

## ***Ramicandelaber*, a new genus of the Kickxellales, Zygomycetes**

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***Ramicandelaber*, a new genus of Zygomycetes is erected to accommodate *Ramicandelaber longisporus* sp. nov. The fungus has hyphal septa with median plugs and forms homologous structures to sporocladia and pseudophialides. These characteristics suggest that it belongs to the Kickxellales, Zygomycetes.**

Key Words—Kickxellales; new genus; *Ramicandelaber*; Zygomycetes.

During a survey of microfungi at the Medicinal Plants Garden of Nihon University, the second author, Shunsuke Hayashi, isolated an unusual fungus from a soil sample. The fungus has hyphal septa with median plugs and special branches and branchlets (sporocladia) bearing subspherical or hemispherical sporogenous cells (pseudophialides). Although these characteristics suggest that the fungus belongs to the Kickxellales, it cannot be accommodated in any known genus of this order. Therefore, a new genus is proposed for this fungus in the Kickxellales, Zygomycetes.

### **Materials and Methods**

A soil sample was collected under leaf litter of evergreen oaks at the Medicinal Plants Garden of Nihon University, Funabashi, Chiba Pref., Japan on 14 July 1996. A small amount of the sample was placed onto Miura agar plates (Miura and Kudo, 1970). Spores of the fungus were isolated using a fine needle made of Elgiloy orthodontic wire, and the isolated spores were grown on Miura agar plates.

For scanning electron microscopy, the fungal materials were prefixed with 0.5% OsO<sub>4</sub> and 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7) and postfixed 1% OsO<sub>4</sub> in the buffer, then dehydrated through a graded ethanol series. After critical drying with a Hitachi HCP-2 apparatus, the materials were coated with platinum-palladium in an ion sputter (Hitachi E-102), then observed with a scanning electron microscope (Hitachi S-2300).

For transmission electron microscopy, the materials were prefixed with Karnovsky's fixation (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7) and postfixed with 1% OsO<sub>4</sub> in the buffer. After dehydration through a graded ethanol series, the fungal materials were embedded in Spurr resin. Ultra-

thin sections were cut with an LKB Ultramicrotome (4800 Ultratome), stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope (JEM-100CX).

### **Taxonomy**

***Ramicandelaber*** Y. Ogawa, S. Hayashi, Degawa et Yaguchi, gen. nov.

Genus Zygomycetum. Saprophyticus. Mycelium ex hyphis hyalinis ramosis septatis compositum. Stolones hyalini, septati, ad apicem rhizoidea efferentes. Sporangio-phora ex parte superiore stolonum oriunda, septata, primo prostrata, ramos verticillatos efferentis, postremo erecta ad septa tertia vel quarta ab rhizoideo segregata, capitula fertilia in partibus segregatis frequenter efferentia. Rami primo ramulos verticillatos efferentes, postea inflati cylindrici vel ellipsoidei ramulos parvos irregulariter efferentes. Ramuli verticillati in ramis juvenilibus sporangio-phorum elongati sursum attenuati et saepe rami vetusti inflati pro sporocladis fungentes. Cellulae sporogenaе (pseudophialides) in sporocladis et capitulis fertilibus genitae, primo subsphaericae, postea deminnescentes et subsphaericae vel hemisphaericae, sporangiola efferentes. Sporangiola tenuiter fusiformia, leviter curvata, non septata, hyalina. Paries sporangiolorum ad sporangiosporam coarctatus. Zygosporae et chlamydosporae ignotae.

Species typica: *Ramicandelaber longisporus* Y. Ogawa, S. Hayashi, Degawa et Yaguchi.

Etymology: Latin, *Ramus*=a branch; *candelaber*=a candlestick.

