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ARTICLE

Dictyostelids: The second major group of slime molds

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Abstract

The dictyostelids (also called cellular slime molds or social amoebae) are among the more abundant and widespread groups of microorganisms in terrestrial ecosystems. Despite this fact, they are totally unknown to most people, who sometimes confuse the dictyostelids with myxomycetes (or plasmodial slime molds). The first dictyostelid (*Dictyostelium mucoroides*) was described by the German Oskar Brefeld in 1869. More than 180 species are now known, and these organisms have been recorded from the high arctic to the subantarctic. The primary microhabitat for dictyostelids is the soil/humus layer on a forest floor, but they also occur in a number of other microhabitats. The purpose of this paper is to provide a comprehensive overview of dictyostelids, including their occurrence, distribution, relationships with other organisms, methods used for isolation, history of their study, and classification. The assemblages of species associated with each major type of ecosystem (tropical forest, temperate forest, boreal forest, tundra, grassland, savanna, and desert) are described. In addition, the compounds produced by dictyostelids and their possible applications are discussed.

Keywords – Biogeography – classification – ecology – history – methods of study – slime molds

INTRODUCTION

The dictyostelids (also called cellular slime molds or social amoebae) are among the more abundant and widespread groups of microorganisms. Despite this fact, they are totally unknown to most people. However, it is now known that dictyostelids play a critical ecological role in nature as well as serving as important model organisms for laboratory studies of cell development and evolution. In addition, the genome of dictyostelids appears to contain genes involving bioactive compounds, including those with possible antibacterial and anticancer properties. However, the latter have yet to be thoroughly investigated.

The purpose of this paper is to provide a comprehensive overview of dictyostelids (Fig. 1), including such basic aspect as their biology and life cycle, their distribution in nature, and what is involved in collecting and studying these organisms. In addition, information is provided on the history of the study of dictyostelids, their classification, biochemistry, relationships with other organisms, and potential role as a potential source for useful compounds. Studies carried out in each of the major types of terrestrial ecosystems are reviewed to indicate what is known about dictyostelid

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biogeography. The references section of the paper includes most of the relevant information that has been published on these organisms since the first species was described in 1869.

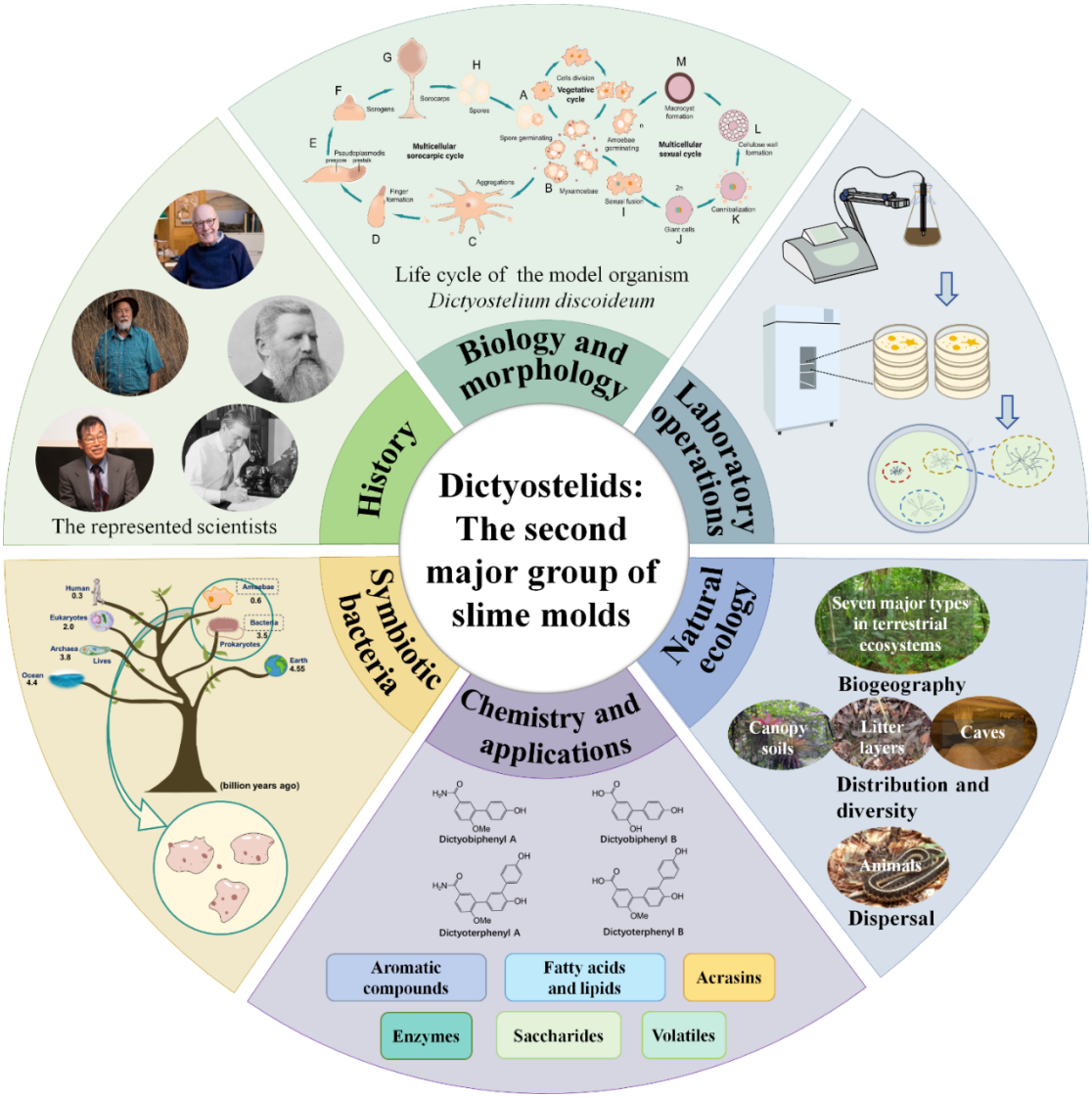


Figure 1 – Conceptual diagram illustrating the comprehensive overview of dictyostelids.

1. Introduction to dictyostelids and history of the people who have studied the group

Dictyostelids were first described by the German Julius Oskar Brefeld in 1869 (Fig. 2A). Brefeld (usually known as Oskar Brefeld) studied pharmacy in Heidelberg and Berlin but then shifted his attention to botany and mycology. He went on to work with the mycologist Anton de Bary at the University of Halle, a pioneering figure in myxomycete research who established foundational concepts of plasmodial behavior and life cycle dynamics in slime molds (de Bary 1859, 1864). While studying the microfungi associated with horse dung, Brefeld observed something that had the same general form of a microfungus but differed in a number of important respects. Later, he was able to characterize and then formally describe this new species as *Dictyostelium mucoroides* (Brefeld 1869). The specific epithet reflects its resemblance to members of the microfungus genus *Mucor* (Raper 1984), while the generic name *Dictyostelium* was derived from the terms *Dictio* (meaning "net") and *stelium* (referring to "tower"), with both of these based on the appearance of the cells making up what Brefeld considered to be the stalk of this new species. Brefeld described the life cycle of this new organism but mistakenly thought that the amoebae that represented the feeding stage fused together to form a plasmodium similar to that found in the myxomycetes (Bonner 2010). His mistake was

corrected by the French mycologist Philippe Édouard Léon Van Tieghem in a paper the latter published in 1880.

Later, Brefeld (1884) went on to describe another new genus and species of dictyostelid. This new species (*Polysphondylium violaceum*) had branched fruiting bodies. Like *Dictyostelium mucoroides*, *P. violaceum* was first isolated from horse dung (Hagiwara 1989). The generic name *Polysphondylium* was derived from the terms *Poly* (meaning "many") and *sphondylium* (the Greek word for "vertebrae" and referring to the regularly spaced whorls of branches arising from the main axis of the fruiting body). The specific epithet was based on the violet color of the sori. The myxomycete genus *Brefeldia*, which produces the largest fruiting bodies known for this group of organisms, was named in honor of Brefeld, as was *Dictyostelium brefeldianum*.

Early efforts to isolate dictyostelids from soil used a number of methods (e.g., van Tieghem 1880, Raper & Thom 1932, Raper 1951), and these yielded additional records from scattered localities in Europe, eastern North America, and other widely separated regions of the world. Some of the dictyostelids isolated were new to science, so the total number of species known gradually increased as time went on.

In 1902, Edgar W. Olive, a student of Roland Thaxter at Harvard University, published a paper in which all of the species of dictyostelids then known to science were described. This paper was the product of the work Olive had carried out for his Ph.D. (Olive 1902), and he made no further contributions to the study of dictyostelids.

Kenneth B. Raper (Fig. 2B) was born in western North Carolina in 1908. He attended the University of North Carolina as an undergraduate and then continued on to earn his M.S. degree at George Washington University in 1931 and his Ph.D. from Harvard University in 1936. In 1952, he accepted a faculty position at the University of Wisconsin in Madison. He would remain in Madison for the rest of his life. Raper's first significant contribution to the study of dictyostelids came in 1933, when he was working in the laboratory of Dr. Charles Thom at the Division of Soil Microbiology of the United States Department of Agriculture. On a visit to Little Butts Gap in the Craggy Mountains of western North Carolina, Raper collected a sample of decaying leaves that he brought back to Thom's laboratory (Raper 1984). When placed into laboratory culture, the sample yielded the isolate of a new species of dictyostelid, which he named *Dictyostelium discoideum*. This new species ultimately turned out to be the most completely studied of all dictyostelids.

Raper's work with the dictyostelids ultimately led to the publication of the first truly comprehensive treatment of the group. This book, entitled *The Dictyostelids* (Raper 1984) was written by Raper with the collaboration of Ann Worley Rahn. Forty years after the book appeared, it still remains the primary source of basic information on dictyostelids. When *The Dictyostelids* was published in 1984, 42 species and five varieties were recognized. At the time the present paper was written, these numbers had increased to 164 species and five varieties. The dictyostelid genus *Raperostelium* was named in honor of Raper.

Prior the appearance of *The Dictyostelids*, a book entitled *The Mycetozoans* and written by Lindsay S. Olive with the technical assistance of Carmen Stoianovitch was published (Olive 1975). This book included a section on dictyostelids that provided basic information on their occurrence, isolation, maintenance, life cycle, and structure along with keys, illustrations, and descriptions for nineteen different species.

John T. Bonner (Fig. 2C) was born in 1920 in New York City. He attended Phillips Exeter Academy, a university preparatory school in New Hampshire, before earning a B.S. in 1941 and an M.A. in 1942 from Harvard University. His career path was interrupted by four years of service with the United States Army Air Forces, but he was able to complete his Ph.D. at Harvard University in 1947. That same year Bonner joined the faculty of Princeton University, first as an assistant professor but ultimately to be named the George M. Moffett Professor of Biology. He spent 42 years on the faculty of the Department of Biology, serving as chair on three different occasions, and then remained active teaching and carrying out research for another twenty years. During his long career, Bonner's work with dictyostelids, especially *Dictyostelium discoideum*, greatly contributed to our understanding of microbial life in general and the behavior of cells in particular. His studies of *D.*

discoideum were a major factor in this dictyostelid becoming an important model organism for studies of the fundamental aspects of cell-to-cell communication, chemotaxis, and the underlying molecular and cellular processes that must have been involved in the transition of the unicellular to the multicellular condition in the evolutionary history of life.

The argument could be made that Bonner's most lasting legacy with respect to the dictyostelids was represented by his writing. He was the author of twenty books on a variety of subjects, including morphogenesis, evolution, and the patterns and processes of life. Among his books were *The Social Amoebae* (Bonner 1959), *The Cellular Slime Mold*s (Bonner 1967), and *The Social Amoebae: The Biology of Cellular Slime Molds* (Bonner 2009). Bonner was an unusually effective communicator, and his books are readily understood by even the casual reader. Like most microorganisms of little or no direct economic value, dictyostelids are unknown to most people, and "slime mold" isn't a particularly attractive name for any organism. Nevertheless, Bonner did as much as anyone else to direct attention towards the dictyostelids.

One of Kenneth Raper's graduate students at the University of Wisconsin was James C. Cavender (Fig. 2D). Cavender was born in Spring Valley, New York, and attended Union College in Schenectady, New York, for his undergraduate degree. After graduation, he went to the University of Wisconsin to work with Raper and received his Ph.D. from the university in 1963. His Ph.D. research centered on the development of a quantitative isolation method for dictyostelids and the use of this method to examine their distribution in nature. Only after a quantitative isolation method that could be replicated in different labs and/or by different individuals became available would carrying out more intensive studies of the ecological distribution and biogeography of dictyostelids be possible. As will be described more fully in another section of this paper, a method fitting this description was described by Cavender and Raper (Cavender & Raper 1965a) and soon began to be referred to as the "Cavender method" for isolating dictyostelids. After a year spent as a post-doc, Cavender was on the faculty at Wabash College in Crawfordsville, Indiana, for five years before moving to Ohio University in Athens, Ohio, where he spent the rest of his academic career. The most amazing thing about Cavender is that he is responsible for describing, either as the only author or as one of two or more coauthors, 126 of the species of dictyostelids now known to science. In addition, the dictyostelid genus *Cavenderia* was named in honor of Cavender.

Cavender himself soon embarked upon a series of surveys that took him from Wisconsin and the deciduous forests of the eastern United States (Cavender & Raper 1965b, 1965c) to other regions of the world such as tropical and subtropical South America (Cavender & Raper 1968), Europe (Cavender 1969a), East Africa (Cavender 1969b), Eastern Canada (Cavender 1972), Southeast Asia (Cavender 1976a, 1976b), Alaska (Cavender 1978), the Southern Appalachians (Cavender 1980), India (Cavender & Lakhanpal 1986), and Japan (Cavender & Kawabe 1989). These surveys greatly expanded what was known about the distribution and occurrence of dictyostelids worldwide. However, there were still regions where little information existed. Beginning in the late 1990s, Cavender collaborated with several other individuals on surveys for dictyostelids carried out in in the Great Smoky Mountains National Park (Landolt et al. 2006a), New Zealand (Cavender et al. 2002), Australia (Landolt et al. 2008), Patagonia (Vadell et al. 2011), Mexico (Cavender et al. 2012), Madagascar (Cavender et al. 2016, 2021, Perrigo et al. 2020), South Africa (Cavender et al. 2018), and Thailand (Vadell et al. 2018). These surveys yielded an appreciable number of new species of dictyostelids, especially from the Southern Hemisphere. Madagascar alone yielded fourteen species of *Heterostelium* new to science.

Three of the individuals most involved in these surveys were one of the coauthors (Stephenson) of this paper, John Landolt, a biologist at Shepherd University in West Virginia, and Edwardo Vadell, a biologist in Buenos Aires, Argentina. Vadell, a talented artist and one of Cavender's former graduate students, was largely responsible for producing the drawings used to illustrate the new species referred to above. However, a number of other individuals were involved in one or more of the surveys, including Diana Wrigley de Basanta, Katherine Winsett, Maria Romeralo, Allison Perrigo, Carlos Lado, and Adam Rollins.

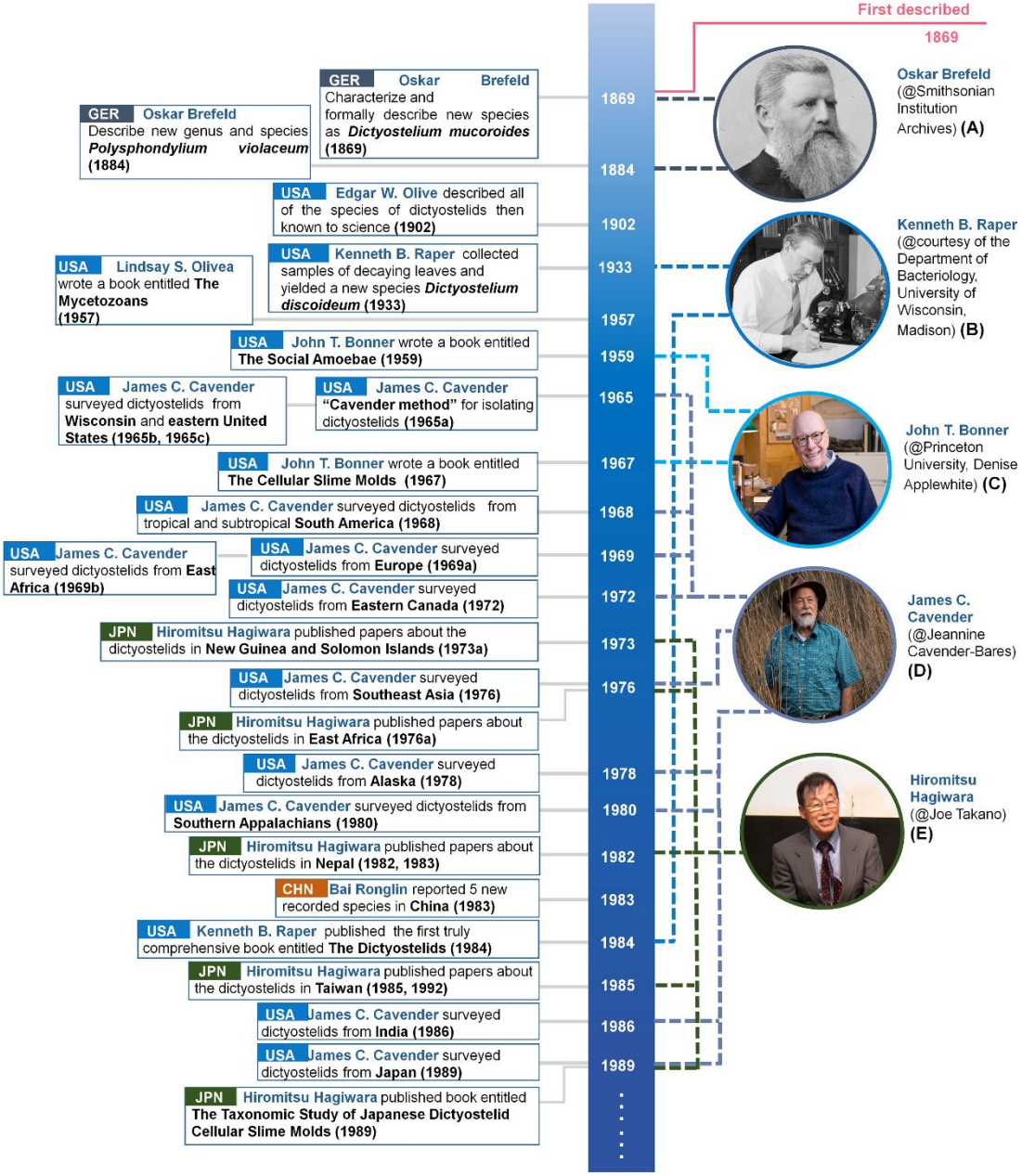


Figure 2 – Timeline of the dictyostelids research progress and the represented scientists who have studied this group since 1869. Abbreviations: GER, German; USA, United States; JPN, Japan; CHN, China.

With the exception of New Zealand, where funding was received from the National Geographic Society, the surveys mentioned above were carried out in the context of major project funded by the National Science Foundation of the United States. The objective of the project (entitled "Global Biodiversity of Eumycetozoans") was to obtain worldwide biodiversity data on the three groups of eumycetozoans—protosteloid amoebae and myxomycetes as well as dictyostelids. Cavender was one of the co-principal investigators on the grant that supported the project. There was no question that the project presented an unparalleled opportunity to carry out surveys in regions of the world that were understudied for all three groups of organisms, and an enormous body of data was generated, as reflected in the publications on dictyostelids cited in this paper.

