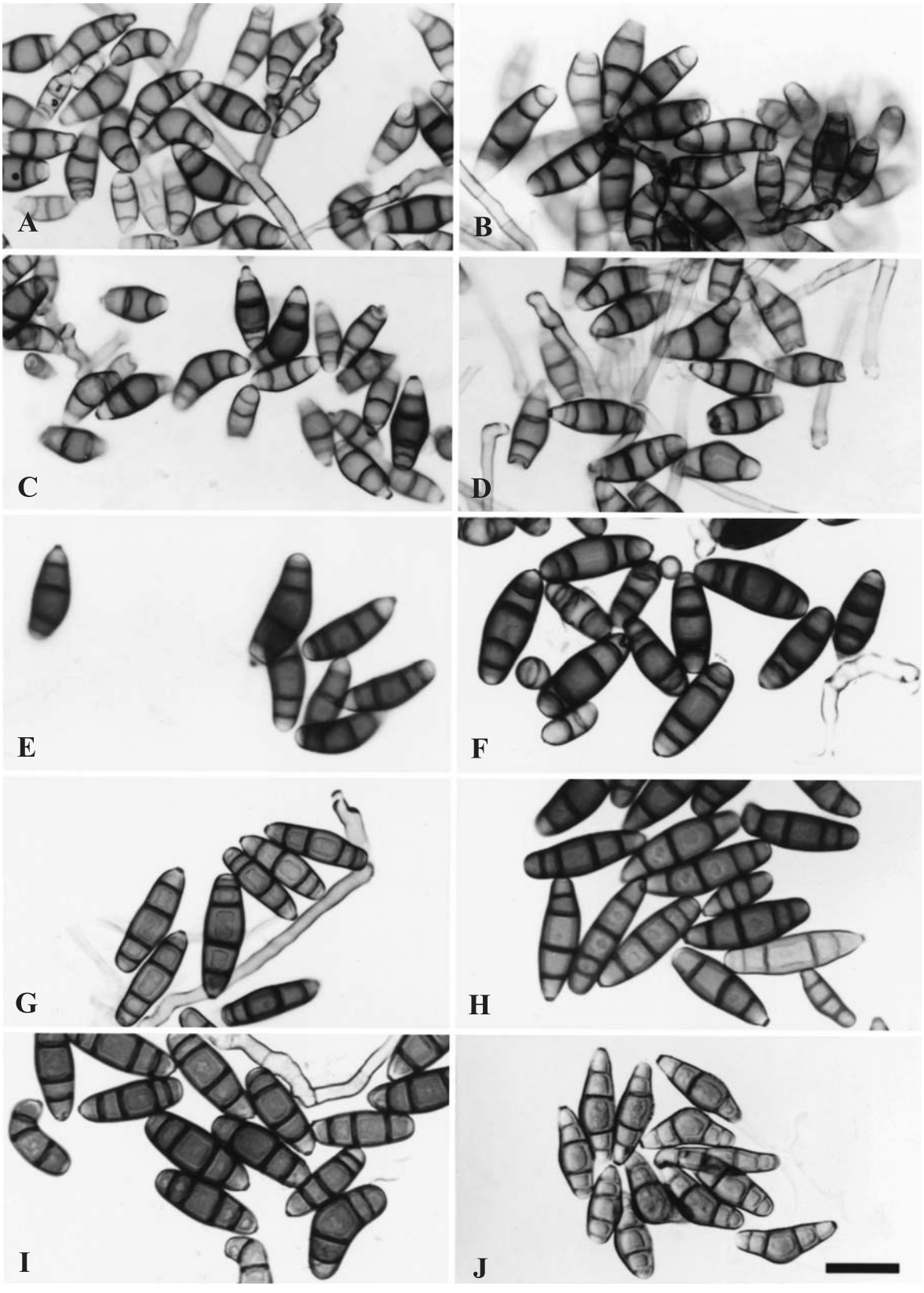

Fig. 1. Conidial morphology of representative isolates on cultural media. **A** *Curvularia geniculata* IMI 69539; **B** *Curvularia affinis* IMI 38975; **C** *Curvularia fallax* IMI 79732; **D** *Curvularia senegalensis* ATCC 24154; **E** *Curvularia inaequalis* IMI 12950; **F** *Curvularia geniculata* ATCC 6671; **G** isolate Cyp ina-g 2-1; **H** isolate Tojin 1-1; **I** isolate Ohi-Akatorii; **J** isolate B77-1. *Bar* 20 μm

