More Than 50 Years with Myxomycetes (Plasmodial Slime Molds): Highlights and Review

Harold W. Keller

Botanical Research Institute of Texas, Fort Worth, Texas 76107, USA

Abstract: My first myxomycete collection of *Dictydium cancellatum* is described. Myxomycete morphospecies concepts are discussed, reference sources given, and criteria and options suggested for the recognition of species new to science. Taxonomic assessment of fruiting body variation is given for *Fuligo septica* and spore ornamentation for *F. megaspora*. Variations of fruiting body characters are discussed for spore-to-spore agar cultures of *Badhamia rhytidosperma* and *B. spinisporum*. A suggested protocol for best taxonomic practice is provided that recognizes the impact of environmental parameters on the plasticity of fruiting body characters using *Cribraria intricata* and *Badhamia rugulosa* as examples. The importance of type collections is discussed, using fine ultrastructure scanning electron microscopy and *Badhamia ovispora* as an example. Monographic publications are emphasized with examples that include *Perichaena brevifila* and *P. reticulospora*. Recent publications documenting spore-to-spore agar cultures with assessment of spore ornamentation and commentary on clustered versus free spores are described.

Key words: Best taxonomic practice; *Badhamia ovispora*; *B. rhytidosperma*; *B. rugulosa*; *B. spinisporum*; *Cribraria intricata*; *Fuligo septica*; *F. megaspora*; moist chamber cultures; morphospecies concepts; *Perichaena brevifila*; *P. reticulospora*; species new to science; spore-to-spore agar culture

1 My fascination with Myxomycetes

My first encounter with Myxomycetes was on a field trip into the mixed hardwood forests near Lawrence, Kansas, while a Master's Degree graduate student in the Department of Botany at the University of Kansas in the early 1960s. Like most

beginner collectors I followed the habitat descriptions in books that directed me to decaying logs or leaf litter as the most productive ground sites.

I discovered and collected stalked sporangia of *Dictydium cancellatum* that covered an extensive area on a well-decayed log as part of the ground litter. Upon closer microscopic examination the spore case resembled a bird cage with dark longitudinal ribs connected by hyaline transverse filaments. There was a ball-like mass of aggregated spores freely suspended inside the spore case that was gradually released as the twisted stalk created movements caused by the slightest air currents or physical touch. This salt-shaker method of spore dispersal is often exhibited by moss capsules, for example, *Atrichum* and *Polytrichum*, which release a few spores over time favoring wind dispersal. The myxomycete genus *Cribraria* also often has weaker flexuous stalks that are twisted and facilitates the spores sifting through the peridial network, hence the name *Cribraria* translated from *cribrum*, meaning a sieve.

What fascinated me the most was the intricate structure of the sporangium that reminded me of the phrase "the biological jewels of nature" and the incredible beauty of the form and function represented by myxomycete fruiting bodies. The display of the iridescent brilliant coloration of the *Diachea* or *Lamproderma* species; the intricate ornamentation of the *Hemitrichia* and *Trichia* capillitial threads; the pattern of spore ornamentation from warted to spiny to bordered reticulate, and also clustered spores; the infinite variety of sizes and shapes of fruiting bodies—all make the study of this group of organisms aesthetically pleasing and challenging to find the words that describe this beauty! Yes, beauty is in the eye of the beholder, but for me, the Myxomycetes have piqued my curiosity for more than 50 years.

2 ICSEM 2 Inaugural Lecture

I was invited to deliver the Inaugural Lecture at the 2nd International Congress on the Systematics and Ecology of Myxomycetes (ICSEM 2) held at the Real Jardín Botánico, Madrid, Spain. My paper was published as part of the proceedings under the title "Biosystematics of Myxomycetes: A Futuristic View" (Keller 1996). There were a number of topics of historical interest highlighted such as information relating to myxomycete collectors and mentors Dr. Travis E. Brooks and Professor George W. Martin (the latter my doctoral dissertation supervisor at the University of Iowa), field collecting associates, importance of myxomycete biosystematics, promotion of professional and public interest, best taxonomic practice, importance of ecological

field observations, importance of collecting, importance of type collections, importance of spore-to-spore cultivation, living cultures a biological standard, importance of monographic works, importance of computerization of mycological collections, importance of DNA sequencing techniques, future directions, and concluding remarks. I highly recommend that beginning students of Myxomycetes read this paper to better understand some of the guiding principles behind myxomycete systematics.

