The Species Problem in the Myxomycetes

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Abstract

Small to microscopic eukaryotic organisms, such as the myxomycetes, often present taxonomic difficulties for the systematist, since they generally have a combination of limited morphological traits, frequent asexuality, poor fossil record, and a widespread dispersal of the morphospecies. Also, a workable taxonomy requires that a single species concept be adopted throughout a group. Thus, in these organisms the options are either a very narrow concept which tries to identify each morphologically or reproductively isolated segment (including asexual lines), or a broad concept based upon a group of related morphotypes and reproductive groups. Both of these approaches are difficult to realize with the classical typological species concept. Therefore, other species concepts have been reviewed as alternatives to the typological concept, but, for the present, these are probably best seen as supplements which can serve to help broaden and define the current morphological species concept. It is suggested that the taxonomist become familiar with the developmental and reproductive biology of the myxomycetes in order to develop a better understanding of the range and causes of morphological variations and the units of evolution in this group. Hopefully, this information will help taxonomists modify the current typologically defined species into a more natural system. Some of the more difficult taxonomic problems, in this group, seem to stem from the presence of high levels of geographically restricted apomictic clonal lines. Since these small genetically isolated populations are capable of independent evolution, they may develop a distinct morphology. This would then be seen as a swarm of polymorphic local populations, which would greatly complicate the taxonomy. Since similar apomictic lines are found in a number of vascular plant genera, a survey of how it is handled in these groups can help to understand the consequences of applying different taxonomic concepts to this problem.

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The Species Problem

A species in the Darwinian natural system is defined as a discrete, reproductively defined collection of populations with a common evolutionary history (METTLER et al. 1988). Since kinship in a coherent group by evolutionary descent is the fundamental criterion for taxa in the natural system, the delimitation of a species often depends upon interpreting available evidence in terms of evolutionary relatedness and separation. However, the application of this definition to taxonomy is difficult, since direct evidence of reproductive interconnections in descent is seldom present and often limited to inferences derived from morphology. This difficulty has been called the species problem: i.e., how do you determine diagnostic levels of similarity (relatedness) and differences (separation) with limited information. Also, since at least some speciation is believed to occur by means of a gradual divergence of populations, there can be no clear cut point in this continuous process between races, subspecies and species. This is an acute problem in the myxomycetes and other eukaryotic microorganisms (SONNEBORN 1957), where the combination of relatively limited morphological traits, frequent asexuality, widespread dispersal of the morphospecies, and a poor fossil record (DOMKE 1952; WAGGONER & POINAR 1992) makes the determination of natural species especially challenging.

Species Concepts

Thus, taxonomists have used a number of species concepts (conceptual level philosophical criteria used to define the species category) in attempts to provide useful guidelines for the delimitation of taxonomically useful (practical level) species. While numerous species concepts have been proposed, the four most applicable to the myxomycetes are the morphological, biological, cladistic, and phenetic concepts.

The morphological species concept is the oldest and still the most commonly used concept. In this concept, morphologically similar individuals are designated a species and are assumed to be separated from other species by morphological discontinuities which are thought to reflect genetic and evolutionary differences. The Linnaean view considers species to consist of individuals conforming to a constant morphological form which varies only within narrow boundaries. Consequently, a single typical specimen (type) can be used to characterize the essential features of the typological morphospecies (CAIN 1957) and, therefore, new species are often described from the study of a few individuals collected in a single locality. The Darwinian view, however, holds that widely separate populations, which appear to be morphologically distinct species, may have character gradients in regions of contact which are indicative of genetic relatedness. For this reason, and the increasing differential powers of SEM and other methods of examining morphological characters it has become difficult to determine if a typological unit is in itself a species or a subordinate group (race) of a larger species. This morphological species concept has proven to be rather inadequate in the myxomycetes, since the sporangia, upon which almost all taxonomy is based, has a relatively simple structure with a limited suite of variations whose phenotypic plasticity is largely unknown. This produces typological taxa based upon a limited number of traits of unknown constancy and thus each taxonomist produces a classification based upon his own intuitive evaluation of these characters. An additional problem is the fairly frequent production of aberrant fructifications due to the disturbances of the developmental process. Without a thorough understanding of phenotypic plasticity, such aberrations may be described as new species or even genera, as in the case of *Squamuloderma nullifila* KOWALSKI which is now considered to be a *Didymium* (FARR 1982). This coincides with an estimation that approximately 50% of all described myxomycete species are known from only the type locality or less than five localities (LADO 2000).

The biological species concept (MAYR 1970) defines species as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from all other such groups". Since reproductively defined populations are the major thrust of the

natural system, the biological species concept should correspond fairly closely to this system. However, two major criticisms are leveled at this concept. First, it applies only to sexually reproducing organisms (asexual or apogamic population can not be classified in this system). Second, the primacy of the interbreeding requirement over all other characteristics can produce seemingly unreasonable results (geographically disjunct populations, which may have fairly distinctive morphological differences, can sometimes interbreed). This problem arises from the fact that reproductive isolation is not always correlated with morphological differentiation. In sympatric (geographically overlapping) and contiguous populations, morphological distinctness is quickly integrated if there are no reproductive barriers; however, in allopatric (geographically separate) populations, which normally do not share a gene pool, morphological differences can accumulate without reproductive barriers. This can lead to biological species designations varying from those consisting of morphologically distinct and geographically disjunct populations (which are interfertile) to those in which the species are morphologically identical, but reproductively isolated (sibling species). Therefore, the application of the biological species concept to the myxomycetes poses some difficulties. First, many morphologically defined species contain both sexual and apogamic strains (CLARK 1995), which are obviously related but do not fit in this concept. Second, these reproductive studies can only be conducted in the laboratory, require considerable time and effort, and only about 10% of the myxomycete taxa can be easily cultivated. Also, culture determinations of reproductive isolation are not always reliable, since some populations will interbreed and form hybrids in culture that would be unviable in nature.

The cladistic species concept defines species as populations which share derived characters (ELDRIDGE & CRACRAFT 1980). Cladists distinguish between primitive (the original trait in the ancestral population) and derived (a change from the original trait which occurs in some of the descendants of the ancestral population) characters and use only derived characters to determine relatedness. These derived characters are then used to construct, using a number of different algorithms, cladograms (hierarchical tree patterns) that are believed to reflect evolutionary relationships. While morphological data can, in theory, be used in cladistic analysis, it is often difficult to distinguish between primitive and derived morphological traits. However, DNA sequence data can be ordered fairly easily and thus have become an important tool in taxonomy, especially in determining relationships at the higher taxonomic levels. Since the generation of DNA sequence data is relatively expensive and technically sophisticated, its use in myxomycete taxonomy has been extremely limited (JOHANSEN et al. 1992) and in the foreseeable future it will probably be restricted to determining order and family relationships.

