

## CHAPTER 3

### Re-evaluation of Optical Microscopic Morphological Characteristics

#### 3.1 INTRODUCTION

The current classification system of the Kickxellales at the generic level is principally based on asexual reproductive morphology (Young 1999). Among the characteristics utilized in the system, the shape and the composition of sporocladia, the arrangement of sporocladia along the sporangiophore/sporophore, and the shapes of sporangiospores are particularly emphasized.

The system would be largely consistent, but some characteristics employed appear to be unsuitable. A typical example is the orientation of pseudopialides of *Martensella* and *Coemansia*; this feature is emphasized as the single criterion for the division of these genera, but Jackson & Dearden (1948) strongly doubted its sufficiency for dividing them. And furthermore, through the present investigation, some morphological features that had never been known in the Kickxellales were introduced by adding several new taxa to the order: for examples, lateral branches and hyphal connections of *Ramicandelaber brevisporus* (tentative name). Thus, in this chapter, morphological characteristics are re-evaluated/evaluated to reconstruct the classification system of the order.

The current taxonomic system of the Kickxellales was constructed without regarding the other kickxellids. However, taking the remarkable similarities in morphology and the close affinities within the kickxellids into consideration, the comparisons of the morphology of the Kickxellales with the possibly homologous structures of other kickxellids would be helpful for the improvement of the system. Thus, each characteristic of the order was evaluated in comparing with that of other kickxellids (Dimargaritales and Harpellales).

#### 3.2 MATERIALS AND METHODS

For the detection of the morphological features of the Kickxellales, the following procedure was employed. Most kickxellalean cultures used for the detection were isolated in this study from nature, and the cultures of species that could not be isolated were obtained from the Centraalbureau voor Schimmelcultures (CBS). All cultures were incubated on 1/2 ME-YE agar, and their features were observed using an optical microscope. Features of the species of which living cultures were not obtainable were referred to the descriptions by other investigators. Morphological terms applied are indicated in Fig. 2-1.

Comparative data of the Dimargaritales and the Harpellales were mainly based on the descriptions of Benjamin (1959, 1961, 1965, 1966), Benny (2001), and Lichtwardt (1986).

### 3.3 RESULTS AND DISCUSSION

#### 3.3.1 Asexual reproductive structures

Asexual reproductive morphology is the main basis of the current taxonomic system of the Kickxellales (Young 1999). In the typical process of the sporogenesis of the order, an asexual spore is borne in a sporangiole formed on a pseudophialide developed from a sporocladium (Figs 1-2, 2-1). Moss & Young (1978) named the total asexual morphology 'coemansoid pattern', and stated that sporangiole, pseudophialide, labyrinthiform organelle, and sporocladium-pseudophialide complex of the Kickxellales were homologues of trichospore, collar region, appendage(s), and generative cell(s) of the Harpellales, respectively.

The asexual reproductive morphology of the Harpellales fundamentally resembles that of the Kickxellales. The major differences between them are that the Harpellales has vegetative mycelia that directly turn into generative cells except in a genus and each of the cell bears a unisporous sporangiole named 'trichospore'.

The close affinities of the asexual apparatus between the Kickxellales and the Dimargaritales were asserted based on morphological and karyological evidence (Benjamin 1966), though they were denied by Moss & Young (1978). The major differences between these orders are that the latter produces two-spored sporangiola on sporogeneous cells called sporiferous branchlets, and lacks pseudophialides (Benjamin 1979) (Fig. 1-2).

#### (1) Coiling of sporangiophores/sporophores

Coiling nature of the fertile region of sporangiophore/sporophore is known in 4 species of 3 genera, *Coemansia nantahalensis*, *Coemansia spiralis*, *Spirodactylon aureum* R. K. Benj. 1959, and *Spiromyces minutus*. Except in the monotypic genus *Spirodactylon*, both coiled and uncoiled species are found in a genus. For example, in the *Spiromyces*, *S. minutus* forms coiled, and *S. spiralis* Benny et R. K. Benj. 1998 forms straight sporophores (Benjamin 1963, Benny & Benjamin in O' Donnell et al. 1998). The coiling nature would be less important at least for the generic level taxonomy of the Kickxellales, although Zycha et

al. (1969) employed the nature as a diagnosis of genera in their key.

