

## CHAPTER 4

### A Grouping of the Kickxellales Based on Optical Microscopic Morphological Characteristics

#### 4.1 INTRODUCTION

The current taxonomic system of the genera of the Kickxellales is essentially based on the concept of Benjamin (1959). He regarded asexual reproductive apparatus, especially sporocladia as the key characters for distinguishing the genera in the order. After Benjamin (1959), several authors have typified the asexual reproductive morphology, particularly sporocladia, mainly based on the septation of sporocladia.

In the earliest typification, Benjamin (1966) distinguished four types of sporocladia: aseptate two types (*Linderina* and *Spiromyces*), tri-septate type (*Kickxella*), and the type with many septa (all remaining genera known at that time: *Coemansia*, *Dipsacomyces*, *Martensella*, *Martensiomyces*, and *Spirodactylon*) (Benny et al. 2001) (Table 4-1). Later, Young (1999) typified the asexual apparatus based on the assortment of (1) whether sporocladia are aseptate or septate, (2) whether the pseudophialides are monosporic or polysporic, and (3) whether sporangiospores are wet or dry, and discriminated three types of them in the order: *Linderina* and *Spiromyces* type, *Spirodactylon* type, and the type for the remaining genera (Table 4-1). More recently, Ogawa et al. (2001) re-categorized it based on the septation and the shape of sporocladia when they added *Ramicandelaber* to the order (Table 4-1). They approved the aseptate type sporocladia (*Linderina*, *Spiromyces*, and *Ramicandelaber*) and the septate type sporocladia (the remaining genera) in the order.

These trials are mere morphological typification and not examined taxonomically. The present study newly added the undescribed taxa to the order. Because asexual reproductive apparatus are the basis of the current classification system, re-evaluation of them is required for the revision of the system. Therefore, in this chapter, sporocladia of the order are evaluated and categorized into groups. Then, the genera of the order are classified into groups based on the total morphology of sporocladia and the other apparatus, and finally, the groups are evaluated taxonomically.

## 4.2 GROUPING METHODS

### 4.2.1 Re-typification of sporocladia

Typification of sporocladia was carried out on the basis of (1) whether sporocladia or pseudophialides generate asexual spores (= whether sporocladia or pseudophialides are the sporogeneous cell), (2) whether sporocladia are septate or aseptate (= whether the sporocladia are polycellular or unicellular).

### 4.2.2 Selection of characters for grouping

To classify the genera into groups, seven characteristics that had been concluded to be appropriate for this purpose in the chapter 3 were employed. The seven characteristics selected here are: (1) whether sporocladia or pseudophialides generate asexual spores (= whether sporocladia or pseudophialides are sporogeneous), (2) whether sporocladia are septate or aseptate (= whether sporocladia are polycellular or unicellular), (3) whether a sporocladial cell generates a single or plural pseudophialides, (4) whether sporogeneous cells are monosporic or polysporic, and (5) whether asexual spores are sporangiospores or conidia. Two features of zygospores; (6) whether the surface of zygospores is ornamented or not, and (7) the number, the size, and the position of globules in zygospores, were treated additionally since zygospores had not been known in all genera.

## 4.3 RESULTS

### 4.3.1 Types of sporocladia

As shown in Figs 4-1, 4-2, sporocladia of the Kickxellales were categorized into five types: *Coemansia* type, *Myconymphaea* type, *Linderina* type, *Spiromyces* type, and *Ramicandelaber* type. Each type was named after the genus that represented it.

#### (a) The *Coemansia* type

The *Coemansia*-type sporocladia are produced in most genera, *Coemansia*, *Dipsacomycetes*, *Kickxella*, *Martensella*, *Martensiomyces*, and *Spirodactylon* (Figs 4-1, 4-2). This type of sporocladia was named after the *Coemansia* since the genus produced the typical one. In this type, the sporocladia are boat-shaped and septate, having a beak-like and sterile apical cell and a stalk, and bearing pseudophialides laterally (Linder 1943; Meyer 1957; Benjamin 1959, 1961). Each cell of sporocladia is uninucleate except in *Kickxella* of which cells have two

or three nuclei (Benjamin 1959, 1966).

(b) The *Myconymphaea* type

The *Myconymphaea*-type sporocladia are produced by *Myconymphaea* (Figs 4-1, 4-2). This type of sporocladia is cylindrical, usually aseptate (= unicellular) but sometimes two-celled with a horizontal septum, develops directly from an apical enlargement of sporangiophore, lacks the stalk and the apical cell, and bears several pseudophialides apically (Kurihara et al. 2001). The sporocladia are usually uninucleate; in the case of two-celled sporocladia, each cell has a nucleus (Kurihara et al. 2001).

(c) The *Linderina* type

The *Linderina*-type sporocladia are constructed by *Linderina* (Figs 4-1, 4-2). This type of sporocladia is large, globose, unicellular, and multinucleate (Benjamin 1966), lacks the stalk and the apical cell, and bears many pseudophialides on the upper surface (Raper & Fennell 1952). Nevertheless both are unicellular, the large, vesiculate, and multinucleate sporocladia of *Linderina* (Benjamin 1966) appear to be essentially different from the uninucleate sporocladia of *Myconymphaea* that aggregate on the vesicle (Kurihara et al. 2001), and thus they were divided into the two types.

(d) The *Spiromyces* type

The *Spiromyces*-type sporocladia are produced by two genera, *Spiromyces* and *Mycoemilia* (tentative name) (Figs 4-1, 4-2). This type of sporocladia is globose, with or without a globose stalk, unicellular, and uninucleate (Benjamin 1966), lacks the apical cell and pseudophialides, and bears several spores directly or on pedicels (Benjamin 1963, Benny & Benjamin in O' Donnell et al. 1998).

