CHAPTER 2

LITERATURE REVIEW

2.1 Myxomycetes

The myxomycetes (plasmodial slime molds) are eukaryotic microorganisms that occur wherever conditions on the earth's surface permit the growth of vegetation but are especially common in forest areas. They can be found, sometimes abundantly, in most terrestrial ecosystems, associated with plants and plant debris (Martin and Alexopoulos, 1969), but some species have been reported from aquatic habitats (Shearer and Crane, 1986). Since at least the mid-17th century, myxomycetes have been known from their fruiting bodies, and their life cycle has also been studied for more than a century (Martin and Alexopoulos, 1969). Although the exact evolutionary affinities of myxomycetes are still debated, these organisms constitute a well-defined and relatively homogeneous taxonomic group (Lado, 2001).

The fruiting bodies produced by myxomycetes are usually small, with a height of no more than 2 mm (0.1–2 mm) (Lado and Pando, 1997; Stephenson, 2003). Because of their small size and the types of situations in which most species occur, myxomycetes tend to be overlooked in nature. However, careful searching of suitable substrates almost invariably reveals several fruiting bodies of myxomycetes in the various microhabitats of a particular ecosystem (Stephenson, 2003).

2.2 General Life Cycle

The life cycle of a typical myxomycetes is distinguished by two trophic stages, one consisting of uninucleate amoebae and another a multinucleate motile

plasmodium, along with a reproductive stage in which a spore-producing fruiting body (or sporocarp) is produced (Martin and Alexopoulos, 1969). The uninucleate structure may be flagellated (swarm cell) or non-flagellated (myxoamoeba) and they feed, grow and multiply by binary fission to produce large populations in the various microhabitats in which they occur. Under unfavourable conditions for growth and metabolism, myxamoebae produce resistant cysts (microcysts). Microcysts can remain viable for long periods of time and are probably very important in the continued survival of myxomycetes in some habitats. Ultimately, two compatible myxamoebae or swarm cells fuse to form a diploid zygote which feeds and undergo repeated mitotic nuclear divisions to gives rise to the multinucleate plasmodium. Plasmodia typically occur in cool, moist, shady places such as within crevices of decaying wood, beneath the partially decayed bark of logs and stumps, and in leaf litter on the forest floor. Under certain suitable conditions, the plasmodium gives rise to one or more fruiting bodies containing spores. Under adverse conditions, a plasmodium may convert into a hardened, resistant structure (sclerotium) which is capable of reforming the plasmodium when favourable conditions return. The fruiting bodies of myxomycetes are spore-bearing, and so are somewhat suggestive of those produced by higher fungi except that they are considerably smaller. The spores of myxomycetes are mostly wind-dispersed and complete the life cycle by germinating to produce one to four haploid, uninucleate amoeboflagellate cells (Martin and Alexopoulos, 1969; Stephenson and Stempen, 1994; Stephenson, 2003). Bacteria are the primary food resources for both feeding stages (myxamoeba and plasmodium); however, plasmodia are capable to feed on yeasts, cyanobacteria, fungal spores and hyphae (Howard and Currie, 1932a,b; Lazo, 1961; Madelin, 1984).
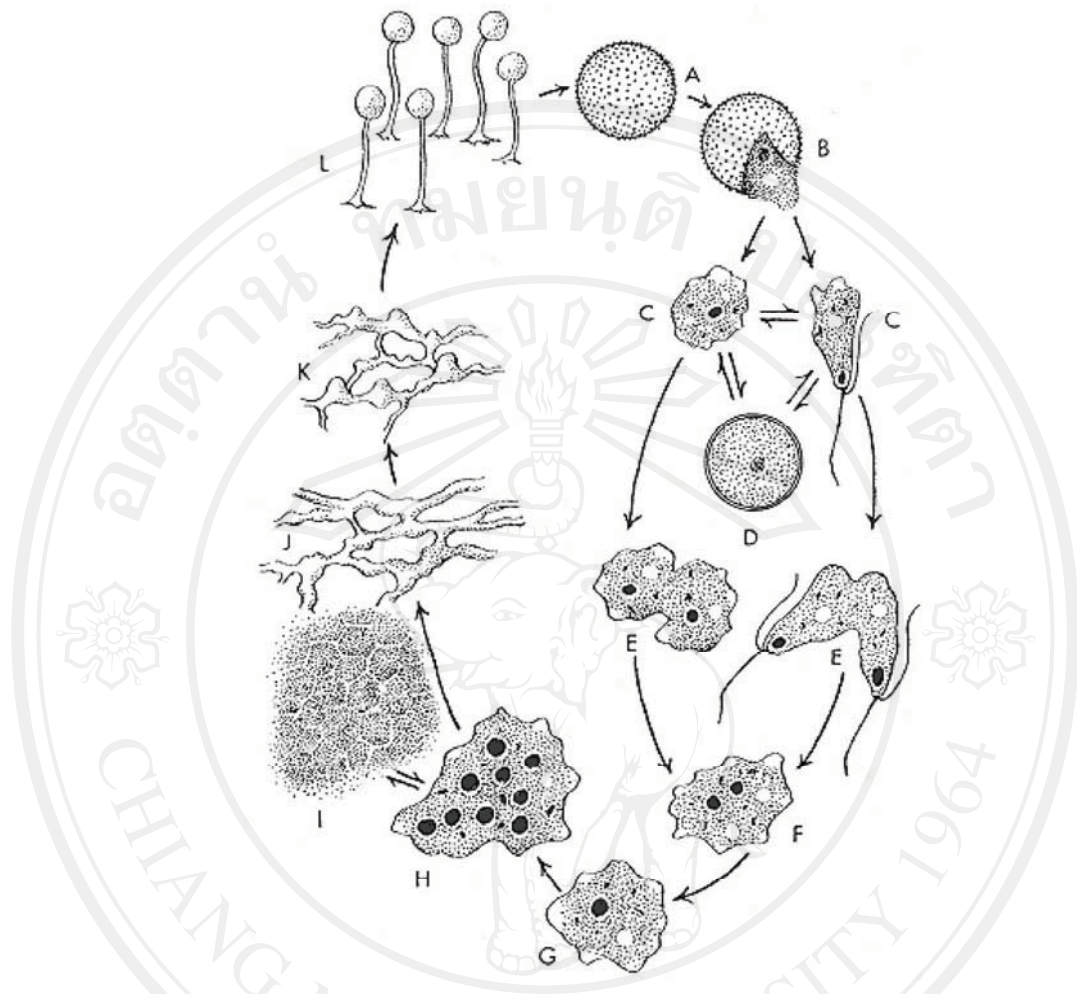
Figure 2.1 Life cycle of a typical myxomycete (Stephenson and Stempen, 1994).