As the function of the coils, Young (1999) has given a striking idea that the coiled phores spring to disperse spores effectively when animal dispersers get a slight touch at the phores. However, no observations that support this idea have been made.

In the Dimargaritales and the Harpellales, such coiled sporangiophores have never been observed except for partly coiled fertile branches of *Dispira*. In short, up to the present, the nature is restricted in the Kickxellales.

## (2) Distribution patterns of sporocladia

In the present taxonomic system, the distribution pattern of sporocladia along the sporangiophore/sporophore is utilized for distinguishing the species of *Coemansia* (Linder 1943). However, the pattern remarkably changes depending on culture conditions such as incubation temperature or media utilized. For instance, *Coemansia aciculifera* bore sporocladia quite sparsely when cultured on nutrient-rich media at relatively high temperature (approximately 25–30 °C).

In the Harpellales, the entire thallus (Harpellaceae) or a part of thallus (Legeriomycetaceae) converts to generative cells (Lichtwardt 1986). Regarding the former family, the distributing pattern of sporocladia is negligible. While on the latter, sporulation experiments using axenic cultures of *Smittium* spp. revealed that the rate of sporulation was variable depending on the composition of media and temperature employed for cultivation of them (El-Buni & Lichtwardt 1976).

In the Dimargaritales, sporiferous branchlets are always produced in mass on the end of fertile branches of sporangiophores.

Because of its instability, the distribution pattern of sporocladia should be treated as a supplementary characteristic in the species-level taxonomy of the Kickxellales.

## (3) Whether pseudophialides or sporocladia are sporogeneous

In most kickxellalean genera, pseudophialides are sporogeneous (Figs 1–2, 2–1), whereas in *Spiromyces* and *Mycoemilia* (tentative name), sporocladia themselves are sporogeneous and bear spores directly or on pedicels without mediated by pseudophialides (see Fig. 2–10–A).

*Spiromyces* has sicyoid sporogeneous cells with or without a septum. Benjamin (1963, 1966) studied the nuclear cycle in the cell and concluded that

the cycle was homologous with that in a sporocladial cell of other genera. Based on the observations, Benjamin (1963) and Benny & Benjamin (in O'Donnell et al. 1998) regarded it as a globose sporocladium with a globose stalk, or a sicyoid sporocladium without septation. *Mycoemilia* (tentative name) produces similar lageniform sporogeneous cells with long necks. The sporogeneous cell is functionally equivalent to both the sporocladium and pseudophialides. Therefore, the application of the term 'sporocladium' to the cell appears to be inappropriate. Coining a term for the cell would be rather better than using the term 'sporocladium' for it, although I also used the term in this article.

In the Harpellales, the cells that correspond with pseudophialides of the Kickxellales are found in *Pteromaktron* Whisler 1963 and called as subsidiary cells. This genus produces sporangiospores on the cells that grow laterally from thalli (Lichtwardt 1973; Fig. 1-2). In other genera, thalli turn into generative cells that bear sporangiospores on necks (collars) of the cells (for example, *Smittium* Poisson 1936), on collar regions (*Genistellospora* Lichtwardt 1972), or directly on the cells (*Harpella* Léger et Duboscq 1929) (Moss & Lichtwardt 1976; Fig. 1-2). Moss & Young (1978) considered that the collar and the collar region were degenerated from the subsidiary cell. In their idea, the evolutionary trend in the Harpellales went toward the loss of the subsidiary cell.