3 Morphospecies concepts in myxomycete taxonomy

There are five other papers that merit special consideration when applying the morphospecies concept to species new to science: (1) "The Species Problem in the Myxomycetes" (Clark 2000); (2) "Species Diversity in Myxomycetes Based on the Morphological Species Concept — a Critical Examination" (Schnittler & Mitchell 2000); (3) "From Morphological to Molecular: Studies of Myxomycetes since the Publication of the Martin and Alexopoulos (1969) Monograph" (Stephenson 2011); (4) "Myxomycete History and Taxonomy: Highlights from the Past, Present, and Future" (Keller 2012); and (5) "Sporophore Morphology And Development in the Myxomycetes: a review" (Clark & Haskins 2014). These papers discuss some of the problems in myxomycete taxonomy posed by the variability of fruiting body types (sporangium, plasmodiocarp, pseudoaethalium, aethalium) and the basic morphological characteristics used in species descriptions that include but are not limited to (1) fruiting body general habit, color, size of overall dimensions, sessile and stalked in the same collection; (2) peridium when present exhibiting dehiscence patterns, thickness, number of layers, amount of calcareous deposition with either granules or crystals, portion forming a network or cup, inner surface markings; (3) columella presence or absence; (4) capillitium presence or absence, amount and branching patterns and attachments, degree of calcification, capillitial threads with cogs, rings, reticulations or elaters smooth or marked with spines or spiral bands, diameter of width; (5) spore color in mass or by transmitted light, ornamentation either smooth, warted, spinulose, spiny, special markings, bordered reticulate, wall thickness, and spores clustered or free, shape, and size in diameter.

Assessment, evaluation, and examples found in species descriptions in books and journal papers will serve to illustrate potential variation of fruiting body characters. *Fuligo septica* is a myxomycete genus and species characterized by an

aethalium fruiting body type and was a species described by Linnaeus in 1780. The myxomycete world monograph by Martin & Alexopoulos (1969) lists *F. septica* with 36 synonyms, including 6 different genera. In the notes following the species description they state:

> "One of the commonest and widely distributed of Myxomycetes. Its extraordinary variability in size, shape, and color is reflected in the numerous names which it has received. The cortex may be very thick or sparse or even lacking, in which case the fruitings appear to be densely clustered and anastomosing sporangia on a common hypothallus, but all have the minutely warted, rather pale spores and there seems to be no way to separate them into coherent subgroups."

Furthermore, the varieties *candida*, *violacea*, *flava*, and *rufa* apparently "do no more than name the color involved" (Martin & Alexopoulos 1969). The color variants are very different in appearance in their striking colors, but an additional suite of distinguishing characters was lacking. However, they also emphasized the following:

> "Unless cultural studies can demonstrate that some of those variations are due to more than a response to conditions under which the plasmodium developed and fruited, they should be disregarded."

Spores of *Fuligo septica* germinate readily in a short period of time, usually within 30 to 90 min (Braun 1971; Keller & Everhart 2010). Fresh aethalia several weeks old gave a higher percentage of spore germination by the split method, forming myxoamoebae and swimming swarm cells as long as free water was present. My students cultured many isolates of *F. septica* from spore to spore on 2% water agar. Yellow phaneroplasmodia fed sterile old fashioned oat flakes were observed and eventually aethalia formed typical of this species. Spores were observed for ornamentation, and at least 100 spore diameters were measured and compared to field collections and found to agree with the species description in Martin & Alexopoulos

(1969): "spores spherical, dull black in mass, purplish, brown by transmitted light, minutely spinulose, 6 – 9 μm in diameter." In other words the spore size and ornamentation were stable in culture suggesting that spore characters were reliable for *F. septica*. In the varieties of *Fuligo* discussed here and in the majority of species (4 of 5) recognized by Martin & Alexopoulos (1969), spores were minutely warted and 6 – 14 μm in diameter. Please note the discrepancy between the species spore ornamentation description and the commentary note that followed (Martin & Alexopoulos 1969).

There was one exception, *Fuligo megaspora*, which merits special consideration. This species was considered rare until Dr. Jean Schoknecht made numerous collections in the Everglades National Park in the state of Florida, USA (Keller & Schoknecht 1989). The spore ornamentation previously was misdescribed using light microscopy (Sturgis 1913; Macbride 1922; Martin & Alexopoulos 1969) as tuberculate arranged in irregular lines, uniformly warted with patches of a close spinulose reticulation, and rough tuberculate to subreticulate markings with the size range of 20 – 22 μm in diameter. This spore ranks as one of the largest in the Myxomycetes and was clearly a *Fuligo* based on the aethalium. This example points out the importance of more accurate species descriptions using scanning electron microscopy that increases the magnification and shows fine structure, in this case, an episporic reticulum with a serrated upper edge (see Figs. 13 and 14 in Keller & Schoknecht 1989). This spore ornamentation is unique in the Myxomycetes. Furthermore, new characters not previously used or recognized sometimes may be helpful in distinguishing species. Granular calcium carbonate ("lime") in the aethalial cortex of *Fuligo* species may show size differences (see Figs. 6 – 10 in Keller & Schoknecht 1989), but this must be tested and compared in different taxa. Spherical calcareous granules ("lime") in the aethalial cortex of *F. cinerea* measure approximately 1 – 1.5 μm in diameter in contrast to that of *F. megaspora* which is more than twice as large at 2 – 4 μm in diameter (Keller & Schoknecht 1989).

Negative culture results often go unreported. Thus, at least 20 mass spore cultures of different *F. megaspora* collections on 2% water agar failed to show spore germination, plasmodial formation, or fruiting body formation (unpublished observations). These starter field collections were fresh (only several months old) but failed to produce spore germination, unlike *F. septica*. Culture work is sometimes frustrating, time consuming, and unrewarding, but more of this kind of research is

needed to evaluate species concepts and morphology.