Zygomycetes, saprobic. Mycelium composed of colorless, branched, septate hyphae. Stolons hyaline, septate, producing rhizoids at the apex. Sporangio-phores derived from the distal parts of stolons, septate,

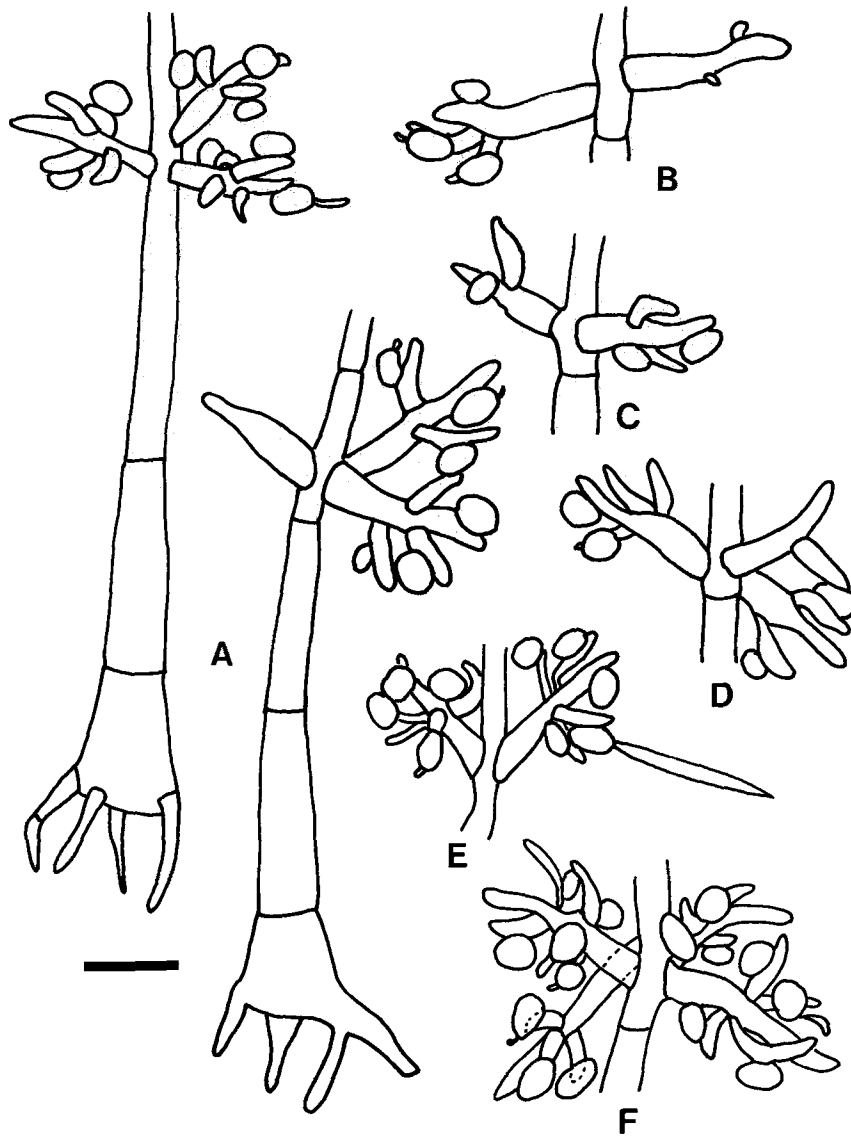


Fig. 1. *Ramicandelaber longisporus* PCNB-1157 (IFO 33179). A. Habits. B-F. Upper parts of sporangiophores showing the development of sporocladia (branchlets on branches of sporangiophores). Sporocladia produce subspherical pseudophialides. Scale bar = 10  $\mu\text{m}$ .

at first prostrated, verticillately branched once or twice by the development of branches, erect in age, often disarticulated at third or fourth septa from rhizoids, often producing fertile terminal heads. Branches verticillately producing branchlets, becoming broad in age, cylindrical or ellipsoidal, irregularly producing shorter branchlets. Sporocladia at first composed of branchlets on branches of sporangiophores, elongate, attenuate distally, often composed of broadened branches of sporangiophores in age. Sporogenous cells (pseudophialides) arising from sporocladia and fertile heads, at first subspherical, in age subspherical or hemispherical, producing sporangia. Sporangia narrow fusiform, slightly curved, aseptate, hyaline. Sporangial wall adnate to the sporangiospore. Zygosporangia and chlamydozoospores not observed.