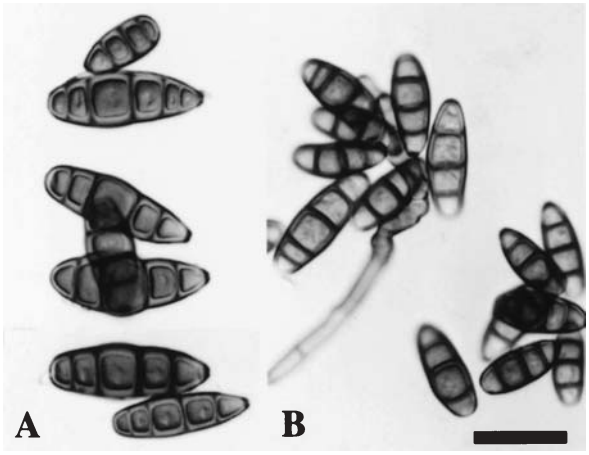

Fig. 2. Comparison of conidial morphology on field material and cultural medium of isolate Yaseiine g. **A** On field substrate; **B** on V-8 juice agar medium. *Bar* 20 μm

If the type materials had no conidia and figures had not been given in the original descriptions, annotation drawings or later illustrations based on type materials were adopted for tentative identifications. *C. senegalensis* and *C. inaequalis* were such cases. The type specimen of *Helminthosporium inaequale* Shear (BPI 450) had only a few conidia (Fig. 4A), which share some common features with those of *C. inaequalis* IMI 12950 authenticated for *H. inaequale*. The specimen of *C. inaequalis* IMI 53747 authenticated for *Acrothecium arenariae* and one more specimen, IMI 106078, had conidia of different shapes. They were straight, oval to broadly fusiform, and somewhat different from those of *C. inaequalis* IMI 12950 (see Fig. 3G, 3H). These figures were coincident with those given by Ellis (1966) but did not agree with those given by Groves and Skolko (1945). *Brachysporium senegalensis* Spegazzini at the Spegazzini Herbarium of La Plata University, Argentina, also has no conidia. Consequently, we followed the descriptions given by Ellis (1966) and Sivanesan (1987). Type materials for *Brachysporium sesami* Sawada deposited at IMI (IMI 31926) and TNS (F-220576) and *Acrothecium falcatum* Tehon deposited at the University of Illinois (Mycological collection no. 14509) agree with *C. geniculata* in their conidial shape and size (see Table 2).

Some herbaria specimens, even the type specimens of described species, were mixed with *C. lunata* or allied "lunata" group members, especially those for naturally collected specimens. In our studies on field-collected materials, we have often observed the similar mixed occurrence of fungi on the same host plants. We believe this is the reason why some early-described species have been abandoned. Thus, *Helminthosporium curvulum* Saccardo 1886 and *Helminthosporium caryopsidum* Saccardo 1886 were excluded by Boedijn (1933).

Crossing experiments with tester isolates

Fertile ascospores, produced when two isolates of opposite mating types were paired, were divided into two mating-type groups of approximately 1:1 ratio. When the authentic cultural isolates *C. geniculata* IMI 69539, *C. senegalensis* IMI 80285b and ATCC 24154, *C. fallax* IMI 79732, and *C. affinis* IMI 38975 were crossed with tester isolates, they all gave fertile ascocarps. Ascospore progenies produced conidia in culture, which showed same morphological variations, as mentioned previously. However, *C. geniculata* IFO 6283, ATCC 6671, and *C. inaequalis* IMI 12950, ATCC 6478, failed to mate with any combinations of tester isolates (Table 4). In this connection, *C. geniculata* IFO 6283 and *C. inaequalis* ATCC 6478 did not produce any conidia.

Field isolates that resembled *C. geniculata* but did not give fertile ascocarps in any combination were divided into three populations by their conidial morphology (see Conidial morphology).

RFLP analyses

All probes are obviously hybridized with the genomic DNA of all species of the group, but the numbers of bands and their relative positions appeared to be somewhat different among the species and populations divided by conidial morphology. When the same fragments were hybridized to chromosomal DNA from a range of species and populations belonging to the "geniculata" group, distinctive restriction patterns were found. In *C. geniculata*, which includes *C. affinis*, *C. senegalensis*, and *C. fallax*, populations with similar cultural characteristics and conidial morphology gave almost identical banding patterns.