In the Dimargaritales, the asexual spores are formed in pairs in sporangiola generated from the cells called sporiferous branchlets (Fig. 1-2). With respect to the affinity to the Kickxellales, Benjamin (1966) considered that the lower spore of the Dimargaritales corresponded to the pseudophialide of the Kickxellales. However, this appears to be a superficial resemblance, since a TEM study has shown that the septa between the two spores of the Dimargaritales are pseudosepta that lack the specific features of the kickxellids' septa (Jeffries & Young 1984).

Varieties of sporogeneous cells also subsist in the Harpellales, and those would occur independently in the Kickxellales and the Harpellales (Moss & Lichtwardt 1976). Moss & Lichtwardt (1976) utilized the variety for the grouping of the harpellalean genera with the attachment pattern of zygosporophores to zygosporangia [see (17) of this section]. In parallel, this feature would be suitable for dividing the kickxellalean genera into groups since it directly involves the asexual reproduction and should be fundamental.

#### (4) Whether sporocladia are septate or aseptate

In the current classification system, whether sporocladia are septate or

aseptate (= whether sporocladia are polycellular or unicellular) has been recognized as one of the characteristics to distinguish genera except for *Coemansia*, *Dipsacomycetes*, *Martensiomycetes*, *Martensella*, and *Spirodaactylon* that produce sporocladia indistinguishable from each other (Benjamin 1966). These five genera and *Kickxella* generate septate sporocladia, whilst *Linderina*, *Mycoemilia* (tentative name), *Myconymphaea*, *Ramicandelaber*, and *Spiromyces* produce aseptate sporocladia. The septate sporocladia become polycellular by septation.

The nature of septate or aseptate is quite stable, though the number of cells composing a sporocladium may be changeable depending on culture conditions. In cases of *Coemansia* species, the polycellular sporocladia degenerated and reduced the number of their component cells when the fungi was incubated on exceedingly nutrient-rich culture media such as Potato Dextrose Agar medium, although the sporocladia never reduced themselves to unicellular.

Concerning other kickxellids, the Harpellales always produces septate generative cells (= sporocladia), and the Dimargaritales lacks sporocladia. Although the sporiferous branchlets of the Dimargaritales are composed of several cells, they appear to fundamentally differ from the septate sporocladia of the Kickxellales because the branchlets become polycellular by budding.

Since the feature appears to be a fundamental one when comparisons are made among the kickxellids, it would be apt for the definition of genera and the division of the groups within the Kickxellales.

(5) Whether a sporocladial cell generates a single or plural pseudophialides

Whether a sporocladial cell generates a single or plural pseudophialides is a characteristic newly focused on in the present study. The single or plural nature is fairly stable in every genus. Sporocladial cells bear plural pseudophialides in most genera, and bear a single pseudophialide in *Ramicandelaber*, while *Spiromyces* and *Mycoemilia* (tentative name) lack pseudophialides.

In many harpellalean genera, sporogeneous cells generate a collar or a collar region, and in the *Pteromaktron*, thalli produce a subsidiary cell (Fig. 1-2). In the Dimargaritales, neither the pseudophialide nor the cell corresponding to it subsists as discussed above (Fig. 1-2).

Since the feature directly involves reproduction and appears to be a fundamental one in comparison with the homologous feature of other kickxellids, it would be adequate to divide the kickxellalean genera into groups.

#### (6) Orientation of pseudophialides

Pseudophialides descend from the underside of sporocladia in many genera including *Coemansia*, while these ascend from the upside of sporocladia in *Kickxella* and *Martensella*. Such a difference in orientation is the single reason for dividing *Coemansia* and *Martensella*. However, the orientation is probably less significant as a taxonomical characteristic, as discussed by Jackson & Dearden (1948). In their idea, *Martensella* raises its pseudophialides in order to cancel out the upside-down conditions since its host corticioid (*Gloiothela* Bres. 1920, Russulales Kreisel ex Kirk et al. 2001, Basidiomycota) is resupinate and adheres to the underside of branches of trees (Jackson & Dearden 1948). Up to the present, however, this topic remains unsolved because the type specimen of the *Martensella* has been lost, and the species has not been rediscovered after the original description (Benjamin 1959).