*Ramicandelaber longisporus* Y. Ogawa, S. Hayashi, Degawa et Yaguchi, sp. nov. Figs. 1-5

Coloniae in agar Miurae floccosae, albae, tarde crescentes, post 14 dies ad 25°C 5.5 cm diam attingentes. Mycelium effusum, ex hyphis hyalinis ramosis septatis 2.5-5  $\mu\text{m}$  crassis compositum. Stolones septati, hyalini, 2.5-5  $\mu\text{m}$  crassi, ad apicem rhizoidea efferentes. Rhizoidea (9-)15-22(-36)  $\mu\text{m}$  longa, basi (1.5-)2-4(-6)  $\mu\text{m}$  crassa. Sporangiophora ex parte superiore stolonum oriunda, septata, primo prostrata, 1-4 ramos verticillatos semel vel bis efferentis, basi 7-10  $\mu\text{m}$  crassa, prope ramis 2-3  $\mu\text{m}$  crassa, postremo erecta, ad septa tertia vel quarta ab rhizoideo segregata, (40-)117-150(-210)  $\mu\text{m}$  longa, basi 6-12  $\mu\text{m}$  crassa, apice 1-3  $\mu\text{m}$  crassa, in partibus separationis frequenter capitula fertilia 3-7  $\mu\text{m}$  diam efferentia. Rami primo

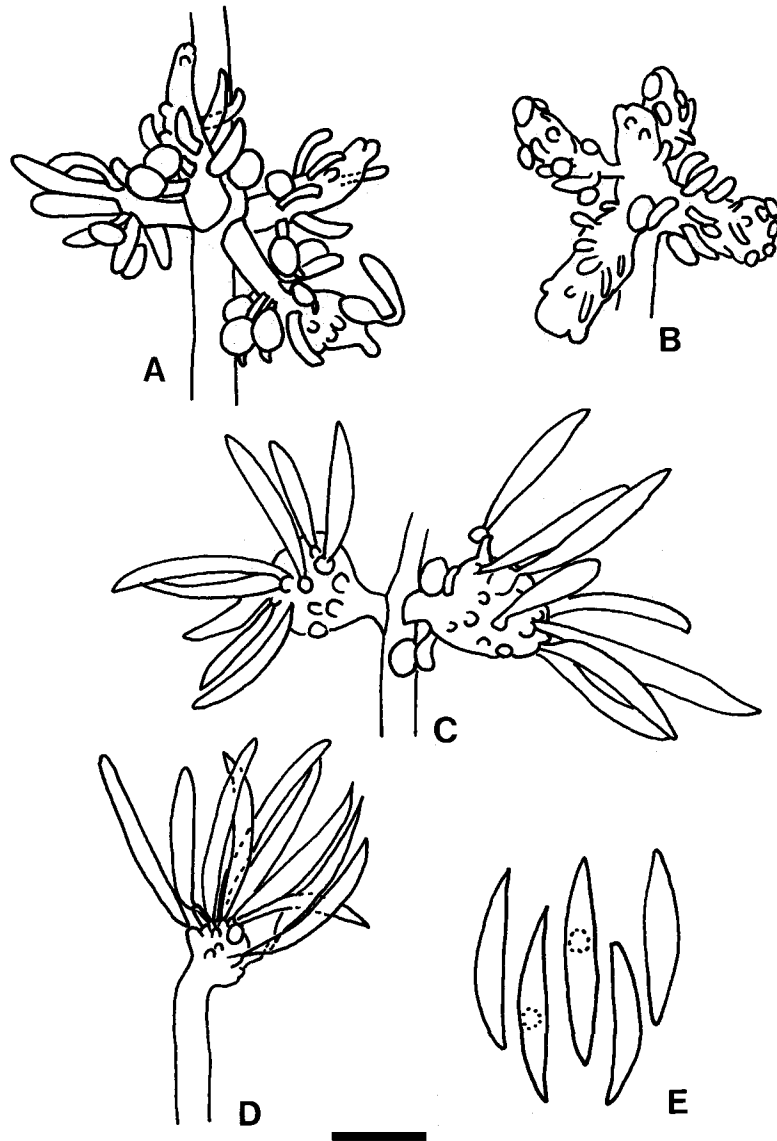


Fig. 2 *Ramicandelaber longisporus* PCNB-1157 (IFO 33179). A-C. Sporocladia in aged cultures. Branches of sporangiophores often become broad and turn into sporocladia producing many small hemispherical pseudophialides on them. D. Fertile heads formed after disarticulation of sporangiophores. E. Sporangiospores. Scale bar = 10  $\mu\text{m}$ .

