CLASSIFICATION OF CORDYCEPS AND RELATED FUNGI – A REVIEW

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ABSTRACT

Cordyceps and related fungi (Hypocreales, Ascomycota) have a long history of interaction with human. This fungal group is well-known for its application in agriculture and medicine. Great interest has been given for this group, especially in their classification and systematics. In this current review, current classification system of Cordyceps fungi is presented under the view of morphology and molecular phylogenetics.

Keywords: *Cordyceps; entomopathogenic fungi classification; molecular phylogeny.*

1. Overview

Cordyceps and related fungi are a special group of fungi within Hypocreales (Ascomycota) that are parasites of insects, *Elaphomyces*, nematodes, and plants (Sung *et al.*, 2007). From morphological and molecular data, this group includes the teleomorph genera of *Cordyceps* Fr. and *Torrubiella*. More than 400 *Cordyceps* species have been described worldwide with the highest diversity in East Asia and Southeast Asia.

Cordyceps fungi are mostly regarded as bio-controls in agriculture and as precious traditional herbals in Vietnamese and Chinese traditional medicines (Gul *et al.*, 2014; Torres and White, 2009). Species within *Cordyceps* have been used widely in agriculture to suppress the activity of harmful insects at all stages including larvae, pupae and mature. The advantage of *Cordyceps* and related fungi lies within their safety for human. Unlike other fungi (e.g. *Aspergillus flavus*), poisons produced from *Cordyceps* selectively kill the hosts without any effects on human body. Popular *Cordyceps* fungi used in agriculture include *Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea*.

In medicine, *Cordyceps* and related fungi have a long history of usage in traditional medicine of Asian countries (Torres and White, 2009; Paterson, 2008; Hodge, 2003). The most well-known of the group is *Cordyceps sinensis* (Đông trùng hạ thảo in Vietnamese). *Cordyceps sinensis* contains a wide range of secondary metabolites including cordycepin, intra-cellular and extra-cellular polysaccharides, adenosine, guanosine, cordymin, etc. Extracts from *Cordyceps sinensis* have different effects including immuno-regulation, anti-tumor, anti-metastatis, anti-oxidation, hypoglycemia and chronical renal dysfunction recovery. Moreover, other *Cordyceps* fungi possess potential therapeutic abilities such as *Cordyceps militaris*, *Cordyceps pseudomilitaris*, *Cordyceps ophioglossoides*, *Cordyceps heteropoda,…*

In recent years, increasing need for *Cordyceps sinensis* in Vietnam has led to the

use of similar species as adulterants due to the similarities between these fungi. With complicated life cycles and the diverse in morphology under different environmental conditions, *Cordyceps* species are easily miss-identified. Moreover, complex classification system of fungi with both sexual (teleomorphic) and asexual (anamorphic) names for one species makes the identification process more troublesome (Sung *et al*., 2007). In this recent review, outlines on the classification of *Cordyceps* and related fungi are presented to provide an overview of the characteristics and morphology of such fungi in the view of traditional morphological-based classification and molecular phylogenetic-based classification. All species names presented in the following sections will be presented in current phylogenetic names in order to create a unified view of the *Cordyceps* group.

2. Morphological-based classification

Cordyceps genus was firstly described systematically in 1818 by Fries (Shrestha *et al.*, 2014). The word "cordyceps" is the combination of the Greek word "cordyle" (club) and the Latin word "caput" (head) which gives the overall description of the stromata of *Cordyceps* species. Based on its cyclindrical asci with thickened ascus apices and filiform ascospores that often disarticulate into part-spores, *Cordyceps* is classified into Clavicipitaceae family within two other entomopathogenic teleomorphic genera *Hypocrella* and *Torrubiella* (Kobayasi, 1941; Kobayasi, 1982; Mains, 1958). *Cordyceps* is distinguished from other genera of the Clavicipitaceae family mainly based on the formation of superficial to completely immersed perithecia on stipitate and often clavate to capitate stromata. Anamorphs of *Cordyceps* include species in more than 25 genera such as *Akanthomyces, Beauveria, Hirsutella, Hymenostilbe, Metarhizium, Nomuraea, Paecilomyes, Polycephalomyes, Isaria,…* (Figure 1)

