

CHAPTER 8

General Discussions

The present investigation is a comprehensive taxonomic study of the Kickxellales *sensu lato* (s. l.) after the interval of 43 years. Through this study, 2 undescribed genera, 4 undescribed species, 2 species that have not been rediscovered after the original descriptions, and several rarely encountered species and many isolates of comparatively common species were discovered. These findings made possible to construct a more reliable taxonomic scheme of the Kickxellales s. l. As a result, the order Kickxellales s. l. was circumscribed and two new orders were proposed.

The Kickxellales s. l. has been considered as "consists of a well-defined and seemingly very natural group of fungi" (Benjamin 1979). Although Young (1968) first noted the specificity of *Spiromyces*, he considered it as a derived character of the genus. Later, O'Donnell et al. (1998) found the heterogeneity of *Spiromyces* by 18S rDNA analyses. But they gave any taxonomic treatments to the order based on the results. The present work based on the optical microscopic morphology, septal ultrastructure, and rRNA genes sequence analyses confirmed and revealed the polyphyly of the Kickxellales s. l., and reached the revision of the current classification system.

The present study also contributed as a floristic survey of the species of the Kickxellales s. l. of Japan since it added 10 species of 5 genera to the floristic list that had included only 5 species of 4 genera (Indoh 1962, Matsushima 1975, Mikawa 1975, Miura 1978, Saikawa 1989, Ogawa et al. 2001). However, even this revised list would be far from the accomplishment, because numerous kinds of potential sources of kickxellalean fungi s. l. remain uninvestigated. Probably much more taxa will be added to the list after detection of these sources. The fact that *Myconymphaea yatsukahoi* was found from an arthropod cadaver, a kind of sources that has not been fully surveyed even in the present study, supports this view.

While the present study solved several taxonomic problems of the Kickxellales s. l., it newly cast light on several issues concerned with nutrition of the members of the order: these are on real microhabitats, nutritional modes, and culture conditions in laboratory.

Up to now, soil and dung of mammals have been the main sources of almost

all isolates of the Kickxellales s. l. However, because soil and dung are mixtures of minute particles, the real microhabitats of the Kickxellales s. l. are hidden in them. Among many substances included in soil, only the root of plants is proved to be the microhabitat of these fungi; several *Coemansia* species occurred on roots of wheat (*Triticum aestivum*, Kwaśna et al. 1999a) and spruce (*Abies alba*, http://www.cbs.knaw.nl/search_fsp.html).

Dead bodies and feces of arthropods are substrata newly focused on through this study. For instance, the cadaver of a dipluran and feces of unknown arthropods provided an undescribed species, *M. yatsukahoi*. Moreover, the species rarely occurred on the roots (Kwaśna et al. 1999a) frequently appeared from soil by the baiting method using dead bodies of arthropods as baits. Particularly, *Coemansia* and *Ramicandelaber* species appeared vigorously in response to shrimp and mealworm baits, respectively. These facts suggest that the cadavers and feces of arthropods are common substrata of kickxellaleans s. l. in nature. During this study, I observed small fragments of these materials included in soil and feces, especially within feces of entomophagous frogs and bats that are known as sources of some kickxellaleans *sensu stricto* (s. str.) (Chien 1994, personal communications from Drs Degawa and Doi). It is possible that these materials supply microhabitats to the kickxellaleans in these feces. Usage of arthropod-derived substances and putative animal dispersal of spores of the Kickxellales s. l. (Benjamin 1979, Young 1999) should be worth emphasizing taking into account that all the members of the Harpellales are parasites of arthropods.

Another important issue revealed in this study is on the inherence of the parasitic nature of the Kickxellales s. l. A considerable number of kickxellaleans s. l. appear to be parasitic, although only three mycoparasitic species have been known up to the present. For the duration of this study, I encountered 4 unculturable kickxellaleans s. l., and the ratio of these species to all species found in the present study (15 species) reached approximately 27 %. At least, some of them should be parasitic because they did not grow on ordinary nutritional media for saprobes. The species of the *Spiromyces* and the *Spirodactylon* that have been regarded as saprobes are suspected to have the mycoparasitic nature based on their particularly poor growth in axenic cultures (Benny et al. 2001). These facts suggest that parasitism is not a rare phenomenon in the Kickxellales s. l. than it has been thought.