Another surprising discovery examining *Fuligo* collections was a specimen deposited at the US National Fungal Collections (BPI) misidentified as *F. megaspora*, but differed in having spores with long spines (see Figs. 11 and 12, Keller & Schoknecht 1989). The conspicuous long spine was different spore ornamentation from any species of *Fuligo*, and the size range in spore diameter of 16 – 19 μm, was much larger than other *Fuligo* species and more in the size range of *F. megaspora*. Did this taxon represent a new species? More collections were needed, but the collection site of Lignumvitae Key Botanical State Park is an isolated island off the coast of the Florida Keys, USA. that can only be reached via water passage. Dr. Schoknecht was able to visit this island several years later and collected a *Fuligo* along the trail. Upon returning to the laboratory, microscopic examination revealed the long spiny spores and a spore diameter of 16 – 19 μm. Scanning electron microscopic (SEM) images highlighted the long spines not seen in any other *Fuligo* species. Unfortunately the collections and SEM negatives were lost, and attempts to find more specimens failed. This taxon to the best of my knowledge has not been described as new to science in the literature, but it would not surprise me that it will be found someday on that remote island or somewhere else in subtropical habitats. The question still remains, however, whether the spiny-spore character alone is enough to describe a new species or whether associated characters will serve to distinguish this taxon. The next generation of myxomycologists hopefully will search for and discover this taxon and evaluate additional morphological characters.

4 Assessment of variation in spore-to-spore agar cultures

Spore to spore cultivation of two *Badhamia* species, *B. rhytidosperma* and *B. spinisporum*, was also instructive in the variable calcium carbonate deposition in the peridium, in the capillitial system, and also in the formation of a calcareous pseudocolumella (Keller & Schoknecht 1989a, 1989b). Both of these species have unique spore ornamentation: *B. spinisporum* with spores spiny on one side and smooth on the other side and *B. rhytidosperma* wrinkled-reticulate on one side and smooth on the other side with a ridge line marking the future site of spore germination. *Badhamia* and *Physarum* are artificial genera that merge into one another, and many species have been transferred back and forth between the two genera based on the amount of calcium carbonate deposition in the capillitial system. *Physarum* is

characterized mostly by a system of hyaline capillitial threads lacking calcium carbonate except for the capillitial nodes, and *Badhamia* is characterized mostly by a network of calcareous capillitial tubules. These two taxa were grown on dung and water agar, and fruiting bodies were compared. Spore ornamentation and size were constant and stable under all conditions of cultivation. Dung cultures had greater concentrations of calcium carbonate in capillital tubules and a more prominent calcareous-filled pseudocolumella when compared to the scanty calcium in agar-developed fruitings (Keller & Schoknecht 1989b).

Premature drying also interferes with normal spore cleavage and development, resulting in aberrant giant spores, and free water or excessive wet surfaces also result in fruiting bodies that lack calcium carbonate deposits and altered shapes (Tamayama & Keller 2013). These same conditions may occur with natural fruitings in the field on different substrata and may give similar results as observed in agar cultures. The best example was *Physarum crateriforme* that had developed on the bark surface of living trees (see Keller & Braun 1999: 129), and the following quotation:

> "Whenever extensive calcareous deposits are present, the white peridium, the columnar columella reaching the upper sporangial apex, and the badhamioid capillitium, coupled with the cylindrical to obovate sporangial shape and black stalk, make this species easy to identify in the field. Under certain conditions it can be troublesome to identify because of extreme variability in sporangial shape and degree of calcification. For example, in some cases the capillitium may consist of hyaline threads lacking calcified nodes and also no apparent columella. These noncalcareous sporangia are often brownish, in contrast to the white color when calcium is present. These fruitings are impossible to identify because spore size and ornamentation fall within the range of many other species."

Persistent long-lasting aethalia of *Fuligo* on ground sites and stalked sporangia of *P. crateriforme* on the bark surface of living trees may undergo physical changes, especially the loss of calcium carbonate deposits due to weathering effects. These morphological changes must be considered by making many collections of the same

taxon in the field over time on different substrata to circumscribe the full variation exhibited.

5 Protocol for best taxonomic practice

There is no substitute for collecting specimens in the field and recording the careful observation of habitats, substrata, seasonal occurrence, and variation of fruiting body characters that develop under different environmental conditions. Field collections made from different geographical locations over longer periods of time, including the observation of plasmodia and the formation of the fruiting bodies on natural substrata, result in more accurate species descriptions for monographic works and species descriptions new to science. Single field collections made on single field forays often result in limited material for type specimens and inadequate species descriptions. The following quotation captures the essence of multiple collections made in multiple locations (Keller 2012).