A consensus bootstrap tree resulting from the RFLP analyses with eight probe–enzyme combinations for 35 isolates is shown in Fig. 5. In each probe–enzyme combination, the constructed topology gave almost the same results with slight differences (data not shown). RFLP type I comprised *C. geniculata*, including *C. affinis*, *C. senegalensis*, and *C. fallax*, although minor differentiation was observed. Some isolates including *C. affinis* IMI 38975 were semibranched. RFLP type II was composed of unidentified species that had slender geniculate conidia with a roughly warted surface. RFLP types V and VI were composed of populations that formed conidia with a warped hilum. RFLP type V was composed of an isolate Cyp ina-g 2-1 and other hilum warping members and RFLP type VI included isolates Tojin 1-1 and Ohi-Akatorii. *C. inaequalis* ATCC 6671 and IMI 12950 made each independent cluster for RFLP types III and IV, respectively. Thus, the differences in conidial morphology correlated well with the results of RFLP analyses.

SEM observations

The surface ornamentation of conidia under SEM was clearly different depending on the specimens observed. Hilum morphology also differed with the species or populations examined. Representative figures are shown in Fig. 6, and the results of SEM observation are summarized below.

1. Conidial type I, which corresponds to RFLP type II. Under SEM, vigorous scalelike warts were observed on

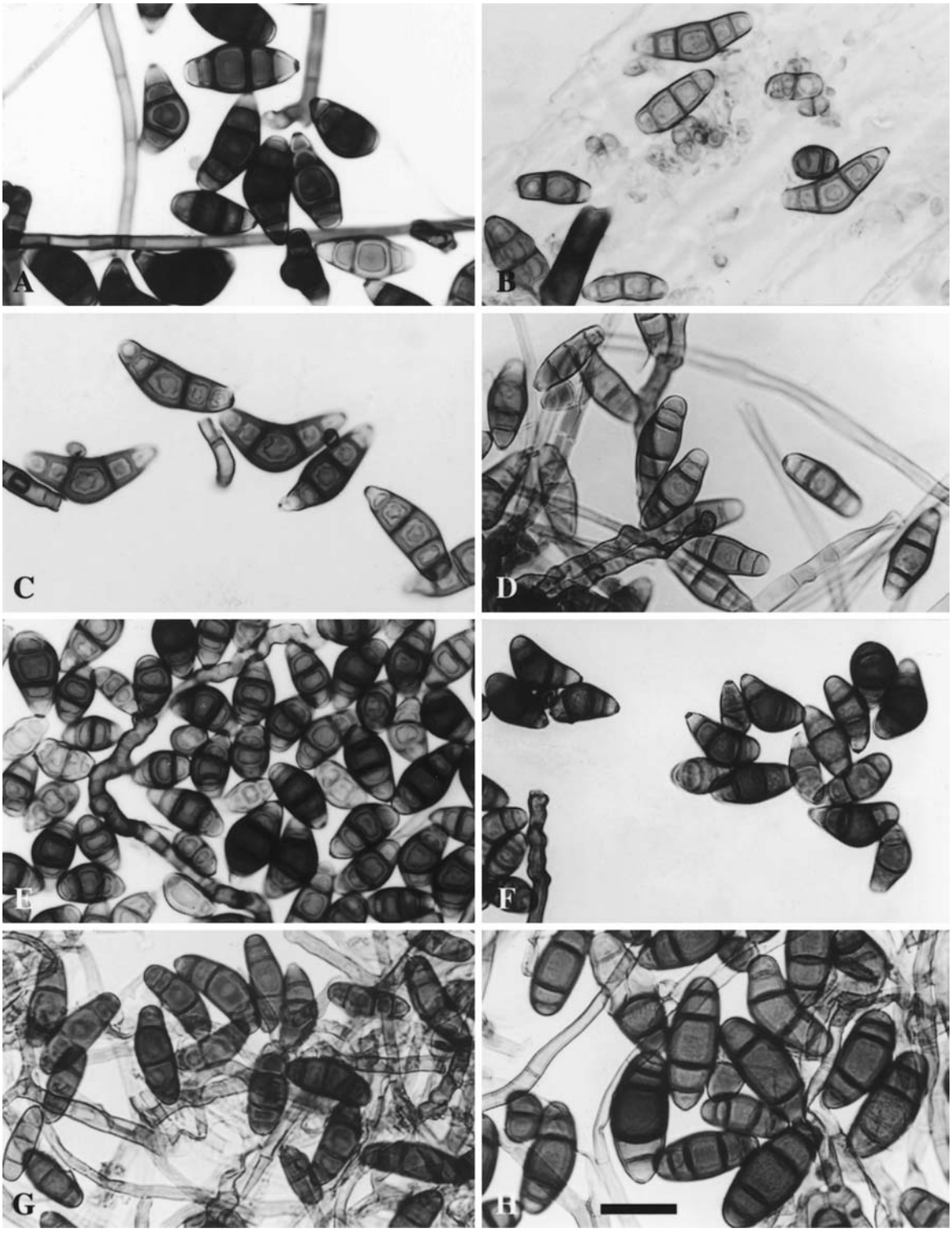

Fig. 3. Conidial morphology of herbarium materials of 4-septate *Curvularia* species. **A** *Curvularia geniculata* IMI 108765; **B** *Curvularia geniculata* UT 176605; **C** *Curvularia affinis* UT 176589; **D** *Curvularia fallax* IMI 110075; **E** *Curvularia senegalensis* IMI 80285b; **F** *Curvularia senegalensis* IMI 91984; **G** *Curvularia inaequalis* IMI 53747; **H** *Curvularia inaequalis* IMI 106078. *Bar* 20 μm