The phenetic species concept defines a species on the basis of overall similarity derived from a quantitative analysis of many characters (DUNN & EVERITT 1982). Pheneticists believe that quantitative measurements which are treated by a set of clearly defined rules, produce an objective taxonomy. These measurements are used to construct, using a number of different algorithms, phenograms (hierarchical tree patterns) that are believed to reflect true relationships if enough characters are utilized. This process is relatively easy to use and can be applied to any codified data but it has been mostly used for morphological and protein (isozyme) characters. However, in practice the selection of traits is not purely objective and the use of different traits can sometimes produce different classification schemes. This concept is also in its infancy in regards to myxomycete taxonomy, with a single paper published by ELHAGE et al. (2000) on the *Didymium squamulosum* (ALB. & SCHW.) FRIES species complex. This procedure seems to be especially difficult to apply to myxomycete morphology since the limited number of traits appear to be highly variable due to the effects of environmental conditions during development.

Since none of these concepts, in itself, seem capable of producing a natural system taxonomy for the myxomycetes, a pragmatic approach using the analyses and limited information derived from these other systems to

supplement morphological studies seems to be the obvious direction for future studies in this group. At a minimum, this approach could help examine and validate various morphological characters in terms of taxonomically meaningful variations.

The Life Cycle and Taxonomy

While almost all taxonomic distinctions are based upon sporangial morphology, there are a number of other important stages in the myxomycete life cycle. The myxamoebal stage, which is derived from the spore by germination and feeds on bacteria, produces clonal uninucleate amoebal cell populations by mitotic division. However, this stage is generally quite uniform throughout the group and probably does not display any morphological variations that are of taxonomic value at the species level. The myxamoebae are also capable of producing flagella (swarm cells) or resistant structures (cysts) under appropriate conditions, but these forms are also quite uniform throughout the group. However, the myxamoebae can also be the gametic stage and are thus directly involved in sexual reproduction and plasmodial formation. Typically, the myxamoeba is haploid and expresses a mating type allele which prevents sexual fusion with a myxamoeba having the same mating type but allowing fusion and zygote formation with another myxamoeba having a different mating type allele. Each species has a multiple allelic mating gene (COLLINS 1979) which encourages outbreeding (since there are a large number of alleles, most myxamoebae from different individuals will also have different alleles and will therefore undergo sexual fusion). Individuals that are able to interbreed are members of the same gene pool (and presumably a species), while those that can not interbreed are in different biological species even if they are morphological identical (sibling species). However, many of the myxomycete isolates grown in culture have proven to be non-heterothallic and presumably apomictic (CLARK 1995). These isolates apparently suppress meiosis and produce diploid or polyploid myxamoebae, which can then convert to the plasmodial stage without mating. The progeny from each of these apomictic individuals are all identical and form clonal lines which are genetically isolated from all of the other apomictic lines and also the sexual isolates. This genetic isolation could result in sympatric populations accumulating independent variations and becoming adapted to very specific environments. This would result in the production of a swarm of related polymorphic microtaxa, some of which could display morphological differences at a level that would greatly complicate the taxonomy of this group. However, these morphologically distinguishable apomictic lines could revert to sexual reproduction (COLLINS et al. 1983) and again blur these differences.

The plasmodium, a diploid coenocytic amoeboid cell feeding on bacteria and organic material, is another major life cycle stage. Although there are a number of different plasmodial types (ALEXOPOULOS 1960; HASKINS & HINCHEE 1974) which have proven useful in differentiating higher level taxa, there are few recognizable plasmodial variations found at the species level, e.g., *Didymium iridis* (DITMAR) FRIES and *D. ovoideum* NANN.-BREM. which are morphologically similar species having brown and yellow plasmodia, respectively (CLARK & STEPHENSON 1994). However, the plasmodium can also be taxonomically useful as a source of readily available proteins and DNA. For example, isozymes isolated from plasmodia have been used in a biosystematic study of the *Didymium squamulosum* complex (ELHAGE et al. 2000). Under appropriate conditions, the plasmodium differentiates into fruiting bodies, of which there are several different developmental types that are also correlated with higher level taxa.

Morphological Information

Although remarkably complex for a protist, compared to vascular plants the sporophore of the myxomycetes is a relatively simple structure with a limited number of components. However, while these components are rather variable for the myxomycetes as a group, their variability within a species is generally not well known nor fully analyzed. Sporophore characters can be divided into three types for convenience of discussion: overall aspects

(type, form, and association), generalized characters (color and lime), and specific component variations (capillitium, peridium, spores, etc.).

The overall aspects of the sporophore include the type (sporangia, pseudoaethalium, aethalium, plasmodiocarp), form (globose, pulvinate, urniform, cylindrical, etc.), and association between the fruiting bodies (scattered, clustered, aggregated, etc.). These sporophore aspects can be very useful in taxonomy but due caution must be used since they can grade into each other and it is not uncommon to find collections in which stipitate sporangia grade into sessile sporangia, sporangia into plasmodiocarps, globose sporangia into pulvinate sporangia, or gregarious sporangia into clustered sporangia. The only solution for this problem is the examination of enough material to understand the range of variations found in each taxon. For example, in a study (CLARK et al. 1999) of 32 isolates of *Physarum compressum* ALB. & SCHW., which is characterized as having gregarious, stipitate, compressed fan-shaped sporangia, some isolates were nearly to completely sessile (sessile sporangia occurred in most isolates). Other isolates produced reniform and occasionally globose shaped sporangial heads and several isolates formed contorted clusters that were identical to the morphological description of *Physarum nicaraguense* MACBRIDE. In culture, plasmodia can form on the Petri dish lid and these "upside down" fructifications are usually sessile and often plasmodiocarpous, whereas the same plasmodium will produce stalked sporocarps in the rest of the culture.

The more general color and lime characters are also rather variable and their underlying basis poorly understood. Sporophore and plasmodial pigments have been investigated in a number of species and a complex variety of organic chemical forms have been reported: including indoles (STELICH et al. 1980), tetrameric acid derivatives (CASSER et al. 1987), carotenoids (CZECZUGA 1980), pteridines (BLACKWELL & BUSARD 1978), and melanins (LOGANATHAN et al. 1989). Thus, simple color may be misleading since two yellow pigments may be due to chemically different compounds, while a yellow and a red pigment may be very closely related. However, a knowledge of the basis of the color can provide useful information in terms of taxonomic problems. For example, the melanins of the Physarales and Stemonitales have been found to be different (LOGANATHAN et al. 1989), and the melanins found in the taxonomically difficult genus *Diachea* are most similar to the Stemonitales melanins (KALYANASUNDARAM & ALI 1989). Also, a chromatographic study of pterdines pigments in multiple collections of four *Hemitrichia* species (BLACKWELL & BUSARD 1978) was able to distinguish between the four species, each of which displayed a number of intraisolate variations. In a similar manner, thin layer chromatography of pigments has provided useful information for differentiating the complex of red *Arcyria* species (REBHAHN et al. 1999). Inherited color variations, within a species, can also occur, as in the brown and cream plasmodial variants in *Didymium iridis* (COLLINS & CLARK 1966, COLLINS & ERLEBACHER 1969), which directly effect sporangial stalk color (CLARK & MIRES 1999). Lime color, in the Physarales, is often used as a taxonomic trait and the basis for these color differences has been examined (ALDRICH 1982). Using a dispersive X-ray spectrometer, he found that lime color differences, in a number of physaraceous species, correlated with the presence of certain inorganic ions (manganese, barium and zinc). Therefore, it is quite possible that the presence or absence of these ions in the environment of the developing sporangium may control the color of the lime in that specimen. For example *P. bilgramii* HAGELST., a blue lime species, crossed with *Physarum globuliferum* (BULL.) PERS., a white lime species, and *P. bilgramii*, when grown in agar culture, has white lime.

