

## CHAPTER 2

### Isolation and Description of Species

#### 2.1 INTRODUCTION

The fungal materials of the Kickxellales accessible at present are fairly insufficient because small numbers of kickxellalean strains are deposited in culture collections. This is due to that most species have been rarely encountered. For example, the Centraalbureau voor Schimmelcultures (CBS), one of the most authoritative culture collections, holds only 21 strains of 11 species of 4 genera excluding the strains entrusted through the present study (as of 29 September 2001, [http://www.cbs.knaw.nl/search\\_fsp.html](http://www.cbs.knaw.nl/search_fsp.html)), that is, living strains of more than half of the known species are unavailable at present. In addition, even the type specimens of several species have been lost. Taking such situation into account, I considered that collecting both the described and undescribed kickxellalean strains as fungal materials was the first priority for contemplating the improvement of the classification system of the order, and thus attempted to obtain them from the field.

For the isolation of the objective fungi, soil and feces of murines were mainly employed as samples, because most members of the Kickxellales have been found from soil or feces of omnivorous rats and mice (Benjamin 1958, 1959, 1961, 1963; Benny et al. 2001; Tables 2-2, 2-3).

In the following part, the results of isolation will be described and the distributions of the species in the field that have been undecided (Table 2-1) will be discussed.

#### 2.2 MATERIALS AND METHODS

##### 2.2.1 Collection and treatment of soil samples

A total of 1012 soil samples were collected all around Japan, from the Hokkaido district to the Yonaguni Island of the Nansei Islands (Table 2-4). The altitude of the localities ranged from 0 m to 2,100 m. Most samples were collected from natural forests; some were from artificial forests and grasslands, and more rarely from arable, seashores, and volcanic deserts. These samples were aseptically put into polyethylene bags and brought back to the laboratory.

The following three methods were adopted for the isolation of the objective fungi from soil.

### (1) Soil plate method

The soil plate method (Warcup 1950) using 5–20% carrot extract agar medium (CaA; Chien 1994) was employed. A small amount of soil was placed in a sterilized petri dish of 9 cm in diameter. About 10 ml of autoclaved and cooled agar medium was poured into the petri dish and mixed with soil particles before it hardened. The plates were kept at room temperature for a month, and observed using a stereoscopic microscope (WILD MZ8, Leica) and an optical compound microscope (Alphaphot-2, Nikon).

### (2) Moist chamber baiting method

A modified moist chamber method (Degawa & Tokumasu 1997) was utilized. An appropriate amount of sample soil was put into a plastic container of 66 mm or 101 mm in diameter, and moistened with distilled water to keep the sufficient humidity. A few sterilized pieces of dried edible shrimps (*Sergestes lucens*) or reared mealworms (larvae and chrysalis of *Tenebrio molitor*, Coleoptera) were placed on the surface of the soil as baits. The containers were kept at room temperature for a month and observed as above.

### (3) Enrichment method

An appropriate amount of sample soil was put into a plastic container of 66 mm in diameter, and 2 ml of 5% aqueous solution of peptone, yeast extract, or soytone was poured on the soil. The containers were kept at room temperature for a month and observed as above.

## 2.2.2 Collection and treatment of fecal samples

One hundred and six fecal samples were collected mainly in the Sugadaira Highland, Nagano Prefecture. Almost all of them were feces of wild mammals, particularly those of field mice and voles (Tables 2-5, 2-6, 2-7). Each sample consisted of 1–50 fecal pellets, and the pellets collected totaled to about 1000.

Almost all feces of murines were collected in a *Miscanthus*-dominant grassland and a deciduous broad-leaved forest. The samples from the grassland were collected successively at the same entrance of a burrow and probably evacuated from the identical individual; since from the habitat, the murine would be a vole (meadow mouse, *Microtus montebelli*) (Kanamori & Ando 1974), and the lifetime of the species is 8 to 12 months and the usage of one burrow is basically restricted

to an individual (Udagawa 1974). Most fecal pellets were treated *in situ*, and rarely treated in the laboratory after brought back pellets that had collected in plastic bags. The following two methods were used to obtain kickxellalean isolates from the feces.

(1) Moist chamber method

Almost all fecal samples, especially dung of mammals were treated by the moist chamber method. Fecal pellets were placed on the surface of *Sphagnum* material wetted and autoclaved that was crammed into a plastic container of 101 mm in diameter as a moisturizer. These containers were placed by a north window and kept for a month, and observed using a stereoscopic microscope (WILD MZ8, Leica).

(2) Direct inoculation method

Minute fecal pellets like those of arthropods were directly inoculated on the surface of Cornmeal agar medium (CMA) or plain agar. The plates were kept at room temperature for a month and checked under a stereoscopic microscope (WILD MZ8, Leica).

### 2.2.3 Isolation and establishment of pure cultures

Kickxellalean fungi occurred on the samples were isolated for obtaining pure cultures. Isolation was carried out by transferring a droplet of sporangiola to the surface of a new agar medium plate using a flamed out fine needle. Various culture media, CaA, shrimp agar medium (Degawa & Tokumasu 1998), and half-strength Malt extract-Yeast extract agar medium (1/2 ME-YE agar, Benjamin 1958) were used for this purpose. Two unidentified cultures were offered from Dr. Degawa.

### 2.2.4 Cultivation and identification of the isolates

All isolates obtained were identified by the following procedure.

These strains were incubated on 1/2 ME-YE agar at 20 °C for 15-20 days. Ten percent CaA and 0.02-0.1% CMA were also applied when the sporulation was poor. Slides were prepared either in the solution of cotton blue in 90% lactic acid (lactic acid-cotton blue) or in 1% aqueous solution of Phloxin. Spore germination in water was observed on microscope slides. These specimens were observed using an optical compound microscope (Microphot, Nikon), and the sizes of individual parts of reproductive apparatus and spores were measured with an ocular micrometer.