Beginning in 1971, well before the Global Biodiversity of Eumycetozoans project, Hiromitsu Hagiwara (Fig. 2E) in Japan published a series of papers that expanded what was known about the distribution and occurrence of dictyostelids in Japan as well as in such other places as East Africa (Hagiwara 1976a), New Guinea (Hagiwara 1973a), Nepal (Hagiwara 1982, 1983a), Taiwan (Hagiwara et al. 1985, 1992a), and the Solomon Islands (Hagiwara 1973b). Hagiwara was born in

Gunma Prefecture in Japan in 1945, and he obtained his academic degree at Hokkaido University. Afterwards, he became affiliated with the Department of Botany of the National Science Museum, Tokyo. His most significant contribution to the study of dictyostelids was his book *The Taxonomic Study of Japanese Dictyostelid Cellular Slime Molds* (Hagiwara 1989). In the book, Hagiwara reviewed previous studies of the group in Japan, described the morphological features of dictyostelids, and provided keys and descriptions of the species then known from the country, a number of which he had described. The drawings in his book are meticulous and established a very high standard for illustrating morphological features of dictyostelids. In the drawings, special attention was directed towards the organization of the cells making up the sorophore, especially at the tip and at the base. Hagiwara also classified dictyostelid aggregation patterns into the mucoroides-type, violaceum-type, minutum-type, and microsporum-type along with recognizing the different growth habitat of sorocarps as solitary, gregarious, clustered, and coremium-like. Hagiwara was responsible for describing more than twenty dictyostelids and one myxomycete (*Arcyria monticola*) as species new to science. The dictyostelid genus *Hagiwaraea* was named in honor of Hagiwara.

In China, research on dictyostelids began in 1983 and has continued since then, as described by Liu & Li (2012). The country, and especially Jilin Agricultural University, have become important centers for studies of dictyostelid ecology and systematics. At the time the present paper was written, there were 59 species and one variety recorded from China. Nine new species and one new variety were reported in studies carried out by (Hagiwara et al. 1985, He & Li 2008, 2010, Liu & Li 2017, Zhao et al. 2017, Liu et al. 2019c, 2019d, Zou et al. 2022). During this same period, An et al. (2013) improved the purified media used to cultivate dictyostelids. Along with morphological characteristics, fatty acid biomarkers have also been used in studies dictyostelid taxonomy in China (An & Li 2017). In addition to taxonomy, ecological distribution (Liu et al. 2020, Zhang et al. 2023a, 2024a, b), associated symbiont bacterial (Liu et al. 2019a, Zhang et al. 2023b, Zhang et al. 2025), developmental features (Sun et al. 2011, An et al. 2018, Zhang et al. 2018), ultrastructural characteristics (Li et al. 2021), genome size (Hou et al. 2019), chemical components and pharmacological activities of different species of dictyostelids have been studied in China (Zhou et al. 2019, Wei et al. 2021).

In addition to the aforementioned, researchers from other regions have also contributed to the growing knowledge of dictyostelid diversity and ecology. In Southeast Asia, Thailand and the Philippines have emerged as active contributors. For instance, Rukseree et al. (2018) conducted a phylogenetic analysis of dictyostelids from Amnat Charoen Province, Thailand, revealing novel lineages within the group. In the Philippines, pioneering studies by Dogma & Blancaver (1965) laid the groundwork for dictyostelid research, followed by recent efforts led by Thomas Edison E. dela Cruz and colleagues. Their work has documented species diversity in Lubang Island and Subic Bay Natural Forest Reserve, integrating ecological and distributional data (Yulo & dela Cruz 2011, 2012; dela Cruz et al. 2011). Similar initiatives are gradually expanding to other regions, though research gaps persist in parts of South America, Africa, and smaller European countries. These collective efforts highlight the global interest in dictyostelids while underscoring the need for broader geographical coverage to fully understand their biogeography and evolutionary dynamics.

2. Dictyostelids as organisms

2.1 Biology: social attributes

Social interactions take place in dictyostelids when in times of starvation, individual amoebae aggregate to form a multicellular mound, containing up to a hundred thousand cells with intuitive logic and goal-directed reciprocity (Schaap et al. 2006, Chen et al. 2018). The aggregate undergoes differentiation and morphogenic changes characterized by the formation of starvation-induced multicellular fruiting bodies which consists of two main cell types. These are (1) spore cells, which are resistant to temperature extremes, desiccation, and digestion, and (2) stalk cells, which form the ancillary structures supporting the mass of spore cells that is formed (Urushihara et al. 2015, Du & Schaap 2022, Kawabe et al. 2023).

One interesting stage is an intermediary structure, the pseudoplasmodium or slug. During this

stage, the multicellular aggregate migrates towards the soil surface in nature, responding to light and heat stimuli in order to locate more favorable locations before ultimately differentiating into a fruiting body. This response to starvation is referred to as development, and by going through this social, multicellular phase, the population dramatically increases its chances of surviving unfavourable environmental conditions (Raper 1984). Interestingly, the slug of the model organism *Dicytostelium discoideum* has some cells, known as sentinel cells, which have detoxification and immune-like functions (Chen et al. 2007, Brock et al. 2016). They can remove bacterial pathogens to protect the presumptive spore population from harm.

Aggregative multicellularity is exemplified by the well-studied development of dictyostelid social amoebae. For example, *D. discoideum* is interesting to students of social and evolutionary biology as well as to developmental biologists (Kjellin et al. 2021). *Dicytostelium discoideum* propagates as a unicellular organism but aggregates upon starvation and forms fruiting bodies with viable spores and dead stalk cells (Zou et al. 2021). Aggregative development exposes dictyostelids to the perils of chimerism, including cheating, which raises questions about how the victims survive in nature and how social cooperation persists (Benabentos et al. 2009). Some aggregation cells (20%) form stalk cells and do not reproduce, and the remaining aggregation cells (80%) develop into spores with reproductive potential (Fets et al. 2010). Aggregation cells in *D. discoideum* altruistically sacrifice their own reproduction in order to aid the success of the cells that become the spores. From an evolutionary point of view, this is apparently built upon a foundation of a kin-selected altruism embodiment in pre-stalk cells (Kaushik et al. 2006).

Unlike most superorganismal societies such as the social insects (Wenseleers & Ratnieks 2006, Ratnieks & Wenseleers 2008), multicellular organisms like the dictyostelids are clonal and all cells are derived from a single progenitor cell. Cooperation is central to many major transitions in evolution, but *D. discoideum* likely evolved strategies for competing in chimeras in nature, and the function of some developmental genes are competitive because different clones commonly co-occur on a very small scale (Fortunato et al. 2003). Studies have found that many mutants “cheat” when facultatively growing in a mixed population, producing more than their fair share of spores in chimaeras and do not contribute their strength to the collective, although cooperating normally when clonal (Strassmann et al. 2000, Gilbert et al. 2007, Santorelli et al. 2008).

2.2 Biology: Agriculture activities

Agriculture (Cohen 2009) in terms of eukaryotes represents an important evolutionary transitional mechanism and is a hotly contested issue. Microorganisms are surprisingly like animals in having sophisticated behaviors such as cooperation, communication, and recognition, as well as many kinds of symbioses. Previously, the perception was that in addition to humans, true agriculture was thought to be restricted to social insects such as termites and ambrosia beetles who practice “fungus-farming” (Morelos-Juarez et al. 2010, Schultz et al. 2015) in which they display a wide range of ecological roles depending on their feeding and nesting habits. However, the study of agricultural evolution has expanded to the social amoebae (Raguso 2013).

In nature, Brock et al. (2011) showed that up to a third of wild *D. discoideum* (“farmer strains”) clones are surprisingly like fungus-growing ants in having sophisticated primitive farming symbiosis behavior that involves carrying bacteria both intracellularly and extracellularly in their fruiting bodies to establish new food crops, while also involving dispersal and prudent harvesting of the crop (Brock et al. 2011, Brock 2011). In addition, Brock et al. (2013) also found that farmer-carried *Burkholderia* inhibited the growth of non-farmer *D. discoideum* clones that could exploit the farmers' crops, suggesting that successful farming is a complex evolutionary adaptation because it requires additional strategies, such as recruiting third parties, to effectively defend and privatize crops.

2.3 Life cycle of dictyostelids

Dictyostelids constitute a prominent and diverse group of soil-dwelling microbes, representing an early evolutionary stage of genetically distinct organisms that exhibit a remarkable feature of aggregative multicellularity (Parfrey & Lahr 2013, Kin & Schaap 2021). Among them, *Dictyostelium*

discoideum and other dictyostelid slime molds are renowned for their intricate life cycle, characterized by alternating phases of aggregation and multicellular development (Romeralo et al. 2010b). This cycle commences with the majority of dictyostelids existing as independent, amoeboid cells known as myxamoebae, which subsist on bacteria, proliferate through binary fission, and multiply. However, when the food supply within their immediate environment dwindles, a remarkable transformation occurs: numerous myxamoebae coalesce to form a cohesive multicellular entity known as the pseudoplasmodium. Within this structure, each cell retains its individual identity while collectively contributing to the formation of one or more fruiting bodies, or sorocarps, that harbor asexual spores. Should conditions be conducive, these spores will germinate, giving rise to fresh myxamoebae, thereby initiating a new cycle of life (Hehmeyer 2019).

2.3.1 Asexual Life cycle

Dictyostelids have a unique asexual life history involving social behavior and division of labor. Spores (Fig. 3H) are produced in a viscous spore mass (spherical sorus) at the top of the sorocarp (Kaushik et al. 2006, Fets et al. 2010). The spores germinate under suitable conditions after maturation (Fig. 3A), and release amoeboid cells "myxamoebae" or "amoebae" (Fig. 3B). In the vegetative portion of the cycle, dictyostelids (acting like animals) proliferate as unicellular myxamoebae and produce actin-rich pseudopods (Guo et al. 2013) that sense, move, and feed as long as enough of their bacterial food or other microorganisms is available (Romeralo et al. 2013a, Boyer 2023). Myxamoebae rely on chemotaxis to respond to the folic acid secreted by bacteria to track, kill, phagocytose and absorb them within their cells (Benghezal et al. 2006, Cosson & Lima 2014). However, upon depletion of available food, numerous single-celled starving amoebae aggregate by the thousands, using the signaling molecules cyclic adenosine monophosphate (cAMP) to regulate a diverse array of multicellular processes (Schaap 2011). With an increase of hunger and acceleration of the aggregation rate, the formation of pseudopods also increases (Guo et al. 2013).

At this point, the obvious cell flow that created the mound of cells elongates, forming what are called "aggregations" (Fig. 3C). Propagating cAMP waves cause the cells to move upward, and the central portion will form an upward-growing finger-like protuberance (Fig. 3D). At this stage, the protuberance is gradually pulled out, aerially projecting as a multicellular structure referred to as a "pseudoplasmodium" (Fig. 3E) or "slug," whether on agar in soil (Alexopoulos & Blackwell 1996, Schaap 2021). The ratio of prespore to prestalk cells stabilizes at about 80:20, regardless of the absolute number of cells in the aggregation (Shaulsky & Kessin 2007). Pseudoplasmodia have the ability to migrate with stalks in some species, but other species do not migrate with stalks. After it undergoes a short period of movement, a small uplifting set of cells (the "sorogen") will be formed and pulled up (Fig. 3F), finally give rise to 1–2 mm high fruiting bodies (sorocarps) (Fig. 3G) (Raper 1984, Hagiwara 1989, Liu et al. 2019c).

The stalks and branches of sorocarps differentiate from the prestalk cells, which form the ancillary structures supporting the spore mass, while the sorus of the sorocarp is differentiated from prespore cells, which are resistant to temperature extremes, desiccation, and digestion (Weijer et al. 1984, Gomer & Firtel 1987, Fets et al. 2010). With the formation of stalks and branches, the prespore cells move from the sorogens to the top of the stalk. They differentiate, reaching the top, into the sorus that contains the fertile spores. The sorus is composed of mucus and spores; the latter are spherical or lemon-shaped, mostly white, but some species are purple, red and golden yellow or cream-colored (Raper 1984, Hagiwara 1989, Sheikh et al. 2018). Once formed, dictyostelid spores are covered by a protective barrier, the spore coat, which is assembled from secreted proteins and cellulose (West 2003). The sorocarps are somewhat microfungus-like, no longer move on the agar plates when mature, and the apoptotic cells in the stalk form a cell wall (Shaulsky & Kessin 2007, Li et al. 2010). In addition to this multicellular mode of asexual reproduction, dictyostelid amoebae also can reproduce asexually by fission (Raper 1984).

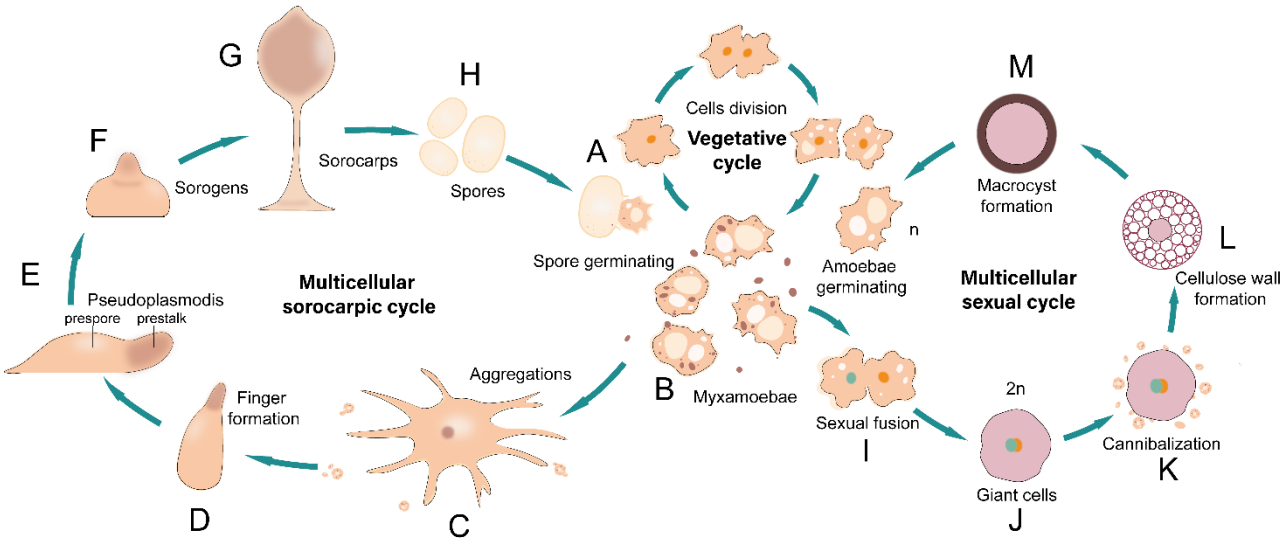


Figure 3 – Life cycle of the model organism *Dictyostelium discoideum*.

2.3.2 Sexual Life cycle

In addition to the multicellular sorocarpic and vegetative cycles described above, a sexual cycle and an encystation cycle have also been observed in many species. Dictyostelid zygotes do not proliferate but grow to a large size by feeding on other cells of the same species, with each zygote ultimately forming a walled structure called a macrocyst (Raper 1984). The sexual phase in social amoebae has a number of striking features. Some species are also known to form macrocysts under specific environmental conditions, such as darkness, low temperature, moisture, and low phosphate, initiated by chemical signals (pheromones, Ca^{2+}), cellular fusion, and phagocytic behaviors. (Raper 1984, Schilde & Schaap 2013, Bloomfield 2018). The macrocyst remains poorly characterized compared to the multicellular fruiting body, which is known to serve dual functions as both the culmination of the sexual cycle and a resistant survival stage. Although macrocysts were probably present in the last common ancestor of dictyostelids, there are many species for which this stage has yet to be observed (Kessin 2001, Romeralo et al. 2012).

The macrocyst resting stage begins with acquisition of fusion competence cells (gamete formation) as the culmination of the sexual cycle (Blaskovics & Raper 1957). They are formed from single dictyostelid amoebae (Fig. 3I) which enter a resting stage. Like the asexual multicellular cycle, interestingly macrocysts also may require environmental factors for induction, particularly darkness (Hirschy & Raper 1964), excess water (Weinkauff & Filosa 1965), and ethylene (Amagai 1984). Once formed, the zygotes attract surrounding myxamebae to form small aggregations that secrete a protecting sheath around each collective group of cells. The zygote progressively increases in size by engulfing and digesting the other cells, hence the term diploid cell, called a “giant cell” (Fig. 3J), and attracts surrounding haploid cells (Fig. 3K) by secreting cAMP. A cellulose wall (Fig. 3L) is then secreted, inside of which the macrocyst matures (Fig. 3M). Division of the giant cell before germination reconstitutes uninucleate cells. The diploid macrocyst nucleus undergoes meiosis, after which a single meiotic product survives to restart haploid vegetative growth.

2.4 Morphology

In the first instance, morphological studies (Fig. 4) began in 1869 with the description of the first species of dictyostelid (*Dictyostelium mucoroides*) by Oskar Brefeld (Brefeld 1869). *Dictyostelium mucoroides* had been known as early as 1863, but at that time, the organism had been mistaken for *Rhizopus nigricans* (Raper 1984). The second dictyostelid genus *Polysphondylium* was established in 1884 by Brefeld with the type species *Polysphondylium violaceum* (Brefeld 1884). A third genus (*Coenonia*) was described in 1884 but has not been reported since and may not be a dictyostelid (Van Tieghem 1884). In the classic morphological classification of the dictyostelids, the

genera *Dictyostelium*, *Polysphondylium*, and *Coenonia* were placed in the family Dictyosteliaceae (Raper 1984). Another family (the Acytosteliaceae), which contains only the single genus (*Acytostelium*) was first reported in 1956 (Raper 1956, Raper & Quinlan 1958). Both families were assigned to the order Dictyosteliales (Raper 1984). All recent morphology-based studies of the dictyostelids have used the family and genus concepts mentioned above (Raper 1984). Raper (1984) summarized those studies carried out prior to the publication of his enormously influential monograph *The Dictyostelids*. Subsequently, Hagiwara described many new species from Japan and summarized these into one publication in 1989 (Hagiwara 1989). Since then, researchers from all over the world have published a large number of new species of dictyostelids.

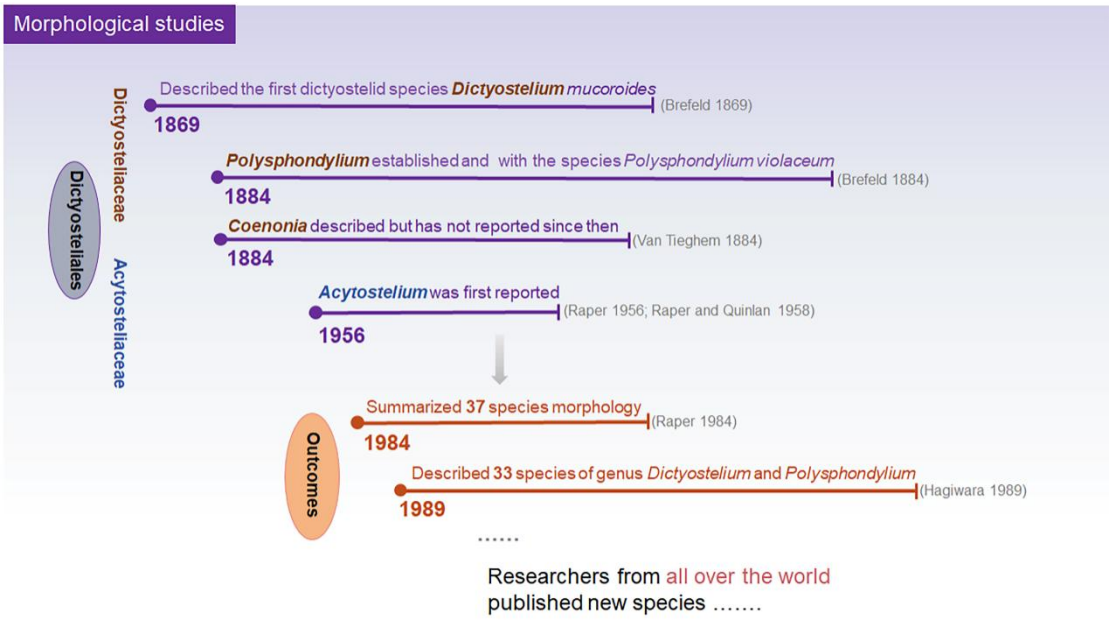


Figure 4 – A timeline of the morphological studies on dictyostelids and outcomes that have been achieved.