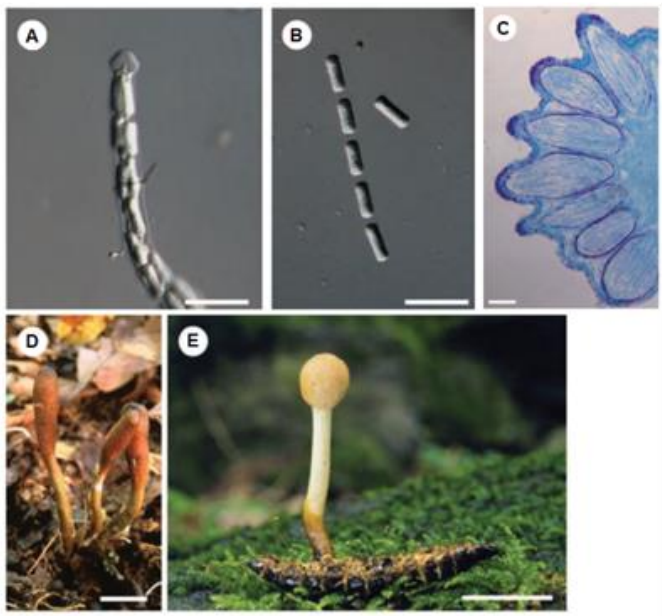

Figure 1. Microscopic and macroscopic morphology of *Cordyceps* fungi (Sung *et al*, 2007). A. An ascus containing filiform ascospores of *Ophiocordyceps variabilis*, B. Part-spores of *Elaphocordyceps subsessilis*, C. Immersed perithecia in stromata of *Cordyceps cardinalis*, D. Clavate stromata of *Elaphocordyceps ophioglossoides*, E. Capitate stromata of *Ophiocordyceps gracilis.*

Despite its long history, classification of fungi based on morphology contains several disadvantages:

Firstly, the observed characteristics are usually simple and the plasticity of *Cordyceps* complicates the identification process (Rehner, 2009).

Secondly, differences in classification often exist among taxonomists (Kobayasi, 1941; Kobayasi, 1982; Mains, 1958). Particularly, Kobayasi proposed three sub-genera for *Cordyceps* including *Cordyceps* subg. *Cordyceps*, *Cordyceps* subg. *Ophiocordyceps*, and *Cordyceps* subg. *Neocordyceps* through the distribution of perithecia and characteristics of asci, ascospores and part-spores. Mains subsequently adopted Kobayasi system with the addition of two new sub-genera (*Cordyceps* subg. *Racemella* and *Cordyceps* subg. *Cryptocordyceps*). The main difference between Kobayasi and Mains is the classification of *Cordyceps* subg. *Ophiocordyceps* and *Cordyceps* subg. *Neocordyceps*. With *Cordyceps* subg. *Ophiocordyceps*, Mains proposed only two species (*Cordyceps blattae* and *Cordyceps peltata*) basing on the lack of thickened ascus apices. Moreover, Mains replaced all species within *Cordyceps* subg. *Neocordyceps* into *Cordyceps* sect. *Cremastocarpon* subsect. *Entomogenae*. Nowadays, the classification system of Kobayasi and Mains are the most widely used for *Cordyceps* fungi.

Thirdly, the classification using hosts might be uninformative (Sung *et al.*, 2007). Host range is different for each *Cordyceps* species. Most species parasite to a single or closely related host species while some can infect a wide range of hosts. However, the host can be immature or severely damaged when the fungi are found. Therefore, using hosts as a taxonomic characteristic can be troublesome.

Fourthly, the existence of a binominal nomenclature of non-lichenized fungi complicates the identification process further (McNeil *et al.*, 2012). For example, *Cordyceps takaomontana* is the teleomophic state of the fungus *Isaria takaomontana*. However, not all teleomorph fungi have their anamophic states and vice versa. Therefore, this nomenclature system is problematic as the number of newly recognized species increases.

In order to overcome the obstacles of morphological-based identification, molecular phylogenetic-based classification is proposed as an alternative method to re-assess the current classification which is mainly dependent on morphology.

3. Phylogenetic-based classification

Early phylogenetic analyses of *Cordyceps* and related fungi within Clavicipitaceae showed contrasting results due to the limited number of taxon sampling and inadequate resolution power of DNA loci chosen for analysis (Sung *et al.*, 2007). Recent publications have shown the paraphyleticity of both *Cordyceps* and Clavicipitacean fungi. Therefore, an effort by Sung *et al.* (2007) using the largest number of samples and DNA loci proved that *Cordyceps* should be classified as a family rather than a genus:

From a 5- and 7-gene phylogenetic analysis including ribosomal, nuclear, and mitochondrial sequences, *Cordyceps* and Clavicipitaceae is reclassified into three new families (Cordycipitaceae, Ophiocordycipitaceae, and Clavicipitaceae sensu stricto) (Sung *et al.*, 2007). Clavicipitaceae s.s. family includes species with fleshy or tough, darkly or brightly coloured stromata or subiculum; superficial to completely immersed, ordinal or oblique in arrangement perithecia; cylindrical asci with thickened ascus apex; cylindrical and multiseptate ascospores which disarticulate into part-spores or non-disarticulate. Clavicipitaceae s.s. teleomorphic genera include *Aciculosporium, Atkinsonella, Balansia, Claviceps, Epichloë, Heteroepichloë, Hypocrella, Metacordyceps*gen. nov., *Myriogenospora, Neoclaviceps, Parepichloë, Regiocrella,* and *Shimizuomyces*, and anamorphic genera include *Aschersonia, Ephelis* Fr., *Metarhizium, Neotyphodium* A.E. Glenn, C.W. Bacon & Hanlin, *Nomuraea*, paecilomyces-like, *Pochonia*, *Sphacelia* Lév., and verticillium-like. *Metacordyceps* contains several species of the morphological *Cordyceps* genus and is characterized as its solitary or multiple, simple or branched

stromata, fleshy or tough, whitish, greenish yellow to greenish stipe that is cylindrical to enlarging in fertile part, cylindrical to clavate fertile part, partially or completely immersed perithecia with ordinal or oblique in an arrangement, cylindrical asci with thickened ascus apex, cylindrical, multiseptate ascospores with or without part-spores at maturity. Anamorphs of *Metacordyceps* include *Metarhizium, Nomuraea,* paecilomyces-like, *Pochonia* genera.

Ophiocordycipitaceae family is characterized by its darkly pigmented or rarely brightly colored, tough, fibrous to pliant and rarely fleshy stromata or subiculum often with aperithecial apices or lateral pads, superficial to completely immersed perithecia with ordinal or oblique arrangement, cylindrical asci with thickened ascus apex, cylindrical, multiseptate, disarticulating or non-disarticulating ascospores. Teleomorphic genera of Ophiocordycipitaceae include *Elaphocordyceps and Ophiocordyceps*, and anamorphic genera include *Haptocillium*, *Harposporium* Lohde, *Hirsutella*, *Hymenostilbe*, paecilomyces-like, *Paraisaria*, *Synglіocladium*, *Tolypocladium,* verticillium-like. *Elaphocordyceps* is identified for its singular or multiple, simple or branched stromata, fibrous to tough and rarely fleshy stipe that is dark brownish to greenish with olivaceous tint and rarely whitish, and cylindrical to enlarging in the fertile part, rhizomorph-like structures that connected the stroma with the host, clavate to capitate fertile part that is rarely undifferentiated, partially or completely immersed perithecia with ordinal arrangement, cylindrical asci with thickened ascus apex, cylindrical, multiseptate ascospores that disarticulate into part-spores upon maturation. *Tolypocladium* is the anamorph genus for *Elaphocordyceps. Ophiocordyceps* includes species with darkly pigmented or rarely brightly colored, tough, fibrous to pliant and rarely fleshy stromata or subiculum often with aperithecial apices or lateral pads, superficial to completely immersed perithecia with ordinal or oblique arrangement, hyaline and cylindrical asci with thickened ascus apex that is rarely fuisoid to ellipsoid, cylindrical, multiseptate, disarticulating or non-disarticulating ascospores. Anamorphs of *Ophiocordyceps* include *Hirsutella, Hymenostilbe, Paraisaria*, *Synglіocladium*.

Cordycipitaceae family includes species with pallid or brightly pigmented, fleshy stromata or subiculum, superficial to completely immersed perithecia which orients at right angles to the surface of the stroma, cylindrical asci with thickened ascus apex, cylindrical, multiseptate, disarticulating or non-disarticulating ascospores. Teleomorphic genera of Cordycipitaceae include *Ascopolyporus, Cordyceps, Hyperdermium, Torrubiella* and anamorphic genera include *Beauveria, Engyodontium, Isaria, Lecanicillium,* mariannaea-like, *Microhilum,* and *Simplicillium. Cordydeps* is characterized by its pallid or brightly pigmented, fleshy stromata or subiculum, superficial to completely immersed perithecia with ordinal arrangement, hyaline, cylindrical asci with thickened ascus apex, hyaline, cylindrical, multiseptate, disarticulating or non-disarticulating ascospores that rarely possesses a thread-like structure connecting the fusiform ends. Anamorphic genera of *Cordyceps* include *Beauveria, Isaria, Lecanicillium,* mariannaea-like, *Microhilum, Simplicillium.*