> "One fact should be stressed. The plasmodium may develop its characteristic fruiting stage in less than 24 h. If this occurs under conditions which cause unduly rapid drying or if repeated rains check the process, great variation may be induced. Under such influences, species which ordinarily have stalks may be sessile or nearly so, or the stalks may be inordinately long; sporangiate species may form plasmodiocarps; aethalioid forms may approach the sporangiate type; the characteristics and disposition of limey secretions may be altered; spore maturation may be checked, resulting in spore-like bodies which are much larger than fully matured spores. Cold weather, and particularly frosts, may induce similar alterations. Such variations are in large part responsible for the extensive synonymy found in the group. Great caution is indicated in describing as new specimens that are the result of such environmental responses. They are not 'abnormal'; they are natural responses of the organisms involved to particular stimuli and must be so regarded. Giving them taxonomic

> status as named varieties serves only to complicate the nomenclature and to extend the meaning of the category variety beyond its legitimate significance" (Martin & Alexopoulos 1969).

I am also reminded of an extensive fruiting of *Cribraria intricata* growing on well decayed decorticated wood of a coniferous log on a moist ground site in a heavily wooded forest — collection *HWK* #2898, Moro Bay State Park, Bradley County, July 8, 1989, State of Arkansas, USA. This fruiting covered several feet beginning at the underside of the log near ground level that was still moist and had various stages of sporangial development including mature long-stalked sporangia. This fruiting consisted of thousands of sporangia that covered the sides of the coniferous log from bottom to top where it had dried more quickly because of more exposure to the drying action of air currents. The stalked sporangia at the bottom had longer stalks and as the sporangia progressed toward the top of the log the stalks became shorter and shorter until some sporangia at the top were almost sessile. This fruiting was the same species and not intermixed with several species that sometimes happens with species of *Cribraria*. This is another example of stalk length variability when subjected to moisture gradients in nature.

Badhamia rugulosa is another example of a species new to science that was collected over a 13 – year period in four states (Florida, Kentucky, Missouri, and Ohio) and was based on 59 ample collections (Keller & Brooks 1975). Since that time additional collections have been made in Arkansas, Tennessee, and Texas. This species was found most frequently on the bark surface of living *Juniperus virginiana* (Eastern Red Cedar) trees and species of *Vitis* (grapevine). The following quotation documents an observational field experiment that records the variation in fruiting body morphology over time. This location is near Fairborn, Greene County, Ohio, USA.

> "A single living *J. virginiana* tree, one among many large red cedar trees, located at John Bryan State Park in the lower picnic grounds was observed over a two-summer period. *Badhamia rugulosa* regularly formed extensive fruitings on this tree after rainy periods. When fructifications were observed immediately following fruiting, they appeared bright orange with the peridium rugulose above. After

> exposure to rains, the calcareous peridium and capillitial system showed varying degrees of bleaching from dull orange to white, eventually the only trace of the former bright orange color being the brownish streaks at the base of the fructifications. The calcareous peridium had often assumed a smoother quite different appearance when compared to unweathered specimens" (Keller & Braun 1999).

There are many other examples that demonstrate this plasticity and variability of myxomycete fruiting bodies, but these will suffice for this discussion.

Moist chamber cultures are often used to survey a given area or to find additional myxomycete specimens from habitats that may be too dry, too wet, or temperatures too cold or have less than ideal environmental conditions to produce fruiting bodies. This culture technique has produced many new distribution records for different countries or specific areas and at the same time has yielded species new to science. However, caution is required because moist chambers often produce aberrant or fewer fruiting bodies and therefore may pose additional problems when single or only a few collections serve as this only source of a species new to science. Too often moist chamber cultures of bark from living trees result in specimens with different collection numbers based on the date harvested from the cultures. However, all of the collections have come from a single tree and a single location and fail to meet the criterion of multiple collections from multiple different locations.

6 The importance of type collections

The literature is full of too many examples of species new to science that lack sufficient quantity of collections to (1) prepare a good species description; (2) a type specimen with adequate fruiting bodies to study with light or scanning electron microscopy; (3) serve to compare closely related species to determine if morphological characters are distinct or intergrade. Therefore, multiple collections from multiple locations will not only provide better source material for selection of a holotype collection but also enough material for isotypes and paratypes. This takes more time and will avoid the "rush to publish" especially if additional collections are obtained on loan from herbaria to compare morphological characters.

The importance of selecting and the study of type specimens cannot be

overemphasized. These are the nomenclatural and morphological standards used for the taxonomy of both fossil and living organisms. Paper descriptions of supposedly species new to science are not always reliable as a substitute for holotype collections. One of the best examples is *Badhamia ovispora* described by Marian Raciborski (1863 – 1917) who was Director of the Department of Botany and the Botanic Garden of the Jagiellonian University in Cracow, Poland (1912 – 1917). Raciborski had followed in the footsteps of the previous director Józef Thomasz Rostafiński (1850 – 1928), also a renowned myxomycologist. The holotype collection was deposited in KRAM Polish Academy of Sciences.