In the Harpellales, both the subsidiary cell and the collar of sporocladia bear the spores laterally or apically, although inside the host's gut, such direction may not play a positive role in spore dispersal.

The Dimargaritales is disregarded on this issue since all members of the order lack pseudophialides.

Solitary employment of this feature appears to be unsatisfactory as the definition of the genera of the Kickxellales, and it would be appropriate to apply the feature for species-level taxonomy.

#### (7) Whether sporogeneous cells are monosporic or polysporic

Whether the sporogeneous cell is monosporic or polysporic is stable in each genus. Most genera including *Ramicandelaber* produce a single spore from a sporogeneous cell (pseudophialide), whereas *Spiromyces* and *Mycoemilia* (tentative name) form plural spores directly on a sporogeneous cell (sporocladium).

The Harpellales bears one spore on the sporogeneous cell (generative cell or more rarely pseudophialide), whereas the Dimargaritales bears plural spores on the cell (sporiferous branchlet).

This nature may correlate the presence/absence of pseudophialides, because pseudophialides and the corresponding cells of the Harpellales are always monosporic, while the sporocladia that lack pseudophialides are certainly polysporic. As well as whether pseudophialides or sporocladia are sporogeneous, this feature would be appropriate for determining the groups of kickxellalean

genera since the feature appears to be elemental in the asexual reproduction of the Kickxellales.

**(8) Whether asexual spores are sporangiospores or conidia**

In almost all species, the asexual spores are sporangiospores that are enveloped with sporangiola (Young 1968, 1970, 1971, 1973a, 1973b, 1974, 1985, 1990), whilst in *S. minutus*, and perhaps also in *Mycoemilia* (tentative name), the spores are conidia that lack the sporangiola (Young 1968). In *Ramicandelaber*, whether spores are enveloped in sporangiola is uncertain.

In the Harpellales and the Dimargaritales, the spores have been confirmed to be sporangiospores. That is, the conidial nature of the *Spiromyces minutus* is unique among the kickxellids. The difference of this nature appears to be taxonomically important, although Young (1968) considered the conidial nature as a secondary-derived characteristic from sporangiospores. Thus, the utilization of this feature for the grouping of the genera appears to be advocated.

**(9) Wet or dry nature of spores**

Most kickxellaleans produce wet spores enveloped in fluid that form spore droplets at maturity, whilst *Spiromyces* and *Spirodactylon* species produce dry spores. Young (1999) suspected that the slime nature of wet spores plays a role to stick to small animals so that the spores will be dispersed by them.

The wet nature is due to the mucilage secreted from the labyrinthiform organelle (Benny & Aldrich 1975) that would structurally correspond to the appendage of the Harpellales (Moss & Young 1978). The labyrinthiform organelle has been found in four wet-spored species, *Dipsacomycetes acuminosporus* R. K. Benj. 1961 (Benny & Aldrich 1975), *Kickxella alabastrina* (Young 1974), *Linderina pennispora* Raper & Fennell 1952 (Young 1968, Benny & Aldrich 1975), and *Martensiomycetes pterosporus* Meyer 1957 (Benny & Aldrich 1975).

The spores of the Harpellales are aquatic and neither wet nor dry, probably because the aquatic conditions allow them to be free from the nature. The spores have appendages that prevent the spores flow out and away from the host's gut, and the appendages are kept in appendage sacs in sporogeneous cell before the liberation of spores (Lichtwardt 1986). The appendage sac is considered to be equivalent to the labyrinthiform organelle of the Kickxellales (Moss & Young 1978), although it never secretes mucilage.