(8-)11-13(-15)  $\mu\text{m}$  longi basi 1.5-3.5  $\mu\text{m}$  crassi apice 1-2  $\mu\text{m}$  crassi ramulos verticillatos efferentes, postea cylindrici saepe inflati 10-18  $\mu\text{m}$  longi 3-11  $\mu\text{m}$  crassi vel ellipsoidei et 8-12  $\mu\text{m} \times 5-8 \mu\text{m}$  ramulos parvos irregulariter efferentes. Ramuli primo elongati attenuati (4-)6-7(-12)  $\mu\text{m}$  longi 1-3  $\mu\text{m}$  crassi, postea leviter deminuentes (2-)5-6(-10)  $\mu\text{m}$  longi 1-2.5  $\mu\text{m}$  crassi. Ramuli verticillati in ramis juvenilibus sporangiophorum elongata sursum attenuata et saepe ramis vetusti inflati pro sporocladis fungentes. Cellulae sporogae (pseudophialides) in sporocladis et capitulis fertilibus genitae, primo subsphaericae 3-7  $\times$  2-5  $\mu\text{m}$ , postea deminuentes subsphaericae et 3-5  $\times$  2-4  $\mu\text{m}$  vel hemisphaericae et 2-3  $\mu\text{m}$  diam, sporangiola efferentes. Sporangiola tenenter fusiformia, leviter curvata, non septata, (33-)40-44(-55)  $\mu\text{m}$  longa, (3-)5-6(-9)  $\mu\text{m}$  crassa,

hyalina. Paries sporangiorum ad sporangiosporam coarctatus.

Holotypus: CBM-FZ-1001; colonia ex siccata in cultura ex solo, Funabashi, Chiba Pref. in Japonia, 14. vii. 1996, a S. Hayashi isolata et ea collectione fungorum Musei et Instituti Historiae Naturalis Chiba (CBM) conservata.

Etymology: Latin, *longisporus* = long spore.

Colonies on Miura agar effuse, floccose, white, relatively slow growing, reaching 5.5 cm diam in 14 d at 25°C. Mycelium composed of hyaline, branched, septate, 2.5-5  $\mu\text{m}$  wide hyphae. Stolons septate, hyaline, 2.5-5  $\mu\text{m}$  in diam, producing rhizoids at the apices. Rhizoids (9-)15-22(-36)  $\mu\text{m}$  long, (1.5-)2-4(-6)  $\mu\text{m}$  in diam at base. Sporangiohores derived from the distal parts of stolons, septate, at first prostrated, verticillately