One hundred measurements were carried out on individual objects, and the values were expressed as 'min.-max. (mean $\pm$ SEM range)'.

Identification and description of the species followed the current taxonomical system of the Kickxellales based on microscopic morphology (Linder 1943, Benjamin 1958, 1959, 1961, 1963, Ogawa et al. 2001). Morphological terms employed in this dissertation are presented in Fig. 2-1 with diagrams. The terms utilized here followed Benjamin (1958).

## 2.3 RESULTS

A total 15 species of 8 genera were found from the soil and fecal samples (Tables 2-8, 2-9). Four undescribed genera, 7 undescribed species, 3 rediscovered species, and 10 species newly recorded to Japan were found in this study. Among these fungi, I succeeded to isolate and cultivate 11 species of 5 genera. The isolates counted up to 268.

### 2.3.1 Soil samples

Twelve species of 5 genera were discovered from the soil samples: *Coemansia aciculifera* Linder 1943, *C. erecta* Bainier 1906, *C. furcata* Kurihara et al. 2000, *C. mojavensis* R. K. Benj. 1958, *C. nantahalensis* C.-Y. Chien 1971, *C. spiralis sensu* Linder 1943, *C. sp. 1*, *Mycoemilia scoparia* (tentative name), *Ramicandelaber brevisporus* (tentative name), *R. longisporus* Ogawa et al. 2001, Kickxellaceae sp. 1, and Kickxellaceae sp. 2. Among them, *C. furcata*, *C. sp. 1*, *Mycoemilia scoparia* (tentative name), *R. brevisporus* (tentative name), Kickxellaceae sp. 1, and Kickxellaceae sp. 2 were undescribed species. Two species, *C. mojavensis* and *C. nantahalensis* were the second records since their original descriptions. *Coemansia spiralis* was the first record in Japan.

Among the species observed, isolates of 9 species of 3 genera were established.

### 2.3.2 Fecal samples

Four species of 3 genera were found from 8 fecal samples. They are *C. aciculifera*, *C. spiralis*, *Kickxella alabastrina* Coem. 1862, and *Spiromyces minutus* R. K. Benj. 1963. Among them, *C. spiralis* was new to Japan.

### 2.3.3 List and comments on the species observed in this study

(1) *Coemansia aciculifera* Linder 1943

*Coemansia aciculifera* occurred all around Japan, and it was revealed to be rather prevalent in forest soil. It frequently appeared from soil samples treated by all the isolation methods used in this study, and also appeared from feces of bat and mouse.

(2) *Coemansia erecta* Bainier 1906

*Coemansia erecta* was relatively common in forest soil in warmer regions of Japan (south of the Kanto district). Its distribution may imply that this species requires warm conditions (higher than 20 °C) for vigorous growth and sporulation. The species was originally recorded from dung (Thaxter in Linder 1943), and subsequently has been recorded from soil and plant debris (CBS list of cultures: [http://www.cbs.knaw.nl/search\\_fsp.html](http://www.cbs.knaw.nl/search_fsp.html)).

(3) *Coemansia furcata* Kurihara, Tokumasu et G.-Y. Chien 2000

*Coemansia furcata* is a newly discovered species in the present study. This species was frequently obtained from soil by all the isolation methods employed in the present study, and appeared to be relatively common in forest soil in Japan and Taiwan (Kurihara et al. 2000).

(4) *Coemansia mojabensis* R. K. Benj. 1958

This is the first record of *C. mojabensis* from Japan. It was isolated from soil samples collected in the Ehime Prefecture. The species had been known only from rat dung collected in a desert of USA (Benjamin 1958) and never been found since its original description.

(5) *Coemansia nantahalensis* G.-Y. Chien 1971

*Coemansia nantahalensis* is new to Japan. This species was rare and recorded only from the soil collected at arable and grasslands using the enrichment method or the soil plate method.

(6) *Coemansia spiralis sensu* Linder 1943

*Coemansia spiralis* is a new record from Japan. The species frequently occurred on forest soil. Although the species had been regarded as being rare

like most of other *Coemansia* species (Kwaśna et al. 1999a), it was frequently isolated from soil in Japan by all the methods employed, especially by the shrimp baiting method. This species was also obtained from a fecal pellet of a field mouse collected from Sugadaira.

Although *C. spiralis sensu* Linder (1943) is different from the original description of Eidam (1887) in producing longer sporangiospores (Chien 1971, Kwaśna et al. 1999a), I followed the description of Linder (1943) since the type specimen of Eidam (1887) has been lost.

(7) *Coemansia* sp. 1

This fungus would be an undescribed species. It was obtained several times from soil of western parts of Japan.

(8) *Kickxella alabastrina* Coem. 1862

This is the second record of *K. alabastrina* from Japan. The species was found from feces of voles collected in Sugadaira. This species has been isolated several times from feces of mammals ([http://www.cbs.knaw.nl/search\\_fsp.html](http://www.cbs.knaw.nl/search_fsp.html)), although the species is rarely encountered in nature (Benny et al. 2001).

Saikawa (1989) employed a strain collected in Sugadaira in 1973 as the material of ultrastructural observation of hyphal septa, and his report was the first record of this fungus from Japan.

(9) *Mycoemilia scoparia* (tentative name)

*Mycoemilia scoparia* (tentative name) is an undescribed species of an undescribed genus. This species was obtained from soil collected in the Hokkaido.

(10) *Myconymphaea yatsukahoi* Kurihara, Degawa et Tokumasu 2001

*Myconymphaea yatsukahoi* is a newly found species in the present study. This species occurred on a dead body of *Metriocampa* sp. (Diplura) collected in Sugadaira.

(11) *Ramicandelaber brevisporus* (tentative name)

An undescribed species of *Ramicandelaber* occurred frequently on forest soils treated by the soil plate method and moist chamber method using mealworms as baits.