A specimen collected by Henry Aldrich (*HA* #13) August 21, 1964, near Nederland, Colorado, Boulder County, USA, on a decaying gymnosperm log was abundant and in excellent condition. The spores were unique in shape (reniform to allantoid, referred to as hotdog-shaped) and with ornamentation as raised plaque-like areas (see Keller et al. 1975, Figs. 5 – 8, 13). Specimens sent to other myxomycologists all resulted in a declaration that this was a species new to science. It took numerous requests and about three years to obtain Raciborski's holotype collection.

Instead of publishing a new species I waited to study the holotype and compare it to Raciborski's published description to determine accuracy: "Spores variable in shape, ellipsoidal, rarely spherical, (14.5 – 16.5) μm × (7.5 – 8.3) μm and smooth" (Keller et al. 1975). Scanning electron micrographs of the holotype spores (see Keller et al. 1975, Figs. 9 and 10) show the elongate hotdog-shape and raised plaque-like areas on the spore surface. These spore characteristics were not included in the Raciborski holotype description. The raised plaque-like spore ornamentation in *HA*#13 can clearly be seen under the oil immersion lens using light microscopy at approximately 1000 × and the hotdog shapes are obvious at lower magnifications (see Figs. 5 – 7, Keller et al. 1975). The freeze-etched preparation of spores from *HA* # 13 shows the raised plaque-like areas in detail (Keller et al. 1975, Fig. 13). The use of higher magnification optics, especially scanning and transmission electron microscopy at 3250 × to 9120 ×, provided ornamentation evidence not seen at lower magnifications. The majority of myxomycete species have spores that are spherical or nearly so, except for *B. ovispora* that has an elongate hotdog shape. This is a rare species seldom collected but recently found by Y. Mourgues at Le Monêtier-les-Bains, a commune in the Hautes-Alpes department in southeastern France, on bark

of a decaying *Fraxinus* log at an elevation of 1500 m and illustrated by Poulain et al. (2011).

7 Monographic concerns and considerations

Monographic publications are becoming a thing of the past. My monograph of the genus *Perichaena* (Keller 1971) included 13 species, and two of these, *P. brevifila* (MycoBank #319341) and *P. reticulospora* (MycoBank #319342), represented species new to science. Please note that *Mycologia* now requires a registered accession number in MycoBank. There was then and even now a trend to publish species new to science as short papers, emphasizing numbers over a single publication with a section of a genus or the entire genus. It was unfortunate my doctoral dissertation was never published as a single publication but was split into several papers, for example, *P. brevifila* (Keller & Brooks 1971), *P. reticulospora* (Keller & Reynolds 1971), and the spore-to-spore cultivation of *P. depressa* and *P. quadrata* (Keller & Eliasson 1992), and several other papers that included different portions of the thesis (Schoknecht & Keller 1977; Keller & Everhart 2008).

The two species new to science were represented by only 12 collections (all in deep compacted leaf litter only in the fall months) from three states Georgia, Kansas, and Virginia in the case of *P. brevifila*, and only a single collection from the type locality for *P reticulospora*. In the latter case, the spores were bordered reticulate and unique for the genus *Perichaena*, and that is true today, even though the number of new taxa have expanded the genus to 26 species (Lado et al. 2009). This means that the total number of species new to science in the genus *Perichaena* has doubled in the last 38 years and that represents quite a remarkable number. Most of the *Perichaena* species new to science more recently have come from previously unexplored semiarid and arid areas of the world (Lado et al. 2009).

The Keller unpublished thesis (1971) had scanning electron micrographs for most of the *Perichaena* species, and in the case of *P. reticulospora* (Keller & Reynolds 1971) these were the first SEMs published of a myxomycete species new to science. This was the beginning of a new era when scanning electron microscopy would begin to usher in the importance of fine structure and detail of spore ornamentation. It has been more than 43 years, and *P. reticulospora* is still only known from the type locality in a secondary forest at the University of San Carlos Biological Station, Philippines. I am certain this species will be found in other

tropical countries of southeast Asia even though it will be difficult to collect in the field because of its small size and occurrence as scattered stalked sporangia on decaying leaf litter on the forest floor. The participants in ICSEM 8 should read the species description of *P. reticulospora* and look for it in tropical forest ecosystems on ground litter.

8 Importance of spore-to-spore agar culture and spore characters

Spore-to-spore agar cultures should be encouraged and attempted. There are many recent examples represented in the papers published by Diana Wrigley de Basanta and Carlos Lado from Madrid, Spain, that should be consulted and followed as a model for future culture work (Lado et al. 2007, *Didymium wildpretii*; Mosquera et al. 2003, *Licea succulenticola*; Wrigley de Basanta & Lado 2010, *Licea eremophila*; Wrigley de Basanta et al. 2009, *Didymium infundibuliforme*; Wrigley de Basanta et al. 2011, *Didymium operculatum*; Wrigley de Basanta et al. 2012, *Physarum atacamense*). The combination of light microscopy of life cycle stages grown in culture and excellent scanning electron micrographs of spores merit special consideration. All of these examples of species new to science were collected in the field then grown in agar culture from spore-to-spore. *Didymium operculatum* spores were described as globose, (9.5) 10 – 11 (– 12.5) μm in diameter, banded reticulate with 9 – 12 meshes per hemisphere and with a second reticulum underneath, visable through the meshes as seen by SEM. These spore characters alone are distinct and would separate this species from all other known described myxomycete species, but more importantly, the spore characters were stable, constant, and reliable as taxonomic key characters.