The presence or absence and type of lime (calcareous deposits) in a species is also an important key character in the myxomycetes. Although calcium is present in the peridium of a number of non-physaraceous species (SCHOKNECHT 1975; NELSON et al. 1977; ALDRICH 1982), it is generally found in a crystalline form only in the Physarales, although under certain conditions calcium oxalate and silicon crystals are found in the peridium of *Perichaena* and *Dianema* species

(SCHOKNECHT & KELLER 1977). In the Physareacae, the lime is present on both the peridium and capillitium and generally contains both calcium and phosphate, while in the Didymiaceae the lime is present only on the peridium and generally contains only calcium (SCHOKNECHT 1975; SCHOKNECHT & KELLER 1989). While other papers (ALDRICH 1982; NELSON et al. 1977) report different results in terms of the phosphate, SCHOKNECHT believes that this is due to their using whole sporangia as an experimental unit as opposed to isolated crystals. The lime is deposited on and in the peridial matrix from the cytoplasm via channels and pores where the final morphology of the lime is determined in part by chemical composition and in part by the pores and matrix structure (SCHOKNECHT & KELLER 1989). The Physaraceae produce globular forms of calcium carbonate with calcium phosphate and the Didymiaceae produce either crystalline calcium carbonate (*Didymium*) or globular (cryptocrystalline) calcium carbonate (*Diderma*). Unfortunately, under moist conditions recrystallization of the lime can occur, which thus camouflages the true nature of the lime crystals.

However, it is the specific component (peridium, capillitium, spores, etc.) variations which provide the bulk of the taxonomic characters in the myxomycetes. The development and use of the electron microscope has been a major advancement in the detection of fine details, in the comparison of structures beyond the resolution of the light microscope and in the determination of development of the fruiting body structural components.

In *Perichaena vermicularis* (SCHW.) ROST. the peridium has been shown (CHARVATS et al. 1973) to be produced by autolysis of the outer layer of cytoplasm and the laying down of wall materials, including the inner peridial ornamentations, under a thin layer, consisting of the slime sheath and excreted materials, to produce the mature peridial wall. This material has been shown, in *Fuligo septica* (L.) WIGGERS, to be composed of protein and carbohydrates with extensive lime deposits (CHAPMAN et al. 1982). The Stemonitales appear to be different in that there is no autophagy and the peridium is a thin layer consisting of the slime sheath with scant or no deposits of wall materials (ROSS 1957; MIMS 1973). The ornamentations on the inner peridial wall has been examined by several investigators (ELIASSON & SUNHEDE 1980; RAMMELOO 1974a) and may provide useful taxonomic characters; however, as in all cases of morphology, care must be taken to insure an adequate determination of the range of variations within each taxon. The use of peridial layers as a taxonomic character also needs to be examined, since these layer may or may not separate at maturity to produce a single, double or triple peridium. The peridium is generally composed of a outer slime sheath layer (presumably the only layer in the Stemonitales and some Trichiales) and a thicker inner layer derived from the cytoplasm which, for example, separates in most *Perichaena* but remains together in *P. vermicularis* and *P. luteolum* (KOWALSKI) GILERT. The latter has been transfered (GILERT 1995) from *Calonema* since it is a *Perichaena* in all other aspects. Also, in the Physarales, free crusts of lime are sometimes considered to be a peridial layer, which adds to the definitional confusion of this structure.

The capillitium is produced by an anastomosing system of tubular cytoplasmic vacuoles into which material is secreted (MIMS 1969, 1973; CHARVATS et al. 1974), although in the Stemonitales part of the capillitium is produced by branching of the columella (MIMS 1973). Several studies (SCHOKNECHT & SMALL 1972; ELLIS et al. 1973) have dealt with details of capillitial ornamentation, which are often used in taxonomy. An interesting finding (ELLIS et al. 1973) in the Trichiales was that there is a continuous variation from solid to hollow capillitia in this order, which brings into doubt the basis for dividing the order into the Trichiaceae and Dianemaceae based on the hollow vs. tubular nature of the capillitial threads.

Spores are cleaved out of the sporangial protoplasm by means of vesicle fusion which produces uninucleate segments of protoplasm around which spore wall material, including the ornamentations, is deposited (MIMS 1972). These spore ornamentations are key taxonomic characters, although they are at the limits of light microscope resolutions in some species.

Therefore, numerous SEM and TEM investigations of spore ornamentation have been conducted (SCHEETZ & ALEXOPOULOS 1971; RAMMELOO 1974b, 1975; DEMAREE & KOWALSKI 1975; HASKINS & MCGUINNESS 1986), and new terminology has been suggested (RAMMELOO 1974b). While these spore ornamentations are considered to be quite constant, they can vary somewhat in different isolates of the same species (GAITHER & COLLINS 1984). In fact, as is the case for most characters, spore ornamentation, size and color may be all alike in one collection but slightly different between collections (MARTIN & ALEXOPOULOS 1969, page 21; ELHAGE et al. 2000). Therefore, a number of isolates must be studied to determine the morphological range of spore characters for a particular species which in certain cases may be quite wide.

Stalk and hypothallus development appear to be similar to peridial development. In most myxomycetes, an outer slime sheath covers a fibrillar tube which is filled with food vacuoles, lime, spore-like bodies or other materials (BLACKWELL 1974; MIMS & ROGERS 1975) and it is produced by the constriction of the basal region of the presporangial primordium. This stalk wall is continuous with the peridium in most cases, although in *Arcyria* it is continuous only with the calyculus since the rest of the peridium consists of only the slime sheath (MIMS & ROGERS 1975). The hypothallus in these myxomycetes is also a continuation of the slime sheath and, in some cases, the stalk wall. In the Stemonitales, the stalk is secreted intra-protoplasmically by the presporangial primordium and is not continuous with the peridium (ROSS 1957, 1973; MIMS 1973). In this group, the hypothallus is the secreted basal portion of the stalk and does not involve a slime sheath. *Diachea leucopodia* (BULL.) ROST. has been found to have a non-stemonitaceous type of stalk development (BLACKWELL 1974) and *D. bulbillosa* (BERK. & BROOME) LISTER has been seen to develop from a bright yellow phaneroplasmodium indistinguishable from the plasmodium of a typical *Physarum* species (SCHNITTLER pers. obs.). These observations are contradictory to the melanin studies which indicate a stemonitaceous relationship (KALYANASUNDARAM & ALI 1989). Therefore, this controversial group still needs more work in order to determine its relationship to the rest of the myxomycetes. Stalk length, color, presence of lime, and internal structures are also used in myxomycete taxonomy, and while these can be useful characteristics (MATSUMOTO & DEGUCHI 1999a, b), they are also often quite variable within a taxon and therefore a determination of the range of variations for a trait is essential when they are used.