(12) *Ramicandelaber longisporus* Ogawa, Hayashi, Degawa et Yaguchi 2001

*Ramicandelaber longisporus* was frequently isolated from forest soils treated by the baiting method with mealworms and the soil plate method.

(13) *Spiromyces minutus* R. K. Benj. 1963

*Spiromyces minutus* was found from a fecal pellet of vole collected in Sugadaira. My attempts to isolate this fungus were unsuccessful since the spores never germinated on any culture media used. The species has only been found from feces of mice and rats and reported several times since its original description (Mikawa 1975, Benny & Benjamin in O'Donnell et al. 1998).

(14) Kickxellaceae sp. 1

An undescribed fungus of the Kickxellaceae grew on the piece of plant debris collected in Sugadaira and kept with soil of the locality. It was apparently an undescribed species, and no proper genus that accommodated the species was existent in the family. The sporangiospores did not germinate and grow on any artificial agar media employed.

(15) Kickxellaceae sp. 2

Another undescribed fungus of the Kickxellaceae occurred on soil samples that contained many plant debris collected in Sugadaira. Obviously this was an undescribed species and appeared to belong to an undescribed genus of the Kickxellaceae. Only one sporangiophore developed, and the spores did not grow on an agar medium employed.

#### 2.3.4 Descriptions of the new taxa

In this part, the newly found genera/species of which aseptic cultures were established are described. Descriptions are based on the microscopic morphology of the taxa.

(1) *Coemansia furcata* Kurihara, Tokumasu et C.-Y. Chien 2000

*Coemansia furcata* Kurihara, Tokumasu et C.-Y. Chien 2000, Mycoscience 41: 579-583. (Figs 2-2, 2-3)

Etym. : From *furcata* (furcate) based on the branching habit of the sporangiophore.

Coloniae in 1/2 ME-YE agaro luteolae, post 10 dies ad 20 °C 52.4 mm diam attingentes. Hyphae vegetativae hyalinae, septatae, 2.0-10.0 (3.9±0.1) μm latae, frequenter gangligerae. Sporangiphora erecta, septata, 7.5-15.5 (12.3±0.2) μm lata, simplicia vel infra furcata, in partibus fertilibus furcata et sporocladia ferentia. Sporocladia asperula, ex stipitibus 10.5-28.0 (16.3±0.4) × 4.0-7.5 (5.4±0.1) μm evolventia, 7-13 (9±0.1) -cellularia, praeter stipites 31.0-56.0 (39.4±0.4) × 5.5-7.5 (6.2±0.0) μm; cellula apicalis sterilis et recurvata, 3.5-12.5 (6.5±0.2) × 1.5-4.0 (2.3±0.1) μm. Pseudophialides lageniformes, e cellulis fertilibus sporocladii lateraliter orientes, latrorsae, 4.5-6.5 (5.5±0.0) × 1.5-2.5 (2.0±0.0) μm. Sporangiola monospora, incolorata, cylindrica, cum latere alio convexo alio leviter concavo, 10.0-17.5 (13.8±0.1) × 2.5-3.0 (2.7±0.0) μm. Sporangiosporae cylindricae, 8.5-13.0 (10.6±0.1) × 1.5-3.0 (2.2±0.0) μm, ad apicem minute cacuminales.

Zygosporae globosae vel oblongae, aliquando anomalae, incoloratae, pachydermae, laevigatae, 21.5-75.0 (42.6±1.1) μm diam, muri 1.0-12.5 (3.5±0.2) μm crassi, globulos hyalinos aliquot vel multum (interdum plus quam 100) 3.0-26.0 (10.1±0.5) μm diam continentes, homothallicae.

**Holotypus:** Lishan, Taichung Pref., Taiwan. From soil of a *Cyclobalanopsis morii* and *Liquidambar formosana* forest, 1 June 1966, collected by H. Indoh and isolated by G.-Y. Chien (TNM F10612-holotypus). Living cultures are deposited in the American Type Cultural Collection, the Centraalbureau voor Schimmelcultures, the Cultural Collection Research Center, Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan, and the Mycological Laboratory, Institute of Biological Sciences, National Taiwan Normal University (F66-124-1), Taipei, Taiwan (ATCC 24540 = CBS 102833 = FIRDI 33645 = F66-124-1-culturae vivae).

Colonies on 1/2 ME-YE agar pale yellow. Vegetative hyphae colorless, septate, 2.0-10.0 (3.9±0.1) μm wide, often with gangliform swellings. Sporangiphores erect, septate, 7.5-15.5 (12.3±0.2) μm wide, unbranched or furcate below, furcate in the fertile part bearing sporocladia, intersporocladial distance 0.5-15.5 (6.3±0.2) μm. Sporocladia asperulate, with stalks of 10.5-28.0 (16.3±0.4) × 4.0-7.5 (5.4±0.1) μm, composed of 7-13 (9±0.1) cells excluding the stalks, 31.0-56.0 (39.4±0.4) × 5.5-7.5 (6.2±0.0) μm, the apical cell sterile and recurved, 3.5-12.5 (6.5±0.2) × 1.5-4.0 (2.3±0.1) μm.

Pseudophialides flask-shaped, 4.5–6.5 ( $5.5 \pm 0.0$ )  $\times$  1.5–2.5 ( $2.0 \pm 0.0$ )  $\mu\text{m}$ , sitting laterally in transverse rows on the fertile cells of sporocladia. Sporangiola monosporic, colorless, cylindrical, 10.0–17.5 ( $13.8 \pm 0.1$ )  $\times$  2.5–3.0 ( $2.7 \pm 0.0$ )  $\mu\text{m}$ , in lateral view one side convex, the other side slightly concave. Sporangiospores cylindrical, slightly pointed at the apex, 8.5–13.0 ( $10.6 \pm 0.1$ )  $\times$  1.5–3.0 ( $2.2 \pm 0.0$ )  $\mu\text{m}$ .