Dictyostelids occur in a diverse array of terrestrial habitats worldwide (Swanson et al. 1999, Liu et al. 2020, Cavender et al. 2021). These organisms have a rich morphological diversity and are usually described by lab culturing methods and identified on the basis of their morphology (Raper 1984, Hagiwara 1989). Surveys for dictyostelids carried out in various areas of world over a century have yielded considerable data and about 200 species, but these organisms remain understudied (Zhang et al. 2023a) and it is likely that numerous additional species have yet to the described (http://www.indexfungorum.org/names/Names.asp).

2.4.1 Morphology: macroscopic morphology

In a primary isolation plate, observations are made under a stereoscopic dissecting microscope to note the location of each early aggregating clone and sorocarp(s) that develops and to mark all locations on the lid of the Petri dish. The macroscopic morphological stages (Fig. 5) in the life cycle, including the growth habit, branching pattern and color of sporocarp (fruiting body), the length, color and size of the sorus, the size and morphology of cell aggregation, and the formation of pseudoplasmodia also are noted. Afterwards, relevant structural features can be photographed, and measurements can be taken and recorded.

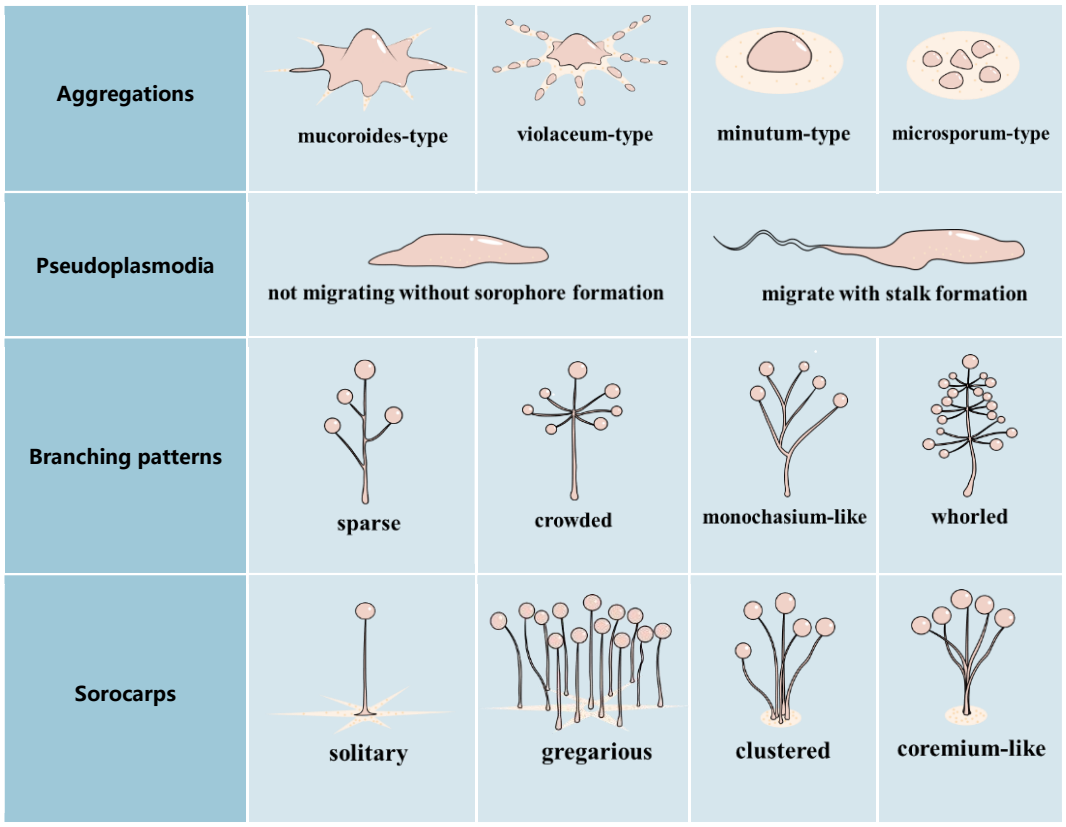


Figure 5 – Macroscopic features used in the identification of dictyostelids (Raper 1984, Hagiwara 1989).

2.4.2 Morphology: microscopic morphology

For identification of a dictyostelid, one should remove a well-developed and complete sporocarp (fruiting body) from a culture while working under a stereoscopic dissecting microscope, place this on a slide with a drop of sterile water, cover the slide, and then seal the latter. The microscopic morphological stages (Fig. 6) in the life cycle, including features such as the shape and size of spores, presence or absence of spore granules, thickness and number in cell lines of in the sorophores, characteristics and width of the sorophore tips and sorophore bases can be observed and measured on the slides by using a light microscope with a 10× ocular and 10, 40, and 100× (oil) objectives. Photographs can be obtained with a color microscope camera, and these can be recorded in a specimen identification file.

3. Methods for isolating and culturing dictyostelids in the laboratory

Because dictyostelids were first identified from dung, they were once thought to be coprophilous. However, it soon became apparent that their primary habitat is the leaf litter decomposition zone of forest soils. Dictyostelids are not restricted to forest soils and also occur in agricultural soils (Agnihothrudu 1956, Stephenson & Rajguru 2010), grassland soils (Smith & Keeling 1968, Rollins & Stephenson 2013), deserts (Benson & Mahoney 1977), and both alpine (Cavender 1983) and arctic tundra (Cavender 1978, Stephenson et al. 1997).

Species of dictyostelid are recognized primarily by the shape, size, color, and (when branches are present) the branching pattern of their sorocarps (spore-bearing structures) (Raper 1984). At present, almost 200 species of dictyostelids have been formally described. Some species appear to be cosmopolitan, whereas others have a more restricted distribution (Swanson et al. 1999). The number of species has been steadily increasing with increased sampling efforts (Zou et al. 2022).

Dictyostelids produce microscopic fruiting bodies (in length) and are rarely observed except in laboratory culture. However, since these fascinating soil-dwelling microorganisms were first isolated

in the laboratory, more and more individuals throughout the world have developed a strong interest in investigating their ecological and geographical distribution (Swanson et al. 1999, Romeralo et al. 2011b, Liu et al. 2020, Cavender et al. 2021) as well as their evolution (Chen et al. 2018, Kin & Schaap 2021). This is possible because of the ease with which dictyostelids can be cultured, identified, and their characteristics enumerated. This entire process will be described below, hopefully to attract more enthusiasts.

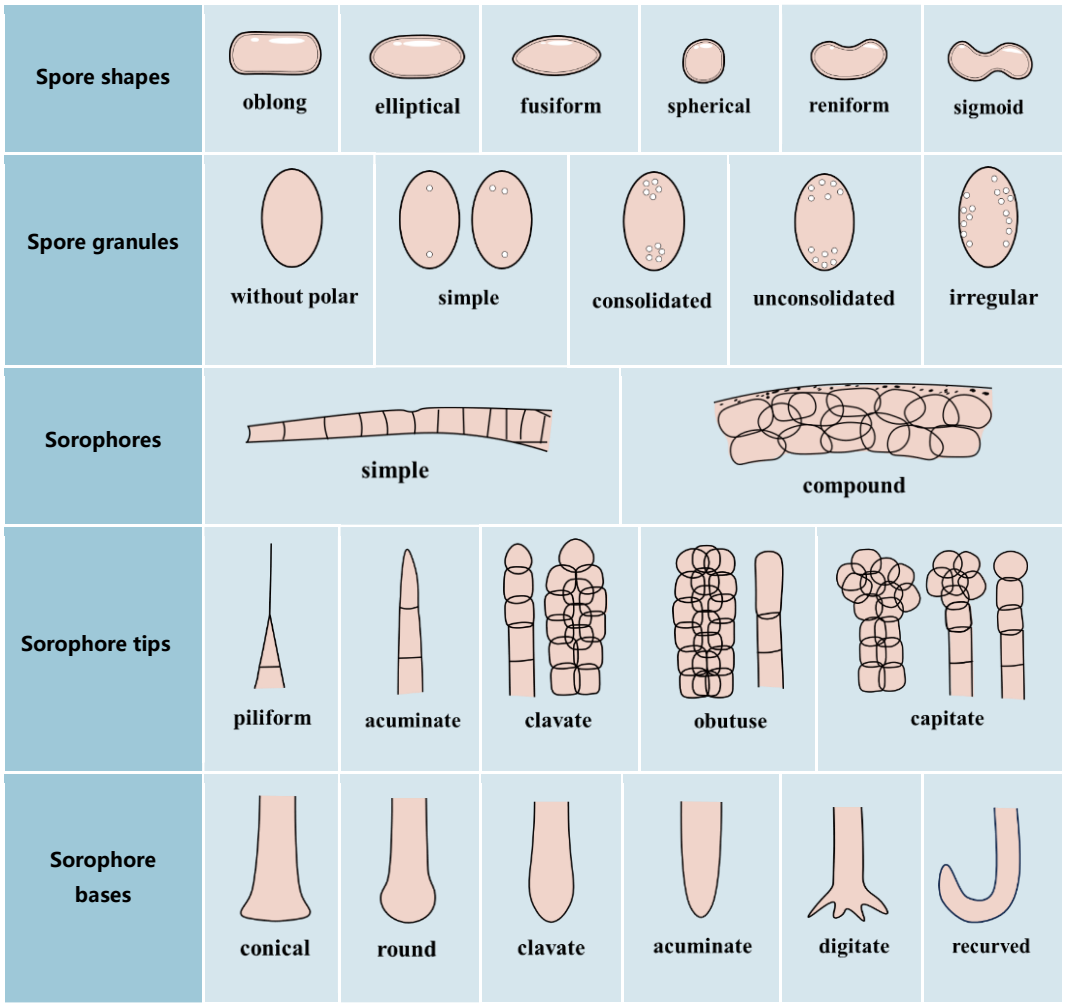


Figure 6 – Microscopic features used in the identification of dictyostelids (Hagiwara 1989, Liu & Li 2017).

3.1 Collecting samples

It is important to note that unlike many other protists, dictyostelids are not known to be wind dispersed (Cavender 1973, Bonner 2009). However, because of their very small size, they can utilize the same food resource and occur together in a spatially limited and clearly defined microhabitat, where the potential for ecological interactions would seem to exist. In spite of their apparent limited potential for dispersal, dictyostelids are usually present and often abundant in the soil and litter microhabitats of all types of terrestrial ecosystems. The process used for collecting samples is described below.

3.1.1 Surface soil

The collecting samples of surface soil is usually based on the requirements of the type of investigation being carried out. If the objective is simply to document biodiversity, samples can be taken randomly at a general locality or within a particular type of ecosystem, habitat or microhabitat. For a more quantitative ecological study, the number of samples to be collected, the spacing of the sites sampled, and the exact method used for collecting samples must be considered. The amount of

material actually collected in published studies has ranged from approximately 10 to 300 g. Sample are usually placed in small plastic bags. Each set of samples should be labeled with the date of collection, vegetation type, elevation, and geographical location. The samples should be transported from the field to the laboratory in a cool box to maintain their integrity during transit.

The procedure involved in collecting sample involves moving aside surface litter (typically consisting of dead leaves), the actual sample is collected from the soil/litter interface zone with a small spoon, twig, knife, or some other item. Each sample should be placed in a plastic bag or other suitable container that allows the sample to remain moist. Samples can be stored in a refrigerator for several days (or longer if necessary), but it is best to process them as soon as possible.

3.1.2 Canopy soil

Canopy soil, the soil-like material that develops beneath mats of epiphytes (both bryophytes and vascular plants) on the branches (and sometimes the trunks) of trees, is a type of microhabitat for dictyostelids found in both temperate and tropical rainforests. Canopy soil, which is highly organic, sometimes often occurs in a thin layer on the top of a branch, and obtaining enough material for a sample may require collecting material from a number of places and then combining it as a single composite sample. Canopy soil has been collected at heights of more than 20–40 m above the ground in tropical forests, but it is sometimes possible to collect samples at a height of no more than a few meters. The greater the height, the greater the difficulty in obtaining samples. Researchers have used a canopy crane, the single rope technique, canopy walkways, and balloons to gain access to heights of several or many meters (Barker & Pinard 2001), with canopy soil collected using identical procedures to surface soil sampling.

3.1.3 Animal dung

Dictyostelids occur on animal dung and were once thought to be primarily coprophilous (Raper 1984). A sample of dung typically consists of small to medium-sized pellets for small animals. These are added to a measured amount of distilled water, broken apart, the resulting suspension diluted as necessary and then plated out. For a quantitative assessment, the pellets are weighed as the first step in processing. For larger animals that produce larger masses of dung, a sample consists of a small amount of material taken from the mass of dung.

3.1.4 Cave soil

One unusual microhabitat for dictyostelids is the soil-like material found in caves. Typically, this is the guano from bats that inhabit the cave in question, but movement of water can transport organic matter into caves. Dictyostelids apparently cannot survive in pure guano, but they can be relatively common in microsites where there is a strong admixture of other types of material. The latter include various types of plant debris, other kinds of detritus, and a mineral matrix that can range from powdery dry dust or gravel to very wet clay mud. As is the case for samples of soil/humus, samples from caves are stored in sterile plastic bags and returned to the laboratory for processing as soon as possible after collection (Landolt et al. 2006b). Very few studies have considered dictyostelids from caves, so there is much yet to be learned.

3.2 Isolation of dictyostelids by laboratory culture

Except under truly exceptional circumstances, dictyostelids cannot be observed directly in the field. Instead, samples of soil collected from the field must be suspended in water at a standard ratio (e.g., 1:10 soil-to-water by weight) to form a soil/water suspension, which is then serially diluted and plated onto low-nutrient agar (such as water agar or hay infusion agar) in Petri dishes. Clones of dictyostelids develop on the surface of the agar from propagules (either spores, microcysts or myxamoebae) that were present in the soil and can be identified and quantified. This “Cavender” method is described in more detail later in this paper.

In the past, many different methods have been used to isolate dictyostelids in the laboratory. The earliest of these methods was described by Raper & Thom (1932), who took samples of soil or decaying vegetation and ground these in a clean mortar and then diluting this material (1:10) with

sterile water. The obtained suspension obtained was added to a mannite agar plate (used for growing *Azotobacter*) and incubated at 16–18 °C for two to three weeks. Once the sorocarps of the dictyostelid being studies had formed, the spores from a sorus (Fig. 7) were removed and implanted onto a fresh substrate by spot or stripe inoculation.

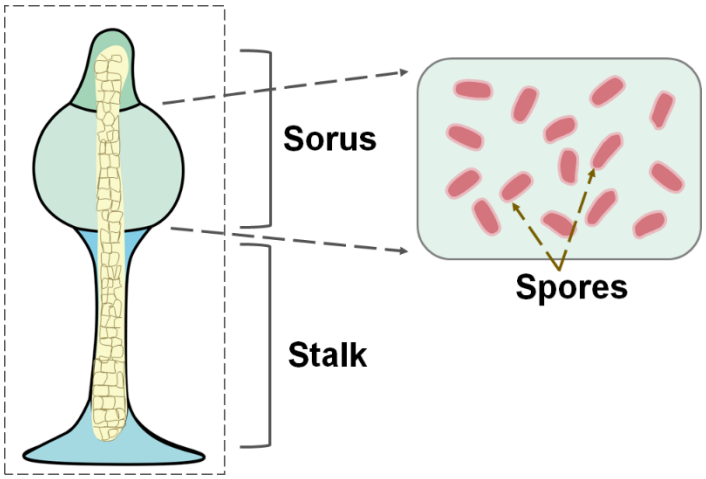


Figure 7 – The fruiting body (or sorocarp) is composed of two main parts. These are the sorus, which contains the fertile spores, and the stalk, which holds the sorus aloft to facilitate spore dispersal.

Over time, there have been improvements. The use of mannite agar was discontinued and replaced with dilute hay infusion agar (Raper 1937, 1951), and the samples were no longer ground in a mortar but shaken in sterilized water for approximately half an hour to disperse dictyostelid spores and myxamoebae. A small amount of the suspension was plated out on dilute hay infusion agar and incubated at 20 to 24 °C for several days, during which it was carefully observed to detect the appearance of initial aggregations of amoebae or sorocarps that had developed from the propagules present in the plates. Each aggregation/sorocarp "clone" (Cavender & Raper 1965a) was marked.

Singh (1946) developed a technique for isolating and studying soil protozoans while studying the distribution of dictyostelids in the soil of Great Britain. He prepared a bacterial ring (which he called a "bacterial circle") with bacteria that had been grown on nutrient agar for 2 to 7 days and added small crumbs of soil or aliquots of a diluted soil suspension to each circle.

Later, Cavender & Raper (1965a) modified this method and obtained semi-quantitative results using what became known as the "Cavender method" (Fig. 8,9), which is useful for comparative studies. Clone quantification is carried out through daily microscopic examination (40× magnification) of each plate, beginning ca 48 hr post-inoculation, with discrete amoebal aggregations and mature fruiting bodies (collectively defined as "clones", Fig. 8) being marked on the plate underside to prevent re-counting. When cultures reach maturity (14 days), three ecological parameters are systematically recorded. These are (1) density (clones/g dry substrate) calculated as total clones divided by sample weight; (2) frequency (%) determined by the proportion of positive plates per sample; and (3) relative density (%) representing each taxon's contribution to the total clone count. Moreover, it is more rewarding than either of the earlier methods with respect to the number of species that may be isolated. In addition, it has the distinct advantage of allowing, for the first time, some quantitative measure of (1) the population of dictyostelids present in the soil of a given forest or other type of ecosystem and (2) the relative abundance of different species in such soils. As such, it provides a means of comparing this information with similar information obtained from other soils collected from similar or markedly different ecosystems. Cavender (1969a, 1969b), Benson & Mahoney (1977), Sutherland & Raper (1978) and others have used this method rather extensively since then.

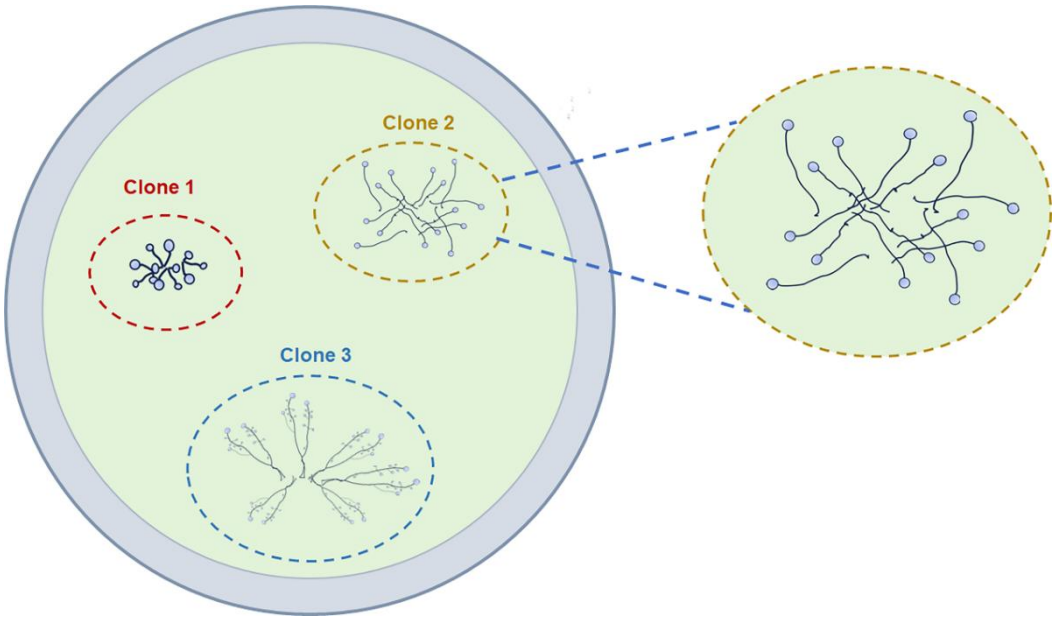


Figure 8 – Improved method for isolating dictyostelids described by Cavender. This method produces semi-quantitative estimates of propagules (spores, myxamoebae, and microcysts). The separation plate shows many recognizable dictyostelid clones, lasting about five days (Cavender & Raper 1965a).