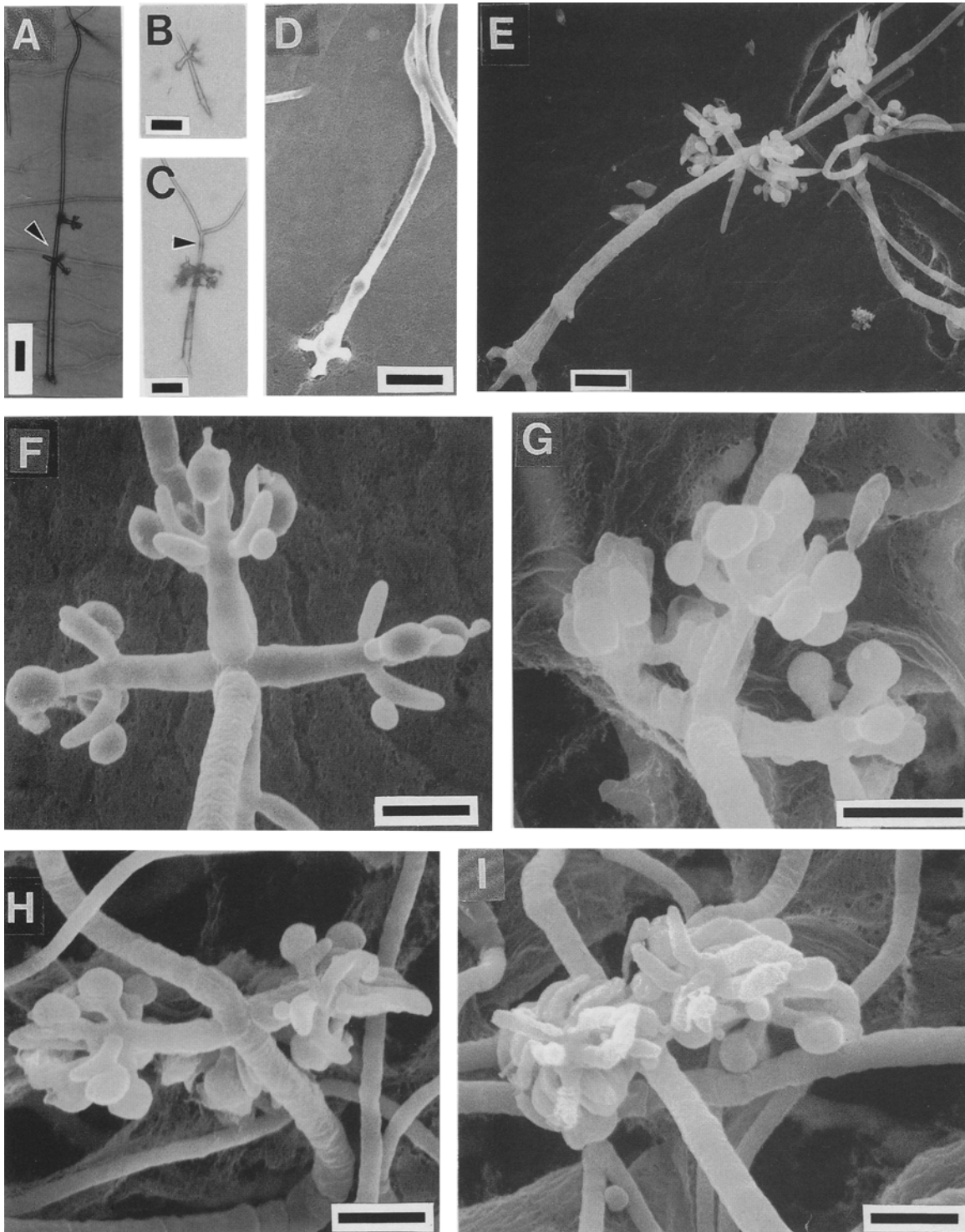


Fig. 3. *Ramicandelaber longisporus* PCNB-1157 (IFO 33179). A–C. Habitus. In aged culture, sporangiophores are often disarticulated at the septum (arrowheads). D. A stolon that has not yet produced sporocladia. E. A habitus. F–I. Sporocladia showing development of pseudophialides and sporangia. Scale bars: A–E = 10  $\mu\text{m}$ . F–I = 5  $\mu\text{m}$ .

branched once or twice by the development of 1 to 4 branches, 7–10  $\mu\text{m}$  in diam at the base, 2–3  $\mu\text{m}$  in diam near branches, in age erect, often disarticulated at the third or fourth septa from rhizoids, (40–)117–150(–210)

$\mu\text{m}$  long, 6–12  $\mu\text{m}$  in diam at the base, 1–3  $\mu\text{m}$  in diam at the apex, often producing fertile heads terminally, the latter 3–7  $\mu\text{m}$  in diam. Branches, (8–)11–13(–15)  $\mu\text{m}$  long, 1.5–3.5  $\mu\text{m}$  in diam at the base, 1–2  $\mu\text{m}$  in diam at the