In the Stemonitales, the columella is an extension, which can be large and branched, of the intra-cytoplasmically secreted stalk (ROSS 1957, 1973; MIMS 1973) and, in the rest of the myxomycetes, it is a intrusion, separated by an extension of the peridium (BLACKWELL 1974), of the stalk into the sporangial space. Again, a knowledge of the development of this structure and the variations found within a taxon are necessary if it is to be used in classification. For example, in many *Didymium* spp. the columella is not so much an intrusion into the sporangial space as it is a folding back of the sporangial space around the stalk apex (WELDEN 1955); therefore, what is usually called the columella in this genus is actually the inside of the peridial wall surrounding the stalk. This process produces an extremely variable structure, such that the morphospecies *D. iridis*, *D. bahiense* GOTTSBERGER, and *D. verrucosporum* WELDEN can all be found in a single biological species (CLARK & MIRES 1999).

Non-Morphological Information

Reproductive Systems

The extensive early studies on myxomycete life cycles and sexuality have been reviewed by COLLINS (1979). In general, the life cycle was found to include haploid myxamobae which carried a heterothallic mating gene that controlled syngamy and plasmodial formation. This gene displayed multiple alleles which functioned to induce outbreeding since any two myxamoebae which differed at their mating type allele could undergo fusion (myxamoebae carrying like alleles could not fuse). However, some isolates of a species had myxamoebae which could form plasmodia without crossing

to a different mating type allele and were presumably diploid or polyploid apomicts. These apomictic lines are apparently derived from the heterothallic isolates by suppression of meiosis during spore formation, since conversions between the two reproductive systems have been shown to occur in the laboratory (COLLINS 1980, COLLINS et al. 1983). This basic life cycle information has been accumulated for many species (Table 1), and this mix of multiple allelic heterothallism and apomictic lines is apparently nearly standard throughout the group (CLARK 1995). Of the 12 species, in 1995, having their reproductive system reported for four or more isolates, four displayed only heterothallism, three only apomixis and the remaining five had mixed systems. At present (2000) 19 species display three heterothallic, five apomictic and 11 mixed systems.

The first report that could be interpreted as indicating the presence of genetical isolated sibling species in the myxomycetes concerned isolates of the *Physarum flavicomum* BERK. morphospecies (HENNEY 1967). However, their presence and nature were established and defined in a series of papers on *Didymium iridis* (COLLINS 1976; BETTERLEY & COLLINS 1983; CLARK & STEPHENSON 1990, CLARK et al. 1991, CLARK & LANDOLT 1993, CLARK 1995) where it was shown that there were numerous, mostly allopatric, sibling species that encompassed not only the *D. iridis* morphospecies but also included all or parts of the *D. bahiense*, and *D. nigripes* (LINK) FRIES morphospecies to produce a morphological and genetic complex (CLARK & MIRES 1999). Two heterothallic biological species, from the Sonoran and Mojave deserts, have also been reported for *Echinostelium minutum* de BARY. These biological species have somewhat larger spores and a more reduced capillitium when compared to the many apomictic isolates found in more temperate areas (CLARK & HASKINS 1998). Sibling species have also been found in the *Didymium squamulosum* morphospecies; however, in this case there appears to be a higher rate of sympatric relationships between the sibling species in this morphologically variable taxon (ELHAGE et al. 2000). These reproductive system findings would seen to indicate that, in general, the myxomycete morphospecies is a complex of genetically independent apomictic lines and allopatric and sympatric sibling species. This genetic independence also allows independent evolution of each of these groups which could result in the accumulation of morphological variations. Some of the apomictic lines, which are generally confined to restricted geographical regions, could accumulate enough morpho-

Table 1. Heterothallic and non-heterothallic reproductive systems.

	1995		2000	
Species	**heterothallic**	**non-heterothallic**	**heterothallic**	**non-heterothallic**
Order Physarales				
Badhamia gracilis	0[a]	1	0	5
Didymium annellus	0	0	0	8
D. difforme	0	9	0	9
D. iridis	27(7)[b]	40	23(9)[c]	55
D. megalosporum	0	0	8[c]	1
D. nigripes	1	4	0[d]	0
D. ovoideum	14	0	14	8
D. squamulosum	0	7	8(5)	28
Fuligo septica	4	6	4	6
Physarum cinereum	1	2	1	7
P. compressum	0	2	1	32
P. flavicomum	5(2)	0	5(2)	0
P. melleum	0	0	0	17
P. polycephalum	12	1	12	1
P. pusillum	3	4	3	4
P. rigidium	4	0	4	0
P. ?straminipes	0	0	3	17
Order Trichiales				
Arcyria cinerea	0	0	0	6
Order Echinosteliales				
Echinostelium coelocephalum	4	0	17	0
E. minutum	0	7	7(2)	35
Order Stemonitiales				
Stemonitis flavogenita	1	7	1	7

[a] Number of isolates reported as having a heterothallic or non-heterothallic reproductive system in the review by CLARK (1995) and at 2000 (not all published).
[b] Numbers in parenthesis indicate the number of biological species which are encompassed in the heterothallic isolates.
[c] For *Didymium iridis*, 5 isolates reported as heterothallic in 1995 have been transferred to *D. megalosporum*.
[d] The isolates reported as *D. nigripes* in 1995 are now considered to be *D. iridis*.

logical variations to be recognized as separate taxa. However, this would result in the naming of multiple microtaxa which would be extremely confusing and add little or nothing to our knowledge of the group. The sibling species are more difficult since they appear to be larger entities often with allopatric geographic ranges. It is also quite likely that in some morphospecies complexes these biological species have accumulated minor morphological differences which could be recognized as different taxa once the underlying genetic information was known.

DNA

The limited DNA sequence data on myxomycete species is taxonomically interesting. The first report (JOHANSEN et al. 1992) indicated that *Didymium iridis* and *Physarum polycephalum* SCHW., species classified in different families of the order Physarales, had undergone a very ancient separation. This has been confirmed by a recent meetings abstract (MILLER & KRISHNAN 1999) where six species were studied: *Didymium iridis*, *D. nigripes*, *Lycogala epidendrum* (L.) FRIES, *Physarum didermoides* (PERS.) ROST., *P. polycephalum* and *Stemonitis flavogentia* JAHN. They found that the *Didymium*, *Physarum* and *Stemonitis* species diverged at about the same time with *Lycogala* having the earliest divergence. This is somewhat surprising, since the stemonitaceous species have been segregated from the rest of the myxomycetes as the subclass Stemonitomycetidae on the basis of plasmodial form and sporangium developmental type (ROSS 1973). While the differences between the *Physarum didermoides* and *P. polycephalum* sequences also indicated an ancient divergence, the *Didymium nigripes* and *D. iridis* sequences indicated a closer relationship. However, the Kerr culture used in this study and designated as *D. nigripes* is generally regarded as a *D. iridis* strain by most researchers in the field (BETTERLEY & COLLINS 1983; CLARK 1995). The major message from this limited DNA sequence data is that the myxomyetes are a very old group and that even species placed in the same genus may have diverged from each other in ancient times.