Note: A, Spore. **B**, Germinating spore. **C**, Unicellular stage, which is either a myxamoeba (left) or a swarm cell (right). **D**, Microcyst. **E–F**, Fusion of two compatible myxamoebae or swarm cells to produce a single cell. **G**, Zygote. **H**, Early plasmodium. **I**, Sclerotium. **J**, Portion of a mature plasmodium. **K**, Beginning of sporulation. **L**, Mature fruiting bodies with spores still enclosed

2.3 Significances of Myxomycetes

Myxomycetes play very important roles in the terrestrial ecosystems by providing ecosystem services and also representing biological resources. As an ecosystem service myxomycetes play a significant role in nutrient cycling by secreting extracellular cellulase and α-amylase (Chung, 1997). Moreover,

myxamoebae are capable of releasing ammonia to plant roots when feeding on bacteria and can cause increases in dry weight and nitrogen content; consequently, mineralization is stimulated and decomposition enhanced (Stephenson and Cavender, 1996). Both of the two trophic stages of myxomycetes are known to feed upon bacteria; and myxomycetes have a significant role in maintaining the natural balance that exists between bacteria and other micro-organisms in the soil environment (Cavender and Raper, 1965). In soil microbial communities, the amoeboid stages of dictyostelids (cellular slime molds) and myxomycetes (plasmodial slime molds), and the plasmodial stage of myxomycetes make up a substantial proportion of the bacterivores present in most soils and this fact seem to suggest that myxomycetes play a key role in the detritus food chain (Feest and Madelin, 1988a,b; Amewowor and Madelin, 1991; Stephenson and Cavender, 1996).

Myxomycetes have been used as biological resources in laboratory studies of various aspects of cellular physiology and biochemistry. For example, the vigorous protoplasmic streaming exhibited by some types of plasmodia represents an ideal situation for studying cellular motility (Stephenson and Stempen, 1994). The major transformation in the life cycle of a myxomycete from uninucleate amoeboflagellate cells to a multinucleate plasmodia and then to fruiting bodies makes these organisms suitable for use as experimental organisms for studies of cell growth and differentiation. Weaver (1976) reported that myxomycetes have been used in cell differentiation studies connected with cancer research since the biochemical changes that take place during the process of the plasmodium differentiating into fruiting bodies are suggestive of the changes that take place when a normal cell becomes cancerous.

Myxomycetes have also developed rather unique secondary metabolites and almost 100 different natural compounds, including lipids, fatty acids, alkaloids, amino acids and peptides, naphthoquinone pigments, aromatic compounds, carbohydrate compounds and terpenoid compounds (Dembitsky *et al*., 2005). An anti-inflammatory substance, which can affect mucous membranes, has been isolated from fruiting bodies of *Lycogala epidendrum* and can be used as external application (Ying, 1987). The fruiting bodies of *Tubifera dimorphotheca* have produced two triterpenoid lactones (Tubiferal-A and B) and one of these (Tubiferal-A) possesses a new compound that exhibited a reversal effect of vincristine resistance against VCR-resistant KB cell lines (Kamata *et al*., 2004). Recently, bisindole alkaloids isolated from myxomycetes have received considerable attention because they have potential for biological activity as small-molecule Wnt signal inhibitors. Kamata *et al*. (2005) reported that bisindole alkaloid compounds were successfully isolated from the naturally occurring fruiting bodies of *Arcyria cinerea* and *Lycogala epidendrum*. Among these compounds, one bisindol alkaloid compound (named bisindoles A12) showed cytotoxicity against cultured tumor cell lines. Kaniwa *et al*. (2007) successfully isolated bisindole alkaloids from the sporocarps of *Arcyria ferruginea*. Furthermore, some species of myxomycetes could be used as a human food source. Examples include young aethalia of *Enteridium lycoperdon* and the plasmodium of *Fuligo septica*, which are collected, fried, and eaten by some indigenous peoples in the state of Veracruz in Mexico (Villarreal, 1983). Moreover, plasmodia and fruiting bodies of some myxomycetes are very beautiful and intriguing such that myxomycetes have become "irresistible models" for photographers and other people encountering them in nature (Stephenson and Stempen, 1994). Myxomycetes feed on

bacteria, fungal spores and the other minute organisms, but they also provide favorable substrates and shelters for various species of fungi and insects (Stephenson and Stempen, 1994).

2.4 Taxonomic study of myxomycetes

2.4.1 Taxonomic status

Kingdom	Protozoa
Phylum	Myxomycotina
Division	Myxomycophyta
Class	Myxomycetes

All of the slime molds have been considered anomalies of nature because it is difficult to determine their taxonomic relationships. They have an animal-like feeding phase that alternates with a fungal-like spore-bearing phase (Katsaros, 1989). Myxomycetes have been known from their fruiting bodies since at least the middle of the seventeenth century, when the first recognizable description of a member of the group (the very common species now known as *Lycogala epidendrum*) was provided by the German mycologist Thomas Panckow. The name given to the group was derived from the Greek words *myxa* (slime) and *myketes* (fungi) and was first used in 1833 by German botanist Heinrich Link, who regarded the myxomycetes as fungi. In 1858 Anton de Bary, who is generally given credit for the first serious studies of the group, concluded that myxomycetes to be more closely related to the protozoans rather than the fungi and proposed the name Mycetozoa (fungus animal). Since Anton de Bary, a number of monographers of the group, including Lister (1894), Hagelstein (1944), and Olive (1975), have perpetuated the name Mycetozoa. Some workers, on

the other hand, followed Link's original interpretation and considered the group to be fungi since myxomycetes produces spores and that is a characteristic common to some taxa of fungi (Macbride, 1899; Macbride and Martin, 1934; Martin, 1949; Martin and Alexopoulos, 1969; Farr, 1976; Nannenga-Bremekamp, 1991). Alexopoulos (1963) regarded Myxomycetes as the phylum Myxomycotina, Division Mycota, and Kingdom Plantae. Ainsworth and Bisby (1971) divided the phylum Myxomycota into four classes including Myxomycetes, Acrasiomycetes, Hydromyxomycetes, and Plasmodiophoromycetes and those belong to Kingdom Fungi. However, the assimilative stage in myxomycetes is morphologically similar to that of an amoeba designated as a myxamoeba. The myxamoeba is a uninucleate, haploid cell which is not enclosed in a rigid cell wall, and ingests its food by means of phagocytosis, and the combination of these behaviors cannot support myxomycetes to be considered fungi. Recently, most myxomycologists and mycologists agree with the classification of myxomycetes as a class in the Phylum Myxostelida, which belongs in the Kingdom Protozoa (Stephenson, 1993; Kendrick 2000; Lado, 2001).