almost the entire surface, especially at the third cell. The hilum was not protruded and was situated at the axial center of the conidium. Under the light microscope, the conidial surface was roughly warted, as in *C. verruculosa*. Isolate B77-1 is typical (see Fig. 6E).

2. Conidial type II-a, which corresponds to RFLP type V. The conidial surface of this member was rather smooth and the scalelike protuberance was not prominent. The hilum was deviated from the axial center, and an unusual warping at the base cell was occasionally observed. Typi-

cal isolates include Cyp ina-g 2-1 (Fig. 6H) and Weeping 1-1 (Fig. 6I).

3. Conidial type II-b, which corresponds to RFLP type VI. The conidial surface of this group was also rather smooth, but a slight scale-like protuberance was observed at the base cell (Fig. 6J). In this group, some conidia were warped at the basal compartment by unequal development of the cell wall, and the hilum was mostly situated in the center. Typical isolates are Tojin 1-1 (Fig. 6G, J) and Ohi-Akatorii (Fig. 6F, K).
4. *C. geniculata* ATCC 6671, which corresponds to RFLP type III. The conidial surface of this isolate was rather smooth, showing no scalelike warts as seen with conidia of isolate B77-1 and *C. geniculata*. The hilum was clearly protuberant (Fig. 6C).
5. *C. geniculata* (including *C. affinis*, *C. senegalensis*, and *C. fallax*), which corresponds to RFLP type I. In this member, the conidial surface was roughly warted. Small and thin scale-like warts were observed on the base and the third cells. The hilum was not protruded.

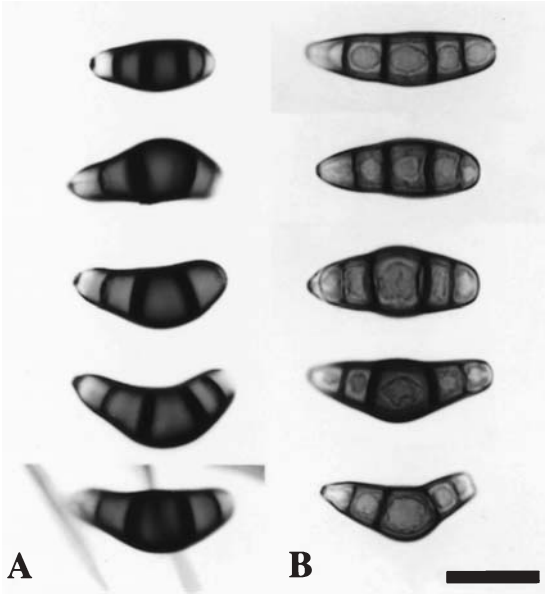

Fig. 4. Conidial morphology of type materials of *Helminthosporium geniculatum* and *Helminthosporium inaequale* deposited at BPI. **A** *H. inaequale* 450; **B** *H. geniculatum* 3485. *Bar* 20 μm

Table 4. Representative examples of results of crossing experiments with isolates from culture collections

Isolate			Mating type	
			× 19A4-1	× 914chijimi
Curvularia geniculata	IMI	69539	F	N
Curvularia geniculata	ATCC	6671	N	N
Curvularia affinis	IMI	38975	F	N
Curvularia fallax	IMI	79532	F	N
Curvularia senegalensis	IMI	80285b	F	N
Curvularia senegalensis	ATCC	24154	F	N
Curvularia inaequalis	IMI	12950	N	N
I54 cu1-1			N	F
19A4-1			F	N
Blue Stem 3			N	F
Yaseiine g			F	N

Rough surface conidia-producing populations, such as isolates B71, B77-1, B110-7-1, B110-7-2, and Awa-Kenjyo, did not produce ascocarps

Hilum warping conidia-producing populations, such as isolates Cyp ina-g 2-1, Tojin 1-1, and Ohi-Akatorii, also did not produce ascocarps

F, Mature ascocarps produced; N, not fertile

Discussion

The conidial characteristics of isolates assignable to *C. geniculata* and closely related species are summarized in Tables 2 and 3 and Figs. 1–3. The isolates showed considerable variations on different culture media used (Fig. 2).