Zygosporcs globose to oblong, sometimes anomalous, colorless, thick walled, smooth, 21.5–75.0 ( $42.6 \pm 1.1$ )  $\mu\text{m}$  in diameter, wall 1.0–12.5 ( $3.5 \pm 0.2$ )  $\mu\text{m}$  thick, containing several to many (sometimes more than 100) hyaline globules of 3.0–26.0 ( $10.1 \pm 0.5$ )  $\mu\text{m}$  in diameter, formed on the surface or buried in the agar medium, homothallic.

Colony diameter on 1/2 ME–YE agar reached 52.4 mm at 20 °C for 10 days.

Other isolates examined: CBS 109359. Mt. Yuwan, Uken, Amami Island, Kagoshima Pref., Japan. From soil of a *Symplocos confusa* and *Symplocos prunifolia* forest, Mar. 1996, collected by R. Machida and isolated by Y. Kurihara. CBS 109358. Sugadaira, Sanada, Nagano Pref., Japan. From soil of a *Pinus densiflora* forest, 23 July 1996, collected and isolated by Y. Kurihara.

Notes: *Coemansia furcata* resembles *C. aciculifera* var. *suhagensis* B. R. Mehrotra & Kakkar 1970 in branching dichotomously in the upper part of the sporangiophore, but clearly distinct in producing higher and wider sporangiophores and sporocladia, and slightly wide, elongate cylindrical sporangiola (Table 2–10).

In 1969, Chien reported this fungus from Taiwan as *Coemansia formosensis* (as '*formosanensis*') –*nomen nudum*, but he has not published it formally since that time. *Coemansia furcata* has been reported in the literature as *C. formosanensis* (Chien 1969) or *C. formosensis* (Chien 1994), but these names were never validly described. Through this survey, I found that this species is widely distributed both in Taiwan and Japan. Therefore, I named it *Coemansia furcata* based on the branching habit of the sporangiophore.

Benjamin (1958) pointed out that the shape of sporangiospores of *Coemansia* spp. and *Kickxella alabastrina* changed in lactic acid, that is, annular thickenings appeared in the spores. A similar alteration also occurred in this species. This suggests that the species has a similar annular thickening of the inner complex of the sporangiospore wall, as shown in *C. aciculifera* (Young 1973a),

*C. mojavensis* (Young 1990), and *K. alabastrina* (Young 1974) by ultrastructural studies.

In water, sporangiola measure  $13.7 \pm 0.107 \times 2.9 \pm 0.02 \mu\text{m}$ , and sporangiospores  $11.1 \pm 0.089 \times 2.5 \pm 0.02 \mu\text{m}$ . The results of dispersion analyzing of the one-way layout (Sokal & Rohlf 1973) showed clear differences in width of sporangiola ( $H = 48.102$ ,  $P < 0.001$ ;  $z = 6.937$ ,  $P < 0.00003$ ), length ( $H = 8.901$ ,  $P < 0.01$ ;  $z = 2.983$ ,  $P < 0.00014$ ) and width ( $H = 105.765$ ,  $P < 0.001$ ;  $z = 10.284$ ,  $P < 0.00003$ ) of sporangiospores in lactic acid-cotton blue to those in water. In contrast, no significant difference was recognized in the length of sporangiola ( $H = 0.682$ ,  $P > 0.05$ ;  $z = -0.826$ ,  $P > 0.05$ ). Therefore, it is necessary to pay attention to this point when we compare the size of various parts of *Coemansia* species.

(b) *Coemansia* sp. 1

*Coemansia* sp. 1 (Fig. 2-4)

[This fungus will be published as *Coemansia ventricosa*.

Etym.: From Latin *ventricosus* (ventricose) based on the shape of the sporangiospore.]

Coloniae in 1/2 ME-YE agar sulphurellae. Hyphae vegetativae hyalinae, septatae. Sporangiphora erecta, septata, simplicia vel furcata. Sporocladia arcuata, asperula, ex stipitibus  $15.5-9.0$  ( $7.3 \pm 0.9$ )  $\times$   $5.0-7.0$  ( $5.8 \pm 0.6$ )  $\mu\text{m}$  evolventia, 7-11 ( $8 \pm 0.6$ ) -cellularia, praeter stipites  $28.0-44.0$  ( $34.3 \pm 2.9$ )  $\times$   $6.5-10.0$  ( $7.5 \pm 0.4$ )  $\mu\text{m}$ ; cellula apicalis sterilis,  $4.5-10.0$  ( $6.7 \pm 0.7$ )  $\times$   $2.5-5.5$  ( $3.7 \pm 0.6$ )  $\mu\text{m}$ . Pseudophialides lageniformes, e cellulis fertilibus sporocladii lateraliter orientes, latrorsae,  $6.5-9.5$  ( $6.9 \pm 0.4$ )  $\times$   $2.5-5.0$  ( $3.2 \pm 0.4$ )  $\mu\text{m}$ . Sporangiola monospora, incolorata, cylindrica, ventricosa, cum latere alio convexo alio leviter concavo,  $10.0-15.5$  ( $12.0 \pm 0.4$ )  $\times$   $2.5-4.0$  ( $2.9 \pm 0.2$ )  $\mu\text{m}$ . Sporangiosporae cylindricae, ventricosae,  $9.5-14.0$  ( $11.2 \pm 0.6$ )  $\times$   $2.5-4.0$  ( $2.9 \pm 0.2$ )  $\mu\text{m}$ .

Zygosporae ignotae.

[Holotypus: Niimi, Okayama Pref., Japan. From soil of a plantation of *Chamaecyparis obtusa*, 1 May 1997, collected and isolated by Y. Kurihara. The type specimen and a living culture will be deposited in the Kanagawa Prefectural Museum and the Centraalbureau voor Schimmelcultures, respectively.]