Cavalier-Smith (1993) suggested placing the myxomycetes into the Kingdom Protozoa according to the molecular phylogeny results on small-subunit ribosomal-DNA sequence analyses. Gene analysis of the elongation factor EF-1A revealed that the clade formed by *Physarum* (myxomycetes), *Dictyostelium* (dictyostelids), and *Planoprotostelium* (protostelids) is the sister group of Animalia and Fungi (Baldauf, 1999). According to DNA and RNA analyses, myxomycetes should be placed within the "crown" clade of eukaryotes (Baldauf, 1999; Baldauf and Doolittle, 1997; Horton and Landweber, 2000), and the phylogenetic tree for myxomycetes was created as shown in Figure 2. 2.
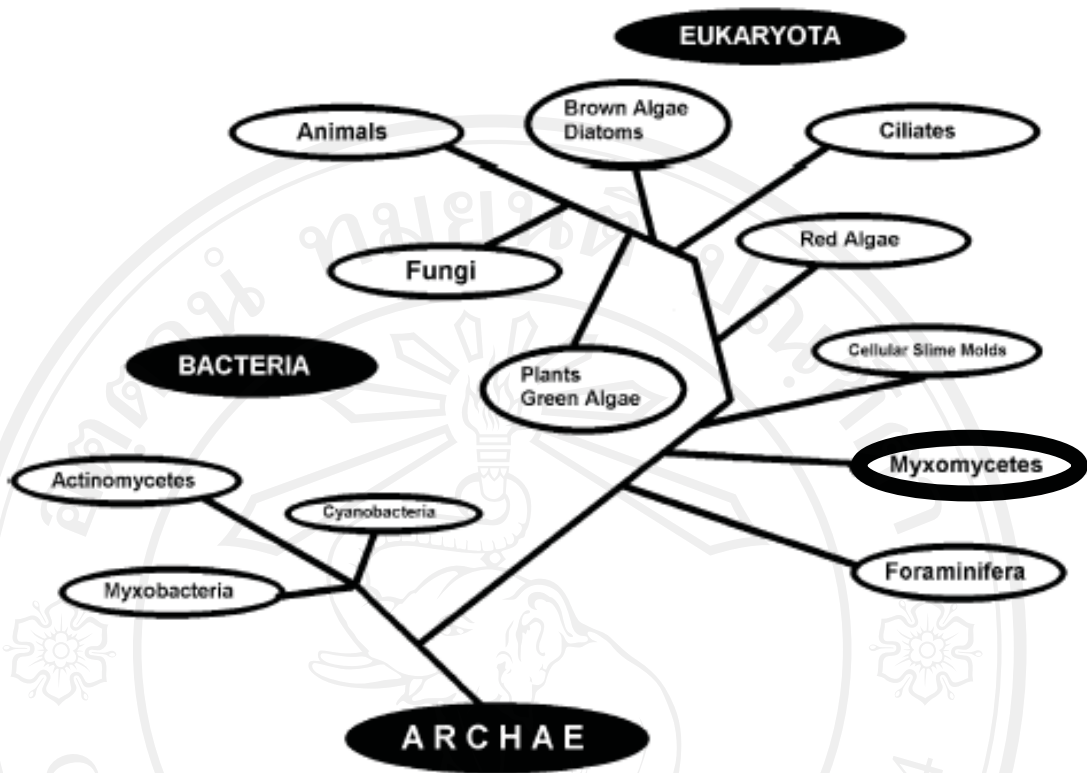
Figure 2.2 Position of myxomycetes on the phylogenic tree (Dembitsky *et al*., 2005).

2.4.2 Classification and Identification

The species concept for myxomycetes is based primarily upon morphology. The type of fruiting body is one of critical features in the morphological identification of myxomycetes. There are four types of fruiting bodies (Figure 2.3) such as,

1. sporangium (Figure 2.3A)

It is a small fruiting body which may be sessile or stalked, with wide variations in color and shape. Sporangia usually occur in groups, since they form from separate portions of the same plasmodium.

2. plasmodiocarp (Figure 2.3B)

A fruiting body of a sessile, branched, ring-shaped, or netted type.

3. aethalium   It is a hemispherical or cushion-shaped sessile fruiting

    (Figure 2.3C)   bodies with variable sizes.

4. pseudoaethalium   It is a fruiting body that consists of a mass of

    (Figure 2.3D)   sporangia tightly packed together to resemble an

                                  aethalium (hence, false aethalium). Pseudoaethalia are

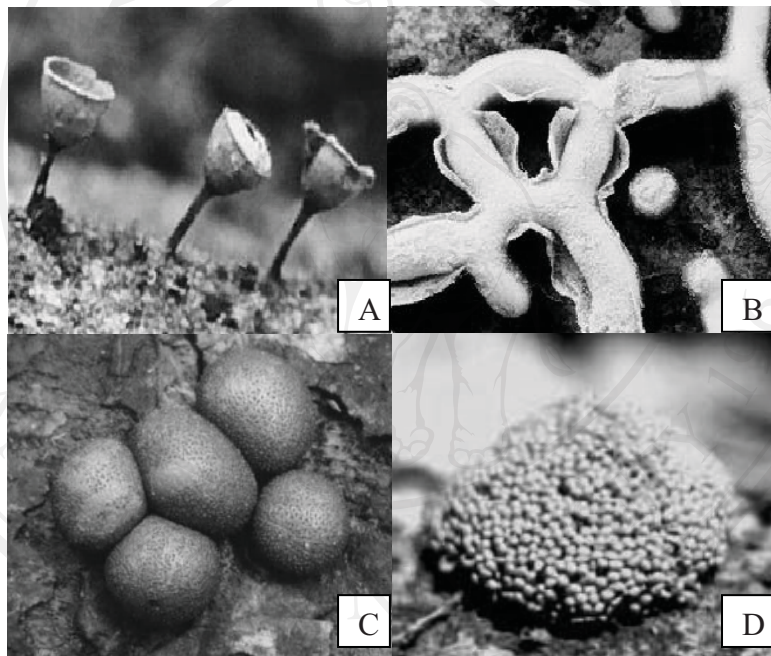   usually sessile, although a few may be stalked.

Figure 2.3 Types of fruiting bodies (http://slimemold.uark.edu/).
Note: A, Sporangium. **B**, Plasmodiocarp. **C**, Aethalium. **D**, Pseudoaethalium.

       In addition to the types of fruiting bodies, the morphological characters of the

major components of fruiting body such as hypothallus, stalk, columella, peridium,

calyculus, capillitium and spores (Figure 2.4) are examined and measured under the

microscopes in order to identify the species (Martin and Alexopoulos, 1969;

Stephenson and Stempen 1994; Stephenson, 2003). Examination and measurements

of the micromorphological structures are observed in mounted slides under the microscope. Reagents such as distilled water, ethanol, aqueous solutions of potassium hydroxide, acetic acid and/or immersion oil are appropriately used if necessary (Stephenson and Stempen 1994). Sometimes morphological features are intensively diagnosed by SEM techniques especially for some confusable myxomycetes species, for example *Diderma niveum* complex (Moreno *et al*., 2003).
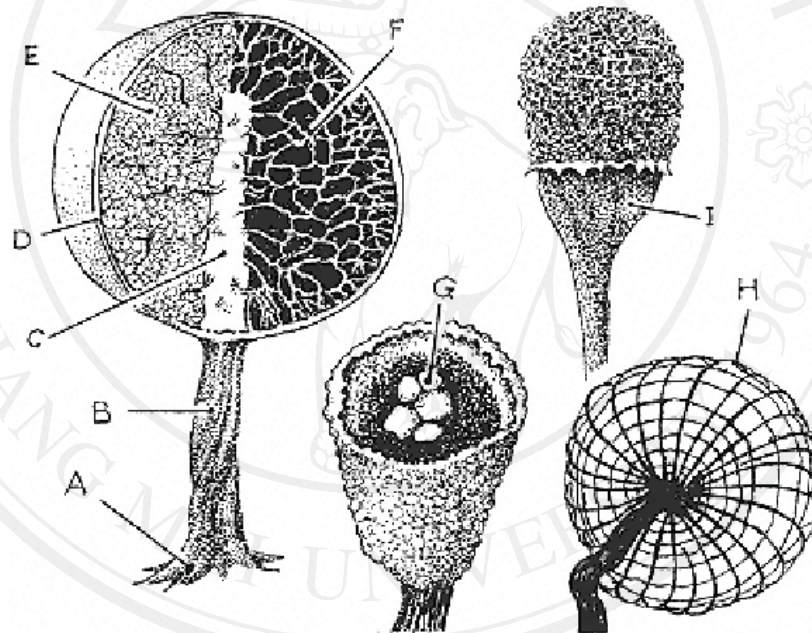

Figure 2.4 Structural components of myxomycete fruiting bodies (Sporangium Types).