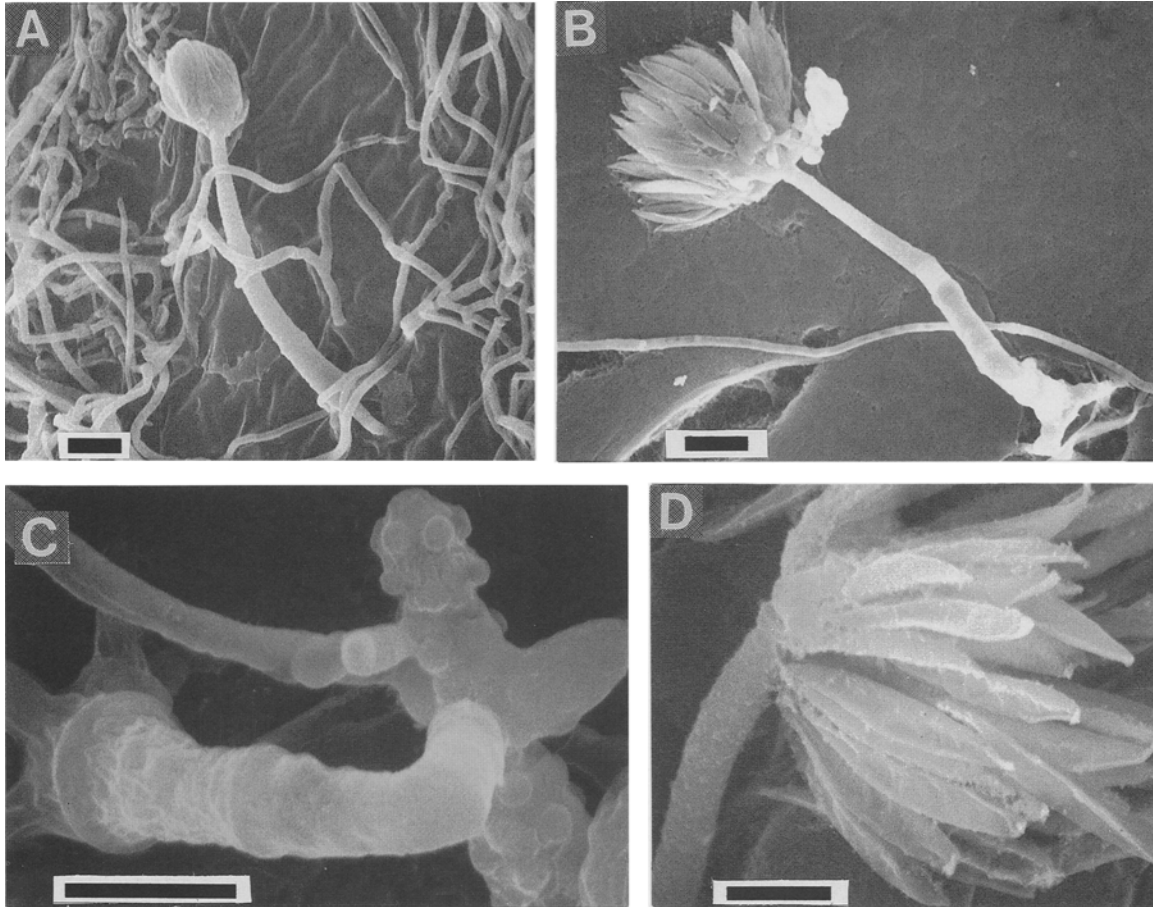


Fig. 4 *Ramicandelaber longisporus* PCNB-1157 (IFO 33179). A, B. Habits in aged cultures. C. Sporocladia in an aged culture. Broadened branches of a sporangiophore turn into sporocladia and produce many hemispherical pseudophialides. D. A fertile head in an aged culture. Scale bars=5  $\mu\text{m}$