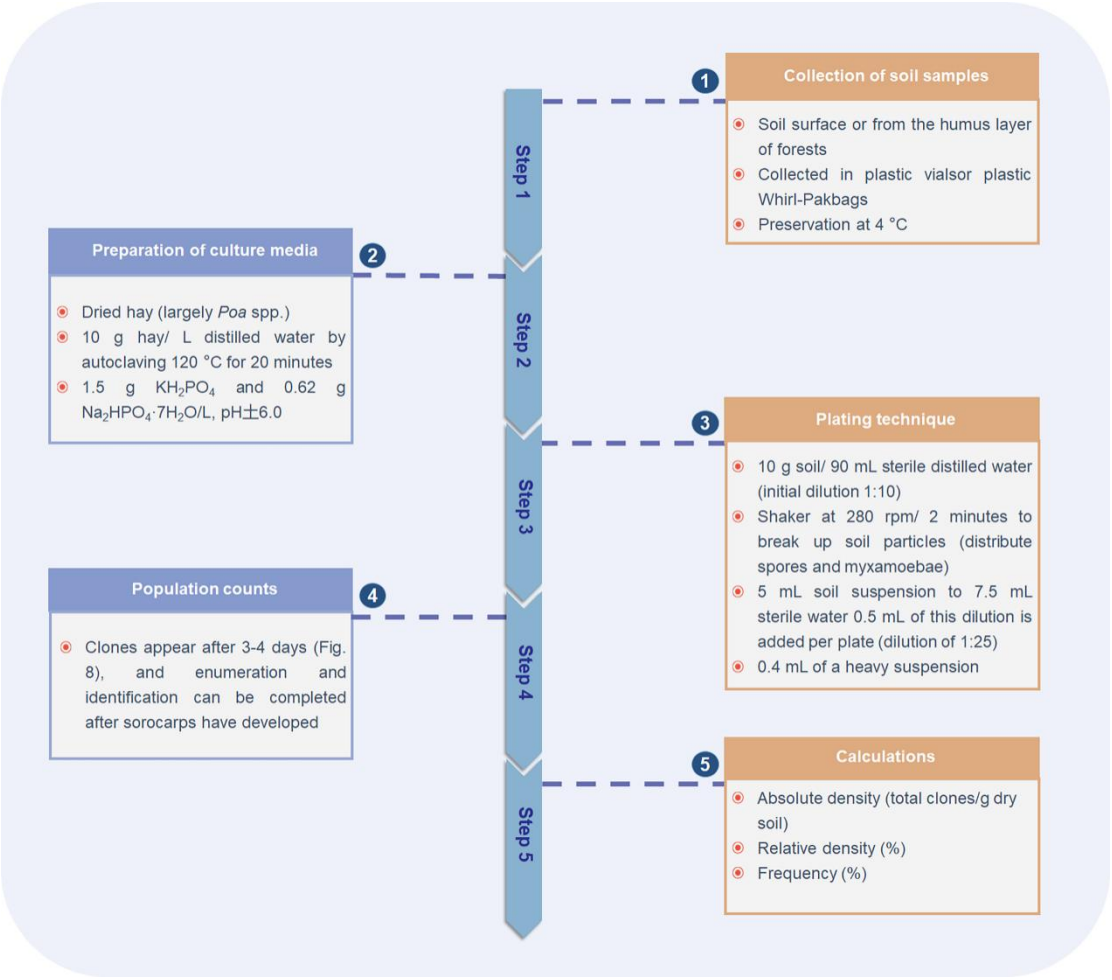


Figure 9 – Schematic diagram of the process for isolating dictyostelids from samples.

The method proposed by Cavender & Raper (1965) for the isolation of dictyostelids has now

been used for almost sixty years. Researchers have published a series of new species, including such examples as four new species (*Cavenderia parvibrachiata*, *C. helicoidea*, *C. ungulata*, and *C. protumula*) from soil/litter samples collected in northern Thailand (Cavender et al. 2022); a new variant and four new species (*Dictyostelium purpureum* var. *pseudosessile*, *D. minimum*, *D. multiforme*, *D. robusticaule* and *Heterostelium recretum*) from soil/animal dung samples collected in Hainan Province, Qinghai-Tibet Plateau, and Jilin Province, China (Liu et al. 2019b, 2019c, Zou et al. 2022); three new species (*H. multibrachiatum*, *D. insulinativitatis*, and *D. barbarae*) from the Sakhalin Island and in the vicinity of Ussuriysk and Vladivostok of Russian Far East; Christmas Island in the Indian Ocean (Liu et al. 2019d, 2020).

The isolation method in all of the studies mentioned above involved the preparation of a soil suspension and determination of the pH of the soil suspension, followed by the inoculating hay infusion agar plates for isolation of dictyostelids. The agar plates were placed at 23 ℃ with a 12 h light and dark cycle for observation (Fig. 10A). In the primary isolation plates, the location of each early aggregating clone and sorocarp(s) that developed was marked. Then, the purified dictyostelids were subjected to morphological observation and photos of their life cycle stages were taken for subsequent identification. Macroscopic morphology was observed using a dissecting microscope. This included observations of myxamoebae, aggregations, pseudoplasmodia, and the size, color, and other features of sorocarps (Fig. 10B). Microscopic morphology was observed using a compound microscope, including spores, sorophore tips and bases, and branches (Fig. 10C). Spores from these isolates were frozen in HL5 media (Cocucci & Sussman 1970) and stored at −80 ℃. Also, soil samples were stored in the herbarium, including for example the Herbarium of the Mycological Institute of Jilin Agricultural University (HMJAU), Changchun, China shown in Fig. 10D.

3.3 Selection of culture medium

Dictyostelids commonly occur in surface soil and humus, where they can selectively feed upon bacteria for growth and survival. So, what types of bacteria do the dictyostelids prey upon? Raper (1937) and Singh (1947a) showed that myxamoebae of the genus *Dictyostelium* could utilize various bacteria as food in laboratory experiments. The choice of the food bacteria should be governed in large measure by the nature of the investigation. If the study focuses on the vegetative stage, especially the feeding habits of amoebae, dictyostelids can be cultured on low nutrient agar together with some large-cell bacterium such as *Bacillus megaterium*. When studying the process of fruiting body formation, it is advisable to have dictyostelids feed upon some easily digestible bacterium such as *Escherichia coli* or *Klebsiella pneumoniae* (Raper 1937). Raper (1937, 1939) found that *E. coli* was the most suitable bacteria for predation by dictyostelids. The reasons for this are that (1) it belongs to the gram negative bacteria and produces moist, non-gummy colonies, which have physical characteristics that are particularly conducive to the feeding of the amoebae; (2) neither excessive acidic nor excessive alkaline byproducts are produced; and (3) it is easy for researchers to identify. In addition, another gram negative bacterium *Klebsiella pneumoniae* has been found to be another suitable nutritional source for dictyostelids. Singh (1947a, b) successfully cultivated *D. mucoroides* and *D. giganteum* using *K. pneumoniae*. However, the studies suggested that bacterial preferences may vary across dictyostelid species. Yulo & dela Cruz (2012) systematically tested multiple gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), gram-positive bacteria (*Micrococcus luteus*, *Bacillus subtilis*, and *Staphylococcus aureus*), and yeasts in Philippine isolates. Their results showed that while *E. coli* remained the most efficient food source, some dictyostelids could partially utilize *P. aeruginosa* and *K. pneumoniae*. Notably, gram-positive bacteria were poorly consumed, and yeasts were barely utilized. These findings align with Raper's early observations on *E. coli*'s physical advantages but also highlight the need for species-specific bacterial selection in experimental designs.

In his book *The Dictyostelids* (Raper 1984) listed 13 agar-based culture media for cultivating dictyostelids (Fig. 11). Among these, one through five are used for observing and studying the vegetative growth of myxamoebae and the process of pseudoplasmodium formation, while six through 13 are used for producing luxuriant growth and development of robust dictyostelids obtained

by cultivating them in association with *E. coli*. Medium 12, dubbed the "SM medium," presumably as an acronym for slime mold medium, probably has been used more than any other.

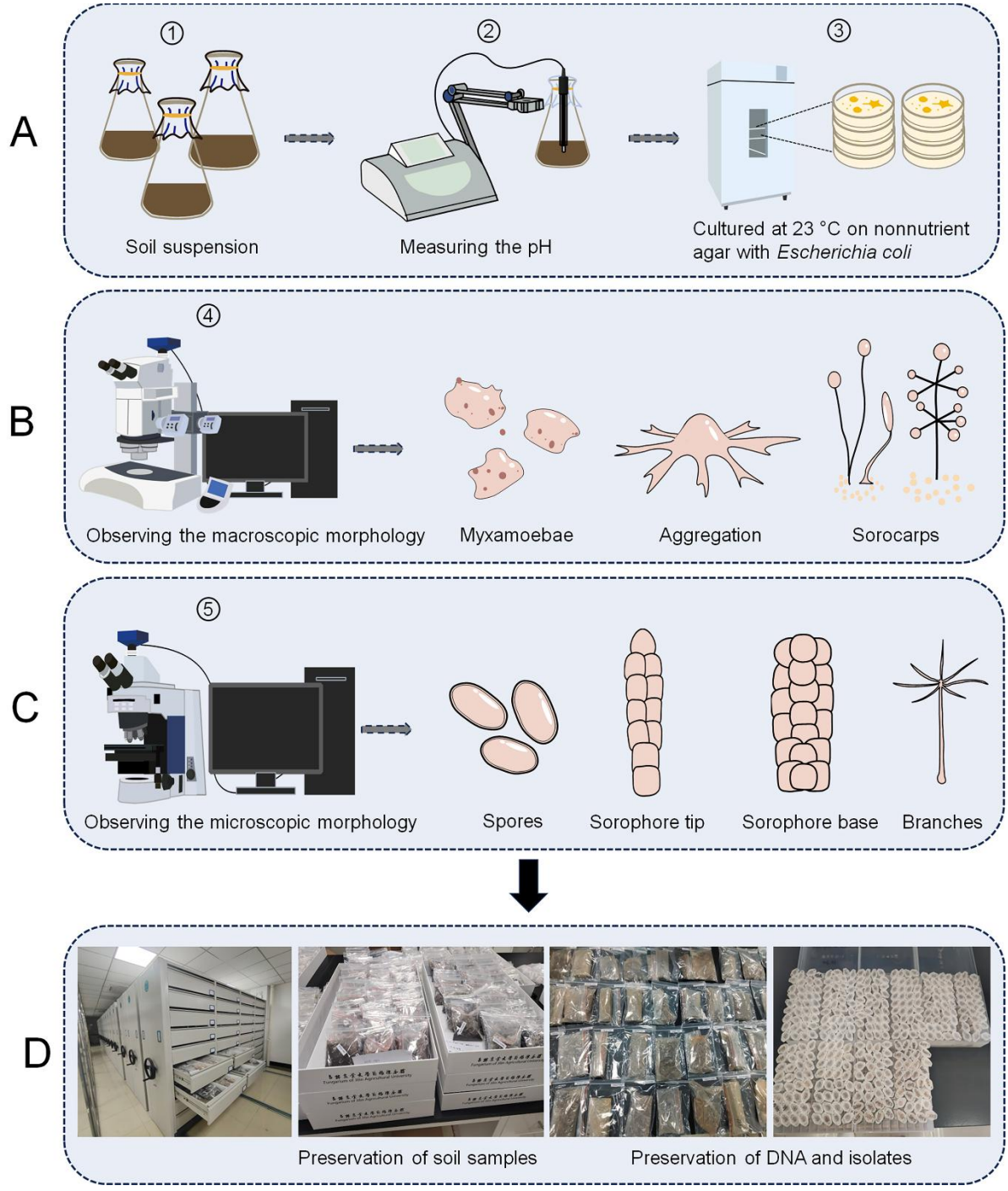


Figure 10 – Laboratory culture: (A) Isolation of dictyostelids from a soil suspension, measuring the pH of the soil suspension, and culturing at 23 °C on non-nutrient agar with *Escherichia coli*. (B) Laboratory equipment for observing the macroscopic morphology of dictyostelids, including myxamoebae, aggregations, and sorocarps. (C) Laboratory equipment for observing the microscopic morphology of dictyostelids, including spores, sorophore tips and bases, and branches of the sorophore. (D) Preservation of soil samples, DNA, and isolates in an herbarium [in this case the herbarium of the Mycological Institute of Jilin Agricultural University (HMJAU), Changchun, China].

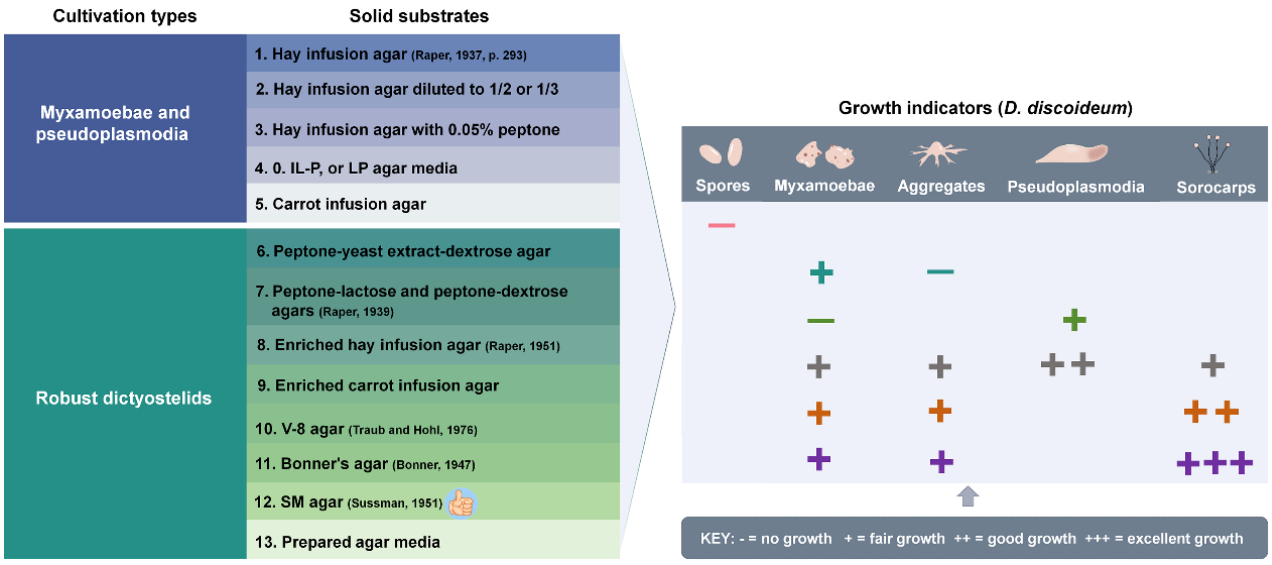


Figure 11 – Culture media and indicators for measuring the growth of *D. discoideum* as listed by Raper (1984).

4. Occurrence of dictyostelids in soil layers and other substrates

When Oskar Brefeld discovered dictyostelids, these organisms were associated with dung. However, it soon became apparent that dung was not their primary habitat. Instead, the primary habitat turned out to be the soil/litter interface zone of the litter layer found on the surface of the ground in forests. The studies that clearly stablished this fact were carried in temperate deciduous forests in Wisconsin in the United States, first by Kenneth Raper and then by Raper and one of his students, James Cavender (Cavender and Raper 1965a, 1965b, 1965c, Raper 1984). Cavender and others extended studies of dictyostelids to other types of forests, including coniferous forests and subtropical/tropical forests, where they found that differences existed in the abundance and diversity of dictyostelids present.

In any forest, dead leaves on the trees and other plants present are destined to fall to the ground. In deciduous forests, leaf fall is seasonal, but this is not the case in all other types of forests. Regardless of when they fall, leaves accumulate on the forest floor as a litter layer where they begin to decompose (Fig. 12A). The litter layer consists mainly of leaves but also includes various other types of plant debris, including twigs, small pieces of dead bark, fruits, seeds, cones, and inflorescences (Stephenson 1989). The leaves of deciduous trees, once on the ground, mostly decompose within a year and do not accumulate to any great extent except in special situations (e.g., redistribution as a result of downslope movement on steep slopes [Orndorff & Lang 1981] or by wind). The litter layer of coniferous forests is typically thick because the leaves (needles) do not readily decompose and do tend accumulate (Fig. 12B). In tropical/subtropical forests, conditions of constantly high temperatures and levels of precipitation promote rapid decomposition of litter, so the litter layer can be very thin and not completely cover the soil surface. This is also the case in dry forests, especially where the trees are evergreen and are characterized by sparce foliage (Fig. 12C).

The litter layer in a forest typically consists of three often relatively distinct layers (or horizons *sensu* Brady 1974). The first layer (O1) consists of that portion of the forest floor litter consisting of largely undecomposed leaves in which the original forms can be recognized by the naked eye (Fig. 12A). The second layer (O2) is the portion of forest floor litter that consists of organic debris partly decomposed to the extent that the original forms cannot be recognized. The third layer (A1) consists of the uppermost portion of the soil itself, which typically consists of soil with admixture of very decomposed organic material (humus) ultimately derived from the O2.

Coauthor Stephenson and John Landolt (Stephenson & Landolt 1996) examined the vertical

distribution of dictyostelids in these three layers for two study areas in the vicinity of University of Virginia Mountain Lake Biological Station in southwestern Virginia. Both study areas were in temperate deciduous forests, but they represented very different situations with respect to vegetation, soils, and microclimate. The first study area was an oak-dominated forest located at an elevation of 1265 m and characterized by highly acidic, poorly developed soils with low levels of major soil nutrients. The second study area was a mixed species forest located at an elevation of 683 m and characterized by only moderately acidic, well-developed soils with relatively high levels of major soil nutrients. Samples for isolation of dictyostelids were taken from a series of soil/litter cores collected from the two study areas. Cores were extracted using an aluminum cork borer with an inside diameter of 2.4 cm. To obtain a core, the cork borer was pressed firmly against the leaf litter layer on the forest floor and enough force applied to cause the borer to penetrate the litter and underlying soil to a depth of approximately 4.5 cm. After the borer had been removed, the intact core was extracted and samples from the portion of the core representing each of the three layers were removed and transferred to small sterile plastic bags. Samples were processed in the laboratory as soon as possible, using the method described by Cavender & Raper (1965a). Samples were not collected from the portion of the core beyond the A1, although there are a few reports of dictyostelids been recorded from depths of 7.5 cm or even greater (Raper 1984).