In the Dimargaritales, *Dispira* and *Tieghemiomycetes* and two species of

*Dimargaris* generate dry spores, and the remaining species of *Dimargaris* bear wet ones (Benjamin 1959, 1965). This means that the wet or dry nature is insufficient as a character to divide the genera of the order. The organelle that corresponds to the labyrinthiform organelle of the Kickxellales or the appendage sac of the Harpellales has not been found both in sporiferous branchlets and in sporangia of the Dimargaritales (Moss & Young 1978).

Regarding these facts, the wet or dry nature of spores appears to be changeable in relation to the manner of spore dispersal and the habitat of species, rather than representing the phylogeny of the species. Thus, it would be less important for the higher-level classification, and usage of this characteristic should be confined to the classification at the species level.

### 3.3.2 Non-reproductive structures

Usage of non-reproductive morphologies such as vegetative hyphae for the classification of fungi is rare owing to the high commonality among fungi resulted from the convergence for efficient absorption of nutrients from the surface of hyphae (Alexopoulos et al. 1996). This is generally concordant in the kickxellids; however, the Kickxellales has several distinctive characters worth contemplating. These are rhizoids, lateral branches, and connections between aerial hyphae. Although the former two belong to sporophore, they are discussed in this part since they do not directly participate in reproduction.

#### (10) Rhizoids of sporangiophores/sporophores

The presence of rhizoid of sporangiophore/sporophore has been used for one of the diagnoses of *C. nantahalensis* and the *Ramicandelaber* species. In these taxa, however, the production of the rhizoids is variable depending on culture conditions. In the case of *R. brevisporus* (tentative name), it drastically changed depending on the media employed; rhizoids developed vigorously on 1/2 ME-YE agar but showed limit extension on 0.1%CMA.

In the Dimargaritales, the rhizoid has been known in two species of *Dimargaris* (*D. cristalligena* Tiegh. 1875 and *D. bacillispora* R. K. Benj. 1959) and a species of *Tieghemiomyces* (*T. californicus* R. K. Benj. 1959), but it has never been known in the *Dispira* species (Benjamin 1959). In this order, the variability in its production has not been reported.

Rhizoids have not been found in the Harpellales. The thallus of the Harpellales prepares a noncellular structure called holdfast to anchor itself to

the inner surface of the guts of their hosts (Lichtwardt 1986). It is not probable that the noncellular holdfast is a homologue of the cellular rhizoid of the Kickxellales and the Dimargaritales, although their functions are similar to each other. Evolutionary, development of rhizoids should result from an adaptation to terrestrial life.

Regarding its variability, the presence/absence of rhizoids appears to play a minor role at the species level taxonomy of *Ramicandelaber*. This treatment is in contrast in the Mucorales where some of the genera distinguished by this feature (Benny et al. 2001).

#### (11) Lateral branches

The presence of lateral branches is a characteristic newly focused on in the present study. Lateral branches originate from sporangiophores/sporophores and support them. The production is observed in *R. brevisporus* (tentative name), and it is highly variable depending on culture conditions. For example, in *R. brevisporus* (tentative name), the frequency of production varied with the media employed; branches are formed on 1/2 ME-YE agar, but not on 0.1% CMA (Figs 2-12-A, B).

Lateral branches have never been known in the Harpellales and the Dimargaritales. Regarding its endoparasitic habitat, the former would be released from the selective forces to bear the lateral branches to physically support its thalli.

On account of its variability, the presence/absence of lateral branches should be treated as an additional characteristic.

#### (12) Connections between aerial hyphae

Connections between aerial hyphae are produced in *R. brevisporus* (tentative name), and no counterpart has been known in other kickxellids yet. In *R. brevisporus* (tentative name), the hook-like connection is regularly produced between two aerial hyphae where they are crossing (Fig. 2-13-G).

Although the hyphal connection is workable as a characteristic at least species level identification, I have no confident idea whether the nature is pertinent to the higher-level classification. Examination of the hypha of unknown lineage of this fungus is needed for the accurate evaluation of this feature.