Following Sung *et al*. (2007), Johnson *et al.* (2009) utilized 5-gene phylogenetic analyses with ribosomal and nuclear regions to investigate the position of the genus *Torrubiella* in relationship with *Cordyceps.* The results refused the monophyly of *Torrubiella* which supported the findings of Sung *et al.* (2007) and proposed two new genera within Clavicipitaceae s.s. family (*Orbiocrella* and *Conoideocrella*). In Cordycipitaceae family, *Torrubiella* showed paraphylic in which *Torrubiella* fungi formed a monophyletic group with *Gibellula* that was separated from other *Cordyceps* fungi and another group that is

separated with the *Gibellula* and *Cordyceps* group. In Ophiocordycipitaceae family, *Torrubiella* formed a monophylic group with *Ophiocordyceps* and therefore was rearranged to this genus.

Since 2012, Article 59 of the International Code of Nomenclature for algae, fungi, and plants has been removed leading to the end of a binominal nomenclature on non-lichenized fungi requiring more phylogenetic studies to be taken to resolve the relationship of *Cordyceps* fungi (McNeil *et al.*, 2012). Subsequently, Kepler *et al.* (2013) proposed a history conserved genus of anamorphic fungi to a teleomorphic state (i.e. *Polycephalomyces*) within Ophiocordycipitaceae family. However, with low support from boostrap, the position of the genus is not stable (Kepler *et al.*, 2012). Another work of Quandt *et al.* (2014) replaced the name of the genus *Elaphocordyceps* to *Tolypocladium*. The authors of this publication also supported the position of *Polycephalomyces* in Ophiocordycipitaceae.

Figure 2 summarizes the latest classification of *Cordyceps* and related fungi in order to give an overall picture of the systematics of this fungal group within Hypocreales.

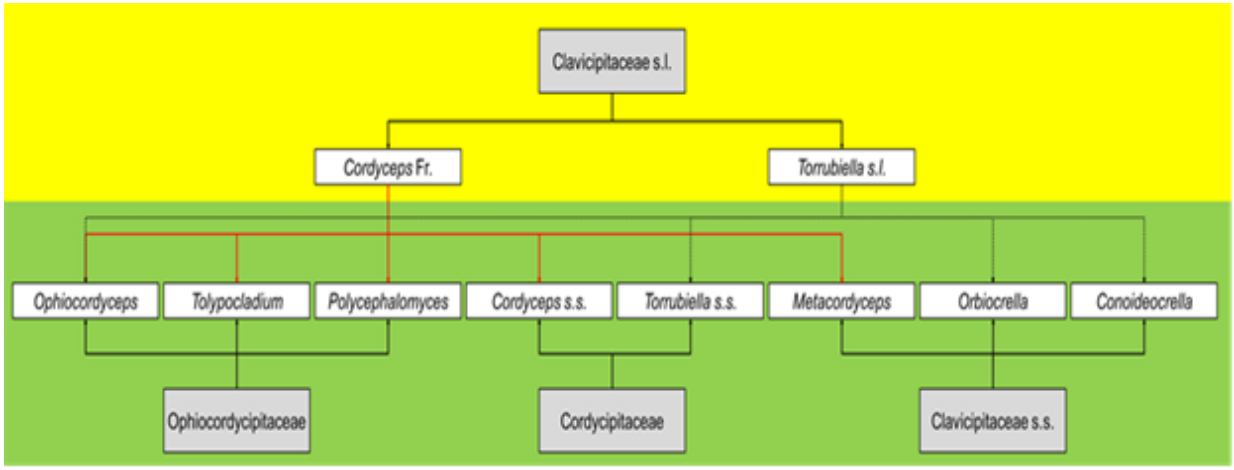

Figure 2. Current classification of *Cordyceps* and related fungi based on morphology (upper part) and molecular phylogenetics (lower part). The families are presented in gray boxes and the genera in white boxes.

4. Conclusion

Cordyceps and related fungi have undergone tremendous changes in their position of the classification system since their first description. Morphological-based classification provided the first framework for the systematics of this fungal group. However, due to subjectivity and lack of quantitative characteristics, morphology-based classification have faced with a number of difficulties.

With the support from molecular data, *Cordyceps* and related fungi are being re-classified into different families of Hypocreales, showing the complexity of this group. Currently, they are classified into three families with established morphological characters guided by phylogenetic studies. Therefore, molecular phylogenetics will be a promising support for traditional classification. However, several issues including the concordance between morphological and phylogenetic classification, the host-parasite relationship within *Cordyceps*, the need for evaluating the quality of the alignment, and the use of ambiguously aligned regions in phylogenetic analysis still need further investigation.

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