Mycoscience 38: 409–420, 1997

Phylogenetic relationships of the hyphomycete genera *Chaetopsina* and *Kionochaeta* based on 18S rDNA sequences

Gen Okada, Akiko Takematsu and Yukiko Takamura*

Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), 2–1, Hirosawa, Wako-shi, Saitama 351–01, Japan

Accepted for publication 16 October 1997

A setiform dematiaceous hyphomycete, *Kionochaeta spissa* was newly collected and isolated from evergreen broad-leaved forests in the southern parts of Japan. Except for its dematiaceous nature, the species is morphologically similar to a nectriaceous hyphomycete, *Chaetopsina fulva*. The morphology and cultural properties of the Japanese isolates of *K. spissa* were described, and the phylogenetic relationships between *Chaetopsina* (*C. fulva* (type species)) and *Kionochaeta* (*K. ramifera* (type species), *K. spissa* and *K. ivoriensis*) were inferred based on nuclear encoded small subunit (18S) rDNA sequences using the neighbor-joining method. *Chaetopsina* and *Kionochaeta* were found to be phylogenetically related to the Hypocreales and Sordariales, respectively. Both should be maintained as separate genus for phylogenetic classification. The morphological resemblance especially between *C. fulva* and *K. spissa* is an example of the convergent evolution.

Key Words—*Chaetopsina fulva*; *Kionochaeta spissa*; mitosporic fungi; phylogenetic relationship; 18S rDNA sequence.

Rambelli (1956) established the anamorph genus *Chaetopsina* Rambelli typified by *C. fulva* Rambelli. The type species is characterized by pale reddish brown setiform conidiophores and compact phialides producing mucoid hyaline ameroconidia. The generic circumscription was gradually modified to accommodate some other species with dark brown conidiophores or lateral branches on the conidiophore (Matsushima, 1971; Rambelli and Lunghini, 1976; Sutton and Hodges, 1976; Persiani et al., 1984). More than 15 species were subsequently ascribed to *Chaetopsina* (Rambelli, 1956; Matsushima, 1971; Rambelli and Lunghini, 1976, 1979; Sutton and Hodges, 1976; Morgan-Jones, 1979; Crane and Schoknecht, 1982; Kirk, 1985; Samuels, 1985; Castañeda, 1986; Wingfield, 1987; Merli et al., 1992; Zucconi and Rambelli, 1993), including moniliaceous and dematiaceous species. Sutton and Hodges (1976) mentioned the heterogeneity among the *Chaetopsina* species with reddish brown (moniliaceous) and dark brown (dematiaceous) conidiophores. Samuels (1985) described 4 new species of *Nectria* Fr. with *Chaetopsina* anamorphs. He tentatively treated *N. chaetopsinae* Samuels as the teleomorph of *C. fulva* and restricted the genus *Chaetopsina* s. str. to anamorphs of the Nectriaceae (Hypocreaceae in Hawksworth et al., 1995) based on the anamorph morphology of these *Nectria* species and the pigment color change reaction of conidiophores. Kirk (1985), moreover, speculated that the teleomorphs for the dematiaceous *Chaetopsina* species were likely to be found in *Chaetosphaeria* Tul. & C. Tul. As a solution to the heterogeneous nature of *Chaetopsina* s. lat., Kirk and Sutton (1985) introduced the anamorph genus *Kionochaeta* Kirk & Sutton for dematiaceous species assumed to be anamorphs of the Sphaeriaceae. They proposed the following new species or new combinations for *Kionochaeta*: *K. aristata* Kirk, *K. malaysiana* Kirk, *K. spissa* Kirk & Sutton, *K. ivoriensis* (Rambelli & Lunghini) Kirk & Sutton, *K. keniensis* (Kirk) Kirk & Sutton, *K. ramifera* (Matsushima) Kirk & Sutton, and *K. virtuosa* (Rambelli & Lunghini) Kirk & Sutton. Kuthubutheen and Nawawi (1988) and Crous et al. (1994) later described another new species of *Kionochaeta*. Rambelli (1987), on the other hand, conducted a bibliographic reassessment of the genus *Chaetopsina* s. lat. in which he rejected the distinction of the genus *Kionochaeta*. He included *Chaetopsina* s. lat. in the Dematiaceae, as Ellis (1971) treated this genus in his book "Dematiaceous Hyphomycetes."

During a survey of microfungi in evergreen broad-leaved forests in Japan, we collected and isolated a *Chaetopsina*-like fungus from plant debris at Kagoshima and Okinawa in Japan. A literature survey and comparison with the type materials allowed us to identify the hyphomycete as *K. spissa*. The species is morphologically very similar to *C. fulva*, but can be distinguished mainly by dematiaceous conidiophores. Although Kirk and Sutton (1985) speculated on the sphaeriaceous

* Present address: Novo Nordisk Bioindustry Ltd., Makuhari Techno Garden CB-6, Nakase 1–3, Mihama-ku, Chiba-shi 261–01, Japan.

affinity of the genus *Kionochaeta*, no teleomorphs have been found for *Kionochaeta* species. Using new Japanese isolates of *K. spissa* and authentic strains of *K. ramifera*, *K. ivoriensis* and *C. fulva*, we carried out a phylogenetic analysis of *Chaetopsina* and *Kionochaeta* based on nuclear encoded small subunit (18S) rDNA sequences by the neighbor-joining method.

Materials and Methods

Strains examined The following strains were used for sequencing or for morphological observation: *K. ramifera* (type species) JCM 9756 (=IFO 9947), *K. spissa* JCM 9817 and JCM 9818, *K. ivoriensis* JCM 9876 (=CBS 374.76, ex-type strain) and *C. fulva* (type species) JCM 9754 (=IFO 8919, ex-type strain) and JCM 9755 (=IFO 8843).

Isolation of DNA To obtain genomic DNA, the strains were cultivated in Difco YM broth at 20–25°C for approximately 7 d. The centrifuged wet hyphae/cells were packed with aluminum foil, frozen at −80°C, then crushed mechanically with a hammer. The genomic DNA was extracted and purified as described by Yotsumoto et al. (1995).

PCR amplification, cloning and sequencing of genomic DNA The DNA of 18S rRNA coding regions from the strains was amplified by the polymerase chain reaction (PCR) method with *Taq* DNA polymerase (Takara *Taq*) and oligodeoxynucleotides 5′-dATCTGGTTGATCCTGCCAGTAG-3′ (designated primer 2F) and 5′-dTTTCACACAGGAAACAGCTATGAC-3′ (designated primer 1794R), which were synthesized on the basis of conserved regions at the 5′ and 3′ termini of eukaryotic 18S rRNAs (Takara). The PCR was performed with a DNA thermal cycler PJ2000 (Perkin-Elmer Cetus) by 25 amplification cycles consisting of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, primer extension at 72°C for 2 min, and the final 7 min extension step necessary to make all DNAs double-stranded with 3′A-overhangs. Amplified 18S rDNA was directly ligated into the plasmid vector pCR™II or pCR™2.1 (3.9 kb), then transformed into One Shot™ INVαF′ competent cells using the Original TA Cloning Kit (Invitrogen). The plasmid DNA was extracted and purified from *Escherichia coli* cultures using the alkaline method of Flexi Prep Kit (Pharmacia Biotech), and the presence of the cloned insert was confirmed by restriction enzyme digestion. The purity and concentration of plasmid DNA solutions were determined by agarose gel electrophoresis and by measuring optical densities of solutions in a capillary cell of 0.5 mm in inside diameter (Shimadzu) with a UV-Visible Recording Spectrophotometer (Shimadzu) at the wavelengths of 280, 260 and 230 nm. For sequencing the total 18S rDNA, denaturated plasmid DNA and eight deoxyoligonucleotides (Table 1) were respectively used as templates and primers in each strain for chain elongation by the dideoxy method (Sanger et al., 1977) with Cy5™ AutoCycle™ Sequencing Kit (Pharmacia Biotech). Sequencing reactions were then carried out with a GeneAmp PCR System 9600 (Perkin-Elmer Cetus). The conditions of the elongation reaction were 17 cycles consisting of denaturation at 95°C for 36 s, annealing at 50°C for 36 s, and extension at 72°C for 84 s; then 13 cycles consisting of denaturation at 95°C for 36 s and extension at 72°C for 84 s; and finally extension for 5 min at 72°C. The sequences of genomic DNA base

Table 1. Primers used in each strain of the *Kionochaeta* and *Chaetopsina* species for amplifying and sequencing 18S rDNA.