### 3.3.3 Sexual reproductive structures (zygospores)

The production of zygospores is the primary feature for defining the phylum Zygomycota. Within the Zygomycota, zygospores are known in all orders except for the Glomales and the Geosiphonales (Zygomycetes) (Very recently, these two orders were removed from the Zygomycota; Schüßler et al. 2001), and the Asellariales and the Eccrinales (Trichomycetes) (Benny 2001, Benny et al. 2001). Since zygospores were not found in all the taxa of the kickxellids, in the subsequent discussion, the feature of each order will be generalized from the knowledge on the species of which zygospores have already been found.

In the Kickxellales, sexual reproduction is only known in 5 genera, whereas in the Dimargaritales, they are known in all the 3 commonly accepted genera. In these two orders, the developmental process and the morphology of zygospores quite resemble each other (Benjamin 1966). Both of the orders typically produce colorless and globose zygospores in zygosporangia by the conjugation of two undifferentiated sexual hyphae (gametangia), and they are homothallic as far as known (Benjamin 1958, 1959). The general resemblance in the sexual reproduction is regarded as an evidence of the close relationships between these two orders, although a few differences in it are the basis of the division of the two orders.

In the Harpellales, zygospores have been found in 9 genera (Benny 2001). Harpellalean zygospores are quite distinct from those of other Zygomycota including the Kickxellales and the Dimargaritales both in the developmental process and in shape (Moss & Young 1978, Lichtwardt 1986). Ultrastructural and karyological studies of the zygospores of the Harpellales have not provided positive evidence for the close affinity of the Harpellales and other kickxellids (Moss & Lichtwardt 1977). Such specificity of the order may result from the adaptation of the Harpellales for dispersal and germination in the hosts' gut and aquatic environment (Moss & Young 1978, Benjamin 1979, Lichtwardt 1986). In this order, zygospores are released to the outside of the gut, and then germinate in the gut of the new host after ingestion by the host (Lichtwardt 1986).

#### (13) The process of the formation of zygospores (zygosporogenesis)

The process of the zygospore formation (zygosporogenesis) of the Kickxellales is indistinguishable from that of the Dimargaritales with some exceptions (Benjamin 1979). In the typical manner of these orders, at the beginning, two undifferentiated hyphae (gametangia) conjugate and form a conjunct cell, and then the cell turns into a zygosporangium and produces a zygospore inside

(Benjamin 1958, 1959, 1961, 1966, 1979). As the zygospore develops, the wall of zygospore thickens, and many globules are made by the successive condensation of the protoplasm. In the Kickxellales, zygosporangia are exceptionally derived from intercalary cells formed near the point of the conjugation in *Coemansia braziliensis* Thaxter ex Linder 1943 and *Coemansia mojavensis* (Benjamin 1958). In the Dimargaritales, an exception is seen in *Dispira* spp. (Benjamin 1959, 1963) and *Dimargaris oblongispora* B. S. Mehrotra & Baijel 1963 (Mehrotra & Baijel 1963); in these species, zygospores are formed at the apex of a stalk jutting on the point of the conjugation. Such appearance reminds us of some harpellalean zygospores that will be described below, although the homology between them has not been examined. Both in the Kickxellales and the Dimargaritales, nuclei in the zygospore have not been observed yet.

Zygosporogenesis of the Harpellales is distinct from other kickxellids. In the Harpellales, a short branchlet (zygosporophore) develops from the conjunct tube between two sexual hyphae (gametangia) or from one of the hyphae after the conjugation, and the zygosporophore bears a zygosporangium on its terminal that produces a zygospore internally (Sato 2001). The zygospore is spherical at first as in other kickxellids; however, it develops to be biconical as it matures (Moss & Young 1978, Lichtwardt 1986). Lichtwardt (1986) considered that the biconical shape of zygospores was the result of adaptation to the method of dispersal, and the shape allowed the zygospores to germinate immediately before their passing through and away from the gut after ingested by new hosts. Zygospores would be better to be biconical than spherical for making transit through the digestive tract of the host after the ingestion (Moss & Young 1978).