For species separation of *C. geniculata*, *C. affinis*, *C. fallax*, and *C. senegalensis*, length/width ratio of conidial measurements and production of columnar stromata is adopted by some authors (Groves and Skolko 1945; Ellis 1966). The length/width values, however, are variable to

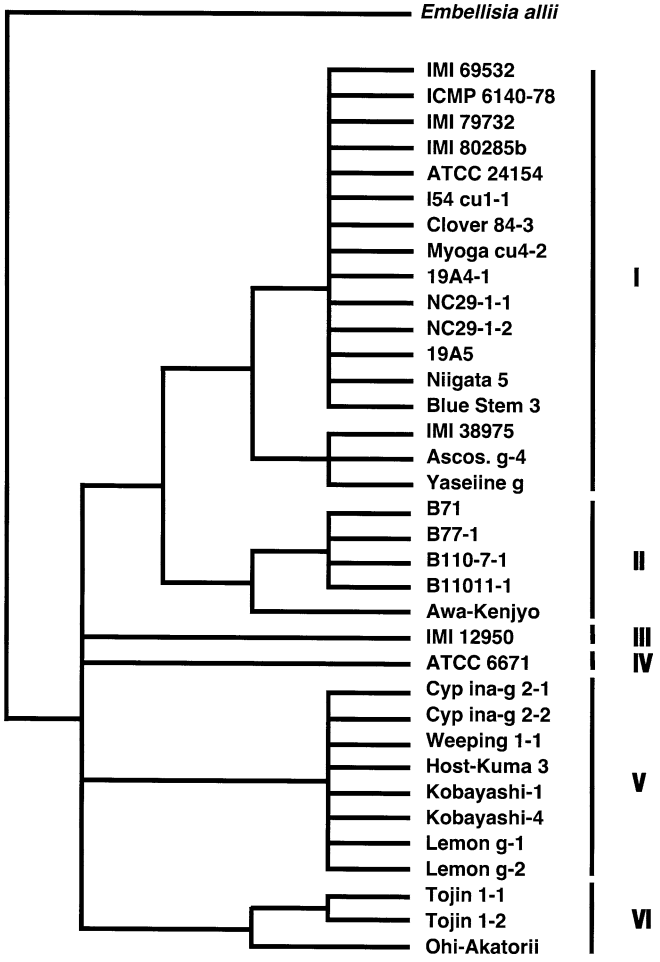

Fig. 5. Bootstrap tree of some members in "geniculata" group of *Curvularia* based on RFLP analyses of two probes with four types of 6-base cutter enzymes