Note: **A**, Hypothallus. **B**, Stalk. **C**, Columella. **D**, Peridium. **E**, Spores. **F**, Capillitium (spores removed). **G**, Pseudocolumella. **H**, Peridial (surface) net. **I**, Calyculus. (Stephenson and Stempen, 1994).

The myxomycetes are considered to represent a taxonomic class (the Myxomycetes) made up of three subclasses. These are the Ceratiomyxomycetidae (with a single order the Ceratiomyxales), the Myxogastromycetidae (with four orders: Echinosteliales, Liceales, Physarales, and Trichiales) and the Stemonitomycetidae

(with single order Stemonitales), as outlined by Martin and Alexopoulos (1969), and Stephenson and Stempen (1994). According to Lado (2001) who made effort to compile a complete monographic treatment of myxomycetes, there are approximately 875 described species of myxomycetes and these have been placed in six different taxonomic orders such as Ceratiomyxales, Echinosteliales, Liceales, Physarales, Stemonitales, and Trichiales (Figure 2.5). On the other hand, some taxonomic treatments (Olive, 1970; 1975; Olive and Stoianovitch, 1979) regarded the members of the Ceratiomyxales distinctly different from members of other orders and placed them with the protostelids, another type of slime mold. Although the exact evolutionary affinities of the myxomycetes are still debated, they constitute a well-defined and homogeneous group (Stephenson *et al*., 2008a). Moreover, the unique taxonomy including identification and nomenclature of myxomycetes within the group is well defined (Hernández-Crespo and Lado, 2005).
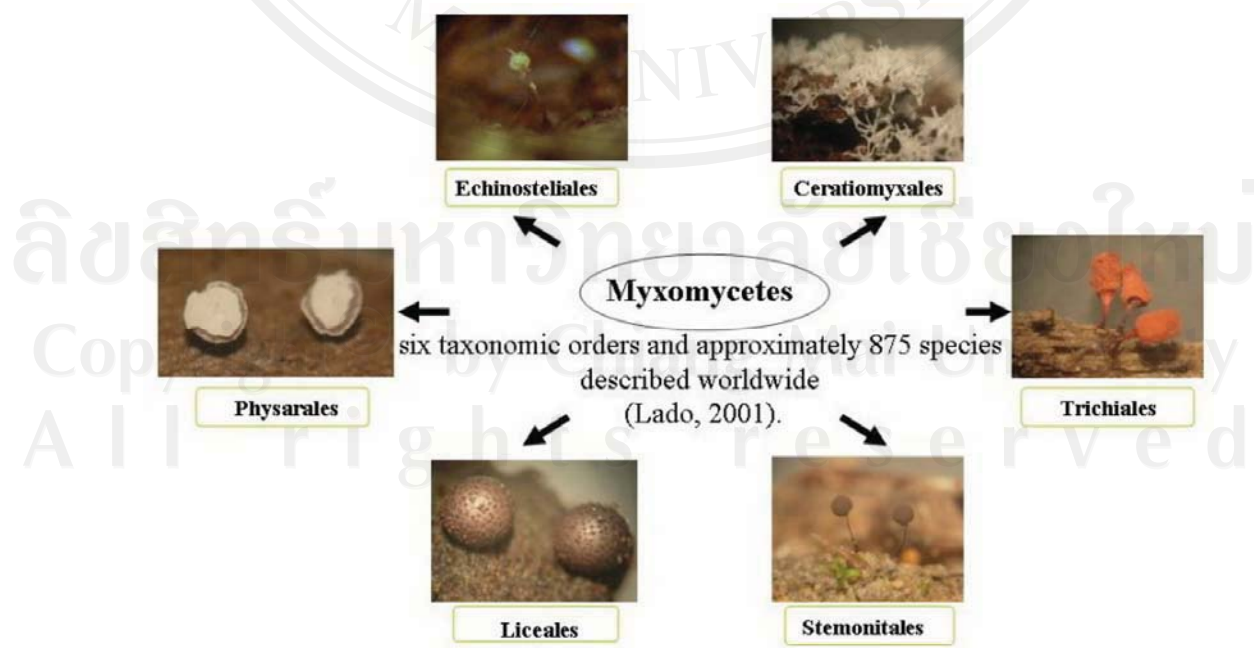

Figure 2.5 Myxomycetes in six taxonomic orders (Adopted from Lado, 2001).

As is the case for many other organisms, dichotomous keys are used for myxomycete identification. Each key consists of a series of couplets, with each couplet made up of a pair of statements composed of one or two more contrasting characters. To identify a particular specimen, one should begin with the key to Orders, and then proceed on the portion of the monograph where taxa belonging to that order are considered. Once reaching the appropriate Order, the process is repeated for additional keys to determine the species (Lado, 2001; Stephenson and Stempen, 1994). The morphological characters of fruiting bodies considered for the purpose of identification are unique to each order. For example, members of order Ceratiomyxales are distinct from the other orders of myxomycetes, with the spores that are externally borne on the outside of fruiting bodies instead of inside. (Stephenson and Stempen, 1994; Stephenson, 2003). The members of order Liceales are quite easy to recognize because of the absence of a columella and true capillitium, but a pseudocapillitium is sometimes present (Alexopoulos, 1969). The prominent characteristic of the order Echinosteliales is the very minute fruiting bodies (< 0.5 mm tall), with a true capillitium often present. The presence of the conspicuously sculptured and sometime highly ornate capillitium and more or less bright colored spores mass distinguish members of order Trichiales from other orders of myxomycetes. The somewhat dark color (brown, purplish brown, violet, or black) of the spore mass is the case for the members of the Physarales and Stemonitales, while the other group (order Ceratiomyxales, Echinosteliales, Liceales, and Trichiales) includes those with spores that are white, pink, pale gray, light brown, rusty brown, yellow, orange, or red (Stephenson and Stempen, 1994). The single most important

character of the order Physarales is the presence of lime (calcium carbonate) deposits

in the peridium, capillitium, or stalk of the fruiting bodies (Stephenson, 2003).