(e) The *Ramicandelaber* type

Production of the *Ramicandelaber*-type sporocladia is confined to the *Ramicandelaber* (Figs 4-1, 4-2). This type of sporocladia is small, cylindrical, and unicellular, develops from fertile branches, lacks stalks and apical cells, and generates a single pseudophialide laterally (Ogawa et al. 2001). Nuclei in the sporocladia have not been observed.

#### 4.3.2 Groups of the genera

The genera of the Kickxellales were classified into three groups: *Coemansia* group, *Spiromyces* group, and *Ramicandelaber* group (Figs 4-1, 4-2). Each group was named after the representative genus of its group.

##### (a) The *Coemansia* group

Among the five types of sporocladia described above, the *Myconymphaea* type and the *Linderina* type exhibit striking similarities to the *Coemansia* type. These two types differ from the *Coemansia* type only in producing unicellular sporocladia, and *Myconymphaea* sometimes bears the septate sporocladia like the *Coemansia* type (Kurihara et al. 2001). These facts suggest that these three types are closely related each other. Consequently, these genera were treat as one, the *Coemansia* group (Figs 4-1, 4-2).

The *Coemansia* group is the largest one that includes *Coemansia*, *Dipsacomycetes*, *Kickxella*, *Martensella*, *Martensiomyces*, *Spirodactylon*, *Myconymphaea*, and *Linderina* (Fig. 4-1). Although the sporangiophores are fairly diverse, the asexual sporogeneous apparatus of these genera are fundamentally the same (Fig. 4-2), and they share the following features: sporogeneous pseudophialides (1), septate (*Coemansia* type) or aseptate (*Myconymphaea* and *Linderina* types) sporocladia (2), plural pseudophialides per sporocladial cell (3), monosporic pseudophialides (4), and sporangiospores (5) (Young 1968, 1970, 1971, 1973a, 1973b, 1974, 1990).

Zygospores are smooth and lack surface ornamentation (6) and contain several to many small globules (7). Zygospores of *Myconymphaea* and *Linderina* have not been observed yet (Fig. 4-2).

##### (b) The *Spiromyces* group

The *Spiromyces* group is composed of two genera, *Spiromyces* and *Mycoemilia* (tentative name). Asexual structures of this group are characterized by producing conidia (5) directly on unicellular sporocladia (1, 2, 4) without intermediated by pseudophialides (3). Based on these unique features, the apparatus have been considered to be distinct from those of remaining genera (Benjamin 1963, Benny & Benjamin in O'Donnell et al. 1998).

Zygospores are also distinctive from those of other Kickxellales and rather resemble those of the Dimargaritales. Zygospores have surface ornamentation (6) and contain a large globule eccentrically (7) (Benjamin 1963, Benny & Benjamin

in O'Donnell et al. 1998).

(c) The *Ramicandelaber* group

The *Ramicandelaber* group comprises a single genus, *Ramicandelaber*. *Ramicandelaber* also owns asexual structures disparate from those of the remaining genera. The sporocladium is unicellular (2) and bears a pseudophialide laterally (3), although this nature changes with aging. The sporogeneous cell is a monosporic pseudophialide (1, 4), and generates an asexual spore on it. A drastic transformation of the apparatus accompanied with aging (Ogawa et al. 2001) remarkably individualizes the genus.

Because zygosporoes of this genus have never been found, their natures are unknown (6, 7).

#### 4.4 DISCUSSION

The asexual reproductive morphology of the Kickxellales has been considered as 'undoubtedly complex' (Young 1999) with showing 'notable diversity' (Benjamin 1966). However, Benjamin (1966) stressed its uniformity within the order based on the morphological and cytological evidence. The present results partly agreed with his opinion. That is, the members of the same group show remarkable similarities each other, while the three groups are quite distinct from each other.

To the *Coemansia* group, three types of sporocladia are accommodated. The unity among the members of the group implies that they have derived from a common ancestral type. The deposition of *Spirodactylon* in this group is supported by molecular analysis in O'Donnell et al. (1998), although they accepted the result with surprise. As a synapomorphy of the *Coemansia* group, O'Donnell et al. (1998) suggested the production of wet spores (except in *Spirodactylon*) on pseudophialides. However, this would be homoplasy since it is also found in the *Ramicandelaber* group.

As putative synapomorphies of the *Spiromyces* group, O'Donnell et al. (1998) mentioned the production of ovoid to ellipsoidal asexual spores and a vesiculate terminal cell of sporocladia with pedicels. However, since *Mycoemilia* (tentative name) lacks the terminal cell and pedicels, the latter should be deleted from the synapomorphies. The production of conidia would be adopted as a synapomorphy of

the group.

Differing from the preceding typifications (Benjamin 1966, Young 1999, Ogawa et al. 2001), the present grouping is founded on the total morphology. In this grouping, the characteristics of sexual reproduction were introduced for the first time, although the grouping was mainly based on the asexual reproductive morphology as in the precedents.

In the Zygomycota, the sexual reproductive morphology is expected to improve the traditional classification systems based on the asexual reproductive morphology because of its evolutionary conservativeness than that of the asexual reproductive morphology (Benjamin 1979). Therefore, the present approach would be effective in constructing a more natural classification system of the Kickxellales than the current system based only on the asexual morphology. In this context, the fact that the zygospore of the *Spiromyces* group rather resembles that of the Dimargaritales than that of other Kickxellales demands further contemplation of the phyletic position of the group. Finding zygospores in the genera of which zygospores have not been known yet, especially *Myconymphaea*, *Linderina*, and *Ramicandelaber* would amend the taxonomic system.