In both study areas, dictyostelids were most abundant in the O2 layer, but the numbers of clones/g were significantly higher (288 versus 1320) for the second study area. Dictyostelids also occurred in the O1 and A1 layers but their numbers were appreciably lower. The occurrence of dictyostelids in the O1 layer was surprising, but the O1 layer sometimes has been recognized as consisting of two sublayers viz. a “dry leaf” layer and a “wet leaf” layer (Raper 1984). These were not distinguished in the study described above, but it seems likely that the dictyostelids isolated would have come from the “wet leaf layer”. Eleven species of dictyostelids were recorded from the two study areas, with the first study area yielding seven species and the second ten species. All of these had been reported in previous studies carried out in the Mountain Lake area (Landolt & Stephenson 1986). Among the more abundant of these were *Dictyostelium mucoroides*, *Polysphondylium violaceum*, and *Raperostelium minutum*. The notable difference in biodiversity and abundance between the two study areas was attributed to the more nutrient-rich soil, the higher pH (3.7 versus 5.1), and the higher biodiversity of the vegetation in the second study area. It is possible that pH may have represented a factor of considerable importance, since a range of 5.0 to 8.0 is usually regarded as optimal for dictyostelids (Kitzke 1951), and the pH in the first study area was outside this range. Since the very earliest studies of dictyostelid distribution were carried out, it has been known that the abundance and biodiversity of these organisms vary from place to place, both on a local scale as shown in this study as well as a global scale. This will be the topic of the next section.

Interestingly, although it is clear that the soil/litter zone of forests represent the primary habitat for dictyostelids, they have been isolated from a number of unusual situations. These include spoiled vegetables, decaying grasses, rotting wood, and the decomposing fruiting bodies of various mushrooms (Raper 1984). However, there are two “other” habitats for dictyostelids that need to be considered in more detail.

In 1996, one of the coauthors and John Landolt were collecting soil samples from a study area in a lowland tropical forest within the Luquillo Experimental Forest in Puerto Rico when they decided to obtain a few samples from the layer of organic matter associated with the larger bromeliads growing on the trunks of trees (Stephenson & Landolt 2011). One notable feature of tropical forests, particularly in the Neotropics, is the abundance and diversity of vascular epiphytes (Fig. 12D). The larger branches and, to some extent, the trunks of trees upon which these epiphytes occur typically develop, at the base of the plant, a layer of dead organic matter derived from decaying epiphytes, partially decomposed tree bark, insect frass, and intercepted litter. This layer of organic matter has become known as “canopy soil” (Fig. 12E). When these samples were processed, they were found to contain dictyostelids. This prompted the two biologists to collect additional samples of canopy from the same forest the following year. This time, samples were obtained from five study sites, each in a different type of forest. Four species of dictyostelids were recovered, and all of these were recorded

from at least two of the study sites. *Dictyostelium purpureum* was the single most abundant species and represented 48% of all clones. Other species recorded were *Dictyostelium firmibasis*, *Heterostelium pallidum*, and an unidentified species of *Dictyostelium* (Stephenson & Landolt 1998).

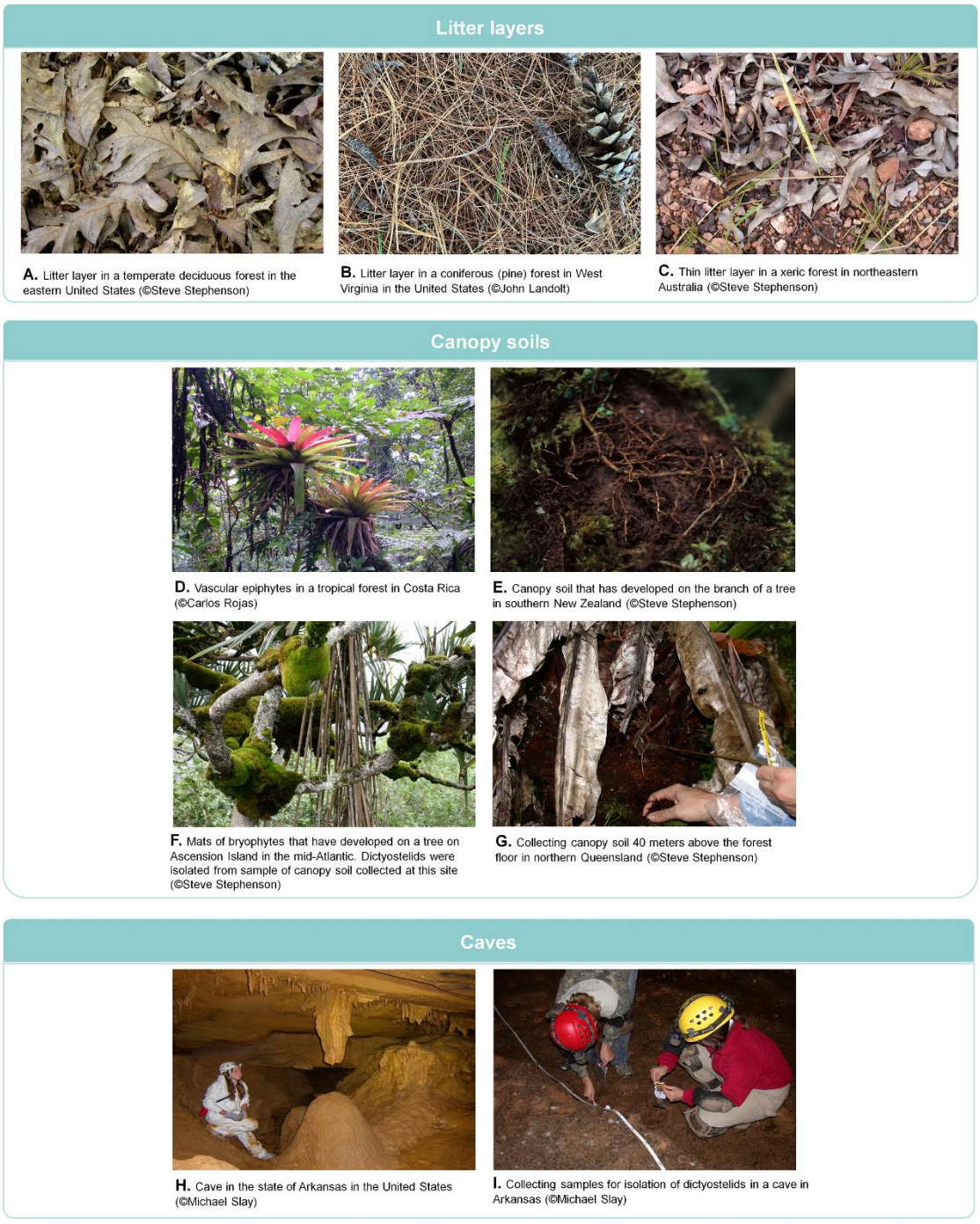


Figure 12 – The three habitat types of dictyostelids in nature, including litter layers (A-C), canopy soils (D-G), and caves (H, I).

In a paper published in 2011, Stephenson and Landolt summarized what was then known about the occurrence of dictyostelids in canopy soil, much of which had resulted from their own studies. They reported that between 1996 and 2008, more than 400 samples collected in 11 different localities around the world had yielded at least 37 different species of dictyostelids. In some localities, samples had been collected from only a single study site but in others samples came from multiple study sites. The 11 localities included a number of different types of tropical forests (e.g., coastal rain forests,

lowland rain forests, montane rain forests, and cloud forests) along with two non-tropical forest types (i.e., a temperate coastal rain forest in the Pacific Northwest of the United States and a montane southern beech forest in New Zealand). In some of these forests, the samples of canopy soil were collected from beneath mats of epiphytic bryophytes (mostly mosses but also including leafy liverworts). As such, canopy soil can be associated with both nonvascular as well as vascular epiphytes (Fig. 12F). In the most mesic forests, it is often a mixture of the two types of epiphytes.

Samples were obtained from three continents (Australia, North America, and South America) as well as Ascension Island, Cuba, the Dominican Republic, New Zealand, and Puerto Rico. Ascension Island was the most isolated locality, since the island is located in the mid-Atlantic a considerable distance from Africa and South America. In northern Queensland in Australia, where access to the upper canopy was provided by a canopy crane, samples of canopy soil were collected more than 40 m above the forest floor (Fig. 12G). Surprisingly, these samples yielded three species (*Acytostelium minutissimum*, *Dictyostelium myxobasis*, and *Polysphondylium australicum*) that were described as new to science. These species are not yet known from ground sites.

Numbers of clones/g varied rather widely among the localities from which samples were obtained. The mean densities for five of the six localities represented by the greatest numbers of samples (>35) were >100 clones/g. The highest value for a single locality was 395 clones/g for a tropical forest in Honduras, whereas the lowest value (10 clones/g) was for a temperate rain forest in southeastern New Zealand. As a general observation, samples of canopy soil collected in the Neotropics appear to be the most favorable for dictyostelids. It seems likely that this can be attributed the abundance of vascular epiphytes.

Although not generally recognized as a habitat for dictyostelids, the data now available provide clear evidence that canopy soil can support a diverse assemblage of species. Moreover, overall abundance (clones/g) is canopy soil is sometimes comparable to those reported for ground sites. In a few instances, dictyostelids have been found to display of species richness and abundance that actually exceed those recorded for samples from ground sites at the same locality.

Stephenson & Landolt (2011) reported that for a tropical forest in Belize, the numbers were 101 clones/g (canopy) versus 60 clones/g (ground). For samples collected in Honduras, the difference was even greater, with 395 clones/g (canopy) and 116 clone/g (ground). These may represent exceptional situations, but they do reinforce the idea that dictyostelids are very capable of surviving in canopy soil.

Dictyostelids are thought to have a very limited potential for dispersal. If this is the case, the question might be asked as to how these organisms can reach what are habitats that are limited in extent, spatially isolated, and sometimes located many meters above the ground. This will be discussed later in this section.

Caves would seem to be a most unusual habitat for dictyostelids. Clearly, the cave environment is very different from the usual environments inhabited by dictyostelids However, but the relatively few studies that have been carried out indicate that their occurrence in caves is not uncommon (Fig. 12H).

The first report of dictyostelids in caves appears to be that of Orpurt (1964) who reported *Dictyostelium mucoroides* and *Polysphondylium pallidum* from a cave located on Eleuthera Island in the Bahamas. Later, Waddell (1982) recovered eight species of dictyostelids Blanchard Springs Cavern in Arkansas in the United States. One of these (*Speleostelium caveatum*) turned out to be new to science.

In what appears to be the extensive study involving a number of different caves, Landolt et al. (1992b) investigated 23 different caves in West Virginia. Nine species of dictyostelids were recorded, and three of these were recovered from least 10 different caves. One of the three species (*Dictyostelium rosarium*) was of particular interest, since it had not been recorded above ground in an earlier extensive study of dictyostelids throughout West Virginia. The occurrence of *D. rosarium* in caves was totally unexpected. Not only was this species recorded from 10 different caves, it was clearly the dominant species present. *Dictyostelium rosarium* (then described as new to science) was first isolated from a sample of surface soil in a xeric mesquite-shrub forest in southern Texas by

Cavender & Raper (1968). Raper (1984) considered it to be a species associated with arid and/or saline soils. Prior to the present study, there had been a previous report of the species from a cave in West Virginia Waddell (1982) but no indication that it was common to sometimes abundant in this habitat.

The study described above was followed by another study Landolt et al. (2006b) directed towards caves in the Ozark region of Arkansas, Missouri, and Oklahoma (Fig. 12I). The data from this study were combined with more limited data for other caves in the Bahamas (Landolt & Stihler 1998), Georgia (Reeves et al. 2000), South Carolina (Reeves 2001), and Puerto Rico (Nieves-Rivera 2003) in addition to few unpublished lists and the earlier study in West Virginia (Landolt et al. 1992b). A total of 123 caves were investigated for the presence of dictyostelids, and samples from 102 (83%) of these yielded at least one species. In West Virginia, the region for which the most data exist, dictyostelids were present in 59 of 61 (95%) caves. Most of the caves considered in this paper were in temperate North America, but dictyostelids were recovered from 11 of 13 (85%) of caves surveyed in Puerto Rico and the Bahamas.

At least 17 species of dictyostelid were recorded from the 123 caves along with a number of isolates that could not be identified to species. *Dictyostelium giganteum D*. mucoroides, *D*. *rosarium*, *D*. *sphaerocephalum*, and *Polysphondylium violaceum* were the most common species, and each was recorded from more than 25 caves. The only species not previously reported from caves was *D*. *caveatum*. Based on the data reported by Landolt et al. (1992b) for caves in West Virginia, numbers of clones/g in samples of cave soil can be very low (<2) or sometimes surprisingly high (>600). In summary, the distribution of dictyostelids in caves appears to be rather patchy, but in the microsites where they do occur, these organisms can exhibit surprising high levels of abundance and biodiversity.

Bats are known to be inhabitants of many of the 123 caves considered in this study. Since dictyostelids depend upon a variety of aerobic bacteria for food, the guano produced by bats almost certainly represents a factor of considerable importance, since other types of organic matter is sparce in caves. However, throughout the entire study, dictyostelids were rarely recovered directly from guano piles. Instead, they were most abundant in the zone just outside the guano pile. Landolt et al. (1992b) analyzed soils from a cave in West Virginia and forest soils from just outside the cave in West Virginia, indicated that the organic matter content was less than <1% for the cave soil and 40% for the forest soils. Levels of calcium, magnesium, phosphorus, potassium, and nitrogen were all higher (sometimes remarkably so) for cave soils. In contrast, the cave soils had a pH of 8.0, where the pH of the forest soils was 3.4. Clearly, cave soils and forest soils represent two quite different environments for dictyostelids. However, it should be noted that cave environment offers two advantages for dictyostelids since it offers constant temperatures and consistency high levels of moisture. Both are favorable for their growth and development.

As was the situation for canopy soil, there must be a way for dictyostelids to be introduced into the cave habitat, and it must be fairly effective to account for their relative abundance. This is another subject that will be addressed later in this paper.

5. Dispersal of dictyostelids

Unlike many other microorganisms, dictyostelids produce spores that appear to have a rather limited potential for dispersal. In the sorus of a sorocarp, the spores are embedded in a mucilaginous matrix that dries and hardens. As a consequence, the spores stand little chance of being dispersed by wind (Cavender 1973). Dictyostelids themselves are motile while they are in their amoeboid vegetive state, but the actual distance traveled is exceedingly small. After these cells aggregate and form a pseudoplasmodium, the latter in some species is capable of migrating a longer distance, but even under optimal conditions, this is no more than a few centimeters. Although dispersal of dictyostelids by water is possible, it probably occurs only under special conditions (e.g., flooding). The obvious question is how does dispersal in dictyostelids occur?

The soil/humus layer of forests, which is the primary habitat for dictyostelids, is also home to numerous small invertebrates. These invertebrates certainly serve to disperse dictyostelid spores

(Cavender 1973, Bonner 1982). In most instances, dispersal undoubtedly involves nothing more than the simple adherence of spores to the body surface of the invertebrate in question. However, dispersal sometimes takes place by ingestion-defecation. Huss (1989) isolated dictyostelids from the gut contents of field-collected earthworms and pill bugs. Suthers (1985) demonstrated that ground-feeding migratory song birds transport dictyostelids between eastern North America and the Neotropics. She isolated eleven species of dictyostelids from feces of ground-feeding eastern North American migratory thrushes, finches, sparrows and warblers, on both their breeding and winter grounds (Fig. 13A). Consequently, long-distance dispersal is possible for dictyostelids under these somewhat unusual circumstances. These likely accounts at least in part for the almost universal distribution of some species of dictyostelids.

In 1992, coauthor Stephenson and Landolt published the results of a study they had carried at the University of Virginia Biological Station in southwestern Virginia. The primary purpose of their study was to assess the distribution patterns of dictyostelids in the soil/litter microhabitat of five different types of forest communities that occur in the general area of the biological station (Landolt & Stephenson 1986). However, they also used this opportunity to examine fecal material collected from nine species of vertebrates that are common and widespread inhabitants of the temperate forests of eastern North America. These included the red-backed salamander, white-footed deer mouse, eastern chipmunk, pine vole, Carolina wren, slate-colored junco, and big brown bat (Fig. 13B).

Fecal material was processed as soon as possible following collection, using standard isolation procedures for dictyostelids. Nine species of dictyostelids were recovered, with each species being recovered from at least three different vertebrates. *Dictyostelium discoideum*, *D. sphaerocephalum*, and *Polysphondylium violaceum* were the three species most frequently isolated from fecal material. All are frequently isolated dictyostelids in the general area of the biological station. Two to five individuals of the three species of birds included in this study were positive for dictyostelids, which provides additional data in support of the results reported by Suthers (1985) and suggests that birds could play an important role in dispersal of dictyostelids over short distances. Clearly, numerous small mammals and salamanders are capable of doing the same thing.

Snakes, lizards, and turtles are widespread and often common inhabitants of both temperate and tropical forests. Although it would seem unlikely that dictyostelid spores would readily adhere to the dry scaly skin of reptiles, Trimble and Stephenson (2021) recovered four species of dictyostelids from wet sterile swabs applied to the ventral surfaces of several different species of snakes, lizards, and turtles (Fig. 13C). These included the three-toed box turtle, western rat snake, eastern garter snake, eastern fence lizard, little brown skink, and timber rattlesnake. The number of positive swabs was low, but dictyostelids were consistently present on all three types of animals. There is little question that these animals have contact with the litter/humus layer on the forest floor as they move about in the forest.

The data obtained by Stephenson & Landolt (1992) would seem to be relevant to the unusual habitats in which dictyostelids occur, as described in the previous section. For example, how are dictyostelids introduced to canopy soil, which can be tens of meters higher than their primary habitat on the ground? As noted above, various animals, ranging from invertebrates to amphibians, small mammals, and birds are capable of dispersing the spores of dictyostelids by means of ingestion-defection. In the temperate or tropical rain forests where canopy soil is best developed, there are several types of animals (small mammals, lizards, salamanders, insects, and snails) capable of making the journey from the forest floor to the canopy. It is theoretically possible for all of these to transport dictyostelid spores, either internally or externally. To our knowledge, this has never been investigated, but Landolt & Stephenson (1997) isolated three species of dictyostelids (Dictyostelium purpureum, D. sphaerocephalum, and Polysphondylium pallidum) from fecal material from three large land snails collected on the Luquillo Experimental Forest in Puerto Rico (Fig. 13D). At this locality, these snails were frequently observed to be present in aerial habitats (e.g., the trunks of trees) well above the ground. This is yet another aspect of dictyostelid ecology that warrants additional study.