Spore ornamentation and spore size appears to be a stable character with less variation as noted in the above examples. However, are these spore characters or any character measurable by DNA techniques? Schnittler & Mitchell (2000) in Table 1 noted clustered-spored species (adhering together in groups of 2 to 40) versus free-spored (single spore) species. They questioned the clustered-spore character as possibly being the result of a single gene mutation. Their discussion noted 28 taxa that have clustered spores listed along with rarity status, number in spore clusters, and possible equivalent species. Nine *Badhamia* species with clustered spores do not have species equivalents in the table, and to the best of my knowledge, there has

been no DNA study to help determine the degree of separation for any of these species. I have collected and studied some of these species (number of collections in parentheses): *Badhamia crassipella* (27), *Didymium synsporon* (22), *Minakatella longifila* (20), *Perichaena syncarpon* (25), and *Physarum synsporum* (14) (Keller & Braun 1999).

Badhamia crassipella is known from the states of California and Washington, U. S. A., and although listed in category 2 by Schnittler and Mitchell (2000) it actually falls in category 3 (more than 20 collections designated as more common taxa) with no known species equivalent. This species was described as new by Whitney and Keller (1982), and the plasmodiocarpous habit distinguishes this species from other members of *Badhamia*. This taxon's clustered spores (4 – 40) do not appear to be a single gene mutation.

Didymium synsporum is a corticolous myxomycete known from the states of Arkansas, Georgia, Kansas, Kentucky, Ohio, and Tennessee, USA, and from the bare bark surface of living *Juniperus virginiana* trees. Spores adhere in firm clusters of 4 – 25 (Keller & Braun 1999). This species is most closely related to *D. difforme* (listed as the equivalent species, (Schnittler & Mitchell 2000), but the capillitium of mostly branching and anastomosing threads differs from the mostly upright, less branching capillitial threads of *D. synsporon* attached above to the peridium and below to the base of the fruiting body.

Minakatella longifila is a corticolous myxomycete only known from the bark of living trees and grapevines located in the states of Illinois, Iowa, Kentucky, Missouri, Ohio, and West Virginia, USA, and in Italy and Japan. It is probably more common and widespread than once thought (Keller et al. 1973; Keller & Braun 1999). There is no equivalent species (Schnittler & Mitchell 2000), but it is listed as a clustered (4 – 14) spored species.

Perichaena syncarpon is found on leaf litter that has accumulated under shrubbery around buildings and among decaying leaves under herbaceous plants and grasses in iris or lily beds. It is known from the states of Iowa and Kansas, USA. The type locality, in Junction City, Kansas, has been destroyed as part of a housing development (Keller 1971). Spores adhere in clusters of 4 – 16; *P. depressa* is listed as the free-spored species equivalent by Schnittler and Mitchell (2000). The pseudoaethalioid to aethalioid fructifications with dehiscence by lobes along prominent ridges or irregular aerolae in *P. syncarpon* differs from *P. depressa* with crowded and

depressed sporangia on decayed wood with circumscissile dehiscence at the margins. There is no species equivalent to *Perichaena syncarpon* in the genus *Perichaena*, suggesting that in this case it is not a single gene mutation.

Physarum syncarpon was described as new to science by Stephenson & Nannennga-Bremekamp (1990) based on two moist chamber developments of bark from a living *Juniperus virginiana* tree from the same locality of Nicholas County, West Virginia, USA. This is an example of a new species based only on moist chamber cultures from a single locality and possibly a single tree. The description is based on line drawings and light microscopy. Indeed, all of the five new species of Myxomycetes (*Arcyria bulbosa*, *Arcyria colloderma*, *Diacheopsis rigidifila*, *Diderma brunneobasalis*, and *Physarum synsporon*) appear to be from a single collection from a single locality (Stephenson & Nannennga-Bremekamp 1990).

It is interesting to note that I had made 13 field-collected specimens during the summers of 1974 to 1976 from six different localities in Adams, Clinton, Greene, and Montgomery counties, Ohio that I had set aside as a possible new species (Keller & Braun 1999). All of these collections had capillitial tubules filled with calcium carbonate, and this clearly suggested a species of the clustered-spored Badhamias. This was ample material to describe a species new to science, but a cursory study of Dr. Travis Brooks' collections of *Badhamia versicolor* revealed several specimens that appeared later to be named *P. synsporon*. Indeed, all of the clustered-spored *Badhamia* species are badly in need of monographic work that includes examination of type specimens and SEM of spore morphology. This represents a possible taxonomic research project to study the Brooks' Collection of Badhamias and the holotype collection of *B. versicolor*.

The category 1 rarity status (Table 1, Schnittler & Mitchell 2000) listed *Calomyxa synspora* (equivalent species: *C. metallica*) and *Trichia synspora* (equivalent species: *Trichia varia*) among others that do appear similar enough to suggest a single gene mutation. None of the 28 different clustered-spored taxa considered have been grown from spore-to-spore in agar culture.