The key to six orders of myxomycetes are as follow (Stephenson, 2003);

Key to the Orders of Myxomycetes

1. Fruiting bodies in the form of erect, simple or branched, usually white but sometimes pink or pale yellow columns; spores attached individually by thread-like stalks **..................** **Ceratiomyxales**

1. Fruiting bodies not as above; spores occurring in a mass within the fruiting body and enclosed (at least during the early stages of development) by a peridium**...........................** **2**

2. True capillitium absent, pseudocapillitium composed of irregular elements sometimes present**.....................................** **Liceales**

2. True capillitium present, pseudocapillitium of more or less uniform elements and usually readily apparent (occasionally considerably reduced or lacking)**.......................................** **3**

3. Fruiting bodies small (usually less than 0.3 mm in diameter or less than 0.5 mm tall), delicate, stalked**.............................** **Echinosteliales**

3. Fruiting bodies larger (usually more than 0.3 mm in diameter or more than 0.5 mm tall), more robust, stalked or sessile**...** **4**

4. Spore mass more or less brightly colored (rarely pallid or light brown); capillitium usually conspicuously sculptured and sometimes highly ornate**...** **Trichiales**

4. Spore mass usually purple brown to black, occasionally pale violet or red brown; capillitium not conspicuously sculptured**...** **5**

5. Lime present in some part of the fruiting body**.....................** **Physarales**

5. Lime absent from all parts of the fruiting body**.....................** **Stemonitales**

The structures and characters that are used for the identification of

myxomycetes by the traditional morphological species concept, such as the overall

character of the well-mature fruiting bodies with their components, the presence or

absence of deposits of lime (calcium carbonate), and spore color in mass (Martin and

Alexopoulos, 1969; Stephenson and Stempen 1994; Stephenson, 2003) are clearly

supported as reliable taxonomic characters by the evidence available from molecular phyloganetic studies. In the first phylogenetic study for the orders of myxomycetes (except order Ceratiomyxales that was regarded as the protostelids in this case) based on molecular data, Fiore-Donno *et al*. (2005) suggested that the Echinosteliales, which produce fruiting bodies with a simple structure, represented the most basal clade of myxomycetes, with two more advanced groups, the first with light-colored spores and consisting of the Trichiales and the (presumably not monophyletic) Liceales, and the second clade having dark spores and made up of the Physarales and Stemonitales (Figure 2.6).
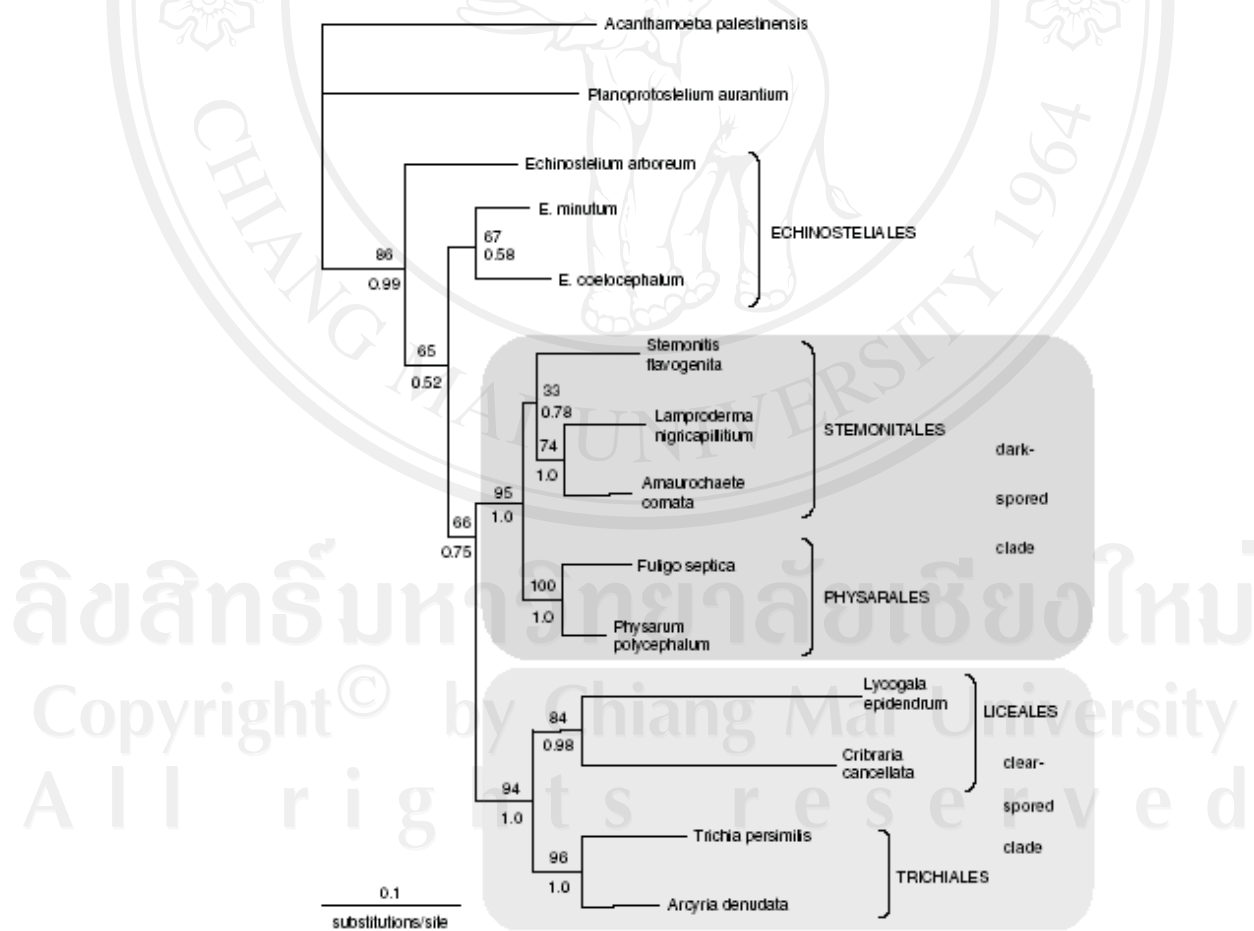

Figure 2.6 Elongation factor 1-alpha (EF1A) phylogeny of Myxogastria (from Fiore-Donno *et al*., 2005).

2.5 Biodiversity and ecological studies

2.5.1 Fruiting seasons

Myxomycetes are commonly associated with the plants and plant debris throughout the world (Stephenson and Stempen, 1994); however, the distribution of myxomycetes in nature is not random because temperature and humidity strongly affect the life of myxomycetes (Martin and Alexopoulos, 1969; Stephenson and Stempen, 1994). It seems that myxomycetes mostly form fruiting bodies at certain periods in the year. In the tropics, the fruiting bodies of myxomycetes appear after a period of precipitation during the rainy season. In temperate regions, fruiting bodies can be found in some abundance beginning in early summer and extending until late fall (Stephenson and Stempen, 1994; Stephenson, 2003). Some species may occur during the entire period of favorable environmental conditions, while some groups of myxomycetes generally appear only during a certain period of the year. For example, a set of data in eastern North America suggested that *Lycogala epidendrum* could appear from early summer through late fall, but the fruiting of *Ceratiomyxa fruticulosa* was common only in summer and the species was absent in the fall (Stephenson, 1988). Moreover, the nivicolous species that occur in alpine regions usually produce sporocarps only during the short period of time when the snowbark is melting back. During the remainder of the summer, the species that found in these alpine regions are the same as those collected at lower elevations in the same region (Stephenson and Shadwick, 2009).

Based on the collections and observations in the field as well as collections obtained from moist chamber cultures in the laboratories, a number of researchers have concluded that myxomycetes displayed seasonal patterns of absolute abundance,

species richness and species diversity (Gray and Alexopoulos, 1968; Stephenson, 1988; Tran *et al*., 2006; 2008). Maimoni-Rodella and Gottsberger (1980) indicated that myxomycetes were rarely found in nature when air temperatures were lower than 14° C, even though there was enough moisture. *Physarum straminipes* showed a more rapid development in moist chambers placed at 20° C than in those placed at 30° (Blackwell and Gilbertson, 1984). More recently, reports of two ecological studies in northern Thailand (Tran *et al*., 2006; 2008) indicated that the fruiting phenology of myxomycetes under natural field condition differed depending upon the time of the year and this was also the case for their seasonal patterns of occurrence on agricultural ground litter and forest floor litter, both in the laboratory cultures and under natural conditions in the field. Tran *et al*. (2006) reported that there were no records of either fruiting bodies of myxomycetes or any evidence of plasmodia during the period of December to April, and a few fruiting occurred during the rest of dry seasons (cool dry and hot dry). But the fruitings of myxomycetes were prominent in the rainy season in Chiang Mai Province, northern Thailand (Tran *et al*., 2006; 2008). Actually, there is no complete explanation for the seasonal distribution records of myxomycetes since the knowledge of this aspect of ecology is still limited and more research is needed in various regions of the world.