Primer	Corresponding position in the 18S rDNA sequence of *Saccharomyces cerevisiae*
PCR primer for amplification of 18S rDNA	
2F: 5′-dATCTGGTTGATCCTGCCAGTAG-3′	2–23[a]
1794R: 5′-dGATCCTTCCGCAGGTTCACC-3′	1794–1775[b]
Primer for sequencing	
Universal primer contained in the AutoCycle Sequencing Kit	
M13(-40): 5′-dCGCCAGGGTTTTCCCAGTCACGAC-3′	
M13Reverse: 5′-dTTTCACACAGGAAACAGCTATGAC-3′	
Synthesized forward primer	
404F: 5′-dGCTACCACATCCAAGGAAGG-3′	404–423
573F: 5′-dCGCGGTAATTCCAGCTCCA-3′	573–591[c]
1270F: 5′-dCATGGCCGTTCTTAGTTGG-3′	1270–1289
Synthesized reverse primer	
581R: 5′-dATTACCGCGGCTGCTGGC-3′	581–564[c]
1332R: 5′-dAAGGTCTCGTTCGTTATCG-3′	1332–1314
1641R: 5′-dACGGGCGGTGTGTAC-3′	1641–1637[c,d]

a) Modified primer of Nishida and Sugiyama (1993).
b) Nishida and Sugiyama (1993).
c) Hendriks et al. (1991).
d) Lane et al. (1985).

Table 2. Species names, gene library accession numbers and strains examined for 18S rDNA sequences.

Species	DDBJ/EMBL/GenBank accession number	Strain number/Source[b)]
Ajellomyces capsulatus	S45469 (X58572)	ATCC 11408
Aniptodera chesapeakensis	U46870	ATCC 32818
Aphysiostroma stercorarium	U32398	ATCC 24747
Auxarthron zuffianum	U29395	UAMH 1875
Balansia sclerotica	U32399	ATCC 16582
Blumeria graminis f. sp. *hordei*	L26253	individual isolate Sui261
Botryosphaeria rhodina	U42476	CBS 356.59
Capronia pilosella	U42473	A. Y. Rossman 1422
Ceratocystis fimbriata	U32418	T. C. Harrington C89
Ceratocystis virescens	U32419	T. C. Harrington C69
Cercophora septentrionalis	U32400	D. Malloch
Ceriosporopsis halima	U47843	J. Kohlmeyer 5473F
Chaetomium elatum	M83257	UCB 81-063
Chaetomium globosum	U20379	ATCC 44699
Chaetopsina fulva	AB003786[a)]	JCM 9754
Claviceps paspali	U32401	ATCC 13892
Cordyceps intermedia	U46881	J. W. Spatafora 31-94
Corollospora maritima	U46871	J. Kohlmeyer 4834
Cryphonectria radicalis	L42442	individual isolate 3K/87
Cudonia confusa	Z30240	UME 29217
Daldinia concentrica	U32402	ATCC 36659
Debaryomyces hansenii	X58053	MUCL 29826
Diaporthe phaseolorum	L36985	F. A. Uecker 458
Diatrype disciformis	U32403	CBS 197.49
Dipodascopsis uninucleata	U00969	UCB 61-016
Dothidea insculpta	U42474	CBS 189.58
Epichloe typhina	U32405	ATCC 56429
Eremascus albus	M83258	UCB 50-026
Eurotium rubrum	U00970	UCB 88-016
Exophiala dermatitidis	X79312 (X79313, X79314)	CBS 207.35
Exophiala mansonii	X78480	CBS 158.58
Geosmithia lavendula	D14405	IFO 7729
Glomerella cingulata	U48427	F. A. Uecker 513
Gyromitra esculenta	Z30238	UME 29221
Halosphaeria appendiculata	U46872	CBS 197.60
Halosphaeriopsis mediosetigera	U32420	ATCC 16934
Hirsutella thompsonii	U32406	ATCC 24874
Hypocrea lutea	D14407	IFO 9061
Hypocrea pallida	U32408	U32408
Hypocrea schweinitzii	L36986	C. T. Rogerson 79-225
Hypocrella sp.	U32409	G. J. Samuels 89-104
Hypomyces chrysospermus	M89993	UCBH 1577345
Hypomyces polyporinus	U32410	ATCC 46844
Hypoxylon atroroseum	U32411	J. D. Rogers
Inermisia aggregata	Z30241	UME 29218
Kionochaeta ivoriensis	AB003787[a)]	JCM 9876
Kionochaeta ramifera	AB003788[a)]	JCM 9756
Kionochaeta spissa	AB003789[a)]	JCM 9817
Kionochaeta spissa	AB003790[a)]	JCM 9818
Leotia lubrica	L37536	not detected
Leptosphaeria bicolor	U04202	ATCC 42652
Leucostoma persoonii	M83259	G. Adams LP8

Lignincola laevis	U46873	J. Kohlmeyer 5180A
Lophiostoma crenatum	U42485	CBS 629.86
Melanospora fallax	U47842	CBS 478.75
Microascus cirrosus	M89994	UAMH 963
Microascus trigonosporus	L36987	Rancho Santa Anna 1942
Monascus purpureus	M83260	ATCC 16365
Morchella elata	L37537	not detected
Nectria cinnabarina	U32412	G. J. Samuels 89-107
Nectria haematococca	U32413	G. J. Samuels 89-97
Nectria viridescens	U44116	ATCC 16217
Neurospora crassa	X04971	not detected
Neocosmospora vasinfecta	U32414	Rancho Santa Anna 1898
Nimbospora effusa	U46877	J. Kohlmeyer 5104A
Nohea umiumi	U46878	J. Kohlmeyer 5103F
Ophiodeira monosemeia	U46879	J. Kohlmeyer 5164A
Ophiostoma piliferum	U20377	T. C. Harrington C300
Ophiostoma stenoceras	M85054	UCB 57-013
Ophiostoma ulmi	M83261	ATCC 32437
Petriella setifera	U32421	ATCC 26490
Pleospora rudis	U00975	UCB 75-001
Pneumocystis carinii	X12708	rat *Pneumocystis* trophozoites
Podospora anserina	X54864	not detected
Protomyces inouyei	D11377	IFO 6898
Pseudallescheria boydii	M89782	UAMH 4304
Saccharomyces cerevisiae	J01353 (M27607)	not detected
Sclerotinia sclerotiorum	X69850	MUCL 11553
Sordaria fimicola	X69851	MUCL 937
Spathularia flavida	Z30239	UME 29216
Sphaerostilbella aureonitens	U32415	G. J. Samuels 83-286
Sphaerostilbella sp.	U32416	G. J. Samuels 82–40
Sporormia lignicola	U42478	CBS 264.69
Sporothrix schenckii	M85053	ATCC 14284
Taphrina wiesneri	D12531 (D01175)	IFO 7776
Thielavia terrestris	U43969	ATCC 26796
Urnula hiemalis	Z49754	UME 30174
Varicosporina ramulosa	U43846	J. Kohlmeyer RVG-113
Xylaria carpophila	Z49785	UME 30349
Xylaria curta	U32417	J. D. Rogers
Xylaria hypoxylon	U20378	ATCC 42768

a) New sequence determined in this study; b) ATCC (American Type Culture Collection), CBS (Centraalbureau voor Schimmelcultures), IFO (Institute for Fermentation, Osaka), JCM (Japan Collection of Microorganisms), MUCL (Mycoteque de l'Universite Catholique de Louvain), UAMH (Univ. of Alberta Microfungus Collection and Herbarium), UCB/UCBH (Univ. of California, Berkeley Collection/Herbarium), UME (Herbariet, Ekologisk Botanik, Umeå Universitet).

pairs were determined by gel electrophoresis using an ALF red™ DNA Sequencer (Pharmacia Biotech) and analyzed with the sequence editing software GeneWorks (IntelliGenetics).