9 Examples of myxomycete monographic papers

Examples of monographic papers published in the 1970s were noted by Keller (1996) that included species descriptions based on extensive field collections, examination of type specimens and representative specimens from different herbaria, and light

microscopy for illustrations. Professor Dr. Donald T. Kowalski who collected in the Cascade mountain range of northern California to Washington, USA, concentrated on the nivicolous myxomycetes (snowline or montane myxomycetes). His monographs of *Lamproderma* (Kowalski 1970), *Lepidoderma* (Kowalski 1971), and *Diacheopsis* (Kowalski 1975) merit special consideration along with the *Echinostelium* monograph by Whitney (1980) that included SEMs. There has been a gap of nearly 40 years, but the best most recent example of a monographic treatment is "*A taxonomic evaluation of the stipitate Licea species*" by D. Wrigley de Basanta & C. Lado (2005). This study included the use of light microscopy, SEM, and the species evaluation and nomenclature based on 21 type specimens. The next generation of myxomycologists would do well to follow these examples of monographs and spore-to-spore cultivation on agar culture.

References

Braun, K. L. 1971. Spore germination time in *Fuligo septica*. The Ohio Journal of Science, 71: 304 – 309.

Clark, J. 2000. The species problem in the myxomycetes. Stapfia,73: 39 – 53.

Clark, J., Haskins, E. F. 2014. Sporophore morphology and development in the myxomycetes: a review. Mycosphere, 5 (1): 153 – 170.

Keller, H. W. 1971. The genus *Perichaena* (Myxomycetes): a taxonomic and cultural study. Ph. D. Dissertation. University of Iowa, Iowa City. 199.

Keller, H. W. 1996. Invited Inaugural Address, Biosystematics of Myxomycetes: A Futuristic View, Second International Congress on the Systematics and Ecology of Myxomycetes (ICSEM2), organized by Real Jardín Botánico, CSIC, Madrid, Spain, Abstract/Volume. 23 – 37.

Keller, H. W. 2012. Myxomycete history and taxonomy: highlights from the past, present, and future. Based in part on the Invited Key note Address given at the Seventh International Congress on the Systematics and Ecology of Myxomycetes (ICSEM 7), Recife, Brazil. Mycotaxon, 122: 369 – 387.

Keller, H. W., Aldrich, H. C., Brooks, T. E. 1973. Corticolous Myxomycetes II: Notes on *Minakatella longifila* with ultrastructural evidence for its transfer to the Trichiaceae. Mycologia, 62: 768 – 778.

Keller, H. W., Aldrich, H. C., Brooks, T. E., et al. 1975. The taxonomic status of *Badhamia ovispora*: a myxomycete with unique spores. Mycologia, 67: 1001 – 1011.

Keller, H. W., Braun, K. L. 1999. Myxomycetes of Ohio: Their systematics, biology and use in teaching. Ohio Biological Survey Bulletin New Series, 13 (2): 1 – 182.

Keller, H. W., Brooks, T. E. 1971. A new species of *Perichaena* on decaying leaves. Mycologia, 63: 657 – 663.

Keller, H. W., Brooks, T. E. 1975. Corticolous Myxomycetes III: a new species of *Badhamia*. Mycologia, 67: 1218 – 1222.

Keller, H. W., Eliasson, U. H. 1992. Taxonomic evaluation of *Perichaena depressa* and *Perichaena quadrata* (Myxomycetes) based on controlled cultivation, with additional observations on the genus. Mycological Research, 96: 1085 – 1097.

Keller, H. W., Everhart, S. E. 2008. Myxomycete species concepts, monotypic genera, the fossil record, and additional examples of good taxonomic practice. Special Volume based on papers presented at the Fifth International Congress on Systematics and Ecology of Myxomycetes. (ICSEM 5). Tlaxcala, Mexico, August 8 – 13, Universidad Autónoma de Tlaxcala. Revista Mexicana de Micología, 27: 9 – 19.

Keller, H. W., Everhart, S. E. 2010. Importance of Myxomycetes in biological research and teaching. Fungi, 3 (1): 29 – 43.

Keller, H. W., Reynolds, D. R. 1971. A new *Perichaena* with reticulate spores. Mycologia, 63: 405 – 410.

Keller, H. W., Schoknecht, J. D. 1989. *Fuligo megaspora*, a myxomycete with unique spore ornamentation. Mycologia, 81: 454 – 458.

Keller, H. W., Schoknecht, J. D. 1989a. Spore-to-spore culture of *Physarum spinisporum* and its transfer to *Badhamia*. Mycologia, 81: 631 – 636.

Keller, H. W., Schoknecht, J. D. 1989b. Spore-to-spore cultivation of a new wrinkled-reticulate-spored *Badhamia*. Mycologia, 81: 783 – 789.

Kowalski, D. T. 1970. The species of *Lamproderma*. Mycologia, 62: 411 – 620.