Proteins

Protein studies have also been very limited in the myxomycetes. The first report on myxomycete proteins (FRANKE & BERRY 1972) dealt with a limited number of physaraceous species and indicated that isozymes were probably taxonomically valid characters in this group. However, it was not until a number of *Didymium iridis* morphospecies isolates were examined (BETTERLEY & COLLINS 1983) that isozyme data relevant to species level taxonomy were found. They worked with 28 isolates, divided by reproductive systems into 19 non-heterothallc (apomictic) isolates and three reproductively isolated mating series (biological species) consisting of seven isolates (from Central America), one isolate (from Kentucky) and one isolate (from Georgia), respectively. They found that the seven isolates in the biological species from Central America shared almost identical isozyme patterns but differed considerably from the isolates of the other two allopatric biological species. These two isolates, which may be sympatric biological species, also differed from each other. All of the non-heterothallic isolates, except for those which had been collected from adjacent sites, also differed from each other and from all three biological species. Isozyme patterns for nine other *Didymium* morphospecies, one to five isolates of each, were also compared for the same set of isozymes and in most cases the pattern fell outside of the range found in the *D. iridis* isolates. Therefore, this research indicated that the *Didymium iridis* morphospecies is divided up into a number of related but genetically isolated apomictic lines and biological species. A recent study of 33 isolates of *D. squamulosum* indicates an even more complex set of relationships among the isolates in this morphospecies (ELHAGE et al. 2000). These isolates consisted of 27 non-heterothallic and three mating series of four (from Costa Rica), one (from Costa Rica) and one (from Puerto Rico) isolates, respectively. A phenogram derived from an analysis of the isozyme data divided the isolates into two branches which partially correlated with two overlapping morphological forms: a short stalked form generally resembling the type description, except that it has minimal lime deposits in culture, and a

longer stalked form approaching the type description of *D. floccoides* NANN.-BREM. & YAMAMOTO. Interestingly, these branches did not correlate with reproductive system differences or the geographical origin of the isolates. The branch with the more typical morphology contained one sexual and 17 non-heterothallic isolates. These isolates, from different regions of Costa Rica and Indonesia, had fairly variable isozyme patterns which did not correlate with their geographical origin, except for a number of non-heterothallic isolates from very restricted local regions. This branch also contained one of the isolates (from Costa Rica) which belonged to the four isolate mating series, i.e., it mated with isolates in the other branch. The branch with the more *floccoides*-like morphology contained five sexual and 10 non-heterothallic isolates. Five of the non-heterothallic isolates were from a single local site and had identical isozyme patterns; however, the other five non-heterothallic isolates and the five sexual isolates were scattered within the branch without regards to geographic origin (various regions of Costa Rica, Puerto Rico and Indonesia) or reproductive system. The three isolates, in this branch, of the four isolate (Costa Rica) mating series were intermixed with the sympatric (Costa Rica) single isolate mating series and two non-heterothallic isolates, and the allopatric (Puerto Rico) single isolate mating series was also adjacent to them in the dendrogram. Apparently, the *D. squamulosum* morphospecies consists of a complex of allopatric and sympatric sexual sibling species and numerous local apomictic lines de-rived from them by suppression of meiosis. Also, while the correlation of morphological differences and isozyme patterns would indicate genetic isolation, the fact that mating can still occur between parts of the two morphological groups would suggest that speciation is not yet complete in this complex. A meetings abstract (CLARK et al. 1999) concerning isozyme patterns in one sexual and 32 non-heterothallic isolates of the *Physarum compressum* morphospecies produced very different results. The isozyme patterns of these isolates produced a phenogram which indicated that, while rarely identical, they were all closely related to each other, including the isolates from various geographic areas (regions of Costa Rica, Puerto Rico, Indonesia and Thailand). While there was some morphological variation between these isolates, especially in terms of the shape of the sporangial head, it was not correlated with any aspect of the isozyme tree or the geographic origin of the isolates. Apparently, this morphospecies consist of a large number of closely related apomictic lines that are probably derived from a relatively small number of sexual isolates by means of meiotic suppression.

Taxonomic Comparisons with Vascular Plant Apomicts

Since apomixis also occurs in the more extensively studied vascular plants, an examination of how this phenomenon is treated in some of these better known genera may help illuminate the current taxonomic problems in the myxomycetes. Flowering plants have certain advantages (size, a large number of easily recognized traits, larger and more complete collections, and more scientists doing taxonomy) over myxomycetes which has produced a better resolution of population and geographic parameters. Thus the size and location of apomictic clones in these genera are often well known and thus biotypes representing stable apomictic clones can be differentiated from non-stabilized recently derived lines. Generally, with this increased knowledge of apomictic vascular plants there has been a tendency toward recognition of these stabilized apomictic clones as species. However, the more frequent the conversion from apomictic to sexual reproduction and back again occurs (and the resulting morphological variability comes closer to a continuum), the more difficult it becomes to distinguish between the apomictic clones. The still unresolved taxonomy of the *Poa pratensis* L. complex, or the concept of "main" and "intermediate" species in *Hieracium* reflects this problem (ZAHN 1987). Another obstacle is the sheer number of apomictic clones, especially in groups which occasionally switch back to sexual reproduction as seen in *Rubus* (brambles), *Hieracium* (dandelions), and the *Rannuculus auricomus* L. aggregate (goldilocks) in Central Europe (Table 2). In *Rubus* only six sexual species

Table 2. Comparison of reproductive systems and current taxonomic approaches in the Myxomycete genus *Didymium* and in the flowering plant genera *Hieracium*, *Rubus*, and *Ranunculus auricomus* agg.

	Didymium	*Hieracium*	*Rubus*	*Ranunculus*
Described taxa	67 species (world) (LADO 2000)	750 main and intermediate species (world) (WEBER 1995)	300-400 sexual, >1000 apomictic species (world) (WEBER 1995)	>650 species (Northern Europe) (ERICCSON 1992)
Reproduction	sexual and apomictic, conversion may occur	sexual and apomictic, conversion occurs regularly, interbreeding between clones	mainly apomictic, conversion and inbreeding can occur	exclusively asexual
Species concepts	typological based on morphology (MATSUMOTO & DEGUCHI 1999); biological for the *Didymium iridis* complex (CLARK & MIRES 1999)	differentiation between main and intermediate (apomictic) species (ZAHN 1987; SCHUHWERK 1996)	apomictic clones with a >50 km diameter range considered species (WEBER 1973, 1996)	four species with apomictic subspecies (MARKLUND 1961, 1965); apomictic clones described as species (ERICCSON 1992)
Traits	5-10[a]	10-20	20-30	10-20
Dispersal	9-12 µm diam airborne spores	0.5-1 mm diam wind dispersed achenes	1-2 cm diam bird dispersed druplets	0.5-2.5 mm diam autochorous or ant dispersed fruits
Range sizes	regional to whole continents; disjunct to continuous	local (isolated rocks) to regional (100-500 km); disjunct to continuous	local to regional; mostly continuous, sometimes disjunct	local to regional; almost exclusively continuous

[a] Number of morphological traits.