Phylogenetic analysis The 18S rDNA sequences used are listed in Table 2 with their accession numbers in the nucleotide sequence databases (GenBank, EMBL and DDBJ). All sequences were aligned using the multiple sequence alignment program CLUSTAL W ver. 1.60, an updated version of CLUSTAL W ver. 1.4 (Thompson et al., 1994). The following phylogenetic analysis was also performed by CLUSTAL W ver. 1.60 using the aligned sequence data set. Sites where gaps existed in the sequences were excluded. Using the two-parameter model of Kimura (1980), distances between the sequences (K_{nuc} values) were calculated. Phylogenetic trees were constructed by the neighbor-joining method (Saitou and Nei, 1987) based on the comparison of 1458 and 751 sites for the long (Fig. 15) and short (Fig. 16) partial sequence data sets, respectively. The topology of the trees was evaluated by a bootstrap analysis (Felsenstein, 1985) of 1,000 random resamplings. The DDBJ homology search system, FASTA ver 3.0, was used (Pearson and Lipman, 1988) for revealing close rela-

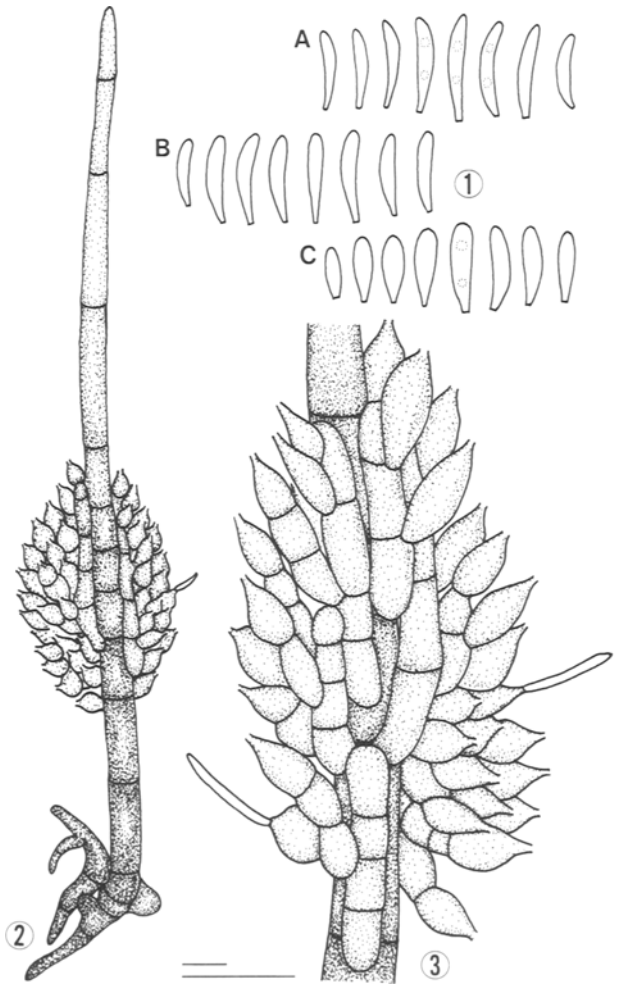
tives to *Chaetopsina* and *Kionochaeta* species in the DDBJ periodically released database.

Description

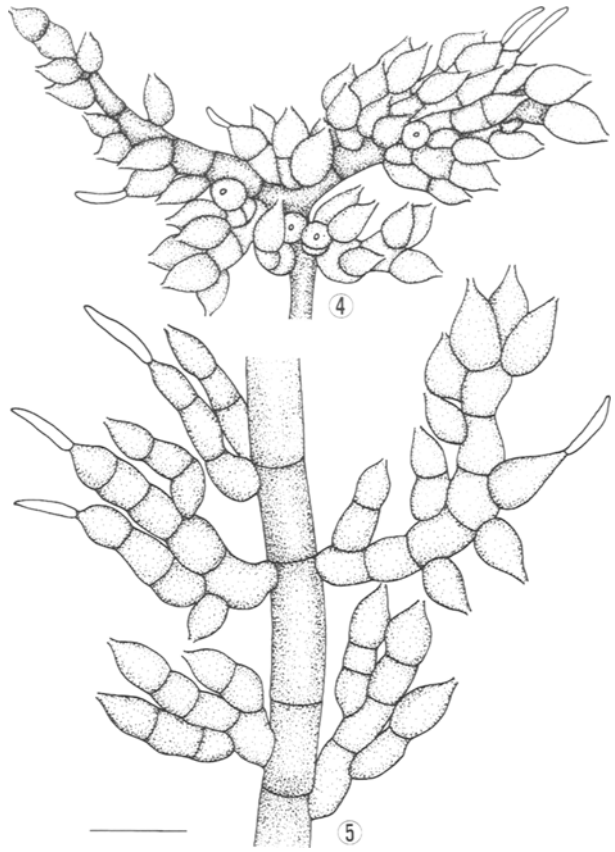
Kionochaeta spissa P. M. Kirk & B. Sutton, Trans. Br. Mycol. Soc. **85**: 715. 1985. Figs. 1–14

Colonies on OA 37–40 mm in diam after 4 wk at 25°C, almost flat, producing little aerial mycelium, velvety in conidiation, blackish dark brown mostly, deep green (30E8; Kornerup and Wanscher, 1978) at the center because of conidiation, with entire or crenate margin; reverse grayish black to black. Conidiophores macronematous, mononematous, solitary, erect, setiform, subulate, straight or slightly curved, 110–250 μm long, 2.5–7.5 μm wide, often swollen at base (up to 10 μm wide), septate, thick-walled, smooth-surfaced, dark brown (6F8–7F8) to yellowish brown (5E8), paler toward the sterile apex, not turning immediately yellow in 100% lactic acid, bearing lateral branches in/below the middle; lateral branches thin-walled, yellowish brown (5E8) to dark brown (6F8), forming phialides in very compact groups. Phialides densely clustered on setiform conidiophores, also produced on non-setiform conidiophores scattered over the whole surface of agar media, ampulliform, monophialidic, thin-walled, yellowish brown (5D5–5E8), 4–8 × 2.5–4 μm, 0.5–1 μm wide at neck. Conidia aggregated into a slimy drop, aseptate, acerose, usually slightly curved, sometimes straight, with a round apex and a narrow truncate base, smooth, hyaline to pale yellow (3A3), sometimes bi-guttulate, 5–9 × 1–1.5 μm; conidial mass (ca. 50 μm long, ca. 40 μm wide) light yellow (3A5), yellowish white (3A2) or very pale greenish yellow when fresh, almost white when old. Optimum temperature for growth 25–30°C on OA and PDA.