Kowalski, D. T. 1971. The genus *Lepidoderma*. Mycologia, 63: 490 – 516.

Kowalski, D. T. 1975. The genus *Diacheopsis*. Mycologia, 67: 616 – 628.

Lado, C., Mosquera, J., Beltra′n-Tejera, E., et al. 2007. Description and culture of a new succulenticolous *Didymium* (Myxomycetes). Mycologia, 99: 602 –

611.

Lado, C., Wrigley de Basanta, D., Estrada Torres, A., et al. 2009. Description of a new species of *Perichaena* (Myxomycetes) from arid areas of Argentina. Anales delJardín Botánico de Madrid, 66S1: 63 – 70.

Macbride, T. H. 1922. The North American Slime-moulds. 2 ed. New York: Macmillan Co. 299.

Martin, G. W., Alexopoulos, C. J. 1969. The Myxomycetes. Iowa City: University of Iowa Press. 561.

Mosquera, J., Lado, C., Estrada-Torres, A., et al. 2003. Description and culture of a new myxomycete, *Licea succulenticola*. Anales del Jardín Botánico de Madrid, 60: 3 – 10.

Poulain, M., Meyer, M., Bozonnet, J. 2011. Les Myxomycètes. Vol 2, éditépar Fédération Mycologique et Botanique Dauphiné-Savoie, Editor. Sévrier, France.

Schnittler, M., Mitchell, D. W. 2000. Species diversity in myxomycetes based on the morphological species concept-a critical examination. Stapfia, 71: 55 – 62.

Schoknecht, J. D., Keller, H. W. 1977. Peridial composition of white fructifications in the Trichiales (*Perichaena* and *Dianema*). Canadian Journal of Botany, 55: 1807 – 1819.

Stephenson, S. L. 2011. From morphological to molecular: studies of myxomycetes since the publication of the Martin and Alexopoulos (1969) monograph. Fungal Diversity, 50 (1): 21 – 34.

Stephenson, S. L., Nannennga-Bremekamp, N. E. 1990. Five new species of Myxomycetes from North America. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, 93 (2): 187 – 196.

Sturgis, W. C. 1913. The Myxomycetes of Colorado, part II. Colorado College Publications Science Series, 12: 435 – 454.

Tamayama, M., Keller, H. W. 2013. Aquatic myxomycetes. Fungi, 6 (3): 18 – 24.

Whitney, K. D. 1980. The myxomycete genus *Echinostelium*. Mycologia, 72: 950 – 987.

Whitney, K. D., Keller, H. W. 1982. A new species of *Badhamia*, with notes on *Physarum bogoriense*. Mycologia, 74: 619 – 624.

Wrigley de Basanta, D., Lado, C. 2005. A taxonomic evaluation of the stipitate

Licea species. Fungal Diversity, 20: 261 – 314.

Wrigley de Basanta, D., Lado, C. 2010. *Licea eremophila*, a new myxomycete from arid areas of South America. Mycologia, 102: 1185 – 1192.

Wrigley de Basanta, D., Lado, C., Estrada-Torres, A. 2011. Spore to spore culture of *Didymium operculatum*, a new Myxomycete from the Atacama Desert of Chile. Mycologia, 103: 895 – 903.

Wrigley de Basanta, D., Lado, C., Estrada-Torres, A. 2012. Description and life cycle of a new *Physarum* (Myxomycetes) from the Atacama Desert in Chile. Mycologia, 104:1206 – 1212.

Wrigley de Basanta D., Lado, C., Estrada-Torres, A., et al. 2009. Description and life cycle of a new *Didymium* (Myxomycetes) from arid areas of Argentina and Chile. Mycologia, 10: 707 – 716.

Harold W. Keller

Professor, Botanical Research Institute of Texas, Texas 76107, USA.

Research area

Tree canopy biodiversity of primarily myxomycetes and fungi. Corticolous myxomycetes from Central and Southeastern USA.

Work experiences

2009 – present Professor Emeritus, University of Central Missouri, Warrensburg;

2006 – 2008 Visiting Professor, University of Central Missouri, Warrensburg;

2008 – 2006 Adjunct Professor of Biology, Central Missouri State University, Warrensburg;

1990 – present Research Associate, Botanical Research Institute of Texas, Fort Worth;

1990 – 1998 Adjunct Associate Professor, Department of Microbiology, University of North Texas Health Science Center, Fort Worth;

1983 – 1990 Associate Professor, Department of Biology, University of Texas at Arlington;

1982 – 1983 Associate Professor, Department of Biological Sciences, University of

North Carolina at Wilmington;

1978 – 1982 Associate Professor, Department of Microbiology and Immunology, Wright State University School of Medicine, Dayton, Ohio;

1971 – 1978 Assistant Professor, Department of Biological Sciences, Wright State University;

1971 National Science Foundation Fellow, "Summer Institute in Systematics V, Origin and Measurement of Diversity" held at Smithsonian Institute, Washington, D. C.

Journal papers, books, book chapters, journal papers at symposia and congress proceedings altogether 108.