2.5.2 Geographical differences

Among the worldwide documented species of myxomycetes, most species are probably cosmopolitan in distribution. However, some species have showed restricted distribution. A few species seem to be confined to the tropics or subtropics while some others found in temperate regions (Alexopoulos 1963; Farr 1976; Martin *et al*.,

1983). For example, such species as *Ceratiomyxa sphaerosperma, Craterium paraguayense* and *Tubifera bombarda* are well-known as tropical myxomycetes whereas *Hemitrichia clavata* is very common in temperate regions (Stephenson and Stempen, 1994).

Over the past two decades, numerous studies of the biodiversity and ecology of myxomycetes have been carried out all over the world. The knowledge of the distribution and ecology in temperate regions is relatively developed, including results from studies in Austria (Singer and Gabriel, 2001), eastern North America (Stephenson *et al.*, 1993), Europe (Moreno *et al.,* 2003), high latitude regions of the Northern Hemisphere (Stephenson *et al.,* 2001), New Zealand (Stephenson, 2003) and Turkey (Sesli and Denchev, 2005). Myxomycetes appear to be particularly abundant in temperate forests, but at least some species apparently occur in any terrestrial ecosystem with plants (and thus plant detritus) present (Stephenson and Stempen, 1994). Some studies have been carried out in tropical regions, for example Costa Rica (Schnittler *et al.,* 2000; Rojas and Stephenson, 2008), Mexico (Lado *et al*., 2003; Estrada-Torres *et al*., 2009), Puerto Rico (Novozhilov *et al*., 2001) and Russia (Novozhilov *et al*., 2006; Kosheleva *et al.*, 2008). Some researchers have emphasized specific regions, including alpine areas (Stephenson and Shadwick, 2009; Ronikier and Ronikier, 2009), and arid regions (Lado *et al.,* 2007; Novozhilov and Schnittler, 2008). However, there have been the very few studies of myxomycetes throughout Asia, with the exception of Japan (Takahashi and Hada, 2009); Hong Kong (Chung, 1997), India (Stephenson *et al*., 1993), Israel (Nissan, 1997) and Taiwan (Chung and Liu, 1996). Even though the available data from the previous studies are still rather limited for some regions, the results do point out that there is a considerable variation

in the distribution patterns of myxomycetes among the different geographical regions. For example, myxomycete biodiversity in tropical forests is greatest in aerial microhabitats while it is greatest in microhabitats associated with the forest floor in temperate regions (Stephenson *et al*., 2008a). Moreover, species compositions among the areas are different since some myxomycetes (*Comatrichia suksdorfii*, *Lamproderma carestiae*, *Lepidoderma carestianum* and *Pgysarum alpinum*) are apparently restricted to alpine areas, while others (for example, *Mucilago crustacean*) are common in high latitudes (Stephenson and Stempen, 1994).

Stephenson (1983) reported that some species of *Comatrichia aequalis*, *Cribraria rufa, Lepidoderma tigrinum, Licea minima*, and *Trichia erecta* are relatively common in high-elevation coniferous forests but are rarely found in the lower elevation forests. Venkataramani and Kalyanasundaram (1986) determined the ecological preferences of calcareous and non-calcareous species in India with respect to elevation, rainfall and temperature. Stephenson *et al*. (1993) carried out a comparative biogeographical study of the mid-Appalachians of eastern North America and India. They reported that myxomycetes show a pattern of latitudinal variation and that the assemblages and distributional patterns were distinctly different in these two regions. Yagiz and Afyon (2006) studied the species diversity of two different regions in Turkey and they reported that the region with the variety of trees seemed to increase the number of taxa of myxomycetes. Rojas and Stephenson (2008) analyzed the assemblages of myxomycetes from different localities in Costa Rica along an elevational gradient. Generally, the data from these previous studies seem to confirm that the assemblages, distribution patterns and communities of myxomycetes are differences in the different geographical localities.

Unfortunately, there have been only a few studies of myxomycetes in south-east Asia, where there have been a limited number of publications in Thailand and only one report from Myanmar with only seven species (Reynolds and Alexopoulos, 1971). Actually, research on myxomycetes in Thailand is severely lacking, with fewer than 200 documented species. The first records of myxomycetes from Thailand were made by Rostrup (1902), who listed *Lycogala epidendrum* and *Stemonitis fusca* from the Koh Chang area. Heim (1962) noted that myxomycetes of Thailand were abundant but gave no details. Reynolds and Alexopoulos (1971) reported 42 species collected from localities in south Thailand (one being undetermined). Siwasin and Ing (1982) reported 34 species that were recorded mostly from northern Thailand, of which 16 were new records for the country. All of the previous studies in Thailand reported only species lists of myxomycetes that occurred anywhere, although mostly in northern parts in Thailand and/or included just a brief note on particular species. Only a couple of most recent studies were directly towards the ecology (distribution and occurrence) of myxomycetes in northern Thailand (Tran *et al.*, 2006; 2008). It was indicated that the fruiting phenology of myxomycetes under natural field condition in mid-elevation forest differed with the time of the year and the substrates on which they occur (Tran *et al.*, 2006), and there were the seasonal patterns of occurrence and distribution on agricultural ground litter and forest floor litter, both in the laboratory cultures and under natural condition in the field (Tran *et al.*, 2008). The data, with a total 100 species of myxomycetes, of which seventy-eight were new records for northern Thailand, reported in these studies provide clear evidence of the high biodiversity of myxomycetes in northern Thailand (Tran *et al.*, 2006; 2008).

2.5.3 Microhabitats relationships

Myxomycetes are common inhabitants in almost all types of the terrestrial ecosystems; however, field observations and collections by many researchers have indicated that the fruiting bodies of particular species tend to be consistantly associated with certain types of substrates (Gray and Alexopoulos, 1968; Stephenson 1989). Some species almost always occur on wood or bark (for example, *Arcyria denudata*) on decaying wood, while others are found more often on dead leaves and other plant debris. The reasons for this microhabitats specificity by myxomycetes are not yet known, but they likely involve many biotic and abiotic factors and their interactions (Stephenson and Stempen, 1994).

The primary microhabitats of myxomycetes are coarse woody debris on the forest floor, the bark surface of living trees, forest floor litter, soil, the dung of herbivorous animals, and dead but still attached plant parts above the ground (Stephenson, 2003). Among the species of myxomycetes that inhabit the different microhabitats, species associated with decaying wood are the best-known since the sizes of the fruitings of myxomycetes typically associated with this microhabitat are large enough to detect in nature (Martin and Alexopoulos, 1969). Various species in such genera as *Arcyria, Lycogala, Stemonitis, and Trichia* are predominantly lignicolous and many of these species are known as the most common taxa of myxomycetes (Stephenson, 2003). There has been some relationship between the decomposition states of woody debris and myxomycetes diversity (Takahashi, 1999; 2004; Takahashi and Hada, 2009), and the number of species and species diversity of the myxomycete community reached the maximum on moderately decayed wood.