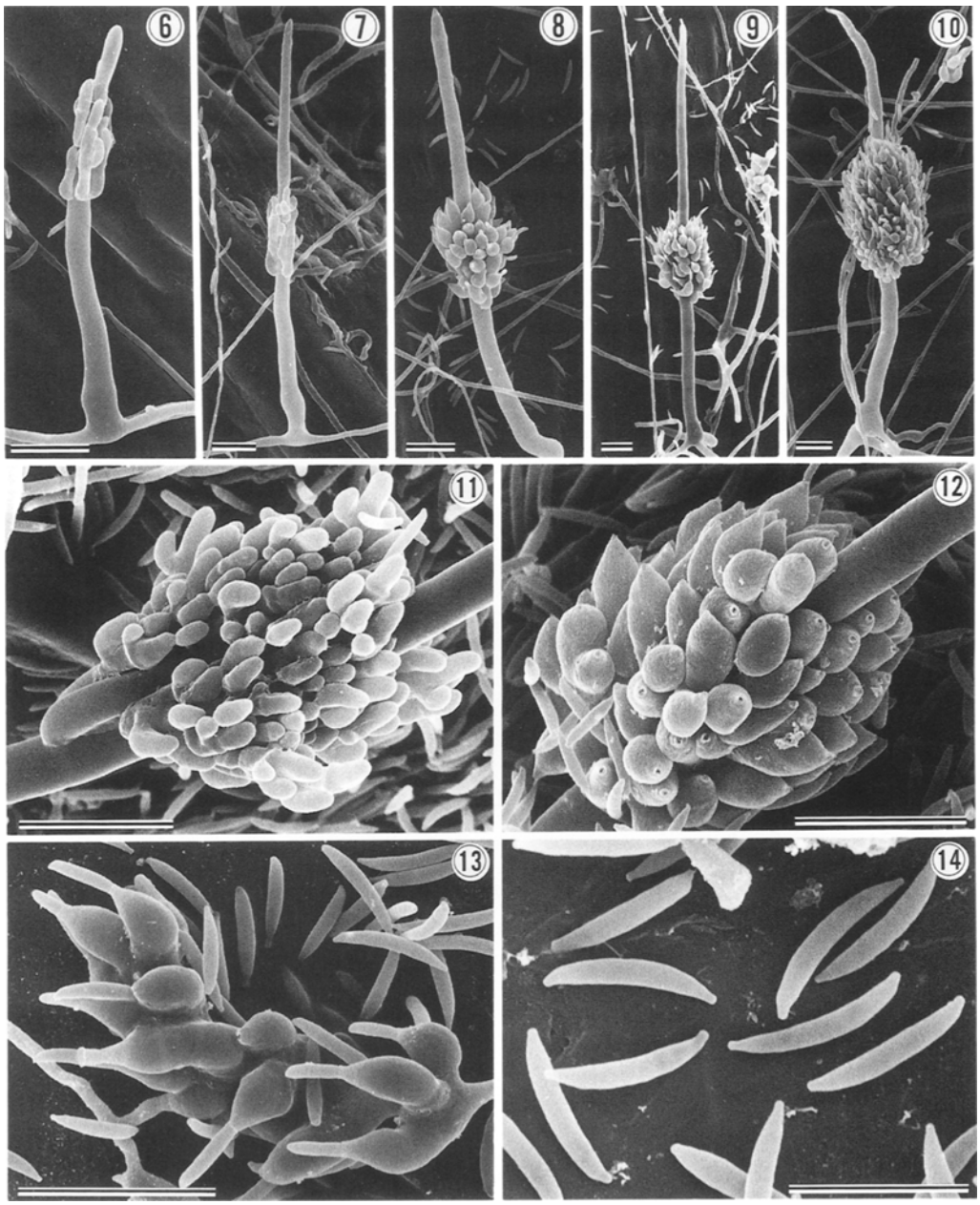
On natural substrate, the morphological features, such as setiform conidiophores (up to ca. 300 μm long) and pale yellow conidia, are similar to those in culture. However, the following differences were observed: 1) the colors of conidiophores and phialides were dark brown to brown (not yellowish as in culture); 2) the conidia were slightly shorter and thicker (5–8 × 1.2–1.8 μm, Fig. 1C) than those in culture (Figs. 1A, B); and



Figs. 1–3. *Kionochaeta spissa*.
1A. Conidia on OA (JCM 9818). 1B. Conidia on OA (JCM 9817). 1C. Conidia on natural substrate (GO 1424). 2. Setiform conidiophore and compactly packed phialides, on CMA with carnation leaves (JCM 9818). 3. Lateral branches from setiform conidiophore forming pigmented phialides, on CMA with carnation leaves (JCM 9818). Short bar=10 μm in Figs. 1 and 3; long bar=10 μm in Fig. 2.



Figs. 4, 5. *Kionochaeta spissa* (JCM 9818).
4, 5. Pigmented phialides produced on non-setiform conidiophores, on CMA with carnation leaves. Bar=10 μm.



Figs. 6–14. *Kionochaeta spissa* (JCM 9818) on CMA with carnation leaves, SEM.
6–10. Developmental stages of conidiophores, lateral branches and phialides. 11. Lateral branches with immature phialides on setiform conidiophore. 12. Mature phialides on setiform conidiophore. 13. Phialides producing conidia on non-setiform conidiophores. 14. Conidia spread on agar medium. Bars = 10 μm in Figs. 6–13; bar = 5 μm in Fig. 14.

3) the fresh conidial drops were yellow in the moist chamber, but completely dried up to become transparent films on herbarium specimens (not dried-up yellow masses as in dried culture).

Specimens examined: G. Okada (GO) 1424 and IMI 374100 (=GO 1425), both on wet decaying bark of an unidentified tree in evergreen broad-leaved forest, leg. G. Okada, Sumiyou, Amami isl., Kagoshima Pref., Japan, 23 January 1995. TNS-F-181992, a dried OA culture derived from JCM 9817, isol. ex GO 1424. TNS-F-181993, a dried OA culture derived from JCM 9818, isol. ex unidentified decaying leaves in evergreen broad-leaved forest, leg. Y. Takamura, Nakama river, Iriomote isl., Okinawa Pref., Japan, 5 May 1995.

Reference specimens examined: IMI 285389 (holotype) and a slide from the type, on leaf litter of *Eucalyptus* sp., leg. P. M. Kirk, Castle forest, Mt. Kenya, Kenya, 25 January 1984.

Results and Discussion

Identification of the Japanese *K. spissa* and on the mor-

phologically similar allies As far as we know, this is the first report on growth of *K. spissa* in culture and the first record of the species from Japan. On the natural substrate, the morphology of the Japanese *K. spissa* (GO 1424 and IMI 374100) agreed well with the holotype (IMI 285389) and the description of Kirk and Sutton (1985). However, there is one different feature in the Japanese *K. spissa*. Especially in fresh materials, vivid yellow conidial drops on setiform conidiophores were observed both on natural substrate and in culture. When the conidial drops were old and completely dried up, they became much paler in color and finally formed a hyaline film. The yellow conidial drops often turned white in old cultures. Based on these phenomena, it is acceptable that Kirk and Sutton (1985) observed only a white conidial mass on the herbarium materials of *K. spissa* collected at Hawaii, Queensland and Mt. Kenya.

The specimen and culture show some slight differences in morphology. For instance, conidia were much shorter and thicker and the colors of conidiophores and phialides were more brown on the natural substrate. It was very difficult to observe the microscopical details of this fungus on the substrate because sticky conidial drops dry up completely and do not rehydrate easily. Therefore, the description and illustrations of the Japanese *K. spissa* in this paper were mainly based on the isolates growing on OA or CMA with sterilized natural substrates (i.e., carnation/banana leaves).

Kionochaeta spissa and *C. fulva* both have non-branched setiform conidiophores (Figs. 2, 3), non-setiform ones in culture (Figs. 4, 5) and other similar morphological features (conidiophore development (Figs. 6–10), conidium and phialide morphology (Figs. 11–14); cf. Onofri and Zucconi (1991) for *C. fulva*). Table 3 lists the diagnostic features of each species from the literature and our own observations, although the definition of *C. fulva* collected or isolated from many different kinds of plant debris differs considerably from researcher to researcher (e.g., Rambelli (1956); Tubaki and Saito (1969); Ellis (1971); Samuels (1985); Onofri and Zucconi (1991); Rambelli et al. (1991a, b)).