apex, verticillately producing branchlets, in age often becoming broad, cylindrical, 10–18  $\mu\text{m}$  long, 3–11  $\mu\text{m}$  in diam, or becoming ellipsoidal, 8–12  $\mu\text{m}$   $\times$  5–8  $\mu\text{m}$ , irregularly producing shorter branchlets. Branchlets at first (4–)6–7(–12)  $\mu\text{m}$  long, 1–3  $\mu\text{m}$  in diam, in age becoming smaller, (2–)5–6(–10)  $\mu\text{m}$  long, 1–2.5  $\mu\text{m}$  in diam. Sporocladia at first composed of branchlets on branches of sporangiophores, elongate, attenuate distally, in age often produced from broadened branches of sporangiophores. Sporogenous cells (pseudophialides) arising from sporocladia and fertile heads, subspherical, 3–7  $\mu\text{m}$   $\times$  2–5  $\mu\text{m}$ , in age becoming smaller, subspherical, 3–5  $\mu\text{m}$   $\times$  2–4  $\mu\text{m}$  or hemispherical, 2–3  $\mu\text{m}$  in diam, producing sporangiola. Sporangiospores narrow fusiform, slightly curved, (33–)40–44(–55)  $\mu\text{m}$  long, (3–)5–6(–9)  $\mu\text{m}$  wide, non-septate, hyaline. Sporangial wall adnate to the sporangiospore.

Hab.: From soil at the Medicinal Plant Garden of Nihon University, Funabashi, Chiba, Japan, 14 July 1996. Holotype(CBM-FZ-1001), a dried culture isolated by S. Hayashi, is deposited in the Natural History Museum and Institute, Chiba, Japan (CBM). A living culture (PCNB-1157) derived from the type is preserved in Institute for

Fermentation, Osaka as IFO 33179.

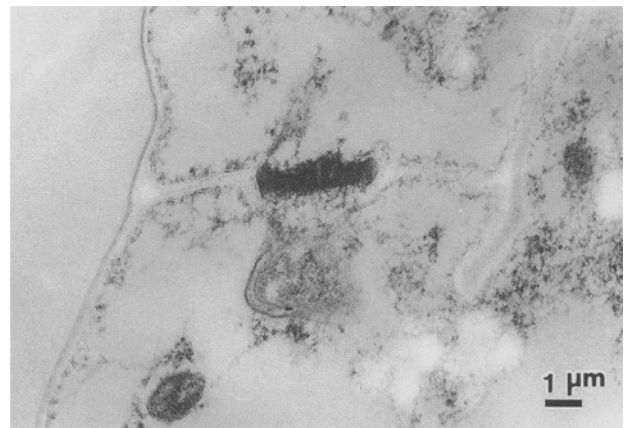


Fig. 5 *Ramicandelaber longisporus* PCNB-1157 (IFO 33179). A section through a septum showing flared central wall and a median plug.

Table 1. Characteristics of sporocladia and pseudophialides of members of the Kickxellales.

	<i>Martensella</i> Coem.	<i>Coemansia</i> Tiegh. & Le Monn.	<i>Kickxella</i> Coem.	<i>Martensiomycetes</i> J.A. Mey.	<i>Spirodactylon</i> R.K. Benj.	<i>Dipsacomycetes</i> R.K. Benj.	<i>Linderina</i> Raper & Fen- nell	<i>Spiromycetes</i> R.K. Benj.	<i>Ramicandelaber</i> Y. Ogawa et al.	
sporocladia	septum	septate						aseptate		
	shape	slightly curved or nearly straight, elongate, usually attenuated distally						ovoid or dome-shaped	recurved by swelling of one side of the cell and constricted subterminally	at first elongate, attenuate, in age broadened cylindrical or ellipsoidal
	arrangement	formed acrogenously becoming laterally disposed by the continued growth of the main axis of sporangiophore	formed verticillately on apical enlargements of sporangiophores	formed umbellately on the ends of branches cymosely produced from the sporangiophores	formed on coiled fertile regions of sporangiophores	arising as lateral outgrowth of branched, septate aerial hyphae	formed alternately and typically arranged in zigzag pattern on the fertile axes of sporangiophores	formed pleurogenously on coiled sporangiophores	at first formed verticillately on branches of sporangiophores, in age, composed of branches of sprangiophores themselves	
pseudophialides	shape	ellipsoidal	ellipsoidal to elongate-ovoid	ellipsoidal to elongate-ovoid	ellipsoidal	ovoid	elongate	ellipsoidal	not formed	at first subspherical, in age subspherical or hemispherical
	arrangement	arranged in transverse rows on upper surfaces of sporocladia	arranged in transverse rows on lower surfaces of sporocladia	arranged in transverse rows on upper surfaces of sporocladia	arranged in transverse rows on lower surfaces of sporocladia	arranged in transverse rows on inner surfaces of coiled fertile regions of sporocladia	arranged in transverse rows on one side of sporocladia	crowded over the surfaces of sporocladia	terminal globose enlargements of sporocladia produce sporangiola instead of pseudophialides	at first formed pleurogenously on the one side of sporocladium, in age crowded over surfaces of broadened sporocladia