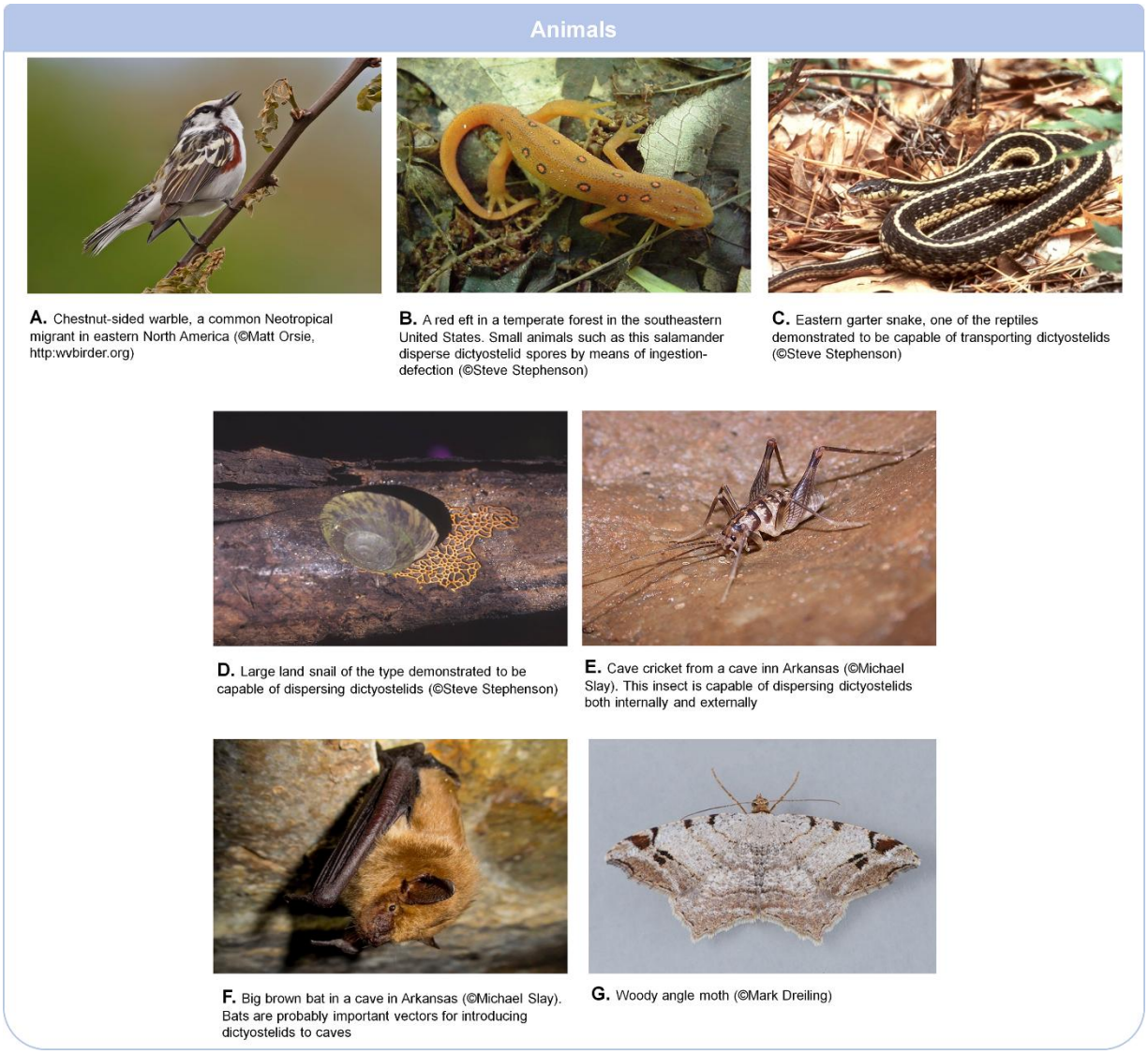


Figure 13 – The dispersal of dictyostelids in nature, including chestnut-sided warble (A), salamander (B), snake (C), snail (D), cricket (E), bat (F), and woody angle moth (G).

Sathe et al. (2010) collected samples of the fresh dung of a number of large mammalian herbivores on the Mudumalai Wildlife Sanctuary in South India (11 N). The animals were the elephant (*Elephas maximus*) spotted deer (*Axis axis*), barking deer (*Muntiacus muntjae*), sambar (*Cervus unicolor*), and gaur (*Bos gaurus*). When these samples were processed for dictyostelids, they yielded at least eight species. *Dictyostelium giganteum* and *Dictyostelium purpureum* were the most commonly encountered, with *D. discoideum*, *Raperostelium minutum*, *D. rosarium*, *Heterostelium pallidum*, and *Polysphondylium violaceum*. Sometimes a given sample produced more than a single species, something that also had been observed by Stephenson & Landolt (1992). Interestingly, Sathe et al. (2010) isolated dictyostelids from yak (*Bos grunniens*) dung collected from a site in the Himalayan Mountains (34º N) located an elevation of 5,300 m.

Sathe et al. (2010) also collected fresh samples of dung from several carnivores i.e. tiger (*Panthera tigris*), wild dog (*Cuon alpinus*), hyena (*Crocuta crocuta*), and panther (*Panthera pardus*) and when processed, these also yielded dictyostelids. For the hyena and panther, no isolates were identified to species, but *Dictyostelium giganteum* and *D. purpureum* were recovered from samples of dung from the tiger. The authors speculated on how dictyostelid spores could have been acquired by the various animals. The fact that dictyostelids could be recovered from fresh dung would appear to suggest that spores would have to have been ingested through the feeding activities of the animal,

passed along through the digestive tract, and then deposited on the ground within dung. Just how spores could be acquired from mammalian herbivores in nature, so they are unlikely to acquire dictyostelid spores in this manner. Presumably, they would have to come into contact in one of the primary habitats in which dictyostelids are found.

The information presented above invariably leads to two questions involving the presence of dictyostelids in caves. How are dictyostelids introduced into caves? What vectors might be involved? Stephenson et al. (2007) studied the cave cricket (*Ceuthophilus gracilipes*) in Arkansas (Fig. 13E). Although these crickets are primarily cave-dwelling, they regularly leave their subterranean habitats to forage in the forest floor litter layer outside the caves. While natural dispersal by carnivores remained problematic, the authors demonstrated that coprophagy (literally "feeding on dung") — thought not to be typical behavior for large animals — enables crickets to disperse dictyostelid spores both through surface adhesion and also via sequential ingestion and defecation processes. Presumably, numerous insects found in tropical forests would be capable of the same thing.

In their study carried out at the Mountain Lake Biological Station in southwestern Virginia, Stephenson and Landolt (Landolt et al. 1992b) isolated dictyostelids from fecal material collected from the big brown bat (Fig. 13F). This was unexpected since this bat feeds entirely upon flaying insects. Moreover, they recorded four different species and their bat fecal samples yielded a surprisingly high total of 570 clones/g. This suggested that bats are capable of transporting dictyostelids and probably represent one important way that these organisms are introduced into caves. Since bats only rarely have direct contact with the forest floor, this suggests that they acquire dictyostelid spores while in flight. Stephenson and Landolt hypothesized that some of the insects upon which the bat feeds are associated with the forest floor litter where they would have abundant opportunity to come into contact with dictyostelids. When these insects take to the air, they carry dictyostelid spores with them. Bats feed upon large numbers of insects each night, so the chances of encountering those insects with dictyostelid spores present would be fairly high. To assess this possibility, an effort was made to isolate dictyostelids from specimens of night-flying moths collected in a light trap placed out in the same general study area where bats were captured. A single isolate of *Dictyostelium discoideum*, one of the more common species in the Mountain Lake area, was recovered from a woody angle moth (*Semiothisa aequiferaria*). This moth (Fig. 13G) is known to pupate in forest floor litter and thus would have ample opportunity to pick up dictyostelid spores.

Although there is no experimental evidence that this takes place, it would be possible for the bats that feed on flying insects to introduce dictyostelids to caves, since many species of bats nest in caves.

Anthropogenic dispersal of dictyostelids is an understudied but potentially important factor accounting for the distribution of these organisms. Perrigo et al. (2012) investigated human footwear as a potential vector of dictyostelids. Eighteen pairs of boots were examined and dictyostelids were isolated from nearly all samples larger than 5.0 g of sample material obtained from these boots. In total, six isolates were representing four species (*Raperostelium*, *D. sphaerocephalum*, *D. leptosomopsis* and a new species of *Heterostelium*) were recovered. Anthropogenic dispersal could very well be responsible for the occurrence of dictyostelids on certain remote oceanic islands as well as biotic islands (e.g., small pockets of unique soils or vegetation in an otherwise homogenous landscape).

There is little question that animal (including humans) can disperse dictyostelids. Since the life cycle of dictyostelids encompasses several different stages, a question remains as to which stage is dispersed. Although it cannot not be discounted completely, it seems unlikely that any of the active stages (myxamoebae, pseudoplasmodia, or sorocarps) would be involved in dispersal. As such, effective dispersal almost surely depends upon spores and/or microcysts being transported by a potential vector.

6. Biogeography of dictyostelids

In 1973, James Cavender published a paper that represented the first effort towards developing

a more complete understanding of global distribution patterns of dictyostelids. He used data on the occurrence of these organisms obtained in twenty-eight countries on five continents. Twenty-nine different species, including five described as new to science, were considered in this paper. He reported that biodiversity is highest in the tropics and decreases with increasing latitude. Eight of the twenty-nine species appeared to be cosmopolitan, eleven species found in temperate regions also occur in the tropics, and approximately half of all species have distributions centered in the tropics and subtropics. Interestingly, the two most widespread and abundant dictyostelids were *Dictyostelium mucoroides* and *Heterostelium pallidum*, the first two species known to science.

Later, Swanson et al. (1999) compiled distributional data on the dictyostelids found in forest soils worldwide and attempted to determine just what factors influence the distribution patterns of these organisms. Sixty-five species of dictyostelids were considered in this study, and their distributions were found to fit into four different categories viz. cosmopolitan, disjunct, restricted, and pantropical. Cosmopolitan species appear to occur in virtually all forests throughout the world. Examples include *Dictyostelium mucoroides*, *Heterostelium pallidum*, *Polysphondylium violaceum*, and *Raperostelium minutum*. Disjunct species occupy similar habitats in widely separated regions, and *D. discoideum* and *D. firmibasis* were listed as two examples. Restricted species were defined as those seemingly confined to a specific region and have rarely or even not recorded anywhere else. Pantropical species, as the name of the category suggests, have distribution patterns centered in or even restricted to the forests of tropical regions. This assemblage of dictyostelids is more biodiverse than the assemblages associated with the other categories. However, the authors also noted that many of the sixty-five species being considered did not display readily discernable distribution patterns.

The main conclusions reached in this study as to the factors that determine differences in the distribution patterns of dictyostelids were the same as those suggested by the results of previous studies. Differences in climate and the resultant vegetation at a particular locality are certainly important factors, since the organic inputs to the primary soil/litter habitat occupied by dictyostelids are ultimately determined by the plants present. In the same way, elevation and latitude are factors because they affect the composition of vegetation, and the type of soil parent material largely determines the pH of the soil that represents a major component of the primary habitat for dictyostelids.

Since these two papers appeared, a considerably larger body of data on the distribution of dictyostelids has become available, and the number of described species is several times greater. The purpose of this section is to review what is currently known about the distribution and occurrence of dictyostelids worldwide.

6.1 Major types of terrestrial ecosystems

Ecologists recognize seven major types of terrestrial ecosystems (Keith et al. 2020). These are tropical rainforests, temperate forests, boreal forests, grasslands, deserts, savannas, and tundras (Fig. 14). The distribution of these seven types (also sometimes referred to as biomes) on the surface of the earth is determined largely by two primary environmental factors (temperature and moisture). There can be considerable variation in the physiognomy and composition of the vegetation for a particular type of terrestrial ecosystem from place to place, both on a regional scale and also on a global scale. However, there are basic underlying features that tend to hold the examples of a single type together.

Tropical rainforests (Fig. 14A) typically receive constant rainfall throughout the entire year, and temperatures remain fairly high and thus are never limiting for the organisms present (Lewis 2006). Most trees in tropical rainforests are evergreen, and vegetation is usually rather lush and dense. Although organic inputs to the soil in tropical rainforests are high, the litter layer may not be well-developed because of the very rapid rate of decomposition. In some areas of the tropics, especially those more distant from the equator, rainfall can be limited since much of it is received during a "rainy season". As a result, the vegetation is noticeably less developed.

Temperate forests (Fig. 14B) are characterized by four distinct seasons (winter, spring, summer, and fall) with major differences among the seasons (Wang 2020). Winters can range from cool to

cold and summers from warm to hot depending upon the latitude at which the forests occur. Temperate forests, especially temperate deciduous forests, are better developed in the Northern Hemisphere than the Southern Hemisphere.

Boreal forests (Fig. 14C), also called taiga, make up the largest terrestrial ecosystem and occur extensive areas of North America, Europe, and Asia (Miller-Schroeder 2005). However, this type of ecosystem is essentially lacking in the Southern Hemisphere because there are no major landmasses at the latitudes where it would be expected to occur. Boreal forests consist mostly of various conifers such as fir, spruce, and larch, but some broadleaf trees (e.g., aspen and birch) are also present. Most of the trees present tend to have needle-shaped leaves and are evergreen. Winters are long and cold, and at the very highest latitudes, the active growing period for plants may be matter of a few weeks. Instead of conifers, the southernmost forests in South America, Tasmania in Australia, and New Zealand tend to be dominated by various species of southern beech, with various mixtures of other broadleaf trees also present. In New Zealand, the latter are mostly podocarps. The southernmost forests in the world (located at the southern tip of South America) are dominated by southern beech.

Boreal-like subalpine forests (Fig. 14D) often occur at higher elevations on mountains located in temperate regions of the world (Knight 1994). Some of the same genera of trees (e.g., spruce) make up the forests present, but the species are different from those found in boreal forests to the north. However, these subalpine forests do share many environmental features in common with boreal forests. Soils in both types of forests are usually fairly acidic and often have low levels of major nutrients.

Deserts (Fig. 14E) by definition receive little rainfall, although the actual amount varies among different deserts (Crawford et al. 1982). The most extremely xeric deserts such as the Namib Desert in southern Africa may not receive any rainfall in a particular year. Vegetation is usually very sparse, and many of the plants present have evolved leaves with a limited surface area (including being reduced to spines) to limit water loss. As a result, the habitat represented by the litter layer is often lacking. Two types of deserts are recognized viz. cool (or cold) deserts and warm (or hot) deserts. A pronounced difference often exists between nighttime and daytime temperatures, especially in warm deserts. The transition between a desert and an adjacent semi-desert, xeric grassland or xerophytic succulent shrubland is usually rather gradual, so defining the exact limits of a desert is often problematic.

Grasslands (Fig. 14F) are dominated by members of the family Poaceae (grasses) although woody plants tend to occur along the streams that flow through grasslands. Although grasses are usually the most abundant plants present, grasslands also contain numerous herbaceous plants. As a result, grasslands sometimes can be more biodiverse than forests (Bond & Parr 2010). Rainfall is limited but enough occurs to maintain an often fairly dense vegetation but one that is subject to burning during dry summers. Small areas of grassland sometimes can be found in otherwise forested regions of the world, and in some places such as the mountains of New Zealand and the Andes of South America, grasslands occur in the subalpine zone.

Savannas (Fig. 14G) resemble grasslands because of the relative dominance of grasses, but unlike grasslands, savannas receive enough rainfall to allow the growth of trees in groups or dotted across the landscape (Wang 2020). The largest and best-known examples of this type of ecosystem are found in Africa, but savannas also occur on other continents. Small areas of savanna-like vegetation sometimes occur in the transition (ecotone) zone between forests and grasslands.

Tundra is the type of vegetation characteristic of the very highest latitudes of the Northern Hemisphere, but it could be argued that true tundra does not exist in the Southern Hemisphere. The vegetation of the subantarctic islands in the Southern Ocean occurs under tundra-like environmental conditions but is markedly different from the tundra of the Northern Hemisphere. Tundra vegetation in the Northern Hemisphere typically consists mostly of low-growing grasses, herbaceous plants, mosses, and lichens, but dwarf willows and birches can be present and sometimes dominant in some types of tundra (Virtanen 2016). Much of arctic tundra is found in areas with permafrost (frozen ground) just beneath the ground surface. Where the landscape is flat, such as on the arctic coastal plain along the northern coast of Alaska, is dotted with small lakes or divided into strange geometric

patterns (Fig. 14H). This is wet tundra as opposed to the dry tundra found on more elevated sites (Fig. 14I). Subarctic alpine tundra (Fig. 14J) is found at the highest elevations on mountains located at lower latitudes. The vegetation is similar to that of northern tundra but there is no permafrost layer present in the soil.

Figure 14 – The seven major types in terrestrial ecosystems of dictyostelids, including tropical rainforests (A), temperate forests (B), boreal forests (C, D), deserts (E), grasslands (F), savannas (G), and tundras (H, I, J).

6.2 Studies of dictyostelids in tropical forest ecosystems

Early studies to document the occurrence and distribution of dictyostelids were mostly confined to the temperate deciduous forests of eastern North America, with very limited data from boreal forests. Until Cavender & Raper (1968) reported the results of a survey carried out in tropical and subtropical areas of North and Central America, there was little information available on the dictyostelids of tropical forests. The survey mentioned above encompassed more than tropical rain forests. Giant cactus forests, thorn forests, mesquite-scrub forests, tropical deciduous dry forests,

hammock forests, seasonal evergreen forests, and cloud forests also were sampled. Most of the study sites from which samples for isolation of dictyostelids were collected fall within the northern Neotropics.

Nine species were recovered from tropical rain forests in Costa Rica (Fig. 15A). The three most abundant dictyostelids were *Dictyostelium mucoroides*, *D. purpureum*, and *Heterostelium pallidum*. Some of the isolates of *D. mucoroides* were described in their paper as a new taxon, *D. mucoroides* var. *stoloniferum*. Numbers of clones/g were relatively low (50 to 600) except for an occasional sample where one or two species were abundant. The authors made note of the occurrence of the then recently described *Hagiwarea lavandulum* (Raper & Fennell 1967), a species that produces sorophores with crampon bases.

Cloud forests occur at the very highest elevations in the tropics of North and Central America. These forests are characterized by very dense vegetation, constantly high levels of moisture, and (because of the elevation at which they occur) temperatures less than the tropical rain forests found lower on the same mountains. Cavender & Raper (1968) recorded seven species from cloud forests, and samples yielded 100 to 500 clones/g. The seven species included two species (*Dictyostelium discoideum* and *Raperostelium minutum*) more characteristic of temperate forests.

Hammock forests, including some examples in Everglades National Park in extreme southern Florida, yielded ten species, including three species (*Hagiwaraea coeruleostipes, H. rhizopodium*, and *H. vinaceofusca*) with crampon bases. Numbers of clones/g ranged from 500 to 600. Overall, *Dictyostelium mucoroides*, *D. purpureum*, and *Heterostelium pallidum* were the usual dominants. *Raperostelium minutum* was not isolated, and *Tieghemostelium lacteum* was recorded for the only time during the entire survey.

Subtropical seasonal evergreen forests yielded the most species (14), and numbers of clones/g ranged between 400 and 1,500. *Dictyostelium mucoroides* and *Heterostelium pallidum* were usually the most abundant, but the assemblage of species present included three of those with crampon bases and several previously undescribed forms (e.g., *Cavenderia deminutiva*).

Tropical deciduous dry forests (Fig. 15B) occur on the Pacific slope of Mexico and Central America. These forests resemble temperate deciduous forests in the physiognomy of the vegetation but are floristically rather different except for the cooccurrence of a few tree genera such as *Quercus*. The two sites sampled yielded seven and eight species, respectively. The first site, located in Costa Rica, had *Dictyostelium mucoroides* and *D. purpureum* as the two most abundant species, while *D. mucoroides* was the single most abundant species at the second site, located in Mexico. *Acytostelium leptosomum* was more common at the latter site than in any other type of forest included in the survey.

Cavender (1969b) then turned his attention to the forests of East Africa, producing the first body of data for the continent of Africa. Samples were collected in Kenya, Tanzania, and Uganda in 1966. Much of East Africa covered by arid savanna, but the full range of vegetation present extends from thorn-scrub to temperate rain forests to subalpine forests. In this study, the primary focus was on areas of forests, which often occur in patches (Fig. 15C). Eight different species were isolated along with an unusual variant of *Dictyostelium mucoroides*. The four most abundant taxa were *Dictyostelium mucoroides*, the unusual variant of *D. mucoroides*, *Dictyostelium purpureum*, and *Heterostelium pallidum*. Numbers of clones/g ranged from 0 to 1,635. Sampling at an elevation above 3,000 m on Mt. Muhavura in Uganda yielded no isolates. The author noted that with increasing elevation, the assemblages of dictyostelids associated with forests in Africa became more temperate in character.