Table 3. Comparison between *Kionochaeta spissa* (JCM 9817, JCM 9818) and *Chaetopsina fulva*.

	Kionochaeta spissa			*Chaetopsina fulva*			*Chaetopsina* cf. *fulva*	
	JCM 9817	JCM 9818	IMI 285389 (Holotype)	JCM 9755	Rambelli (1956)	Ellis (1971)	Samuels (1985) Nature	Samuels (1985) Culture
Conidiophore								
Length	(160–)190–250[b)]	(110–)150–250	ca.190–250	(200–)250–320	120–275	280	200–230	(107–)125–165(–175)
Width	5–7.5	(2.5–)4–5	–	5–10	7.5–8	5–8	–	–
Base	5–10	(3–)5–7.5	6.25–7.5	7.5–12.5	–	15–20	10–15	10–12
Color	dark brown	dark brown	dark brown	red brown	brown	brown	red brown	red brown
Phialide								
Length	4–7	(4–)5–7(–8)	–	4–7	–	7–15	(10–)15–20(–22)	(10–)15–20(–22)
Width	3–4	2.5–3(–4)	–	3–4	–	3–4	2.5–3.0	1.5–2.0
Neck	1	(0.5–)1	–	1	–	1	0.5–1.0	1
Color	dark brown	dark brown	brown	hyaline	hyaline[a)]	hyaline[a)]	hyaline[a)]	hyaline[a)]
Conidium								
Shape	acerose, with a round apex and a narrow truncate base; straight or curved		narrowly clavate; straight or curved	cylindrical, with rounded ends; straight	cylindrical, with rounded ends; straight[a)]	cylindrical, with rounded ends; straight[a)]	oblong; straight or curved[a)]	oblong to cylindrical; straight or curved[a)]
Length	5–9	5–9	5–7	10–16(–20)	7.5–10.8	7–11	(5.0–)5.6–7.2(–8.5)	(6.5–)9.3–17.3(–22.0)
Width	1–1.5	1–1.5	1–1.25	1.5–1.8	1–1.5	1	1–1.3(–1.7)	2.0–2.5(–3.0)
Color	hyaline to pale yellow	hyaline to pale yellow	hyaline	hyaline	hyaline	hyaline[a)]	hyaline	hyaline
Substrate	bark	dead leaves	*Eucalyptus* sp. leaf litter	—[c)]	*Cedrus deodara*, *Laurus nobilis*, *Carpinus* sp., *Quercus* sp.	dead leaves, soil	*Collospermum hastatum*	
Locality	Amami isl.	Iriomote isl.	Kenya	—[c)]	Italy	Italy, Canada	New Zealand	

a) Observed in the illustrations in the literature. b) All dimensions shown in μm. c) Probably isolated from *Pinus densiflora* in Japan (c.f., Tubaki and Saito, 1969).

Phylogeny based on 18S rDNA sequences

1) Selection of the examined species and strains of *Chaetopsina* and *Kionochaeta* for the sequencing study For comparing the 18S rDNA sequences, we tried to use ex-type or authentic strains of the type species of *Chaetopsina* and *Kionochaeta* as well as some other species: i.e., *C. fulva* (type species) JCM 9754 (ex-type strain), *K. ramifera* (type species) JCM 9756, *K. ivoriensis* JCM 9876 (ex-type strain) and *K. spissa* JCM 9817, JCM 9818. Because the ex-type strain of *K. ramifera* (MFC-2983) has unfortunately died (personal communication from K. Matsushima), we used an authentic IFO strain of the species. Except for the two JCM strains of *K. spissa*, there appear to be no other strains of the species in the culture collections listed by the World Federation for Culture Collections (WFCC). Although the ex-type strain of *C. fulva* (JCM 9754) did not produce setiform conidiophores in culture, the conidia produced on non-setiform conidiophores, hyphal growth rate at 15–30°C (data not shown here) and other cultural properties were almost the same as those of another authentic strain of the species (JCM 9755) that produced abundant setiform conidiophores (Table 3; cf. Tubaki and Saito, 1969). Although Samuels (1985) tentatively treated *N. chaetopsinae* as the teleomorph of *C. fulva* (cited as *Chaetopsina* cf. *fulva*), we know of no available culture or sequence data of *N. chaetopsinae*.

2) Phylogenetic relationships of *Chaetopsina* and *Kionochaeta* in the Pyrenomycetes The color differences of the conidiophores between *Chaetopsina* and *Kionochaeta* were supposed to be important by Samuels (1985) and Kirk and Sutton (1985). In culture, the colonies of *C. fulva* were pale yellow (JCM 9754, JCM 9755) to orange (Rambelli et al., 1991b), and those of *Kionochaeta* species (*K. ramifera*, JCM 9756; *K. ivoriensis*, JCM 9876; *K. spissa*, JCM 9817, JCM 9818) blackish brown. As mentioned below, the colony colors also reflect the phylogenetic relationships of both genera based on 18S rDNA sequences (Figs. 15, 16).

Excluding 47 positions at the 5′ and 3′ termini, which are complementary to the primers that amplify genomic DNA, we determined the following 18S rDNA sequences corresponding to nucleotide positions 24-1774 of *Saccharomyces cerevisiae* Meyen ex Hansen: 1748 bp in *C. fulva* (JCM 9754), 1749 bp in *K. ramifera* (JCM 9756), 1748 bp in *K. spissa* (JCM 9817), 1751 bp in *K. sppisa* (JCM 9818) and 1751 bp in *K. ivoriensis* (JCM 9876). For representatives of the Nectriaceae, we could find only short partial 18S rDNA sequences in the nucleotide sequence databases. Therefore, we first created a NJ tree using nearly full sequences of a few hypocreaceous fungi with other groups of the Ascomycota for inferring the phylogenetic relationships of *Kionochaeta* and *Chaetopsina* (Fig. 15). To search further the closest relatives to these two genera, we then built another NJ tree using mainly short partial sequences of the Pyrenomycetes (Fig. 16). Based on 18S rDNA sequences, *Chaetopsina* and *Kionochaeta* were clearly separated (Figs. 15, 16), basically supporting Kirk and Sutton's conclusion (Kirk and Suttn, 1985). As Samuels (1985) mentioned, *C. fulva* was included in a clade of the Hypocreales (Figs. 15, 16). All the examined species of *Kionochaeta*, on the other hand, clustered with the sordariaceous sister group (Figs. 15, 16 (may be a different family than the Sordariaceae)). Therefore, the genus *Kionochaeta* may be related to the Sordariales, not the Hypocreales. Using the DNAML program in the PHYLIP package ver. 3.51c, we analyzed the same data set used for Fig. 15 by the maximum likelihood method. A trial reached the same conclusion that *Chaetopsina* and *Kionochaeta* were phylogenetically related to the Hypocreales and Sordariales, respectively (ML tree not shown here). The homology search system, FASTA ver. 3.0, was also used to reveal close relatives to *C. fulva* and *Kionochaeta* species. When we used the nearly full sequences of *Kionochaeta* and *Chaetopsina* species, the FASTA showed that three *Kionochaeta* species and *C. fulva* were closely related to *Sordaria fimicola* (Roberge) Cesati & de Notaris and *Hypocrea lutea* (Tode) Petch, respectively. Partial sequences of *C. fulva* and the three *Kionochaeta* species, corresponding approximately in length and position to the partial pyrenomycete sequences in the data bases, were also used in FASTA searches. The search results with partial and complete *Kionochaeta* sequences were the same. The closest candidate sequences to the *C. fulva* partial sequences, however, included *Geosmithia lavendula* (Raper & Fennell) Pitt, *Melanospora fallax* Zukal, and several hypocrealean fungi. Ogawa et al. (1997) recently reported that *G. lavendula* was phylogenetically related to the Hypocreaceae (Nectiaceae). *Melanospora fallax*, which clustered with *C. fulva* (Fig. 16), was found to be a hypocrealean fungus based on 18S rDNA sequences (Spatafora and Blackwell, 1994). In the strict sense, the molecular results conflict with Kirk's speculation (Kirk, 1985) that the teleomorphs of the dematiaceous species of *Chaetopsina* (i.e., *Kionochaeta* species) were likely to be found in *Chaetosphaeria*. Although the taxonomic disposition of the genus *Chaetosphaeria* is still uncertain (e.g., Sphaeriales (Ainsworth et al., 1971), Sphaeriaceae (Kirk and Sutton, 1985; Sphaeriaceae s. str. being treated as the Xylariaceae in Hawksworth et al., 1995), Trichosphaeriaceae in the Sphaeriales (Hawksworth et al., 1983), Lasiosphaeriaceae (*Chaetosphaeria* may not belong here) in the Sordariales (Hawksworth et al., 1995)), Kirk's phylogenetic speculation on *Kionochaeta* was found to be mostly true. *Zanclospora* Hughes & Kendrick and some allies (cf. DiCosmo et al., 1983) seem to be related morphologically and phylogenetically because they have *Chaetosphaeria* teleomorphs (Kendrick et al., 1979). Although we used only a few sequences of members of the Ophiostomatales (Figs. 15, 16), Diaporthales (Figs. 15, 16) and Xylariales (Fig. 15), it is safe to presume that *Chaetopsina* and *Kionochaeta* are phylogenetically distinct from these pyrenomycetes.