Zygospores of the Harpellales contain a nucleus, and zygosporophores and the conjugated hyphae contain three nuclei in total (Moss & Lichtwardt 1977). Based on these observations, Moss & Lichtwardt (1977) considered that the nucleus of zygospores was haploid and it had migrated through the subtending zygosporophore after the meiotic division of a diploid nucleus. The time of meioses of the Harpellales is contrastive to that of the Mucorales. In the latter, meioses are carried out within zygospores during germination (Alexopoulos et al. 1996).

The developmental process would be inadequate as a characteristic for infra-ordinal classification of the Kickxellales since it is common in the Dimargaritales, and may support the close affinity between these two orders.

#### (14) Surface ornamentation of zygospores

Most kickxellaleans produce smooth zygospores without surface ornamentation (Benjamin 1958, 1959, 1961), whilst the species of *Spiromyces* and *Mycoemilia* (tentative name) produce punctulate zygospores (Benjamin 1963, Benny & Benjamin in O'Donnell et al. 1998) that remind us of those of Dimargaritales rather than those of the Kickxellales.

In the Dimargaritales, zygospores are punctulate in most species, although smooth in some species (Benjamin 1959, 1961, 1965). Benjamin (1963) was the first to state the resemblance in the surface ornamentation of *S. minutus* and some dimargaritalean species.

In the Harpellales, no such ornamentation has been found either on zygospores, zygosporangia, or the conjunct cells (Lichtwardt 1986). Perhaps the absence of surface ornamentation assists smooth passage of zygospores through the digestive tract when ingested by hosts.

The presence/absence of ornamentation is taken up for distinguishing the genera of the Mucorales (Benny et al. 2001). Compared to other kickxellids, in the Kickxellales, this feature appears to be of taxonomic value as in the Mucorales, and thus it is invested with a diagnosis for classifying the genera into groups.

#### (15) Pigmentation of zygospore walls

Whether zygospore wall is pigmented or not is fundamentally constant in each order. In most Kickxellales, zygospores are colorless or slightly yellowish (Benjamin 1958, 1959, 1961). Both in the Dimargaritales and the Harpellales, zygospores are colorless (Benjamin 1959, 1961, 1965; Lichtwardt 1986). Among the kickxellids, only *Spiromyces* and *Mycoemilia* (tentative name) produce indisputably pigmented zygospores (Benjamin 1963, Benny & Benjamin in O'Donnell et al. 1998).

The pigments that exhibit the dark color of the zygospore of *Spiromyces* and *Mycoemilia* (tentative name) have not been identified yet. In the Mucorales, melanin (Grove 1976) and sporopollenin (Hawker & Gooday 1968, Gooday 1973) have been identified as the materials that color zygosporangia darkly (Schipper et al. 1975, Kirk 1977), and a precursor of sporopollenin in young zygospores,  $\beta$ -carotene, is found to be the cause of the yellowish color of them (Gooday et al. 1973). The dark and yellowish color of the Kickxellales might be derived from these pigments as in the Mucorales.

If this prediction is true, the pigmentation of the kickxellalean zygospores may not be mere coloring but reflects some function and extensive taxonomic value.

because in the Mucorales,  $\beta$ -carotene is shown as the precursor of the sexual hormone (trisporic acid) (Werkman & van den Ende 1973). Indeed, this feature is utilized for the discrimination between genera of the Mucorales (Benny et al. 2001). For the classification of the Kickxellales, I propose adopting this feature for the grouping of the genera since the dark pigmentation in *Spiromyces* and *Mycoemilia* (tentative name) is exclusive among the kickxellids.

#### (16) Contents of zygospores

Zygospores of the Kickxellales and the Dimargaritales contain globules derived from the protoplasm (Benjamin 1958, 1959) and these contents would act as reserve structures. In almost all Kickxellales, mature zygospores contain many globules since the aggregation of globules stops before the entire accomplishment, whereas in *Spiromyces* and *Mycoemilia* (tentative name) and in the Dimargaritales, all the globules subsequently aggregate during the zygosporogenesis, and then a single (or sometimes several) large globule(s) locates eccentrically in mature zygospores.

