

## ABSTRACT

The order Kickxellales is a relatively small fungal group that belongs to the class Zygomycetes of the phylum Zygomycota. The members of the order are mainly saprobes and inhabit in soil or dung of herbivorous or omnivorous mammals, and only a few species are mycoparasites. Most members of the order are rarely discovered.

For a long time, the Zygomycota has been regarded as a monophyletic group in the Fungi derived from a chytrid-like ancestor that has flagellate cells in its life cycle. During the last decade, many researchers have investigated the phylogenetic relationships within the Zygomycota and between the fungal phyla by using molecular techniques. They revealed that the Zygomycota is paraphyletic and makes a cluster joined with the Chytridiomycota (van de Peer & De Wachter 1997), several clades subsist in the Zygomycota-Chytridiomycota cluster (O'Donnell et al. 2001, Tanabe et al. 2000), and the Kickxellales constructs an independent clade with the Dimargaritales of the same class and the Harpellales of the class Trichomycetes in the Zygomycota-Chytridiomycota cluster (Tanabe et al. 2000). Therefore, the Kickxellales has attracted our attention, and precise taxonomic studies of the Kickxellales are required to clarify the phylogenetic relationships between the above orders.

No comprehensive taxonomic studies of the order have been carried out since Benjamin (1959), while several new taxa were added to the order during such a long absence of the investigation (Kurihara et al. 2000, Ogawa et al. 2001). Due to the inclusion of these newly added taxa, the current classification system lacks the consistency. Therefore, in this study, I aimed to construct a more consistent taxonomic system of the Kickxellales.

For this purpose, first of all, I collected kickxellalean isolates from nature since only a few fungal materials were available at that time. As a result, 15 species of 8 genera of kickxellalean fungi were found, and the establishment of 268 cultures of 11 species of 5 genera including 2 undescribed genera and 5 undescribed species was succeeded. By using these cultures, the Kickxellales was reconsidered taxonomically. Cultures of tropical species that could not be isolated in the present study were obtained from a culture collection.

For the reconstruction of the classification system of the Kickxellales, the following strategy was employed. First, all kickxellalean genera were classified into groups based on the optical microscopic morphology. Second,

these groups were evaluated based on the septal ultrastructure and ribosomal DNA (rDNA) sequence analyses, respectively. And finally, from these results, taxonomical conclusions were drawn.

Optical microscopic morphology of the Kickxellales provides us enormous information because of its complicate structures. Actually, the morphology is the basis of the definition of all the taxa of the order. Therefore, a grouping of the genera was made based on the morphology, and as a result, *Coemansia* group, *Spiromyces* group, and *Ramicandelaber* group were recognized in the Kickxellales *sensu* Benjamin (1979).

The septal structure is regarded as an important feature when we consider the phylogenetic relationships within the Fungi since it is relatively conservative evolutionarily (Alexopoulos et al. 1996). The Kickxellales and its phylogenetically close relatives are distinctive from any other fungal groups in having an exclusive septal structure. Since the septal structures would be significant to clarify the Kickxellales, the morphological groups were evaluated based on the structures. Through the investigation, some differences in the shape of plugs were shown between the groups, although the total septal structures were fundamentally common in the Kickxellales and its related orders.

Currently, rRNA gene sequence analyses have provided us a lot of information on the fungal taxonomy independent of morphological characteristics (McLaughlin et al. 1995). Large comparative data are accessible because the sequences of the genes were revealed in many fungi including the representatives of most kickxellalean genera (O'Donnell et al. 1998). In addition, the rDNA sequences can determine the clades within the Kickxellales (O'Donnell et al. 1998, Tanabe et al. 2000). Therefore, the morphological grouping was detected based on the 18S and 28S rDNA sequence analyses. As a result, *Coemansia* clade, *Spiromyces* clade, and *Ramicandelaber* clade were recognized in the Kickxellales. The genera that consist of each clade were equal to the genera that form each morphological group.

In conclusion, three groups: *Coemansia* group, *Spiromyces* group, and *Ramicandelaber* group were recognized in the order. These groups were morphologically and phylogenetically distinct enough from each other and from any other fungal groups including the Dimargaritales and the Harpellales. Thus, I concluded that each of the three groups should be regarded as an independent order, and proposed the division of the Kickxellales *sensu* Benjamin (1979) (= Kickxellales *sensu lato*) into the Kickxellales *sensu stricto*, the Spiromycetales

(undescribed, tentative name), and the Ramicandelaberales (undescribed, tentative name).

Historically, Benjamin-(1963) and Ogawa et al. (2001) stretched out the range of the Kickxellales when they described *Spiromyces* and *Ramicandelaber*, respectively. By this treatment, the range of the Kickxellales recurs to the original one, and the opinion of O'Donnell et al. (1998) that the *Spiromyces* should be taxonomically separated from the other members of the order is realized.