Seven years after the paper by Cavender appeared, additional data on the dictyostelids of Africa were provided by Hagiwara (1976a), based on a study carried out in 1974 on Mt. Margheruta (0º23' N and thus essentially on the equator) in Uganda. One hundred and forty-four samples were collected along an elevation gradient on the mountain that extended from approximately 1,070 m to 4,510 m. A total of 144 strains were recovered from the samples, although those samples collected at the very highest elevations did not yield any dictyostelids. Taxa recorded from this study were *Dictyostelium purpureum, Polysphondylium violaceum*, *Raperostelium minutum*, *R. monochasioides*, and six unidentified species of *Dictyostelium*.

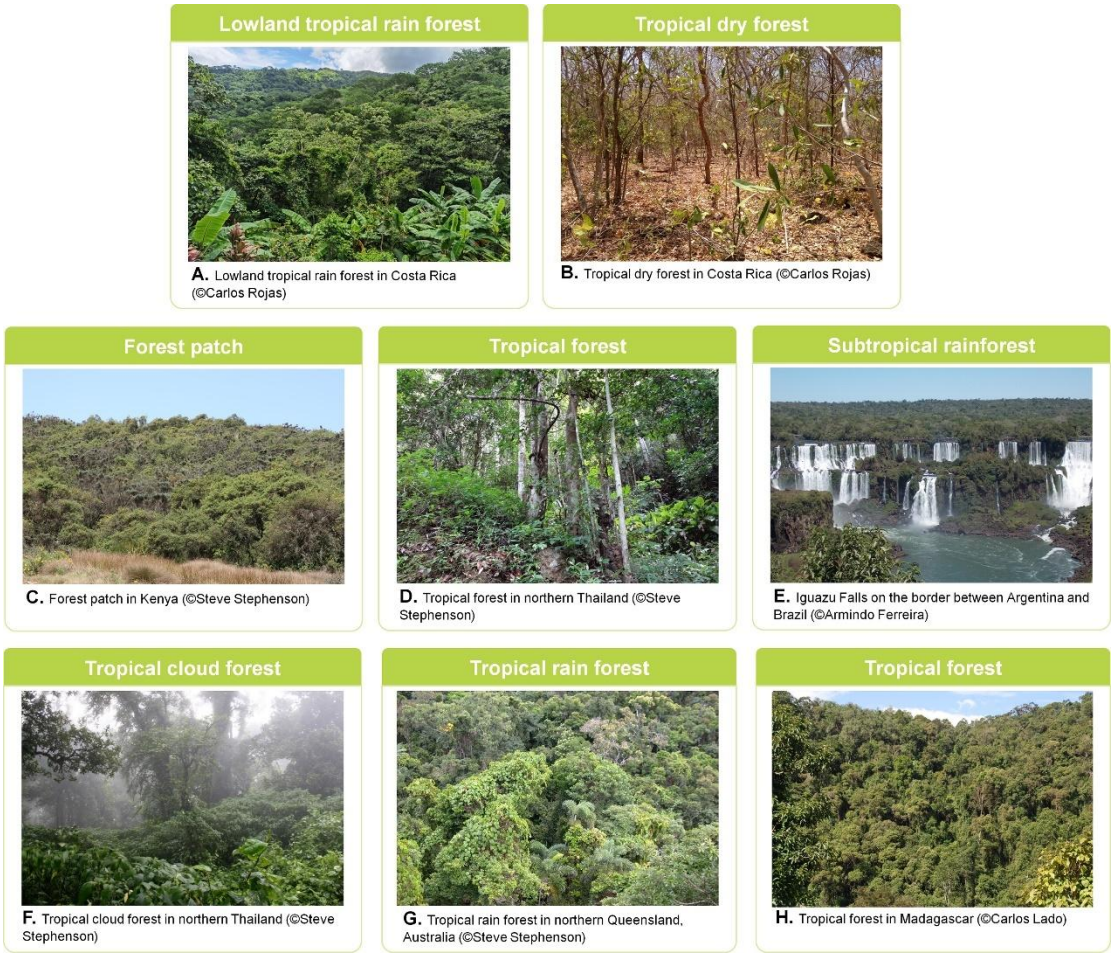


Figure 15 – The habitat types in tropical forest ecosystems of dictyostelids, including lowland tropical rain forest (A), tropical dry forest (B), forest patch (C), tropical forest (D), subtropical rainforest (E), tropical cloud forest (F), tropical rain forest (G), and tropical forest (H).

In 1995, Peter Njuguna, a graduate student at the University of Arkansas, collected a few samples at several sites in the Aberdare Mountains (0.4º S) of Kenya. Ten species of dictyostelids were identified from the isolates recovered from these samples, including *Coremiostelium polycephalum, Dictyostelium aureum*, *D. giganteum*, *D. leptosomum*, *D. mucoroides*, *Heterostelium tenuissimum*, and *Raperostelium minutum*. An appreciable number of other isolates could not be assigned to any described species, which suggests that the true biodiversity of dictyostelids in the tropical forests of Africa remains unknown (Cavender et al. 2010, Landolt et al. 2011).

In August of 2006, Jolanda Roux of the University of Pretoria collected samples for isolation of dictyostelids from site sites in Kruger National Park (24º S). These samples yielded six already described species (*Coremiostelium polycephalum*, *Dictyostelium giganteum*, *D. purpureum*, *D. sphaerocephalum*, *Hagiwaraea lavandula*, and *Polysphondylium violaceum*) along with along with isolates of at least five taxa that could not be referred to any described species (Cavender et al. 2010).

The first major study of dictyostelids in tropical rain forests in the Paleotropics was carried out by Cavender (1976b), who collected samples from thirteen study sites located in Singapore, Malaysia, Thailand, Indonesia, and the Philippines in southeast Asia (Fig. 15D). Sixteen species were recorded from the 2,998 clones appearing in primary isolation plates. Five species (*Dictyostelium mucoroides*, *D. purpureum*, *Heterostelium pallidum*, and *Polysphondylium violaceum* along with an unidentified species of *Dictyostelium*) were very common and widespread across all of the collecting sites Four other species (*Acytostelium globosum*, *Coremiostelium polycephalum, Hagiwaraea lavandula*, and *H. rhizopodium*) were much less common but still present in about half of the

collecting sites. All other species were localized in their distribution. The most significant finding from this study was that the six most common species in the tropical forests of Southeast Asia have comparable levels of abundance in northern Neotropical forests half a world away. Therefore, some dictyostelids clearly display a pantropical distribution.

Cavender & Lakhanpal (1986) included a number of collecting sites in subtropical and tropical forests in their survey of the dictyostelids of India. Seven collecting sites in tropical forests (ca 30º N) yielded the same species as temperate forests except for the notable absence of *Raperostelium minutum*. The mean number of clones/g for tropical forests was 547. Biodiversity of subtropical forests was lower, with only nine species being recorded. The mean number of clones/g (559) was essentially the same as for tropical forests. In addition to the absence of *R. minutum*, two other species (*Hagiwaraea vinaceofusca* and *R. tenue*) recorded for tropical forests were not isolated from samples collected in subtropical forests.

Landolt & Stephenson (1991) collected soil samples for isolation of dictyostelids from tropical rain forests in eastern Peru during the 1989 field season. Samples were collected from both primary and second forests. At least eleven species were recovered, nine of which apparently represented new records for the Amazon Basin. In addition, nine isolates were assigned to two as yet undescribed species, designated as *Dictyostelium* sp. A and *Dictyostelium* sp. B. Numbers of clones/g were low, with a mean value of 63 for all samples. The five most abundant species were *Dictyostelium mucoroides*, *D. purpureum*, and *Heterostelium pallidum*, the same three species reported by Cavender & Raper (1968) as the usual dominants in the tropical and subtropical forests of the northern Neotropics. Four of the remaining species are among those with digitate or discoid bases, but three of these were isolated only once. Especially noteworthy were the first records of *Dictyostelium macrocephalum* and *Raperostelium monochasioides* from the Neotropics.

Evidence of the very high biodiversity of dictyostelids in tropical rain forests was provided by Vadell (1993), who recovered 35 different taxa from soil samples collected from a semievergreen rain forest in Guatemala. Collecting was carried out in and around the ancient Mayan ruin of Tikal. This total included three species (*Heterostelium asymetricum*, *H. colligatum*, and *H. tikaliensis*) new to science. The thirty-five taxa represented the largest number of different dictyostelids obtained from a single forest anywhere in the world. As such, it represented a true “hot spot” for dictyostelids.

Landolt & Wong (1998) carried out a survey for dictyostelids on three (the big island [Hawaii], Kauai, and Oahu) of the Hawaiian Islands. Prior to their survey, there had been few reports of dictyostelids from any part of Hawaii. The authors collected 194 samples of soil from 20 collecting sites. Eight described species, one variety, and one species that was undescribed at that time were recovered. The totals recorded for the three different islands were comparable (five to six species). *Heterostelium pallidum* was the species most frequently isolated from the samples. Numbers of clones/g were low throughout, ranging from 0 to 73, with an appreciable number of samples yielding ten or fewer clones/g.

Swanson et al. (2004) studied ecological succession in assemblages of dictyostelids associated with variously-aged lava flows on the island of Hawaii. Samples of soil were collected from lava flows ranging in age from 67 to about 10,000 years. A total of eleven described species and two undescribed species were recovered from these samples. *Cavenderia aureostipes*, *Dictyostelium capitatum*, *D. impicatum*, and *D. mucoroides* were the most abundant species present, making up 96% of all clones. Mean numbers of clone/g ranged from 5 to 350. The most important finding from this study was that dictyostelid biodiversity and abundance appeared to decline with increasing age of the lava flow and thus the initial establishment of a primary habitat that could support these organisms.

Cavender et al. (2013) reported additional information on the dictyostelids of seasonal rainforests of Guatemala and the neighboring country of Belize. Sampling carried out in these forests yield 10 new taxa (nine species and one variety) of small species of dictyostelids, including *Raperostelium capillare*, *R. filiforme*, *R. reciprocatum*, and *Tieghemostelium unicornutum*. As will be described in another section of this paper, this particular paper represented a turning point in the study of dictyostelids associated with tropical forests, since SSU rDNA sequence analysis was used

to distinguish among morphologically similar species. Previously, only morphological and developmental (e.g., streaming behavior and patterns of aggregation) could be used for species differentiation. DNA sequence analysis narrowed the species concept applied to dictyostelids well beyond what was used in the Raper monograph (1984). Most subsequent studies of dictyostelids have incorporated this molecular approach to facilitate identification.

Stephenson et al. (1999) studied the dictyostelids associated with five tropical forest types (tabonuco forest, secondary tabonuco forest, palo Colorado forest, palm forest, and cloud forest) located along an elevation gradient in the Luquillo Experimental Forest (18º N) in Puerto Rico. Thirteen taxa of dictyostelids were recorded, but four of these could not be referred to any described species. *Dictyostelium mucoroides* and *Heterostelium pallidum* and were the two most abundant species, and the predominately temperate *Raperostelium minutum* was the most surprising record. Numbers of both species and clones/g were highest in the tabonuco forests at the low end of the elevation gradient, and no dictyostelids were recovered from the cloud forest at the highest elevation.

During 1995 and 2003, collecting was carried out in the Atlantic subtropical rainforest in the general vicinity of the Iguazú Falls (25º S) located on the border between Brazil and Argentina (Fig. 15E). This site turned out to be yet another "hot spot" for dictyostelids and yielded an amazing total of 13 species new to science (Vadell & Cavender 1995, 2007, Vadell, 2003). Among these were *Cavenderia macrocarpum*, *Dictyostelium dichotomum*, and *Heterostelium arachnoideum*. The large number of undescribed species from this locality suggested that the latter was yet another "hot spot" for dictyostelids in the Southern Hemisphere. Later studies, the more important of which are discussed in this paper, also reinforced the notion that biodiversity of dictyostelids in some regions of the Southern Hemisphere is exceedingly high. Not all of these are in tropical rain forests.

Cavender et al. (2013) collected samples for isolation of dictyostelids at 23 localities throughout Costa Rica, including examples of all of the major forest types. Collecting sites ranged in elevation from 10 to 3,000 m. More than 3,300 clones representing at least 24 described species and a number of isolates that could not be referred to any described species were recovered from the samples. *Dictyostelium mucoroides*, *Heterostelium pallidum*, and *Polysphondylium violaceum* were consistently present, as would have been expected based on the results reported in other studies carried out in tropical forests. Interestingly, cloud forests were characterized by the higher number of species, which contrasts the data reported earlier by Cavender & Raper (1968). As part of this study, a phylogenetic analysis of 18S rDNA sequences was carried out on 12 different isolates of the predominantly temperate *Dictyostelium discoideum* from Costa Rica, Mexico, and other localities scattered throughout the world.

In 2008, coauthor Stephenson collected 40 samples at four localities in northern Thailand to assess the biodiversity of these organisms in that region of the world. These samples yielded at least 15 species of dictyostelids. These included *Acytostelium* sp., *Coremiostelium polycephalum*, *Dictyostelium giganteum*, *D. mucoroides*, *D. purpureum*, *D. sphaerocephalum*, *Hagiwaraea lavandula*, *Heterostelium candidum*, *H. pallidum*, *Polysphondylium violaceum*, and *Tieghemostelium lacteum* along with forms that were not morphologically consistent with any described species. Later, these were described as five species (*Cavenderia aureostabilis*, *C. bhumiboliana*, *C. protodigitata*, *C. pseudoaureostipes*, and *C. subdiscoidea*) new to science based on a combination of morphological characteristics and their phylogenetic positions (Vadell et al. 2018). This study further confirmed the high biodiversity of dictyostelids in the forests of northern Thailand and the overall similarity of the assemblage of species present with that characterize Neotropical forests. Moreover, as discussed at some length in the paper, the study provided evidence of a locality where relatively rapid evolution and speciation in dictyostelids appears to have occurred in very small area of a tropical cloud forest near the summit of the Doi Inthanon (18º N, 2,560 m) in Doi Inthanon National Park. This site is characterized by cool temperatures, unusually high levels of organic matter, and consistently wet conditions (Fig. 15F).

Four years later, Cavender et al. (2022) described four new species (*Caverderia helicoidea*, *C. parvibrachiata*, *C. protumla*, and *C. ungulata*) from the set of isolates that had been recovered from the same small collecting site on Doi Inthanon (mentioned above) during the sampling carried out in

2008. The sequences obtained for these four species appear to indicate that they are very closely related and probably evolved *in situ*. This is a situation similar to what was reported by Cavender et al. (2005) for several morphologically similar species collected in a single small collecting site (a boggy area in a grass bald) located at higher elevations in the Great Smoky Mountains National Park in the United States.

Rukseree et al. (2018) collected soil samples from two districts (Phana and Mueang) in Amnat Charoen Province (15º N) in northeastern Thailand for a study of the phylogenetic diversity of dictyostelids. These samples produced 73 isolates of dictyostelids, from which 18S rDNA sequences were obtained. Mean numbers of clones/g were 106 and 238 for samples collected in the two districts. Forty eight percent of these isolates could be assigned to the genus *Dictyostelium*, with *Cavenderia* and *Raperostelium* next in abundance. Only two other genera of dictyostelids were identified from these sequences, and one of these (*Heterostelium*) was represented by only a single isolate. These data suggest that the assemblages of dictyostelids are not especially biodiverse at the level of genus in northern Thailand.

Seephueak & Petcharat (2014) collected samples of litter from 10 rubber tree plantations in southern China and processed these for dictyostelids. Ten different species were recovered. These were *Cavenderia macrocarpa*, *C. microspora*, *Dictyostelium dichotomum, D. mucoroides D. rosarium, Heterostelium pallidum*, *H. multicystogenum*, *Polysphondylium violaceum*, *Raperostelium minutum,* and *Tieghemostelium menorah.* The authors noted that samples collected from area where herbicides were used had much lower numbers of clones (15 to 33 clones/g versus 123 to 255 clones/g) than the areas in which herbicides were not used.

Liu et al. (2019a, 2019b) and Liu & Li (2012) reported four species of dictyostelids from Yunnan and Hainan provinces, respectively, in China. These were a *Heterostelium colligatum*, new record for China, and the new variant *Dictyostelium purpureum* var. *pseudosessile* from the Xishuangbanna Tropical Botanical Garden in Yunnan Province. This new variant *D. purpureum* var. *pseudosessile* was also isolated from samples collected in the Qixianling Hot Springs National Forestry Park in Hainan Province. *Dictyostelium gloeosporum*, another new record for China, was recorded from samples collected on Limu Mountain in Hainan Province, and *D. sphaerocephalum* was recorded from Wuzhi Mountain in Hainan Province.

Dictyostelids also have been reported from the tropical rain forests of Taiwan. For example, Hagiwara et al. (1985) listed *Dictyostelium macrocephalum* from Maopitou and Kenting of Taiwan. Hagiwara et al. (1992a) collected three samples for isolation of dictyostelids in Checheng, 21 samples in Kenting, six samples in Maopitou, and nine samples in Oluanpi. These samples yielded at least 11 species of dictyostelids. These included *D. macrocephalum* and *D. purpureum* in Checheng; a member of the *Acytostelium leptosomum* complex, *Cavenderia aureostipes*, *Coremiostelium polycephalum*, *D. macrocephalum*, *D. purpureum*, *Hagiwaraea lavandula*, a member of the *Heterostelium pallidum* complex, *Polysphondylium violaceum*, and *Raperostelium monochasioides* in Maopitou; and *C. polycephalum*, *C. aureostipes*, *D. macrocephalum*, a member of the *D. brefeldianum* complex, *D. purpureum*, a member of the *H. pallidum* complex, *H. lavandula*, and *R. monochasioides* in Oluanpi. Yeh & Chien (1983) isolated *Heterostelium pallidum* in the Hengchun Garden, located in Pingtung Taiwan.

Tropical rain forests occur in the northernmost portion of Queensland, Australia (Fig. 15G). During the period of 2001 to 2006, Stephenson and Landolt collected samples for isolation of dictyostelids from more than 30 localities and/or habitats in Australia, with a primary focus on northern Queensland. These samples included samples of "canopy soil" as well as ground soil samples. When processed, these samples yielded a total of 697 clones. Many of these could be assigned to described taxa, including such cosmopolitan species as *Dictyostelium giganteum*, *D. mucoroides*, *Heterostelium pallidum*, and *Polysphondylium violaceum*. However, a significant number of others turned out to be species new to science, which were described by Landolt et al. (2008). The new species were *Cavenderia boomeransporum*, *C. myxobasis*, *Heterostelium australicum*, *H. flexuosum*, *H. granulosum, H. rotatum,* and *H. stolonicodeum*. All of the new species except for *H. granulosum* (Western Australia) and *H. flexuosum* (Victoria) were from tropical rain

forests in northern Queensland. Interestingly, *C. myxobasis* and *H. australicum* were isolated from samples of canopy soil collected ca 40 meters above the ground.

In the survey of the distribution and occurrence of dictyostelids carried out in South Africa in 2006, the primary focus was on forests where these existed. Well-developed forests are uncommon in much of South Africa, so many of the collecting sites were in patches of forests or woodlands surrounded by savanna. During the entire survey samples were collected from 31 different study sites, although only 24 of these were positive for dictyostelids. A total of 881 clones were recovered from the positive samples, with a mean density of 210 clones/g. Eighteen different species were recorded, including six that were later described as new to science. These included *Cavenderia fulva*, *Hagiwaraea tenebrica*, and *Raperostelium cymosum*. The specific habitat listed for the collecting site for all of these new species was listed as either "subtropical forest" or "tropical woodland," which suggests that the assemblage of dictyostelids associated with tropical forests in South Africa has a seemingly unique component. Overall, for all collecting sites, *Dictyostelium giganteum* and *D. mucoroides* were the two most abundant species recorded in South Africa (Cavender et al. 2018).