Colonies on 1/2 ME-YE agar somewhat sulphur-yellow. Vegetative hyphae colorless, septate. Sporangiphores erect, septate, branched or rarely unbranched. Sporocladia arcuate, asperulate, with stalks of 5.5-9.0 (7.3±0.9) × 5.0-7.0 (5.8±0.6) μm, composed of 7-11 (8±0.6) cells excluding the stalks, 28.0-44.0 (34.3±2.9) × 6.5-10.0 (7.5±0.4) μm, the apical cell sterile, 4.5-10.0 (6.7±0.7) × 2.5-5.5 (3.7±0.6) μm. Pseudophialides flask-shaped, 6.5-9.5 (6.9±0.4) × 2.5-5.0 (3.2±0.4) μm, sitting laterally in transverse rows on the fertile cells of sporocladia. Sporangiola monosporic, colorless, cylindrical, ventricosus, 10.0-15.5 (12.0±0.4) × 2.5-4.0 (2.9±0.2) μm, in lateral view one side convex, the other concave. Sporangiospores cylindrical, ventricosus, 9.5-14.0 (11.2±0.6) × 2.5-4.0 (2.9±0.2) μm.

Zygospores not found.

Other isolates examined: CBS 109371. Nishikata, Touwa, Yashiro Island, Yamaguchi Pref., Japan. From soil of an *Ilex rotunda*(?) and *Myrsine seguinii* forest, 4 Jan. 1999, collected and isolated by Y. Kurihara. CBS 109362. Yashima, Takamatsu, Kagawa Pref., Japan. From soil under a shrub of *Ligustrum japonicum* and *Quercus acutissima*, 29 Dec. 1998, collected and isolated by Y. Kurihara.

Notes: *Coemansia* sp. 1 resembles *C. mojavensis* in producing ventricose sporangiospores, but the present species is distinguishable from the latter in producing longer and arcuate sporocladia and more ventricose and broader sporangiospores (Table 2-11).

The strain described as *C. erecta* in Matsushima (1975) is suspected to be *C. sp. 1*. According to Matsushima (1975), his strain has arcuate sporocladia and ventricose sporangiola, and these are characteristic of *C. sp. 1* (Table 2-12). This issue, however, remains unsolved since I could have no chance to observe the strain.

(3) *Myconymphaea yatsukahoi* Kurihara, Degawa et Tokumasu 2001

*Myconymphaea* Kurihara, Degawa et Tokumasu 2001, Mycol. Res. 105: 1397-1402.

Etym.: From Greek *Mycō-* (fungal) and *Nymphaea* (water lily), based on its resemblance to a water lily flower under wet conditions (see Fig. 2-6-B).

Sporangiophora erecta, septata, furcata vel simplicia, asperula, 1-nonnulas partes fertiles vesiculosas formantia in his, sporocladia catervatim formata in superis hemisphaeriorum. Sporocladia cylindrica, unicellularia vel aliquando didyma, pseudophiales ferentia. Pseudophialides lagenariae, cum collis longis, sporangiola ferentes in collis. Sporangiola monospora, incolorata, cylindrica et acicularia. Sporangiosporae cylindricae et aciculares.

Holotypus: *Myconymphaea yatsukahoi* Kurihara, Degawa et Tokumasu.

Sporangiophores erect, septate, branched or unbranched, asperulate, producing one to several fertile parts. The apical end of sporangiophores enlarged, forming a subglobose to oblate vesicle bearing sporocladia massed on the upper hemisphere. Sporocladia cylindrical, composed of one (sometimes two) cell(s), bearing pseudophialides. Pseudophialides flask-shaped, with long necks, bearing a single sporangiole. Sporangiola monosporic, colorless, cylindrical and aciculate. Sporangiospores cylindrical and aciculate.

*Myconymphaea yatsukahoi* Kurihara, Degawa et Tokumasu 2001, Mycol. Res. 105: 1397-1402. (Figs 2-5, 2-6, 2-7)

Etym.: Named after the collector of the dipluran, Dr. Yatsukaho Ikeda. 'Yatsukaho' is a classic Japanese word that means the conspicuously long and fruitful spikes of cereals. In addition, it also reflects a unique feature of the fungus to bear abundant long sporangiospores.

Coloniae in 1/2 ME-YE agaro eburneae-luteolae. Hyphae vegetativae hyalinae, septatae, 1.5-4.0 ( $2.4 \pm 0.0$ )  $\mu\text{m}$  latae. Sporangiphora erecta, septata, 10.0-15.0 ( $12.6 \pm 0.1$ )  $\mu\text{m}$  lata, furcata vel simplicia, asperula, 1-nonnulas partes fertiles vesiculosas formantia in his 5.5-26.5 ( $15.4 \pm 0.5$ )  $\times$  12.5-25.0 ( $19.2 \pm 0.3$ )  $\mu\text{m}$ , 9-25 ( $16 \pm 0.4$ ) sporocladia catervatim formata in superis hemisphaeriorum. Sporocladia cylindrica, unicellularia vel aliquando didyma, 17.0-38.0 ( $25.0 \pm 0.4$ )  $\times$  9.0-15.5 ( $12.2 \pm 0.1$ )  $\mu\text{m}$ , 2-6 ( $4 \pm 0.1$ ) pseudophiales ferentia. Pseudophialides lagenariae, 22.0-29.5 ( $25.6 \pm 0.2$ )  $\times$  7.5-10.5 ( $9.1 \pm 0.1$ )  $\mu\text{m}$ , cum collis longis 7.5-12.5 ( $10.2 \pm 0.1$ )  $\mu\text{m}$  longis, sporangiola ferentes in collis. Sporangiola monospora, incolorata, cylindrica

et acicularia, leviter sigmoidea, 89.0–130 (113±0.9) × 6.5–8.0 (7.1±0.0) μm. Sporangiosporae cylindricae et aciculares, 78.0–115 (97.4±0.8) × 5.0–7.0 (6.0±0.1) μm.