(biological species in the sense of this paper) are known, with two of them (*R. ulifolius* and *R. canescens*) forming an agamous complex with about 300 pseudogamous biotypes which occasionally hybridize and form new biotypes (WEBER 1973, 1995). An artificial criterion was introduced to make the taxonomy workable and exclude singular biotypes; with only those having a range extending 50 km in diameter being treated as species (WEBER 1996).

This concept seems to work in *Rubus*, since the biotypes inhabit lowlands and the lower mountains and have seeds dispersed by small songbirds. It cannot work for *Hieracium* since its seeds are wind dispersed and many biotypes inhabit isolated rocky outcrops which often produce highly disjunct ranges. As in *Rubus*, the sexual species (main species) hybridize, but frequent switchbacks to sexual reproduction produce an infinitely large number of "intermediate species" which are often identical with clones forming local populations (SCHUHWERK 1996). In the *Ranunculus auricomus* aggregate hybridization is rather rare since the underlying genetic mechanism includes a dominant, but lethal apospory allele A^-, which favors apomictic biotypes (A^+A^-) besides a few sexual species (A^+A^+). Dispersed by ants, short distance vectors, and with almost exclusive apomictic reproduction, goldilocks are a good example of an apomictic complex (MARKLUNG 1961, 1965). Theoretically, every viable population which represents an apomictic clone could be considered a species, and consequently an increasing number of often locally occurring taxa have been recently described (ERICCSON 1992; HÖRANDL & GUTERMANN 1998).

Even for genera like *Didymium*, which are in general easily grown in culture, our understanding of myxomycete reproductive systems which include apomixis does not match that of comparable vascular plant genera. In addition, myxomycetes have two features which make the way towards a natural system even more difficult: small air-borne spores which can at least theoretically reach every point on earth (long distance dispersal); and apomictic-sexual conversions are probably of frequent occurrence (produces a morphological continuum). Currently in *Didymium* a biosystematic species concept (CLARK & MIRES 1999), that pools

both sexual and apomictic biotypes on the basis of reproduction and morphology, exist besides the traditional typological concept with many morphospecies. In the *D. iridis* complex [here understood to include *D. nigripes* (LINK) FRIES] three species are recognized (CLARK & MIRES 1999), whereas at least 30 morphospecies are described in the complex (LADO 2000). Numerous other myxomycete genera, like the often nivicolous species of *Lamproderma*, also seem to consist of many biotypes. Since, the species of *Lamproderma* can not be grown in culture we have no information concerning their reproductive system. Consequently, only the typological species concept exist for this genus of 36 species of which 14 have been described after 1980 (LADO 2000). In this genus with its comparatively rich pattern of morphological characters, it is likely that apomictic clones have already been described at the species level.

Undoubtedly, the same tendency, as seen in vascular plants, of describing stabilized apomictic biotypes at the species level will occur with an increasing knowledge of myxomycete distribution especially if it is supported by DNA sequencing evidence. To make such a taxonomy workable for non-specialists of the group, an additional concept of biosystematic species of common origin (for apomictic clones) and the ability to interbreed when reproducing sexually (for biological sibling species) would be highly desirable. Under the current code of botanical nomenclature (GREUTER et al. 1994), a possible approach would be to describe stabilized apomictic biotypes at the species level, but treat the groups of biological sibling species and their related apomictic clones as taxonomic aggregates.

Summary

Myxomycete morphospecies are a mix, which varies with the species, of heterothallic sexual isolates consisting of one or more biological sibling species and non-heterothallic isolates divided into numerous related apomictic lines. Thus, widespread cosmopolitan species with considerable morphological variation, such as *Didymium iridis* and *D. squamulosum*, are species complexes consisting of related sibling species (often geographically based) and a swarm of micro-endemic apomictic lines (little evidence of widespread lines). These sibling species and apomictic lines display a fairly wide range of morphological variations, and since each is capable of an independent accumulation of random neutral mutations and specific adaptations, some of them can appear to be morphological unique (especially if the full range of the taxon has not been documented). Other morphospecies, such as *Physarum compressum*, can be quite uniform, both morphologically and genetically, even when divided into numerous genetically isolated taxa.

The fine division of the reproductive system, capability for long distance spore dispersal, and limited morphological repertory produces many overlapping taxa, some of which consist of small geographically restricted populations. While the biological sibling species should be described as separate species, if they are distinct enough to be recognized, the delimitation and description of the apomictic microtaxa, especially on a morphological basis, is probably impossible in most cases due to a lack of characters and disjunct ranges. Therefore, the taxonomic practice, seen in vascular plants, of treating apomictic clones at the species level will be much more difficult in the myxomycetes and probably should not be undertaken except in those cases where they are morphologically distinct and very well known.

However, since almost all identification and taxonomic decisions will continue to be made on the basis of morphological characters, we need to determine which criteria will insure the production of the best taxonomy. Two fairly recent reviews by ELIASSON (1977) and KELLER (1996), respectively have called for the inclusion, whenever possible, of experimental studies in myxomycete taxonomy and have also indicated that the careful study of numerous collections in the context of a wider group (genus) is good taxonomic practice. To this I wish to add that a basic understanding of myxomycete biology and development can help the taxonomist understand the cause and range of morphological variations, and also that a proper understanding of population structure is necessary for the delimitation of a

natural taxonomic system. Now that we know that a majority of individual myxomycete isolates are clonal apomicts that can independently accumulate variations, we have an explanation for the high number of rare myxomycete species which have been described and the concurrent long running disagreements between lumpers and splitters. This and the recent increases in our knowledge of myxomycete biology and genetics has made a reliance on a strict Linnean typological concept indefensible. That is not to say that types are not necessary or good taxonomic practice; in fact, they are still needed and can be quite useful if used as a part of a critical evaluation of taxa (CASTILLO et al. 1997). Therefore, it is suggested that taxonomists undertake the naming of new morphospecies with due care, and that they base their descriptions on a reasonable number of sporophores (as many as possible) collected from a number of different areas (as widespread as possible), combined with a basic understanding of the population and developmental biology of the myxomycetes.

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Literature

ALDRICH H. (1982): Influence of inorganic ions on color of lime in the myxomycetes. — Mycologia 74: 404-411.

ALEXOPOULOS C. J. (1960): Gross morphology of the plasmodium and its possible significance in the relationships among the myxomycetes. — Mycologia 52: 1-20.

BETTERLEY D. & COLLINS O. R. (1983): Reproductive systems, morphology, and genetic diversity in *Didymium iridis* (myxomycetes). — Mycologia 75: 1044-1063.

BLACKWELL M. (1974): A study of sporophore development in the myxomycete *Protophysarum phloigenum*. — Archiv für Mikrobiologie 99: 331-344.

BLACKWELL M. & BUSARD A. (1978): The use of pigments as a taxonomic character to distinguish species of the Trichiaceae (myxomycetes). — Mycotaxon 7: 61-67.

CAIN A. (1957): Animal species and their evolution. – Harper & Row, New York.

CASSER I., STEFFAN B. & STEGLICH W. (1987): The chemistry of the plasmodial pigments of the slime mold *Fuligo septica* (myxomycetes). — Angewandte Chemie 28: 586-587.