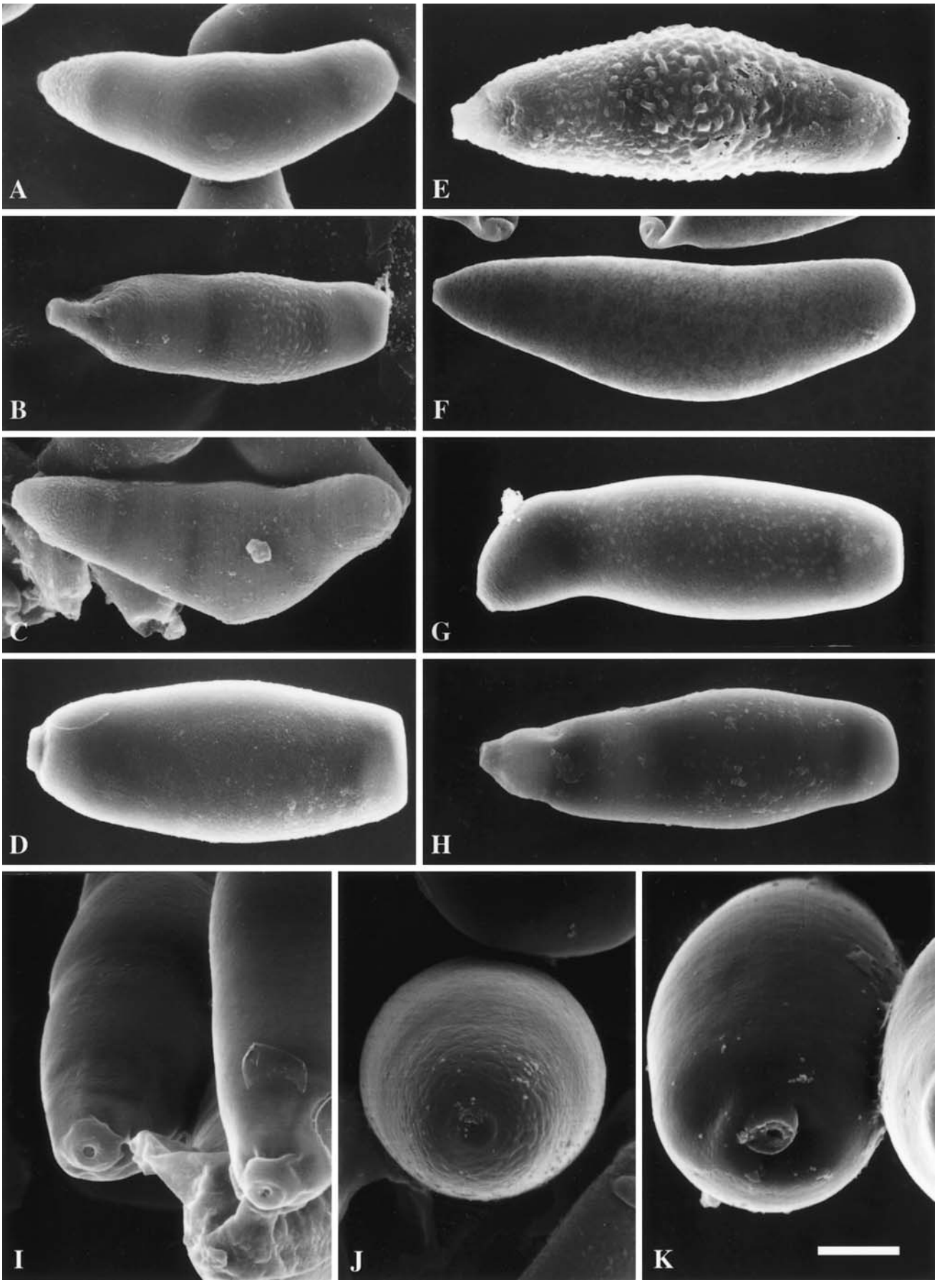

Fig. 6. Scanning electron microscopy (SEM) of conidia produced by some members in "geniculata" group of *Curvularia* used in this study. **A** *Curvularia geniculata* IMI 69539; **B** isolate Yaseiine g; **C** *Curvularia inaequalis* IMI 12950; **D** *Curvularia geniculata* ATCC 6671; **E** isolate B77-1; **F** isolate Ohi-Akatorii; **G** isolate Tojin 1-1; **H** isolate Cyp ina-g 2-1; **I** isolate Weeping 1-1; **J** isolate Tojin 1-1; **K** isolate Ohi-Akatorii. *Bars* **A**, **B**, **D**, **G**, **H** 5.0 μm; **C** 4.3 μm; **E** 3.25 μm; **F** 6.0 μm, **I**, **K** 3.0 μm

some extent with the media on which the conidia were produced (see Tables 2, 3).

Unfortunately, columnar stromata production is incidental, and the ability for production is easily lost by prolonged subcultures for most isolates (data not shown). This structure is strongly correlated to teleomorph production, because the ascocarps are often produced on the tip of columnar stromata. When two compatible isolates were grown on the same plate, fluffy thin straight mycelia were produced abundantly on the stromata that looked like trichogene (data not shown). Thus, the production of columnar stromata is not a suitable characteristic for species separation in these members of the genus.

When the herbarium materials of *C. geniculata*, *C. affinis*, *C. fallax*, and *C. senegalensis*, including the type specimens, were examined, it was difficult to distinguish one from another. Morphological variations among these specimens might suggest that the diverse conidial morphology in the same species is a common event. Variations found in the same isolate by different cultural conditions also supported this conclusion (see Fig. 2, Table 3). No conidium was found on the type specimen of *Brachysporium senegalensis*. However, the figures accompanying the type specimen suggested the species was identical with *C. geniculata*, treated previously as *C. senegalensis*.

In crossing experiments, the isolates of four species hitherto known as *C. affinis*, *C. fallax*, and *C. senegalensis* including authenticated isolates, are fertile with tester strains of *C. geniculata* (see Table 3). The conidial morphology of these ascospore progenies was indistinguishable from *C. geniculata*.

Variability in conidial morphology has also been recognized in some species in the genus *Curvularia*, such as *C. verruculosa* (Tsuda and Ueyama 1983), *C. lunata* (Tsuda et al. 1985), and particularly *C. pallescens* with *C. leonensis*-type conidia (Tsuda and Ueyama 1983). Morphological instability of conidia in the same isolate and within a species might be a common feature in this genus (Tsuda 1992).