Zygospores ignotae.

Holotypus: Sugadaira, Sanada, Nagano Pref., Japan. From a cadaver of *Metriocampa* sp., 26 June 1995, collected by Y. Ikeda and isolated by Y. Degawa (KPM-NC0007581-holotypus). A living culture is deposited in the Centraalbureau voor Schimmelcultures (CBS 109376-cultura viva).

Colonies on 1/2 ME-YE agar ivory-white to pale yellow, 1–1.5 cm high. Vegetative hyphae colorless, septate, 1.5–4.0 (2.4 ± 0.0) μm wide. Sporangiphores erect, septate, 10.0–15.0 (12.6±0.1) μm wide, branched or unbranched, asperulate, producing one to several fertile parts. The apical end of sporangiphores enlarged, forming a subglobose to oblate vesicle 5.5–26.5 (15.4 ±0.5) × 12.5–25.0 (19.2±0.3) μm, bearing 9–25 (16±0.4) sporocladia massed on the upper hemisphere of the vesicle. Sporocladia cylindrical, composed of one (sometimes two) cell(s), 17.0–38.0 (25.0±0.4) × 9.0–15.5 (12.2±0.1) μm, bearing 2–6 (4±0.1) pseudophialides. Pseudophialides flask-shaped, 22.0–29.5 (25.6±0.2) × 7.5–10.5 (9.1±0.1) μm, with long necks 7.5–12.5 (10.2±0.1) μm long, bearing a single sporangiole. Sporangiole monosporic, colorless, cylindrical and aciculate, slightly sigmoid, 89.0–130 (113±0.9) × 6.5–8.0 (7.1 ±0.0) μm, immersed in fluid and easily detached at maturity. Sporangiospores cylindrical and aciculate, 78.0–115 (97.4±0.8) × 5.0–7.0 (6.0±0.1) μm, at germination, one to several pseudosepta often formed and (1–)2–3(–5) germ tubes developed laterally.

Zygospores not observed.

Notes: *Myconymphaea yatsukahoi* does not appear to be an obligate or facultative parasite of dipturans since it exhibits abundant saprophytic growth on a relatively simple artificial medium such as CMA. Moreover, this fungus was observed to grow on frass inside a decaying log near the type locality a year after it was first isolated. Efforts to obtain a culture from this second collection were unsuccessful.

This species differs from all known kickxellalean species in having the

following features: (a) essentially unicellular sporocladia with plural pseudophialides on apical vesicles of sporangiophores, (b) conspicuously long, aciculate sporangiola and sporangiospores, (c) pseudosepta formed at germination, and (d) unique features of sporangiophore septal plugs. Thus, I proposed *Myconymphaea* as a new genus for the fungus.

Although its sporangiophore septal plug has a prominent protuberance on the upper side that superficially resembles that of *Dimargaris cristalligena* (Dimargaritales), I conclude that *Myconymphaea* can be accommodated in the order Kickxellales, because, like other kickxellalean species, it produces sporangiospores within unispored sporangiola, it grows as a saprobe, and its septal plugs are stable in both acidic stains and 3% KOH.

The intermediate features of the plug of this fungus require evaluation as to whether the morphological features of plugs at the microscopic level are appropriate characteristics to distinguish the Kickxellales from the Dimargaritales. Further observations on the plugs, including ultrastructural observations, would clarify this issue.

(4) *Mycoemilia scoparia* (tentative name)

*Mycoemilia* (tentative name)

Etym.: From Greek *Myco-* (fungal) and *Emilia*, representing the appearance of the fungus similar to the flower of *Emilia sonchifolia* (Asteraceae).

Hyphae vegetativae septatae. Sporophora erecta, septata, simplicia vel furcata, 1-nonnulas partes fertiles formantia. Apex sporophorum paulo inflatus, sporocladia catervatim formans. Sporocladia lagenaria, cum collo longo, sporas catervatim formantia. Sporae hyalinae fusiformes.

Zygosporae globosae, punctulatae, cum muro crasso, globulum hyalinum continentes.

Holotypus: *Mycoemilia scoparia* (tentative name)

Vegetative hyphae septate. Sporophores erect, septate, branched or unbranched, producing one to several fertile parts. The apex of sporophore slightly enlarged, bears 4-8 ( $6 \pm 0$ ) aggregated sporocladia. Sporocladia lageniform, bearing spores apically. Spores colorless, fusiform, deciduous,

immersed in fluid at maturity.

Zygosporae spherical, punctulate, with thick wall, containing a hyaline globule.

*Mycoemilia scoparia* (tentative name) (Figs 2-8, 2-9, 2-10, 2-11).

Etym.: From Latin *scoparius*, representing the broom-like appearance of sporophore.

Coloniae in 1/2 ME-YE agar ochraceae vel pallentes venaceobrunneae, velutinae. Hyphae vegetativae hyalinae, septatae, 2.5-6.5 ( $3.4 \pm 0.1$ )  $\mu\text{m}$  latae. Sporophora erecta, septata, simplicia vel furcata, 1-nonnulas partes fertiles formantia. Cellulae sporophororum 45.0-148 ( $111 \pm 2.2$ )  $\times$  2.0-4.0 ( $2.8 \pm 0.0$ )  $\mu\text{m}$ . Apex sporophorum paulo inflatus, 4-8 ( $6 \pm 0$ ) sporocladia catervatim formans. Sporocladia lagenaria, 14.5-21.5 ( $17.6 \pm 0.2$ )  $\times$  2.5-4.3 ( $3.5 \pm 0.0$ )  $\mu\text{m}$ , 4-11 ( $7 \pm 0$ ) sporas catervatim formantia in collo. Sporae hyalinae, fusiformes, 7.0-9.0 ( $8.1 \pm 0.1$ )  $\times$  2.5-3.0 ( $2.8 \pm 0.1$ )  $\mu\text{m}$ .