Kionochaeta ramifera possesses lateral branches on the conidiophore (Matsushima, 1971; Sutton and Hodges, 1976; Persiani et al., 1984), and *K. ivoriensis* (Rambelli and Lunghini, 1976) and *K. spissa* (Kirk and

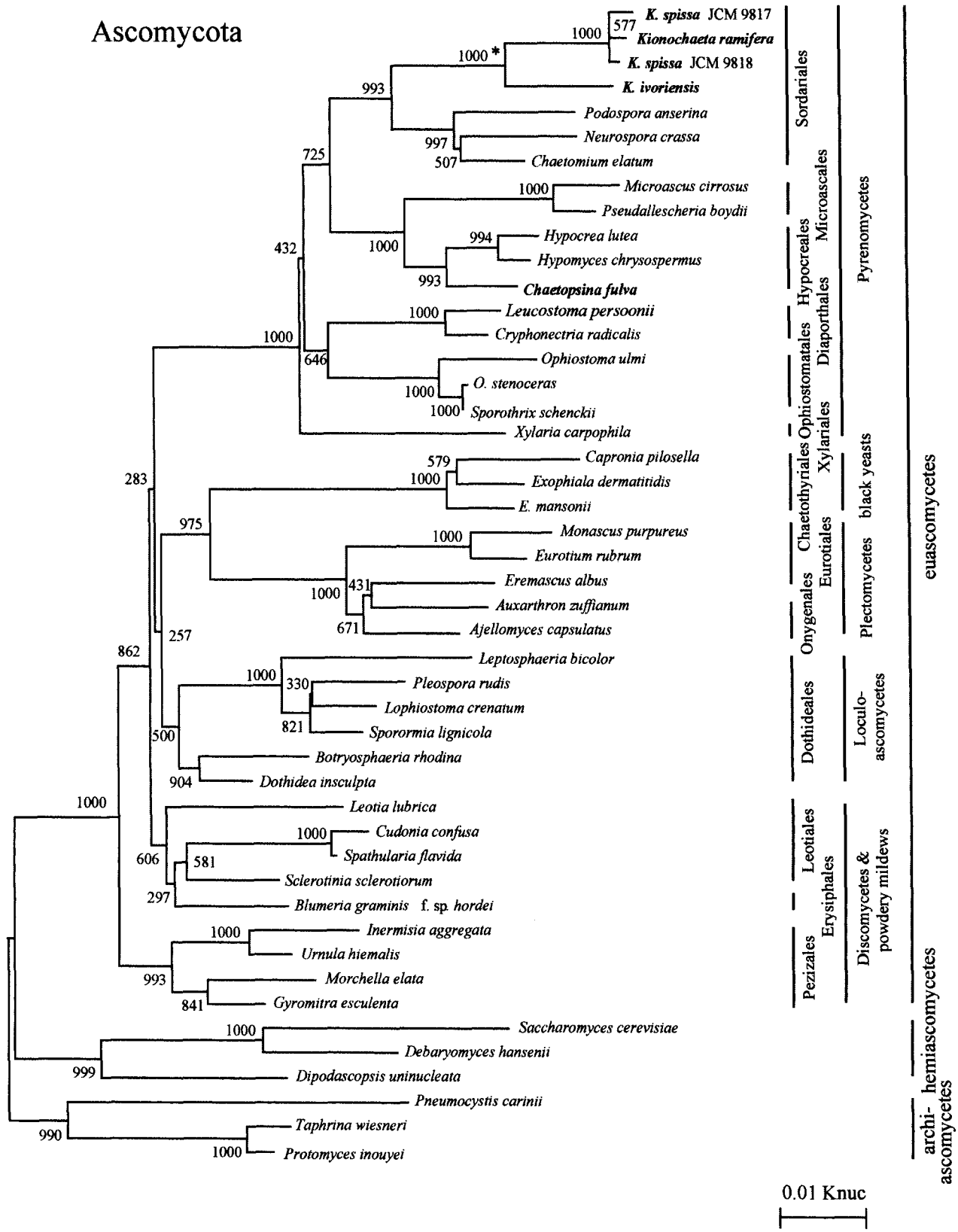


Fig. 15. 18S rDNA sequence-based phylogenetic tree drawn by the neighbor-joining method, showing the dispositions of *Kionochaeta* and *Chaetopsina* in the Ascomycota. *: Bootstrap values were calculated from 1,000 replications.