CASTILLO A., MORENO G., ILLANA C. & LAGO J. (1997): A critical study of some Stemonitales. — Mycological Research 101: 1329-1340.

CHARVATS I., CRONSHAW J. & ROSS I. (1974): Development of the capillitium in *Perichaena vermicularis*. — Protoplasma 80: 207-221.

CHARVATS I., ROSS I. & CRONSHAW J. (1973): Ultrastucture of the plasmodial slime mold *Perichaena vermicularis*. II. Formation of the peridium. — Protoplasma 78: 1-19.

CHAPMAN C., NELSON R. & ORLOWSKI M. (1982): Peridium of the acellular slime mold *Fuligo septica*: Structure and composition. — Experimental Mycology 6: 195-199.

CLARK J. (1995): Myxomycete reproductive systems: Additional information. — Mycologia 87: 779-786.

CLARK J. & HASKINS E. F. (1998): Heterothallic mating systems in the *Echinostelium minutum* complex. — Mycologia 90: 382-388.

CLARK J. & LANDOLT J. (1993): *Didymium iridis* reproductive systems: Additions and meiotic drive. — Mycologia 85: 764-768.

CLARK J. & MIRES A. (1999): Biosystematics of *Didymium*: The non-calcareous, long-stalked species. — Mycotaxon 71: 369-382.

CLARK J. & STEPHENSON S. L. (1990): *Didymium iridis* reproductive systems: New additions. — Mycologia 82: 274-276.

CLARK J. & STEPHENSON S. L. (1994): *Didymium ovoideum* culture and mating system. — Mycologia 86: 393-396.

CLARK J., COLLINS O. R. & TANG H.-C. (1991): *Didymium iridis* mating systems: Partial compatibility between mating series. — Mycologia 83: 210-213.

CLARK J., IRAWAN B. & STEPHENSON S. L. (1999): Biosystematics of *Physarum compressum*. — Abstracts of the Third International Congress on the Systematics and Ecology of Myxomycetes: 67

COLLINS O. R. (1976): Heterothallism and homothallism: A study of 27 isolates of *Didymium iridis*, a true slime mold. — American Journal of Botany 63: 138-143.

COLLINS O. R. (1979): Myxomycete biosystematics: Some recent developments and future research opportunities. — Botanical Review 45: 145-201.

COLLINS O. R. (1980): Apomictic-heterothallic conversion in a myxomycete, *Didymium iridis*. — Mycologia 72: 1109-1116.

COLLINS O. R. & CLARK J. (1966): Inheritance of the brown plasmodial pigment in *Didymium iridis*. — Mycologia 58: 743-751.

COLLINS O. R. & ERLEBACHER B. (1969): Effects of two muta-

tions on production of a red plasmodial pigment in the myxomycete *Didymium iridis*. — Canadian Journal of Botany **15**: 1245-1247.

COLLINS O. R., GONG T., CLARK J. & TANG H.-C. (1983): Apomixis and heterothallism in *Stemonitis flavogenita* (myxomycetes, Stemonitales). – Mycologia **75**: 614-622.

CZECZUGA B. (1980): Investigations on carotenoids in fungi VII. Representatives of the myxomycetes genus. — Nova Hedwigia **32**: 347-354.

DEMAREE R. & KOWALSKI D. (1975): Fine structure of myxomycetes with clustered spores. — Journal of Protozoology **22**: 85-88.

DOMKE W. (1952): Der erste sichere Fund eines Myxomyceten im baltischen Bernstein. — Mitteilung Geologie Staatsinstitut Hamburg. **21**: 154-161.

DUNN G. & EVERITT B. S. (1982): An introduction to mathematical taxonomy. — Cambridge University Press, Cambridge.

ELDRIDGE N. & CRACRAFT J. (1980): Phylogenetic patterns and evolutionary process: Method and theory in comparative biology. — Columbia University Press, New York.

ELHAGE N., LITTLE C., CLARK J. & STEPHENSON S. L. (2000): Biosystematics of the *Didymium squamulosum* complex. — Mycologia **92**: 54-64.

ELIASSON U. (1977): Recent advances in the taxonomy of myxomycetes. — Botaniska Notiser **130**: 483-492.

ELIASSON U. & SUNHEDE S. (1980): External structure of peridium, pseudocapillitium and spores in the myxomycte genus *Lycogala* ADANS. — Botaniska Notiser **133**: 351-361.

ELLIS T., SCHEETZ R. & ALEXOPOULOS C. J. (1973): Ultrastructural observations on capillitial types in the Trichiales (myxomycetes). — Transactions of the American Microscopic Society **92**: 65-79.

ERICCSON S. (1992): The microspecies of the *Ranunculus auricomus* complex treated at the species level. – Annale Botanici Fennici **29**: 123-158.

FARR M. L. (1982): Notes on myxomycetes III. – Mycologia **74**: 339-343.

FRANKE R. & BERRY J. (1972): Taxonomic applications of isozymes patterns produced with disc electrophoresis of some myxomycetes, order Physarales. — Mycologia **64**: 830-840.

GAITHER T. & COLLINS O. R. (1984): Comparative SEM observations of sporophore characteristics in three species of *Didymium* (myxomycetes, Physarales). — Mycologia **76**: 650-664.

GILERT E. (1995): Taxonomic evaluation of the myxomycete *Calonema luteolum*. — Mycological Research **99**: 311-316.

GREUTER W., BARRIE F. R., BURDET H. M., CHALONER W. G., DEMOULIN V., HAWKSWORTH D. L., JORGENSON P. M., NICHOLSON D. H., SILVA P. C., TREHANE P. & MCNEILL J. (1994): International code of botanical nomenclature (Tokyo Code), adopted by the Fifteenth International Botanical Congress, Yokohama, Aug.-Sept. 1993 – Regnum Vegetabile **131**, Utrecht.

HASKINS E. F. & HINCHEE A. (1974): Light- and ultramicroscopic observations on the surface structure of the protoplasmodium, aphanoplasmodium, and phaneroplasmodium (myxomycetes). — Canadian Journal of Botany **52**: 1835-1839.

HASKINS E. F. & MCGUINNESS M. (1986): Comparative ultrastructural observations of spore wall structure in six species of *Echinostelium* and three species of Eumycetozoa. — Mycologia **78**: 613-618.

HENNEY M. (1967): The mating type system of the myxocmycete *Physarum flavicomum*. — Mycologia **59**: 637-652.

HÖRANDL E. & GUTERMANN W. (1998): Der *Ranunculus auricomus*-Komplex in Österreich. I. Methodik, Gruppierung der mitteleuropäischen Sippen. – Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie **120**: 1-44.

JOHANSEN S., JOHANSEN T. & HAUGLI F. (1992): Extrachromosomal ribosomal DNA of *Didymium iridis*: Sequence analysis of the large subunit ribosomal RNA gene and sub- telomeric region. — Current Genetics **22**: 305-312.

KALYANASUNDARAM I. & ALI M. (1989): Taxonomic note on the myxomycete genus *Diachea*. — Mycological Research **93**: 235-237.