Zygosporae globosae, 27.5-35.5 ( $32.0 \pm 0.2$ )  $\mu\text{m}$  in diam, punctulatae, cum muro crasso 2.5-4.5 ( $3.3 \pm 0.1$ )  $\mu\text{m}$ , globulum hyalinum et excentricum continentis.

[Holotypus: University of Hokkaido, Sapporo, Hokkaido, Japan. From soil with caterpillar worm collected under a shrub, 1 May 1997, collected by N. Kotaka and isolated by Y. Degawa. The type specimen will be deposited in the Kanagawa Prefectural Museum. A living culture is deposited in the Centraalbureau voor Schimmelcultures (CBS 109375-cultura viva).]

Colonies on 1/2 ME-YE agar ocher to pale vinaceous brown, velvety. Vegetative hyphae colorless, septate, 2.5-6.5 ( $3.4 \pm 0.1$ )  $\mu\text{m}$  wide. Sporophores erect, septate, branched or unbranched, producing one to several fertile parts. Sporophore cell 45.0-148 ( $111 \pm 2.2$ )  $\times$  2.0-4.0 ( $2.8 \pm 0.0$ )  $\mu\text{m}$ . The apex of sporophore slightly enlarged, bears 4-8 ( $6 \pm 0$ ) aggregated sporocladia. Sporocladia lageniform, 14.5-21.5 ( $17.6 \pm 0.2$ )  $\times$  2.5-4.3 ( $3.5 \pm 0.0$ )  $\mu\text{m}$ , bearing 4-11 ( $7 \pm 0$ ) spores apically. Spores colorless, fusiform, in lateral view one side convex, the other slightly concave, 7.0-9.0 ( $8.1 \pm 0.1$ )  $\times$  2.5-3.0 ( $2.8 \pm 0.1$ )  $\mu\text{m}$ , the ratio of length to width (l/w ratio) 2.2-3.5 ( $2.8 \pm 0.0$ ), deciduous, immersed in fluid at maturity.

Zygosporae spherical, 27.5–35.5 ( $32.0 \pm 0.2$ )  $\mu\text{m}$  in diameter, punctulate, with brownish thick wall of 2.5–4.5 ( $3.3 \pm 0.1$ )  $\mu\text{m}$ , containing an hyaline and eccentric globule.

Notes: Unlike *Spiromyces* species, *M. scoparia* (tentative name) produces the lageniform sporocladia produced acrogenously in mass and bears wet and fusiform spores on the sporocladia. The sporogeneous apparatus of *M. scoparia* (tentative name) superficially resemble a hyphomycete genus *Stachybotrys* Corda 1837, however, this fungus obviously produces zygosporae in the similar way of *Coemansia aciculifera* (Benjamin 1958) and the septal structure particular to the kickxellids.

The phenomenon that the shape of asexual spores changes in lactic acid was not observed in this species.

(5) *Ramicandelaber brevisporus* (tentative name)

*Ramicandelaber brevisporus* (tentative name) (Figs 2–12, 2–13)

Etym.: From Latin *brevi-* (short-) and *spora* (spore), representing the short spore in comparison with the type species of the genus, *Ramicandelaber longisporus*.

Coloniae in 1/2 ME-YE agar relative tarde crescentes, candidae. Hyphae vegetativae hyalinae, septatae, 2.0–5.0 ( $2.8 \pm 0.1$ )  $\mu\text{m}$  latae. Sporophora erecta, septata, cum rhizoideis et ramis lateralibus, 1–3 partes fertiles in his. Rhizoidea enata cellula basilari sporophori, 12.5–138 ( $50.9 \pm 2.7$ )  $\times$  2.0–5.0 ( $3.1 \pm 0.1$ )  $\mu\text{m}$ . Ad partem fertilem, 3–13 ( $8 \pm 2.5$ ) rami fertiles verticillate enati gibba sporophori. Rami fertiles 15.0–34.0 ( $25.8 \pm 0.4$ )  $\times$  2.5–4.5 ( $3.5 \pm 0.1$ )  $\mu\text{m}$  lati in basi, 2–9 ( $5 \pm 0.2$ ) sporocladia efferentes. Sporocladia cylindrica, eseptata, 6.5–10.0 ( $7.8 \pm 0.1$ )  $\times$  2.0–2.5 ( $2.4 \pm 0.0$ )  $\mu\text{m}$ , pseudophialidem subglobosam cum collo brevi formantia, 4.0–6.5 ( $4.9 \pm 0.0$ )  $\times$  2.5–5.0 ( $3.5 \pm 0.1$ )  $\mu\text{m}$ . Sporae hyalinae, tenui-fusifformes, acuminatae, 17.5–25.0 ( $21.0 \pm 0.2$ )  $\times$  3.0–4.0 ( $3.8 \pm 0.0$ )  $\mu\text{m}$ .

Zygosporae ignotae.

[Holotypus: Mihara-Ohike, Hachijyo Island, Tokyo, Japan. From soil under a plantation of *Cryptomeria japonica*, 1 May 1999, collected by Y. Degawa and isolated by Y. Kurihara. The type specimen will be deposited in the Kanagawa Prefectural

Museum. A living culture is deposited in the Centraalbureau voor Schimmelcultures (CBS 109374-cultura viva).]