Our RFLP analysis results indicated that intraspecific polymorphism was slight and that interspecific diversity was very prominent (see Fig. 5). The results concurred with the conclusions based on the morphological characteristics and the crossing experiments. Concludingly, the synonyms of *C. geniculata* are summarized as follows.

Teleomorph:

Pseudocochliobolus geniculatus (Nelson) Tsuda, Ueyama et Nishihara 1977

≡*Cochliobolus geniculatus* Nelson 1964

Anamorph:

Curvularia geniculata (Tracy et Earle) Boedijn 1933

≡*Helminthosporium geniculatum* Tracy et Earle 1896

=*Brachysporium sesami* Sawada 1959

=*Curvularia affinis* Boedijn 1933

=*Curvularia fallax* Boedijn 1933

=*Curvularia senegalensis* (Speg.) Subram. 1956

≡*Brachysporium senegalensis* Spegazzini 1914

=*Curvularia falcata* (Tehon) Boedijn 1933 (as *flacata*)

≡*Acrothecium falcatum* Tehon 1919

Some isolates that did not mate with standard mating-type isolates of *C. geniculata* showed apparently different conidial morphology (see Fig. 1). The isolates, whose conidia were somewhat slender and pale in color with a rough epispore (Figs. 1J, 6E), might belong to a distinct species because no such ornamentation is observed on the conidia of *Curvularia geniculata* and allied species. We checked the conidial morphology of the isolates in detail under SEM. The surface ornamentation was clearly different from any of the "geniculata" group members examined (Fig. 6). These isolates are properly assignable to *C. coicis* Matsushima 1975. However, the name is a later homonym of *C. coicis* Castellani 1952. Thus, the name should be given as a distinct species.

Curvularia matsushimae (Matsushima) Tsuda, comb. nov.

≡*Curvularia coicis* Matsushima. Icones Microfungorum a Matsushima lectorum. pp. 41–42, pl. 83–5, 85–5, 1975.

As the type specimen is not available, we tentatively designate PRI-8601 in KYO as the lectotype, and also deposit the living culture from the lecto-type in IFO.

The hilum warping population was divided into two subpopulations on the basis of differences in DNA homology. The ornamentation of the outer surface of the conidia is rough, and small warts were visible under SEM in one population (RFLP type V) but not in another population (RFLP type VI); also, the center axis of the conidia is somehow different between the two populations (Fig. 6I, J). Therefore, the two populations should be treated as separate species.

One population might be *C. inaequalis* or its closely related species. Unfortunately, we could not trace enough conidia of that species on the type materials. Groves and Skolko (1945) mentioned that it was difficult to distinguish the species from *C. geniculata*, and recognized only the difference in length/width ratio between the two species. Conidia of *H. inaequale* have a length/width ratio of 2.4 and are more dumpy (Figs. 2, 9, and 15 in Groves and Skolko 1945), which clearly differ from conidia of *C. inaequalis* depicted by Ellis (1966, 1971). The conidial measurements given previously are different among different authors (e.g., Braverman 1966). Conidial morphology of isolate IMI 12950, authentic for *H. inaequale*, and of isolate IMI 53747, authentic for *Acrothecium arenariae*, fits designated characteristics given by Ellis (1966). Their conidia resemble those of *C. geniculata* ATCC 6671. However, *C. inaequalis* IMI 12950 and *C. geniculata* ATCC 6671 were separately situated in different clusters in our RFLP analyses.

When the distal end of the conidial cell is enlarged disproportionately, the hilum may be not situated at the axial center of the conidium. Conidia with such morphological characteristics were frequently observed in some field isolates, which were different from the conidia produced by *C. geniculata*. *Curvusporium ribaldii* Corbetta (Fig. 6 in Corbetta 1963) very closely resembles these populations. This species should be treated as a member of *Curvularia*, as follows.

Curvularia ribaldii (Corbetta) Tsuda, comb. nov.
≡*Curvusporium ribaldii* Corbetta, Riso n. 3, p. 28, Fig. 6, 1963.

Acknowledgments We wish to express our sincere thanks to curators of herbaria IMI, BPI, UT, TNS, Spegazzini Herbarium of La Plata University, and the University of Illinois, for loan of the specimens. We also thank Dr. Agnes Onion for gift of culture isolates used in this study. SEM observations were undertaken by the courtesy of Dr. H. Ohba, Aburahi Laboratories of Shionogi Pharmaceutical Company; we thank him for his cooperation. We are also grateful to an anonymous reviewer for reviewing the manuscript with critical comments.