KELLER H. (1996): Biosystematics of myxomycetes: A futuristic view. — Abstracts of the Second International Congress on the systematics and Ecology of the myxomycetes: 23- 37.

LADO C. (2000): Nomenmyx. A nomenclatural taxabase of myxomycetes. – Manuscript, Real Jardin Botanico CSIC, Madrid.

LOGANATHAN P., PARAMASIVAN P. & KALYANASUNDARAM I. (1989): Melanin as the spore wall pigment of some myxomycetes. — Mycological Research **92**: 286-292.

MARKLUNG G. (1961): The *Ranunculus auricomus* complex in Finland. I. Diagnose und Fundortslisten einiger Sippen des *R. auricomus* L. coll. (s. str.). – Flora Fennica **3**: 1-128.

MARKLUNG G. (1965): The *Ranunculus auricomus* complex in Finland. II. Diagnosen und Fundortslisten einiger Sippen von *R. fallax* (W. & GR.) SCHUR, *R. monophyllus* OVEZ., und *R. cassubicus* L. — Flora Fennica **4**: 1-198.

MARTIN C. W. & ALEXOPOULOS C. J. (1969): The myxomycetes. — University of Iowa Press, Iowa City.

MATSUMOTO J. & DEGUCHI H. (1999a): Two new species of *Didymium* (Physarales, myxomycetes) from Japan. – Mycotaxon **70**: 153-161.

MATSUMOTO J. & DEGUCHI H. (1999b): Taxonomic studies of the genus *Didymium* from Japan. — Abstracts of the Third International Congress of the Systematics and Ecology of Myxomycetes: 75.

MAYR E. (1970): Population, species, and evolution. — Belknap Press, Harvard University Press, Cambridge.

METTLER L., GREGG T. & SCHAFFER H. (1988): Population genetics and evolution. 2nd ed. — Prentice Hall, Englewood Cliffs.

MILLER D. & KRISHNAN U. (1999): Phylogenetic analysis of myxomycetes using DNA sequence alignment of

nuclear and mitochondrial rDNAs and RNA editing site location. — Abstracts of the Third International Congress on the Systematics and Ecology of myxomycetes: 40.

MIMS C. (1969): Capillitium formation in *Arcyria cinerea*. — Mycologia **61**: 784-798.

MIMS C. (1972): Spore-wall formation in the myxomycete *Arcyria cinerea*. — Transactions of the British Mycological Society **59**: 477-481.

MIMS C. (1973): A light and electron microscope study of sporulation in the myxomycete *Stemonitis virginiensis*. — Protoplasma **77**: 35-54.

MIMS C. & ROGERS M. (1975): A light and electron microscopic study of stalk formation in the myxomycete *Arcyria cinerea*. — Mycologia **77**: 638-649.

NELSON R., SCHEETZ R. & ALEXOPOLOS (1977): Elemental composition of *Metatrichia vesparium* sporangia. — Mycotaxon **5**: 365-375.

RAMMELOO J. (1974a): Ornamentation of the peridium inner side in the Trichiaceae (myxomycetes), as seen with the scanning electron microscope. — Bulletin de la Societe Royale de Botanique de Belgique **107**: 291-304.

RAMMELOO J. (1974b): Structure of the epispore in the Trichiaceae (Trichiales, myxomycetes), as seen with the scanning electron microscope. — Bulletin de la Societe Royale de Botanique de Belgique **107**: 353-395.

RAMMELOO J. (1975): Structure of the epispore in the Stemonitales (myxomycetes) as seen with the scanning electron microscope. — Bulletin de la Nationale Plantentuin van Belgie **45**: 301-306.

REBHAHN M.-A., SCHNITTLER M. & LIEBERMANN B. (1999): Taxonomic relevance of pigment patterns in *Arcyria* species (Trichales, myxomycetes). – Nova Hedwigia **69**: 415-427.

ROSS I. (1957): Capillitium formation in the Stemonitaceae. — Mycologia **49**: 809-819.

ROSS I. (1973): The Stemonitomycetidae, a new subclass of myxomycetes. — Mycologia **65**: 477-485.

SCHEETZ R. & ALEXOPOULOS C. J. (1971): The spores of *Badhamia gracilis* (myxomyctes). — Transactions of the American Microscopical society **90**: 473-475.

SCHOKNECHT J. (1975): SEM and X-ray microanalysis of calcareous deposits in myxomycete fructifications. — Transactions of the American Microscopic Society **94**: 216-223.

SCHOKNECHT J. & KELLER H. (1977): Peridial composition of the white fructifications in the Trichiales (*Perichaena* and *Dianema*). – Canadian Journal of Botany **55**: 1807-1819.

SCHOKNECHT J. & KELLER H. (1989): Peridial calcification in the myxomycetes. — In: CRICK R. (ed.): Origin, evolution, and modern aspects of biomineralization in plants and animals. Plenum Press, New York: 455-488.

SCHOKNECHT J. & SMALL E. (1972): Scanning electron microscopy of acellular slime molds (Mycetozoa-myxomycetes) and the taxonomic significance of surface morphology of spores and accessory structures. — Transactions of the American Microscopic Society **91**: 380-410.

SCHUHWERK F. (1996): Kommentierte Liste der bayerischen *Hieracium*-Arten. Teil I. – Berichte Bayerischen Botanischen Gesellschaft **66/67**: 137-152.

SONNEBORNE T. M. (1957) Breeding systems, reproductive methods and species problems in Protozoa. – In: MAYR E. (ed.): The species problem. American Association for the Advancement of Science, Washington D.C.: 155-324.

STEGLICH W., STEFFAN B., KOPANSKI L. & ECKHARDT G. (1980): Indole pigments from the fruiting bodies of the slime mold *Arcyria denudata*. — Angewandte Chemie **19**: 459- 460.

WAGGONER B. & POINAR G. (1992): A fossil myxomycete plasmodium from eocene-oligocene amber of the Dominican Republic. — Journal of Protozoology **39**: 639-642.

WEBER H. E. (1973): Die Gattung *Rubus* L. (Rosaceae) im nordwestlichen Europa vom Nordwestdeutschen Tiefland bis Skandinavien unter besonderer Berücksichtigung Schleswig-Holsteins. – Mitteilungen der AG für Floristik in Schleswig-Holstein und Hamburg **22**: 1-504.

WEBER H. E. (1995): 4. *Rubus*. In: HEGI G. (ed.): Illustrierte Flora von Mitteleuropa, 4(2A), Spermatophyta, Angiospermae: Dicotyledones 2(2). Blackwell Scientific, Berlin: 284-595.

WEBER H. E. (1996): Former and modern taxonomic treatment of the apomictic *Rubus* complex. – Folia Geobotantica et Phytotaxonomica **31**: 373-380.

WELDEN A. (1955): Capillitial development in the myxomycetes *Badhamia gracilis* and *Didymium iridis*. — Mycologia **47**: 714-728.

ZAHN K.-H. (1987): *Hieracium* L. — In HEGI G. (ed.): Illustrierte Flora von Mitteleuropa, 6. Angiospermae, Dicotyledons 4(4). Parey Verlag, Berlin, Hamburg: 1182-1351.

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