## Discussion

Until the present study, the Kickxellales comprised eight known genera: *Kickxella* Coem., *Martensella* Coem., *Coemansia* Tiegh. & Le Monn., *Martensiomycetes* J. A. Mey., *Spirodactylon* R. K. Benj., *Dipsacomycetes* R. K. Benj., *Linderina* Raper & Fennell, and *Spiromycetes* R. K. Benj. (Benjamin, 1959, 1961, 1963). These genera are characterized by special branches (sporocladia), which produce sporogenous cells called pseudophialides (Benjamin, 1959, 1979). *Ramicandelaber longisporus* produces branches and branchlets that bear subspherical or hemispherical sporogenous cells from which sporangiola arise. These branching structures and sporogenous cells seem to correspond to sporocladia and pseudophialides of the Kickxellales, respectively.

Benjamin (1959, 1979) further specified that the Kickxellales fungi develop flared septa with median cavities occluded by biconvex or biumbonate plugs. Using a transmission electron microscope, Young (1969) showed that the septum of *Linderina pennispora* Raper & Fennell has a biconvex plug seated in a groove running

round the surface of the septal pore. The section through a hyphal septum of *R. longisporus* shows the same septal structure (Fig. 5) as *L. pennispora*.

From the above evidence, it seems reasonable to ascribe *R. longisporus* to the family Kickxellaceae, Kickxellales, Zygomycetes, although we could not observe zygospores which are characteristics of this class.

The morphological characteristics of *Ramicandelaber* are clearly distinct from those of other members of the Kickxellales. Only *Ramicandelaber* produces stolons and forms rhizoids at their apices.

On the basis of Benjamin's monographs (1959, 1961, 1963), Table 1 summarizes the morphological characteristics of sporocladia and pseudophialides, making clear the unique features of *Ramicandelaber* among the Kickxellales.

Sporocladia can be classified into two types: septate and aseptate. *Kickxella*, *Martensella*, *Coemansia*, *Martensiomycetes*, *Spirodactylon* and *Dipsacomycetes* have septate sporocladia. These sporocladia are slightly curved or nearly straight, elongate and usually attenuated distally, although their arrangements on sporangiophores vary.

On the other hand, *Linderina* and *Spiromyces* have aseptate sporocladia. The sporocladia of *Linderina* are ovoid or dome-shaped and produce pseudophialides crowded over their surfaces. Those of *Spiromyces* are sessile, constricted subterminally and the terminal parts produce unispore sporangia directly without forming pseudophialides.

The most conspicuous characteristic of sporocladia of *Ramicandelaber* is the morphological change that accompanies its growth. At first, young sporocladia are formed verticillately on branches of sporangiophores (Figs. 1, 3F–G). In age, branches of sporangiophores themselves turn to broadened sporocladia (Figs. 2A–C, 4C).

A similar change with aging is also seen in the formation of pseudophialides of *Ramicandelaber*. At first, the subspherical pseudophialides are formed on the young sporocladia (Figs. 1, 3F–G). Later, many hemispherical ones are formed on the aged sporocladia (Figs. 2B–C, 4C). The septa of hemispherical pseudophialides on the aged sporocladia are obscure. The many pseudophialides crowded over aged sporocladia look like the pseudophialides of *Linderina*. The obscurity of septa of aged pseudophialides implies a resemblance to the terminal enlargements of sporocladia of *Spiromyces*. The

aseptate sporocladia of *Ramicandelaber* suggest that the fungus may be related to *Linderina* or *Spiromyces*. Analysis of the rDNA sequence of *R. ceriforus* should clarify its phylogenetic relationships with other members of the Kickxellales.

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