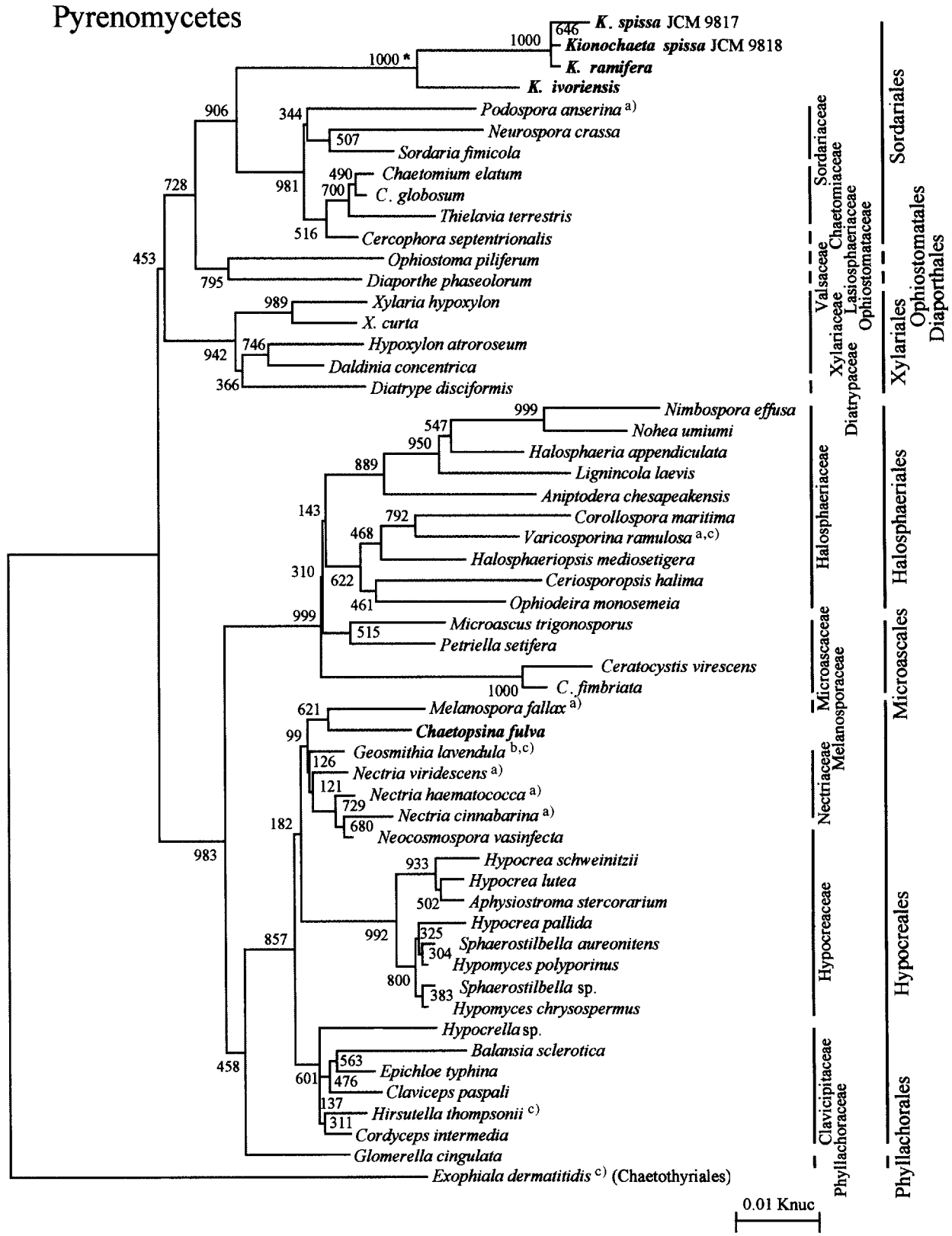


Fig. 16. 18S rDNA sequence-based phylogenetic tree drawn by the neighbor-joining method, showing the dispositions of *Kionochaeta* and *Chaetopsina* in the Pyrenomycetes. a) Family used in NCBI. b) Family used in this paper referring to Ogawa et al. (1997). c) Mitosporic species. *: Bootstrap values were calculated from 1,000 replications.

Sutton, 1985) have non-branched conidiophores. In contrast, the morphology of the compact fertile regions of *K. ramifera* and *K. spissa* are more similar to each other than to *K. ivoriensis*. The Japanese isolates of *K. spissa* are slightly different in conidium productivity, but they are morphologically very similar. It is very interesting that two strains of *K. spissa* (JCM 9817, JCM 9818) are close to a strain of *K. ramifera* (JCM 9756) with 18S rDNA similarities of 99.8%, 99.9% and 99.7% between JCM 9817 and JCM 9818, JCM 9756 and JCM 9818, JCM 9756 and JCM 9817, respectively. Morphology of the branched/non-branched setiform conidiophores in *Kionochaeta* might have less phylogenetic value based on 18S rDNA sequence, and further research is required on DNA-DNA hybridization among these related species (Stackebrandt and Goebel, 1994).

In addition to the species of *Chaetopsina* and *Kionochaeta* with non-branched conidiophores (*C. fulva* and *K. spissa*), we can find similar parallelism among the species with lateral branches on the conidiophore (e.g., *C. splendida* Sutton & Hodges and *K. ramifera*). These are presumably examples of morphological convergence between *Chaetopsina* and *Kionochaeta*.

3) Phylogeny on other ascomycetes In Fig. 15, there are three major lineages in the Ascomycota as shown in the taxonomy browser of the NCBI WWW Entrez (Taylor, 1995): i.e., the archiascomycetes, hemiascomycetes and euascomycetes (Nishida and Sugiyama, 1994). In the euascomycetes, we also found polyphylogenesis, especially in the Discomycetes which includes the Erysiphales (Pezizales vs. Leotiales and Erysiphales; Gargas and Taylor, 1995). As Berbee (1996) recognized two representative groups in the Loculoascomycetes (Dothideales and Pleosporales), we also found the same two lineages in the Dothideales. *Capronia pilosella* (Karst) Müller et al. formed a clade with black yeasts (*Exophiala dermatitidis* (Kano) de Hoog and *E. mansonii* (Castellani) de Hoog) in the Chaetothyriales, which is also associated with the Plectomycetes (Eurotiales and Onygenales). In contrast with the systematic arrangement of the genus *Capronia* Sacc. in the Dictionary of the Fungi (Hawksworth et al., 1995), our results agreed with Haase et al. (1995), Untereiner et al. (1995) and Berbee (1996) in which *Capronia mansonii* (Schol-Schwarz) Müller et al. and *C. pilosella* cluster with the members of the Chaetothyriales (black yeasts) and Plectomycetes rather than the Loculoascomycetes based on 18S rDNA sequence data. The group of the Chaetothyriales and Plectomycetes clustered with the Dothideales at the bootstrap value of 26% in Fig. 15. When the sequence data of *Leucostoma persoonii* (Nitschke) von Höhnel were omitted from the same dataset, the group of the Chaetothyriales and Plectomycetes clustered with the Pyrenomycetes at the bootstrap value of 53% (not shown here). Although there are recent morphological studies on some *Capronia* species associated with *Exophiala/Phialophora* anamorphs (Untereiner, 1995; Untereiner et al., 1995), other members in the Chaetothyriales need to be reevaluated with respect to their affinities to the Plectomycetes (Spatafora, 1995; Spatafora et al., 1995).

Acknowledgements——We thank Dr. L. Zucconi and Dr. K. Ohta for sending some valuable reprints on *Chaetopsina* and advising A.T. on cloning techniques, respectively. One of the authors (G.O.) wishes to thank to Prof. Emeritus H. Indoh, Prof. Emeritus K. Tubaki, Drs. S. Tokumasu, Y. Ogawa, K. Ando, A. Nakagiri and T. Aoki for their encouragement, and also to the curators of IFO and IMI for supplying cultures or specimens of *C. fulva* and *K. spissa*. Helpful comments were provided by Drs. K. A. Seifert, M. Suzuki, M. Takashima, T. Sugita, M. Tamura, and two anonymous reviewers. This work was supported in part by grants to G.O. from the Harada Sekizen foundation and the Indoh foundation.

Literature cited

Ainsworth, G. C., James, P. W. and Hawksworth, D. L. 1971. Ainsworth & Bisby's dictionary of the fungi, 6th ed. Commonwealth Mycological Institute, Kew.

Berbee, M. L. 1996. Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol. Biol. Evol. **13**: 462–470.

Castañeda, R. F. 1986. Deuteromycotina de Cuba. Hyphomycetes IV. Inst. Invest. Fund. Agric. Trop. (Alejandro de Humboldt), Habana.

Crane, J. L. and Schoknecht, J. D. 1982. Hyphomycetes from freshwater swamps and hammocks. Can. J. Bot. **60**: 369–378.

Crous, P. W., Wingfield, M. J. and Kendrick, W. B. 1994. *Kionochaeta pini* sp. nov. and *Verrucophragmia splendens* gen. nov. from leaf litter in South Africa. Mycologia **86**: 447–450.

DiCosmo, F., Berch, S. and Kendrick, B. 1983. *Cylindrotrichum*, *Chaetopsis*, and two new genera of Hyphomycetes, *Kylindria* and *Xenokylindria*. Mycologia **75**: 949–973.

Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39**: 783–791.

Gargas, A. and Taylor, J. W. 1995. Phylogeny of discomycetes and early radiations of the apothecial Ascomycotina inferred from SSU rDNA sequence data. Exp. Mycol. **19**: 7–15.