Colonies on 1/2 ME-YE agar relatively slow growing, pure glossy white. Vegetative hyphae colorless, septate, 2.0-5.0 ( $2.8 \pm 0.1$ )  $\mu\text{m}$  wide. Hook-like connections regularly produced between two aerial hyphae where they are crossing. Sporophores erect, septate, with rhizoids and lateral branches, bearing 1-3 fertile part. Rhizoids developed from a basal cell of the sporophore, 12.5-138 ( $50.9 \pm 2.7$ )  $\times$  2.0-5.0 ( $3.1 \pm 0.1$ )  $\mu\text{m}$ . Lateral branches erect, septate, developing from just on the septum of sporophore cell, with rhizoids. At fertile part, 3-13 ( $8 \pm 2.5$ ) fertile branches verticillately develop from an enlarged part on sporophore. Fertile branches 15.0-34.0 ( $25.8 \pm 0.4$ )  $\times$  2.5-4.5 ( $3.5 \pm 0.1$ )  $\mu\text{m}$  wide at base, bearing 2-9 ( $5 \pm 0.2$ ) sporocladia near the apex. In old culture, fertile branches become enlarged and finally globose. Sporocladia cylindrical, aseptate, 6.5-10.0 ( $7.8 \pm 0.1$ )  $\times$  2.0-2.5 ( $2.4 \pm 0.0$ )  $\mu\text{m}$ . Pseudophialides formed on a sporocladium from its lateral side, develops in parallel to the sporocladium, subglobose, 4.0-6.5 ( $4.9 \pm 0.0$ )  $\times$  2.5-5.0 ( $3.5 \pm 0.1$ )  $\mu\text{m}$ , with short neck, develops a spore on the neck. Spores colorless, slender fusiform, acuminate, 17.5-25.0 ( $21.0 \pm 0.2$ )  $\times$  3.0-4.0 ( $3.8 \pm 0.0$ )  $\mu\text{m}$ , l/w ratio 5.0-7.2 ( $5.8 \pm 0.1$ ), deciduous and immersed in fluid at maturity.

Zygosporoes not found.

Other isolate examined: CBS 109373. The Cape Muroto, MurotoMisaki, Kouchi Pref., Japan. From soil under a tree of *Ficus superba* var. *japonica* in a coastal shrub zone of *Quercus phillyraeoides*, 30 Dec. 1998, collected and isolated by Y. Kurihara.

Notes: *Ramicandelaber brevisporus* (tentative name) is readily distinguished from *R. longisporus* by its much shorter spores (Table 2-13). The spores were not stainable with lactic acid-cotton blue at maturity. The phenomenon that the shape of spores changes in lactic acid known in *G. aciculifera* (Benjamin 1958) was not observed. The presence or absence of the lateral branch was changeful depending on culture conditions.

The best sporulation of *R. brevisporus* occurred on 0.1% CMA, but this fungus seldom sporulated on Egg medium of Ayers (1933), 1/2 ME-YE agar, 5% Pablum agar medium, and 3% Pablum dextrose agar medium.

## 2.4 DISCUSSION

This study succeeded in obtaining many isolates of the Kickxellales. These isolates broadened our knowledge of the taxonomy and the distribution of the order.

### 2.4.1 Diversity and prevalence of the Kickxellales

Through the survey, several undescribed taxa were discovered in spite of the narrow research area limited to Japan. The percentage of the undescribed species (7 species) to all species found in this study (15 species) reached 47%. This implies that the Kickxellales would be a larger and much diverse fungal group than we have imaged. This survey also demonstrated that some kickxellaleans such as *Coemansia* species are widely distributed in soil, although they had been reported rather infrequently.

### 2.4.2 Geographic distribution of kickxellalean genera

Kirk (1993) predicted that the distributions of two genera, *Kickxella* and *Martensella* were limited only in the temperate zone, and those of other five genera, *Dipsacomyces*, *Linderina*, *Martensiomycetes*, *Spirodactylon*, and *Spiromyces* were restricted to the tropics. However, I found that the 'tropical' and 'temperate' genera were concurrent at the place. The 'tropical' genus *Spiromyces* and the 'temperate' genus *Kickxella* were observed at the same time in the Sugadaira Highland where a cool temperate climate dominates. The fact implies that the distributions of some kickxellaleans are wider than previously estimated. Probably their confined distribution known up to the present is caused by that they are hardly discovered.

Kirk (1993) also stated that the Kickxellales had been relatively well researched in the temperate regions compared with those from the tropics. However, this should be also reconsidered because recent works (Kurihara et al. 2000, 2001; Ogawa et al. 2001) and the present study report many undescribed kickxellalean taxa from the temperate regions of Japan and Taiwan. Thus, in the temperate zone, we should advance the research on the Kickxellales, in addition to that in the tropics.

### 2.4.3 Evaluation of feces as the sources of the Kickxellales

Feces of omnivorous rats/mice have been regarded as the best source of kickxellaleans (Benjamin 1959, Benny et al. 2001). The present study confirmed it also in feces of voles, and elucidated that the sedentary of murines facilitated

collecting their feces repeatedly at the same plot. In addition, the small uniform size of the feces made handling easy. From the series of observations of the feces collected at the same point and might be evacuated by an individual, freshness of feces and the temperature for incubation were found to affect the occurrence of the objective fungi. Fresh feces kept at relatively low temperature (ca 5-15 °C) provided the optimum results. The fact that some kickxellalean species require cool conditions for abundant sporulation (Benjamin 1958, Chien 1994) might account for this phenomenon.

Feces of other animals, especially those of arthropods and entomophagous bats have also been recommended as the sources of kickxellaleans (personal communications from Drs Degawa and Doi). Through this study, two kickxellalean species were found from these feces (*M. yatsukahoi* from arthropodous frass and *G. aciulifera* from bat guano) even though only a small number of samples were examined. On the basis of the facts, these feces appear to be proper sources of kickxellaleans, although examination on more feces is needed for elucidating it.

Although feces would be an appropriate resource for isolating kickxellaleans, in the present study, I could encounter them only 8 times through the detection of 106 samples composed of nearly 1,000 fecal pellets. Kickxellaleans seem to be rare in nature indeed.