In the Harpellales, zygospores contain highly electron-opaque cytoplasm owing to the existence of glycogen granules (Moss & Lichtwardt 1977), but the globose contents found in the Kickxellales and the Dimargaritales have never been observed.

Adoption of the configuration of contents within sexual spores is found in infra-general classification of water molds, *Saprolegnia* Nees 1823 (Saprolegniales, Oomycota, Stramenopila), although now they are not recognized as the members of the Fungi.

#### (17) The attachment point of suspensors to zygospores

In the Kickxellales, both opposed and apposed dispositions of the suspensors to zygospores are found in a genus. Taking the *Coemansia* for example, *C. aciculifera* has apposite suspensors, while suspensors of *C. mojabensis* are opposite (Benjamin 1958). This nature would depend on the origin of zygosporangia.

In the Dimargaritales, this nature is almost identical with that in the Kickxellales except in *Dispira* spp. and *D. oblongispora* (Benjamin 1959, 1963; Mehrotra & Baijel 1963) of which zygospores have a single stalk. It has not been concluded whether the stalk is the outgrowth from the conjunct cell as found in the Harpellales (Moss & Young 1978, Lichtwardt 1986, Sato 2001) or the upper part

of one of the two fertile hyphae on which the tip of another hypha conjugates as found in *C. braziliensis* and *C. mojavensis* (Benjamin 1958).

In the Harpellales, the attachment position of the zygosporophore is used for recognizing four types of zygosporangium (Moss et al. 1975). This typification has been discussed phylogenetically in correlation of the presence of pseudophialide or its corresponding structure (Moss & Lichtwardt 1976).

For the taxonomy of the Kickxellales, this characteristic does not appear to be important for the generic level taxonomy, and should be treated in species-level taxonomy. This treatment is in contrast in the Mucorales: in the order, whether the suspensors of zygosporangium are opposed or apposed is one of the characteristics for dividing families (Benny et al. 2001).

### 3.4 CONCLUSIONS

The comparisons of the morphological features among the kickxellids were great help for the assessment of the features of the Kickxellales, and would improve the current taxonomic system of the order. The comparisons were somewhat difficult, because each order, especially the Harpellales, has adapted its morphology to each peculiar habitat (Moss & Young 1978, Benjamin 1979, Lichtwardt 1986). The morphological features of the Kickxellales are categorized as the discriminative characteristics of each taxonomic level (Table 3-1).

Among the characteristics discussed, the following would be relevant to the definition of genera and the division of the groups within the Kickxellales: (3) whether pseudophialides or sporocladia are sporogeneous, (4) whether sporocladia are septate or aseptate (= whether polycellular or unicellular), (5) whether a sporocladial cell generates a single or plural pseudophialides, (7) whether the sporogeneous cells are monosporic or polysporic, (8) whether asexual spores are sporangiospores or conidia, (14) surface ornamentation of zygosporangia, (15) pigmentation of zygosporangium walls, and (16) contents of zygosporangia (Table 3-1, criterion A). The numbers in parentheses indicate sections of results and discussion of this chapter.

To the classification of species, the following characteristics would be applicable: (1) coiling of sporangiophores/sporophores, (4) the number of cells that composing a sporocladium when the sporocladium is polycellular, (6) orientation of pseudophialides, (9) wet or dry nature of spores, and (10) rhizoids of sporangiophores/sporophores (Table 3-1, criterion C).

The entire process of the zygosporangium formation (13) appears to be inapt for

dividing taxa within the Kickxellales since it is common to the Dimargaritales. The following two features, (2) distribution patterns of sporocladia and (17) the attachment point of suspensors to zygosporangia are not employed for the definition of the taxa due to their unsteadiness.