In addition, within a species, both the cultivatable and unculturable

strains present. Torrey (1921) first reported this phenomenon in *Kickxella alabastrina* and postulated that obligate parasitic strains subsist in the species, as discussed by Benjamin (1958). I verified this phenomenon in *Ramicandelaber* spp. and *K. alabastrina*. These unculturable strains may be parasitic.

Discoveries and cultivation of parasitic kickxellaleans s. l. would facilitate us to resolve the phylogenetic relationships within the Kickxellales s. l. and within the kickxellids, because the missing link taxa may subsist in these parasites. This is probable since the ancestral form of the kickxellids is presumed to be parasitic (Benjamin 1966). Recently, PCR amplification techniques make it possible to analyze DNA sequences of such non-cultivable species (Mitchell et al. 1995) and determine their phylogenetic positions. However, we should keep trying to cultivate them since axenic cultures yield further and more precise studies of the species (Benny & O' Donnell 2000) that expand our knowledge and enable to amend taxonomic schemes, as demonstrated in the present study.

On the evolution within the Kickxellales s. str. and s. l., several schemes have been proposed. Linder (1943) described that *Coemansia* and *Martensella*, and *Kickxella* had respectively originated from two different species of *Syncephalis* Tiegh. & G. Le Monn. (Zoopagales, Zygomycetes). Nowadays, however, his idea is hard to be accepted because of the exclusive monophyly of the Kickxellales s. str. Later, Moss & Young (1978) considered that the order has progressed toward the reduction of the septa between sporocladial cells and then those between sporocladia and pseudophialides in its evolution. In their ideas, the ancestor of the order had produced *Coemansia*-like asexual apparatus, and then, from the ancestor, *Spiromyces* evolved through *Linderina* (Moss & Young 1978). However, O' Donnell et al. (1998) supported the opposite direction in the evolution in accordance with the 18S rDNA sequence-based trees when they suggested the exclusion of *Spiromyces* from the Kickxellales. In their proposal, the evolution of sporocladia proceeded from aseptate to septate, and the *Coemansia* type asexual apparatus is the most advanced one in the order. More recently, Gottlieb & Lichtwardt (2001) pointed out the possibility of a polytomous radiation among the kickxellids.

The rRNA gene sequence-based trees of the present study allocated aseptate sporocladia to all the three orders of the Kickxellales s. l. (Kickxellales s. str., Spiromycetales, and Ramicandelaberales), while most members of the

Kickxellales *s. str.* and the all species of the Harpellales produce septate sporocladia. Among them, the Ramicandelaberales is excluded from this context due to its phylogenetic position distant from the others. Within the group that consists of the Kickxellales *s. str.*, the Spiromycetales (tentative name), and the Harpellales, the septate sporocladia appear to be the prototype. Through the evolutionary process, the fusion of component cells of sporocladia might individually occur in the Spiromycetales, *Linderina*, and *Myconymphaea*, and as a result, they show a convergence in producing aseptate sporocladia. An increase in the number of pseudophialides that described as an evolutionary trend within the group by O' Donnell et al. (1998) appears to be limited to the Kickxellales *s. str.*

The ancestor of the kickxellids appears to be endowed with sporocladia, pseudophialides, and sporangiospores, though either the sporocladia are septate or aseptate is uncertain for the time being; and then, the ancestor may have variously differentiated in each order. The extensive base substitution of the 18S rRNA gene of the Dimargaritales (Tanabe et al. 2000) may reflect the remarkable differences in morphology of this order from that of the other kickxellids.

The evolutionary processes in the Kickxellales *s. str.*, the Kickxellales *s. l.*, and the kickxellids appear to be complicated beyond our expectations. Discoveries of the unknown relatives of the kickxellids including the missing links would fill many gaps in our knowledge that cause the ambiguity in the phyletic relationships between the kickxellids (Moss & Young 1978) and may loose these entangled processes. Probably these expectations will be realized since the majority of the extant fungal species remain undiscovered (Hawksworth 1991, 2001).