Haase, G., Sonntag, L., van de Peer, Y., Uijthof, J. M. J., Podbielski, A. and Melzer-Krick, B. 1995. Phylogenetic analysis of ten black yeast species using nuclear small subunit rRNA gene sequences. Antonie van Leeuwenhoek **68**: 19–33.

Hawksworth, D. L., Kirk, P. M, Sutton, B. C. and Pegler, D. N. 1995. Ainsworth & Bisby's dictionary of the fungi, 8th ed. International Mycological Institute, Egham.

Hawksworth, D. L., Sutton, B. C. and Ainsworth, G. C. 1983. Ainsworth & Bisby's dictionary of the fungi, 7th ed. Commonwealth Mycological Institute, Kew.

Hendriks, L., De Baere, R., van de Peer, Y., Neefs, J., Goris, A. and De Wachter, R. 1991. The evolutionary position of the rhodophyte *Porhyra umbilicalis* and the basidiomycete *Leucosporidium scottii* among other eukaryotes as deduced from complete sequences of small ribosomal subunit RNA. J. Mol. Evol. **32**: 167–177.

Kendrick, B. and DiCosmo, F. with the unitunicate and bitunicate committees of Kananaskis-II. 1979. Teleomorph-anamorph connections in Ascomycetes. In: The whole fun-

gus, the sexual-asexual synthesis, (ed. by Kendrick, B.), pp. 283–410. National Museum of Natural Sciences, National Museums of Canada and the Kananaskis Foundation, Canada.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**: 111–120.

Kirk, P. M. 1985. New or interesting microfungi XIV. Dematiaceous hyphomycetes from Mt Kenya. Mycotaxon **23**: 305–352.

Kirk, P. M. and Sutton, B. C. 1985. A reassessment of the anamorph genus *Chaetopsina* (Hyphomycetes). Trans. Br. Mycol. Soc. **85**: 709–717.

Kornerup, A. and Wanscher, J. H. 1978. Methuen handbook of colour, 3rd ed. E. Methuen, London.

Kuthubutheen, A. J. and Nawawi, A. 1988. Two new species of *Kionochaeta* (Hyphomycetes) and *K. ramifera* from Malaysia. Trans. Br. Mycol. Soc. **90**: 437–444.

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. and Pace, N. P. 1985. Rapid determination of 18S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. USA **82**: 6955–6959.

Matsushima, T. 1971. Microfungi of the Solomon islands and Papua-New Guinea. Published by the author, Kobe.

Merli, S., Garofano, L., Rambelli, A. and Pasqualetti, M. 1992. *Chaetopsina nimbae*, a new species of dematiaceous Hyphomycetes. Mycotaxon **44**: 323–331.

Morgan-Jones, G. 1979. Notes on Hyphomycetes. XXXI. *Chaetopsina auburnensis* sp. nov. Mycotaxon **8**: 411–416.

Nishida, H. and Sugiyama, J. 1993. Phylogenetic relationships among *Taphrina*, *Saitoella*, and other higher fungi. Mol. Biol. Evol. **10**: 431–436.

Nishida, H. and Sugiyama, J. 1994. Archiascomycetes: detection of a major new lineage within the Ascomycota. Mycoscience **35**: 361–366.

Ogawa, H., Yoshimura, A. and Sugiyama, J. 1997. Polyphyletic origins of species of the anamorphic genus *Geosmithia* and the relationships of the cleistothecial genera: Evidence from 18S, 5S and 28S rDNA sequence analyses. Mycologia **89**: 756–771.

Onofri, S. and Zucconi, L. 1991. Scanning electron microscopy of conidiophore development and conidiogenesis in *Chaetopsina fulva*. Mycotaxon **41**: 451–457.

Pearson, W. R. and Lipman, D. J. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**: 2444–2448.

Persiani, A. M., Zucconi, L. and Onofri, S. 1984. Hyphomycetes rari o interessanti della foresta tropicale. III. Contributo alla conoscenza del genere *Chaetopsina* Rambelli. Mic. Ital. **2**: 11–14.

Rambelli, A. 1956. *Chaetopsina*, nuovo genere di ifali demaziacei. Atti Accad. Sci. dell Ist. Bol. **3**: 1–6.

Rambelli, A. 1987. A bibliographic reassessment of the genus *Chaetopsina*. Micol. Ital. **1**: 7–13.

Rambelli, A. and Lunghini, D. 1976. "*Chaetopsina ivoriensis*" a new species of dematiaceous Hyphomycetes. Giorn. Bot. Ital. **110**: 253–258.

Rambelli, A. and Lunghini, D. 1979. *Chaetopsina* species from tropical forest litter. Trans. Br. Mycol. Soc. **72**: 491–494.

Rambelli, A., Pasqualetti, M. and Lunghini, D. 1991a. Variabilità morfologica in tre ceppi di *Chaetopsina fulva*. Micol. Veget. Mediterranea **6**: 11–15.

Rambelli, A., Zucconi, L. and Pasqualetti, M. 1991b. Variabilità morfo-dimensionale di due ceppi di *Chaetopsina fulva* su differenti matrici vegetali. Giorn. Bot. Ital. **125**: 907–917.

Saitou, N. and Nei, M. 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425.

Samuels, G. J. 1985. Four new species of *Nectria* and their *Chaetopsina* anamorphs. Mycotaxon **22**: 13–32.

Sanger, F., Nicklen, S. and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**: 5463–5467.

Spatafora, J. W. 1995. Ascomal evolution of filamentous ascomycetes: evidence from molecular data. Can. J. Bot. **73** (Suppl. 1): S811–S815.

Spatafora, J. W. and Blackwell, M. 1994. Cladistic analysis of partial ssrDNA sequences among unitunicate perithecial ascomycetes and its implications on the evolution of centrum development. In: Ascomycete systematics; problems and perspectives in the nineties, (ed. by Hawksworth, D. L.), pp. 233–242. Plenum Press, New York.

Spatafora, J. W., Mitchell, T. G. and Vilgalys, R. 1995. Analysis of genes coding for small-subunit rRNA sequences in studying phylogenetics of dematiaceous fungal pathogens. J. Clin. Microbiol. **33**: 1322–1326.

Stackebrandt, E. and Goebel, B. M. 1994. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. **44**: 846–849.

Sutton, B. C. and Hodges, C. S. 1976. *Eucalyptus* microfungi: Some setose hyphomycetes with phialides. Nova Hedwigia **27**: 343–352.

Taylor, J. W. 1995. Making the Deuteromycota redundant: a practical integration of mitosporic and meiosporic fungi. Can. J. Bot. **73** (Suppl. 1): S754–S759.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**: 4673–4680.

Tubaki, K. and Saito, T. 1969. *Endophragmia alternata* sp. nov. and other hyphomycetes on *Pinus* leaves in Japan. Trans. Br. Mycol. Soc. **52**: 477–482.

Untereiner, W. A. 1995. Fruiting studies in species of *Capronia* (Herpotrichiellaceae). Antonie van Leeuwenhoek **68**: 3–17.

Untereiner, W. A., Straus, N. A. and Malloch, D. 1995. A molecular-morphotaxonomic approach to the systematics of the Herpotrichiellaceae and allied black yeasts. Mycol. Res. **99**: 897–913.

Wingfield, M. J. 1987. A new name for *Phialocephala illini*. Mycotaxon **30**: 149–156.

Yotsumoto, Y., Oohara, A. and Mikawa, T. 1995. A convenient method for extraction and purification of DNA from filamentous fungi. Microbiol. Cult. Coll. **11**: 19–21. (In Japanese.)

Zucconi, L. and Rambelli, A. 1993. A new species of *Chaetopsina* from tropical forest litter. Mycotaxon **48**: 5–12.