By using the productive technique of moist chamber cultures, the myxomycetes occurring on the bark surface of living trees have received relatively greater attention than ever before and more than 100 species of corticolous myxomycetes have been reported from the field and moist chamber cultures (Mitchell, 1980). Some species in certain genera, for example *Echinostelium*, *Licea*, and *Macbrideola*, seem to restrict to the bark microhabitats; however, some species known from the bark are also reported from other microhabitats. Such species as *Calomyxa metallica, Comatricha fimbriata, C. nigra, Enerthenema papillatum,* and *Perichaena chrysosperma* are known to be common in moist chamber cultures prepared with the pieces of back (Stephenson and Stempen, 1994). There have been considerable variations in myxomycetes assemblages reported that appear to correspond to the different characteristics of the bark such as bark texture, pH, water-holding capacity and nutrient concentrations (Stephenson, 1989). Moreover, the presence of epiphytic lichens and bryophytes on bark surface seems to increase the number of myxomycetes species present (Stephenson and Stempen, 1994). Many ecological studies of myxomycetes have directed their primary focus to the assemblage of species associated with bark (for example, Snell and Keller, 2003; Everhart and Keller, 2008; Everhart *et al.,* 2009). Recently, Ndiritu *et al.* (2009) discussed that the assemblages of myxomycetes associated with ground bark was very different from the species collected from bark from aerial sites.

Although a few studies have focused on leaf litter as a microhabitat for myxomycetes (Gray and Alexopoulous, 1968; Härkönen, 1981; Stephenson, 1989; Stephenson *et al.,* 1998; Tran *et al.*, 2008), some myxomycetes have been recorded as consistently associated with litter. *Diderma effusum, Dider. testaceum, Didymium*

melanospermum, Lamproderma scintillans, Physarum bivalve, and *Phy. cinereum*
have been collected almost exclusively from the forest floor litter and assumed to be
litter-inhabiting (Stephenson and Stempen, 1994). Some studies (Stephenson 1989;
Stephenson *et al.*, 1998) that have examined the assemblages of species found on
litter have pointed out the fact that the myxomycetes occurring on litter are not the
same species found in other microhabitats in the same forest community. Moreover,
some species display an affinity for the leaf litter of different tree species (Evenson,
1961; Blackwell and Gilbertson, 1980; Tran *et al.*, 2006). For example, *Stemonitis
herbatica* and species of *Didymium* have been reported to be restricted to the litter of
broad-leaved trees while *Cribraria microcarpa* and member of the genera *Arcyria*
exhibit a preference for coniferous litter (Härkönen, 1981; Stephenson, 1989).
Myxomycetes also exhibit different patterns of distributions on the forest floor litter
and agricultural litter, and the species of the genera *Stemonitis* are more likely to
occur in the forest than in agricultural sites. In contrast, species in the order *Trichiales*
are exceedingly common in agricultural litter (Tran *et al.,* 2008). Aerial litter was also
the more productive microhabitats for myxomycetes, and the levels of species
richness and associated community on aerial litter were not same as that for
myxomycetes on ground litter (Ndiritu *et al.*, 2009).

In fact, there are numerous other substrates that would be interesting to
consider as potential microhabitats for myxomycetes, since the assemblage of species
associated with each microhabitat tends to be distinct (for example Stephenson, 1988;
1989; Stephenson and Stempen, 1994; Stephenson *et al.,* 2008a). The characteristics
of a high moisture content, large microbial populations, and nutrient richness make
dung a favorable microhabitat for myxomycetes (Hudson, 1986) and at least 80

species have been recorded from dung (Eliasson and Lundqvist, 1979), and a few species seem to be predominant on it (Eliasson and Keller, 1999). The myxomycetes found in soils have been studied by a number of workers (Thom and Raper, 1930; Indira, 1968) and various species of the genera *Didymium* seem to be abundant in soils (Feest and Madelin, 1988a,b). Furthermore, the presence or abundance of myxomycetes in the soils has been evaluated by using the enumeration technique (Feest and Madelin, 1985a,b) and molecular fingerprinting techniques (Kamono and Fukui, 2006) and they reported that the majority of myxomycetes recovered from soil proved to be *Didymium* species. The distribution of myxomycetes on the inflorescences of Neotropical herbs was reported by Schnittler and Stephenson (2002), and the specimens that collected on this microhabitat differ in some features (such as color and size of fruiting bodies) from those of same species that are collected from more typical microhabitats (Stephenson *et al.,* 2008a). There are some records for climbing lianas as potential substrates for myxomycetes (Lado *et al*., 2003; Nieves-Rivera *et al.,* 2003; McHugh, 2005). However, only one study has examined the assemblages of myxomycetes in the liana microhabitat and 65 species were recorded (Wrigley de Basanta *et al*., 2008). Stephenson *et al*. (2008b) documented the assemblages of myxomycetes associated with the twig microhabitat in forests and woodlands throughout the world. The first intensive study of myxomycetes on cacti and succulent plants was carried out by Estrada-Torres *et al*. (2009) who documented cacti and succulent plants as a very productive microhabitat, with a set of data yielding 104 species and one variety of myxomycetes. In addition to plants and plant debris, a living animal, the lizard *Corytophanes cristatus*, was reported to carry the myxomycete *Physarum pusillum* on its body (Townsend *et al*.,

2005). Since all of these different microhabitats would seem to represent potential microhabitats for myxomycetes (Wrigley de Basanta *et al*., 2008), a more detailed examination of the various types of plant and plant debris would be needed in order to develop a more complete understanding of the overall biodiversity assessment of myxomycetes.

2.6 Problems for the studies of myxomycetes

Until recently, almost all of the studies of myxomycetes, including taxonomy, diversity and ecology have relied on the morphological species concept on only one stage of their lifecycle—the fruiting body (or sporocarp). This is because of several reasons: the unicellular myxoamoeba stage is too small to be detected in nature and only the plasmodium and sporocarp can be observed directly in the field (Stephenson and Stempen, 1994). Moreover, the nature of plasmodia is such that they are often hidden in the substrates in which they occur and plasmodia also lack meaningful taxonomic characters (Stephenson *et al*., 1993). Even though sporocarps have been used as indicators of myxomycetes, studies of myxomycetes carried out in the past were based only on the direct observation of myxomycetes in the field under natural conditions. Moreover, sporocarps are usually produced from plasmodia only under certain conditions (Stephenson, 2003) and most species of myxomycetes have quite fragile sporocarps, which tend to last for only a short time (Stephenson *et al*., 2008a). This resulted in a poor understanding of myxomycetes with associated with some microhabitats such as the bark surface of living trees (Stephenson and Stempen, 1994), and carrying out fairly complete biodiversity assessments of myxomycetes is often difficult.