References

Boedijn KB (1933) Ueber einige phragmosporen Dematiazeen. Bull Jard Bot Buitenzorg (Ser 3) 13:120–134

Braverman SW (1966) *Curvularia geniculata* on *Sambucus chinensis*. Plant Dis Rep 50:488–489

Castellani E (1952) Una seccume fogliare del *Coix lacryma-jobi*. Riv Agron Trop Subtrop 45:159–165

Corbetta G (1963) Ricerche su alcuni funghi isolati da cariossidi riso e di *Echinochloa crusgalli*, riferibili ai generi *Helminthosporium*, *Brachysporium*, *Curvularia* e *Curvusporium* n.g. Riso 3:13–30

Corbetta G (1964) Rassegna delle specie del genere *Curvularia*. Riso 4:3–23

Ellis MB (1966) Dematiaceous Hyphomycetes. VII. *Curvularia*, *Brachysporium*, etc. CBA Mycol Pap 106:1–57

Ellis MB (1971) Dematiaceous Hyphomycetes. Commonwealth Mycology Institute, Kew

Groves JW, Skolko AJ (1945) Notes on seed borne fungi. III. Can J Res Sect C Bot Sci 23:94–104

Matsushima T (1975) Icones Microfungorum a Matsushima lectorum. Published by the author, Kobe

Nakada M, Tanaka C, Tsunewaki K, Tsuda M (1994) RFLP analysis for species separation in genera *Bipolaris* and *Curvularia*. Mycoscience 35:271–278

Nelson RR (1964) Perfect stage of *Curvularia geniculata*. Mycologia 56:777–779

Shear CL (1907) New species of fungi. Bull Torrey Bot Club 34:305–317

Shimizu K, Tanaka C, Tsuda M (1997) Cloning of *Brn1*, a reductase gene involved in melanin biosynthesis in *Cochliobolus heterostrophus*. J Gen Appl Microbiol 43:145–150

Shimizu K, Tanaka C, Peng Y-L, Tsuda M (1998) Phylogeny of *Bipolaris* inferred from nucleotide sequences of *Brn1*, a reductase gene involved in melanin biosynthesis. J Gen Appl Microbiol 44:251–258

Sivanesan A (1987) Graminicolous species of *Bipolaris*, *Curvularia*, *Exserohilum* and their teleomorphs. CAB Mycol Pap 158:1–261

Somal BS (1976) A key to the species *Curvularia*. Ind J Mycol Plant Pathol 6:59–64

Spegazzini CL (1914) Fungi Nonnulli Senegalensis et Canarienses. An Mus Nac Hist Nat Buenos Aires 26:116–134

Subramanian CV (1953) Fungi Imperfecti from Madras. V. *Curvularia*. Proc Ind Acad Sci Sect B 38:27–39

Subramanian CV (1956) Hyphomycetes. II. J Ind Bot Soc 35:446–494

Tracy SM, Earle FS (1896) New species of fungi from Mississippi. Bull Torrey Bot Club 23:205–211

Tsuda M (1988) Variability of conidial morphology in *Curvularia* species. In: Abstracts, 5th International Congress of Plant Pathology, Kyoto, Japan, 1988, p 139

Tsuda M (1992) Identification of graminicolous *Helminthosporium* sensu lato and *Curvularia* species in Japan. 3. *Bipolaris* and *Curvularia* (in Japanese). J Antibact Antifung Agents 20:437–448

Tsuda M, Ueyama A (1975) Culture condition and the formation of perfect state of *Helminthosporium oryzae*, *Cochliobolus miyabeanus*. Ann Phytopathol Soc Jpn 41:447–452

Tsuda M, Ueyama A (1982) *Pseudocochliobolus verruculosus* and variability of conidium morphology. Mycologia 74:563–568

Tsuda M, Ueyama A (1983) *Pseudocochliobolus pallescens* and variability of conidium morphology. Mem Coll Agric Kyoto Univ 122:85–91

Tsuda M, Ueyama A (1987) Some plant pathogenic fungi and their teleomorph. In: Sugiyama J (ed) Pleomorphic fungi: the diversity and its taxonomic implications. Kodansha/Elsevier, Tokyo/Amsterdam, pp 181–220

Tsuda M, Ueyama A, Nishihara N (1977) *Pseudocochliobolus nisikadoi*, the perfect state of *Helminthosporium coicis*. Mycologia 69:1109–1120

Tsuda M, Nagakubo T, Taga M, Ueyama A (1985) Sexuality for the teleomorph formation and conidial variability in *Curvularia lunata*. Trans Mycol Soc Jpn 26:27–39

Upsher FJ (1975) Fungal biological flora. 3. *Curvularia* Boedijn. Int Biodeteriol Bull 11:24–30