One way to overcome these situations, at least in part, is to culture myxomycetes in the laboratory with the use of what are referred to as moist chamber cultures. This technique was first described by Gilbert and Martin (1933) who collected bark from living trees to demonstrate to their classes the growth of algae in moist chamber cultures and discovered that the cultures they prepared also yielded the development of mature myxomycete sporocarps. This accidental discovery resulted in the description of several new myxomycete species, and since that time it has been used at any time of the year and in any part of the world (Stephenson and Stempen, 1994). The moist chamber culture technique has been widely used to supplement field collections of myxomycetes in numerous studies (e.g. Stephenson and Stempen, 1994; Lado *et al.,* 2007; Novozhilov and Schnittler, 2008; Rojas and Stephenson, 2008; Wrigley de Basanta *et al*., 2008). However, the plasmodia that appear in such cultures sometimes do not grow well and no sporocarps are produced. On the other hand, the cultivation of myxomycetes on various types of agar cultures has been attempted in order to get a wide range of myxomycetes in cultures to serve as primary research organisms in laboratories for many research purposes around the world. However, universal cultivation techniques have not been established for myxomycetes, and therefore, they can apply only to a few species. For example *Badhamia utricularis*, *Physarella oblonga*, *Diachea splendens*, *Lamproderma splendens*, *Calomyxa metallica*, *Leocarpus fragilis*, *Amaurochaete comata*, *Perichaena vermicularis*, *Metatrichia vesparium*, and *Mucilago crustacea* have been reported in agar culture (Haskins, 2008).
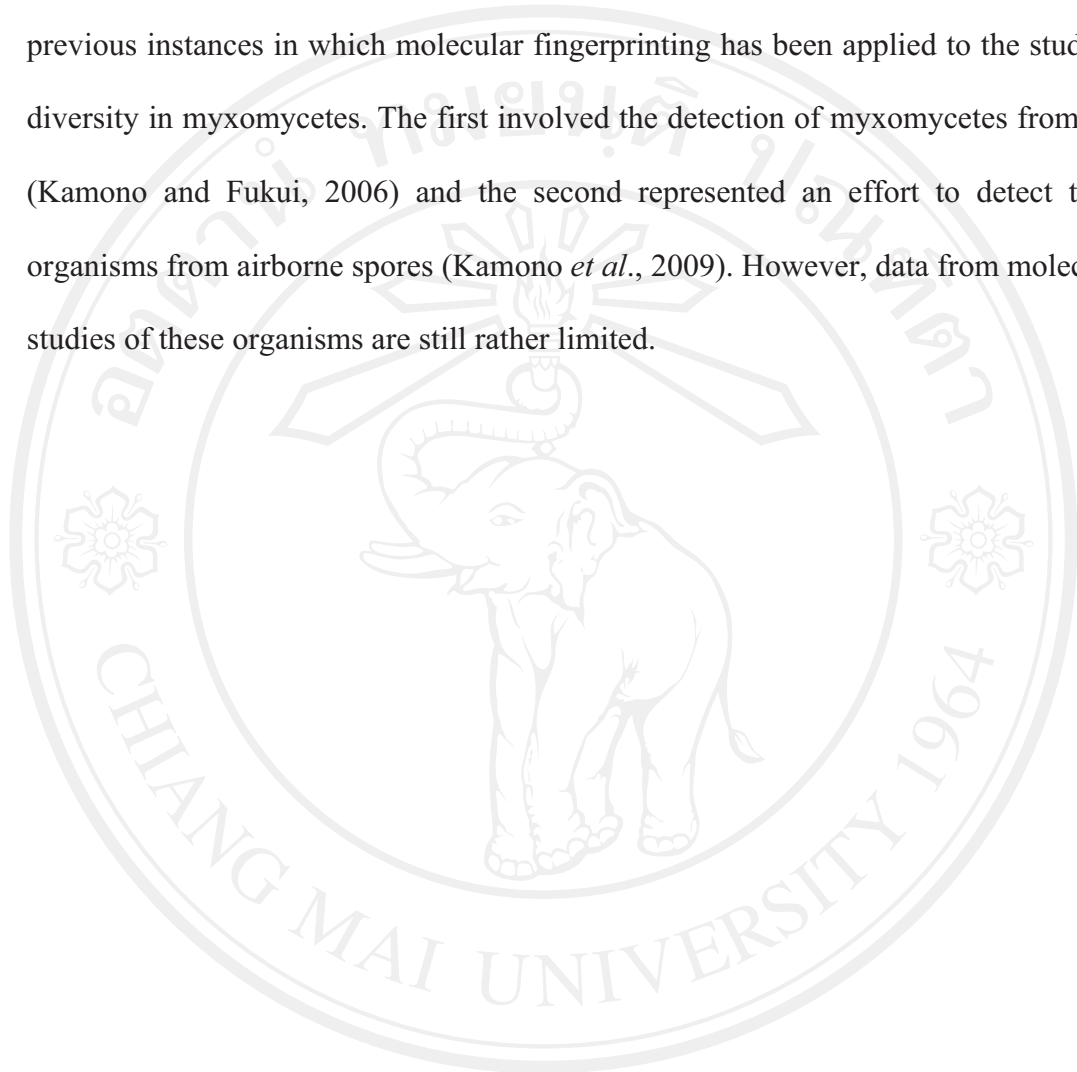
Even though the morphological identification of myxomycetes is relatively constant, and readily observable, inherent genetic and environmental variations may

sometimes create difficulties in preparing precise morphological descriptions (Clark *et al.*, 2003). In addition, Clark (1995) indicated that myxomycetes have a relatively limited number of morphological traits; moreover, geological distribution and frequent asexuality can make species definitions especially difficult. Therefore, when using morphological characteristics only, it may be difficult to avoid making overlap in species identifications. On the basis of evidence from molecular studies, some workers (Baldauf and Doolittle, 1997; Baldauf *et al.,* 2000) suggested that myxomycetes would have a significant evolutionary history with other eukaryotes. However, due to the fragile nature of the fruiting body, fossil records of the group are exceedingly rare (Stephenson *et al.*, 2008a). Consequently, there is little known concerning the genetic diversity of myxomycetes (Cavalier-Smith, 1993).

A few studies have attempted to quantify plasmodia in the soil by using a simple enumeration technique in order to evaluate the abundance of myxomycetes (Feest and Madelin, 1985a,b; 1988a); however, there is still a need to develop more effective methods to characterize myxomycete biodiversity and ecology in the environment. Stephenson *et al.* (2008a) suggested that direct environmantal sampling with the use of molecular techniques had considerable potential value for revealing the hidden taxa or population such as myxoamoebae and plasmodia of myxomycetes.

However, the literature available on most aspects of the molecular biology of myxomycetes is not very extensive. Virtually all early studies were directed towards the model species *Physarum polycephalum*. Recently, a number of studies (Rusk *et al.,* 1995; Horton and Landweber, 2000; Martin *et al.,* 2003; Fiore-Donno *et. al.*, 2005; 2008; Kamono and Fukui, 2006) have demonstrated that it is possible to design myxomycete-specific primers. A couple of studies (Fiore-Donno *et. al.*, 2005; 2008)

have provided some data relating to phylogenetic analysis among the higher orders and within the dark-spored members of myxomycetes. There have been only two previous instances in which molecular fingerprinting has been applied to the study of diversity in myxomycetes. The first involved the detection of myxomycetes from soil (Kamono and Fukui, 2006) and the second represented an effort to detect these organisms from airborne spores (Kamono *et al.*, 2009). However, data from molecular studies of these organisms are still rather limited.