The first comprehensive survey of the dictyostelids of Madagascar was carried out in 2009. A total of 52 samples were collected from 20 collecting sites in 18 localities. These samples yielded a total of only 401 clones. However, these 401 clones represented 30 different species of dictyostelids, and seventeen of the species were new to science. Four additional clones could not be identified. The very low total number of clones reflected the fact that in several collecting sites every clone appearing in a primary isolation plate was a different species. Fourteen of the new species were members of the genus *Heterostelium* (Cavender et al. 2016). Three of these (*H. ampliverticillatum*, *H. cumulocystum*, and *H. pseudoplasmodiofascium*) were listed as having been isolated from samples collected in tropical forests. The authors noted that the total number of species recorded from Madagascar exceeded the total (25 species) known from all of mainland Africa at the time their study was carried out Cavender et al. (2010).

The vegetation of Madagascar ranges from tropical rain forests (Fig. 15H) to semi-desert. For the purposes of the survey, collecting sites were assigned to two categories (moist sites and xeric sites). Moist sites included subhumid tropical forests, primary montane tropical forests, gallery forests, and high elevation grasslands. Xeric sites included dry forests, succulent shrublands, and dry grasslands. Both abundance and biodiversity of dictyostelids were higher in the moist sites. It was noted that larger species of dictyostelids (e.g., *Dictyostelium giganteum* and *Polysphondylium violaceum*) were more commonly associated with xeric sites while smaller species (e.g., *Cavenderia deminutivum* and *D. implictum*) were commonly associated these mesic sites. Overall, *D. purpureum* was the single most widespread species.

Numerous groups of terrestrial organisms are known to reach their highest level of biodiversity in tropical forests. Based on the data on dictyostelids accumulated thus far, there seems to be little question that they adhere to this same pattern. Although several major attempts have been made to document more completely the species associated with all types of tropical forests, there are still regions of the world that warrant more study. This is particularly true for the Amazon Basin of South America, Africa, and Southeast Asia. Limited information on dictyostelids exists for all three regions, but it is clearly insufficient to document the assemblage of species present. A relatively larger body of data is available for northern Queensland, but the high percentage of new species identified thus far strongly suggests that additional surveys are warranted.

6.3 Studies of dictyostelids in temperate forest ecosystems

Serious studies of the distribution and occurrence of dictyostelids in temperate forest ecosystem began with Kenneth Raper in the 1950s and continued as a collaboration between Raper and his student James Cavender in the 1960s and beyond (Cavender & Raper 1965c). The first forests studied were in the general region of Wisconsin where the University of Wisconsin is located (Fig. 16A). In a paper published in 1965, they presented data on the species of dictyostelids present and their relative abundance in nine different forest types (mixed mesophytic, beech-maple, maple-basswood, oak-hickory, oak-chestnut, oak-pine, hemlock-white pine-northern hardwood, boreal,

and bottom hardwood) found in eastern North America. These data were based on an extensive set of samples (typically ten per collecting site and from four to eleven examples of the forest type in question). When these samples were processed nine species of dictyostelids were recovered. Four species (*Dictyostelium mucoroides*, *Heterostelium pallidum*, *Polysphondylium violaceum*, and *Raperostelium minutum*) were consistently present and usually dominant for a particular forest type. Five other species (*Acytostelium leptosomum*, *Coremiostelium polycephalum*, *D. discoideum*, *D. purpureum*, and *Tieghemostelium lacteum*) tended to be secondary components of the assemblages of dictyostelids associated with the nine forest types.

Numbers of species recorded for the nine forest types varied from only two for the boreal forest to all nine for mixed mesophytic and oak-hickory. Numbers of clones ranged from very low to high (<50 clones/g in boreal forests to >1,000 clones/g in oak hickory forests). However, the most significant aspect of this study is that it clearly demonstrated that species of dictyostelids display differences in their distribution patterns in response to differences in the habitats potentially available to them, with the environmental parameters (type and amount of the organic input from the plants present, moisture level, pH, and microclimate) associated with a particular forest type actually defining their habitat. Perhaps somewhat surprising, the authors found that more xeric forests (e.g., oak-hickory) appeared to more favorable for dictyostelids than more mesic forests (e.g., beech-maple).

This single study served as the starting point for similar studies in other examples of deciduous forest ecosystem as well as studies carried out in other types of terrestrial ecosystems. In one paper, Cavender & Raper (1965c) commented that *Raperostelium minutum* appeared to display a pattern of increasing importance with increasing latitude, and the effect of latitude on assemblages of dictyostelids is now well established.

In many respects, the changes that occur with increasing (or decreasing) elevation are similar to those associated with changes in latitude. This was demonstrated by Cavender (1980) in a study carried out in the Southern Appalachians of the United States, which is one of the most biodiverse regions of temperate forests anywhere in the world. He sampled nineteen study sites in seven different forest types that ranged in elevation from 230 to 1,800 m. Twelve described species of dictyostelids, including one species (*Acytostelium subglobosum*) not previously known from North America, and several unknown isolates of *Dictyostelium* were recovered from the study sites. *Coremiostelium polycephalum* was the only species listed as characteristic of forests of eastern North America that was not recorded in the study described above. As a general observation, the most favorable habitats for dictyostelids were found to occur at elevations between 590 and 820 m.

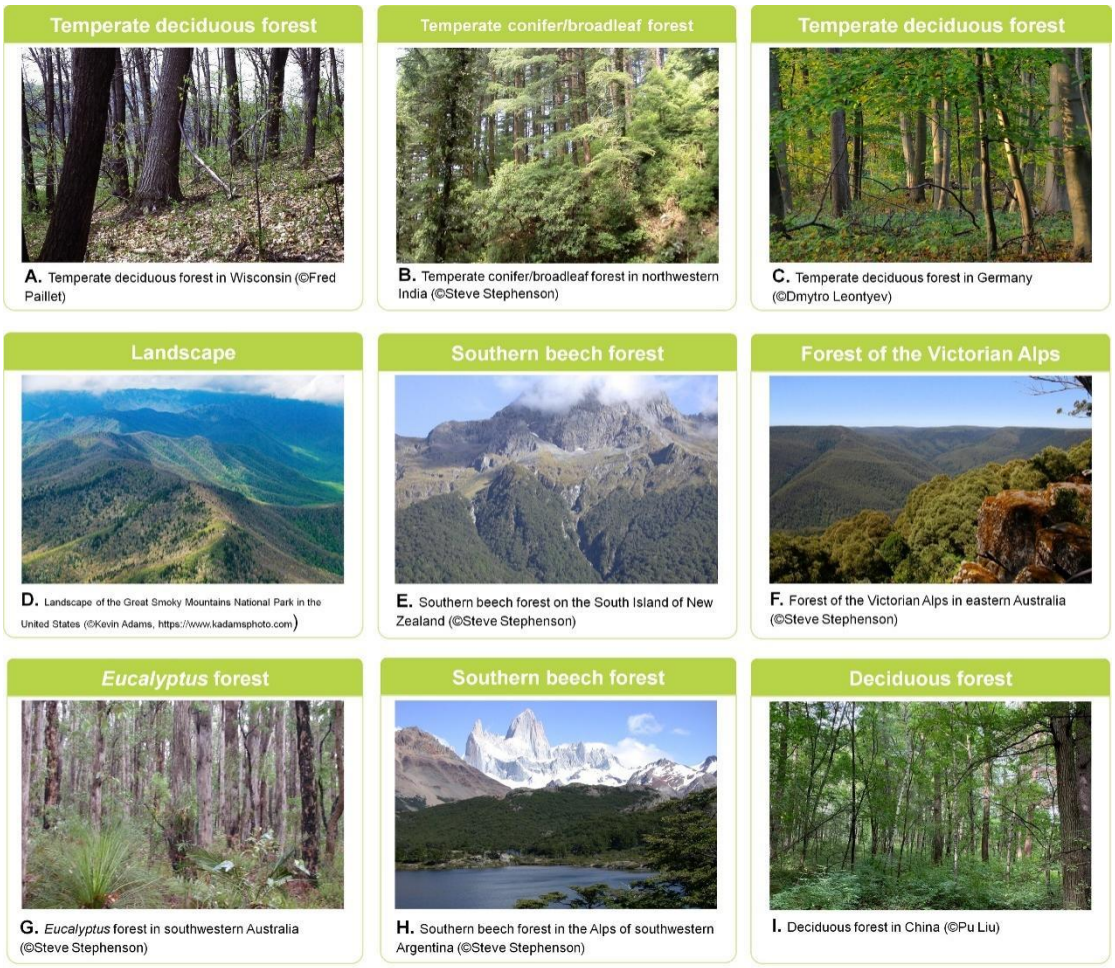


Figure 16 – The habitat types in temperate forests ecosystems of dictyostelids, including temperate deciduous forest (A), temperate conifer/broadleaf forest (B), temperate deciduous forest (C), landscape (D), southern beech forest (E), forest of the Victorian Alps (F), *Eucalyptus* forest (G), southern beech forest (H), and deciduous forest (I).

In any earlier study carried out in the western United States, Mishou & Haskins (1971) determined the occurrence and distribution of dictyostelids in the state of Washington. The authors divided the state into ten regions on the basis of geography, climate, and the predominant vegetation. Samples for isolation of dictyostelids were collected in all ten regions, which ranged from temperate rain forest to cold desert. Ten taxa were recorded, including *Cavenderia deminutiva*, *Dictyostelium discoideum*, *D. mucoroides*, a branched variant of *D. mucoroides*, *D. rosarium*, *Raperostelium minutum*, and *Tieghemostelium lacteum*. *Dictyostelium mucoroides* was the most widespread and abundant species. The eastern portion of the state of Washington is characterized by higher rainfall and thus more moist conditions than the western portion. Numbers of densities reflected this fact, with absolute densities significantly higher in the eastern portion.

Cavender & Lakhanpal (1986) provided the first data on the distribution and occurrence of dictyostelids in the forests of India (Fig. 16B). A total of 190 samples were collected from twenty-six sites ranging in elevation from 100 to 2,700 m and encompassing a number of different forest types. The majority of the sites were in the state of Himachal Pradesh (ca 31º N) in northwestern India, but two were in southern India (13º N). The samples yielded 6,353 clones, and twelve different species were identified. The most abundant species across India was *Heterostelium pallidum*, while *Cavenderia aureostipes*, *Dictyostelium mucoroides*, *Polysphondylium violaceum*, and *Raperostelium minutum* also represented significant components of the community. Six collecting sites in cool temperate forests yielded eight species, and nine species were recorded from seven collecting sites in warm temperate forests. Clone abundance showed marked variation between

forest types, with cool temperate forests yielding a mean of 881 clones and warm temperate forests 520 clones. The same five core species dominated both ecosystems, though differential abundance patterns emerged: *Cavenderia aureostipes* and *Polysphondylium violaceum* attained higher densities in warm temperate forests, whereas *D. mucoroides* proliferated more abundantly in cool temperate forests. In their paper, the authors included a key and descriptions of all of the species of dictyostelids then known from India. They also commented that overall biodiversity of dictyostelids in India was lower than anticipated, and the assemblage of species recorded appeared to display some similarity with the assemblage present in East Africa.

The first extensive study of dictyostelids in the temperate forests of Europe was carried out by Cavender (1969a), who collected samples for isolation of dictyostelids in the Netherlands, West Germany, Switzerland, France, Spain, Portugal, Italy, and (what was then) Yugoslavia (Fig. 16C). Only six species were recovered from these samples, which was lower than the total (nine species) that had been listed for eastern North America by Cavender & Raper (1965c). *Dictyostelium mucoroides* was the most abundant species and the only species recorded from several collecting sites. *Dictyostelium violaceum* was next in abundance and shared dominance with *D. mucoroides* at some collecting sites. *Heterostelium pallidum* and *Raperostelium minutum* were widely distributed but much less frequent than *D. mucoroides*. *Acytostelium leptosomum* and *Tieghemostelium lacteum* were isolated only once.

The next extensive study of dictyostelids in Europe was carried out in Switzerland (Traub, 1972, Traub et al. 1981a, 1981b). In this study, which took place during the period of 1972 to 1979, samples were collected from (1) the deciduous forests that are the predominant forest type at lower and intermediate elevations and (2) the coniferous forests dominated by spruce, larch, and pine found at higher elevations in the Alps. Samples collected at fifteen different sites yielded 7,266 clones, and a total of sixteen species were identified, including three (*Dicytostelium fasciculatum*, *Heterostelium filamentosum*, and *Synstelium polycarpum*) described as new to science. *Dictyostelium mucoroides* was found to be the overwhelming dominant in all the forest types investigated, with *Polysphondylium violaceum* and *Raperostelium minutum* also abundant in deciduous forests. These three species made up 87% of the clones isolated from soil samples. In coniferous forests, *Cavenderia aureostipes* and *S. polycarpum* were characteristic of coniferous forests. The general pattern was for numbers of species to be highest in the mesic beech forests at lower elevations, with lower numbers in the coniferous forests located at the highest elevations sampled. In their paper, the authors provided detailed descriptions of the patterns of distribution of eleven species associated with the various forest types in Switzerland.

In 1993, twenty-four study sites in deciduous, coniferous, and mixed coniferous forests in southern, central, and western Germany were sampled for dictyostelids as reported by Cavender et al. (1995). When processed for dictyostelids, these samples yielded 18 species, one of which (*Dictyostelium germanicum*) was described as new to science. The two most common were *Dictyostelium mucoroides* and *Polysphondylium violaceum*. Four species (*Cavenderia microsporum*, *D. capitatum*, *D. implication*, and *Heterostelium candidum*) originally described from Japan were recorded for the first time from Europe. The assemblages of dictyostelids associated with coniferous forests and deciduous forests were found to be distinctly different, with the latter characterized by higher numbers of species and clones/g. Overall, the number of species in Germany was comparable to those of Ohio/West Virginia in North America and the cool temperate region of Japan. The authors provided images, descriptions, and a key to the dictyostelids recorded for Germany.

Studies of the distribution and occurrence of dictyostelids of Japan began in the late 1960s (e.g., Lee 1971, Yamada et al. 1969) but were greatly expanded by Hagiwara (Hagiwara 1971, 1973b, 1974, 1978, 1979, 1983b) from 1971 onwards. Hagiwara, who also made significant contributions to the taxonomic concepts used for dictyostelids, described a number of species (e.g., *Cavenderia delicatum*, *D. firmibasis*, and *Heterostelium tenuissimum*) new to science.

In 1987, Cavender & Kawabe (1989) collected samples for isolation of dictyostelids at 41 sites in six major localities in Japan as one component of ecological and biogeographical study of these

organisms. The collecting localities were located within examples of the three major forest types (evergreen, deciduous, and mixed conifer-deciduous) found in Japan. A total of 144 samples were collected. These yielded 4,371 clones representing twenty-six different species. At that time, this was the largest number known for any region of comparable size in the temperate zone. *Dictyostelium mucoroides* was the most widespread and abundant species, with *Polysphondylium violaceum* ranking second. The three major forest types differed with respect to the numbers of species recorded, with mixed conifer-deciduous forests characterized by the highest number (23) and evergreen (12) the lowest. Perhaps the most important contribution of this study is that it demonstrated that the assemblage of species of dictyostelids present in Japan (and by extension eastern Asia) was compositionally similar to that found in eastern North America. As such, dictyostelids would appear to show evidence of the same "Grayan disjunction" pattern noted for vascular plants and least to some extent higher fungi (Vasilyeva & Stephenson (2010).

Two more limited studies of the dictyostelids in the eastern United States were those of (Landolt & Stephenson 1989) for the state of West Virginia and Landolt & Stephenson (1986) for the Mountain Lake area of southwestern Virginia. In the first of these, samples were collected from 21 study sites, including examples of all major forest types of the state, and these forest types ranged in elevation from 145 to 1,210 m. Twelve species were recovered. The mean number of species isolated at a given study site was 6.6, and the average density (clones/g) was 140. Several of the more widely distributed and abundant species were found to have distribution patterns that display a response to elevation. For example, *Dictyostelium purpureum Polysphondylium violaceum*, and *Raperostelium minutum* became less important with increasing elevation, whereas *D. discoideum* and *D. mucoroides* became more important. In general, numbers of clones recovered in this study were highest in soils at lower elevations and tended to decrease with increasing elevation. In the second study, carried out in forests located at elevations >1,100 m, six described species were recorded along with two species that may represent forms new to science. Numbers of clones/g were low, ranging from 16 to 96. Interestingly, *Dictyostelium discoideum* was the most abundant dictyostelid in three of the study sites from which samples were collected.

During the period of 1989 to 1993, Landolt & Stephenson (1995) studied the dictyostelids associated with mixed hardwood forests in five different watersheds on the Fernow Experimental Forest (39º N) in eastern central West Virginia. On thirteen different dates during the five years, ten samples were collected in each of the four watersheds. Sampling was carried out on as many as four different dates in a single year, with the number of dates ranging from one to four (mean = 2.6 per year). A total of 520 samples were processed for dictyostelids, which is likely to have been the largest number ever collected from a single locality. These yielded twelve species of dictyostelids. This total included species known to be common in the forests of the eastern United States (Cavender & Raper 1965c) as well as two species (*Heterostelium candidum* and *H. tenuissimum*) that are uncommon. The mean numbers of species recorded for a particular watershed for all thirteen dates ranged from 6.2 ± 1.4 to 7.9 ± 1.9, which suggests that that biodiversity remained relatively constant during the entire sampling period. Mean numbers of clones/g recorded for the four watersheds were somewhat more variable, ranging from 82 to 612 when all watersheds and all dates were considered. However, the means for three of the four watersheds for all sampling dates were rather similar (326, 336, and 379), which indicated that the density of dictyostelids remained rather stable. The fourth watershed had the lowest mean numbers for both species (6.2 ± 1.3) and numbers of clones/g (193). Presumably, this was the result of some undetermined environmental factor to which dictyostelids respond. The most important result of the study was that it provided evidence that collecting at least ten samples for isolation of dictyostelids at a given locality in a temperate deciduous forest appears to provide a reasonable "snapshot" of the assemblage of the species present.

Some of the collecting sites in the Southern Appalachians used by Cavender and mentioned above are located in the Great Smoky Mountains National Park, which became a focal point for biodiversity studies of all groups of organisms during the ATBI project mentioned earlier in this paper (Fig. 16D). This was especially true for dictyostelids. During the period of 1993 to 2004, 412 samples for isolation of dictyostelids were collected from twenty-five study areas located throughout

the park. These samples yielded 2,300 different clones. At the beginning of the ATBI, twelve species of dictyostelids were known from the park. By end of the period of intensive sampling, eleven additional described species had been added to this total, including two species (*Dictyostelium fasciculatum* and *Synstelium polycarpum*) that were new records for North America. Moreover, fifteen or more forms that might represent species new to science had been recovered (Landolt et al. 2004). Ten of these were formally described by Cavender et al. (2005). All ten were small species (<2 mm total height) and were recovered mostly from acidic soils and at higher elevations. This brought the total number of species of dictyostelids recorded from the park to more than thirty, which is comparable to the totals reported for some areas of the tropics of Central America (Vadell & Cavender 2007). The distribution patterns and ecology of dictyostelids in the Great Smoky Mountains National Park were described in a paper by Landolt et al. (2006a).

In another more limited study, Landolt et al. (2009b) collected 167 samples for isolation of dictyostelids from seventeen different study sites throughout the state of Arkansas. Thirteen species were represented among the 2,082 clones recorded from the samples. *Heterostelium pallidum* was the overwhelming dominant and made up more than fifty percent of all clones tallied. Among the other species was *Hagiwaraea rhizopodium*, a species characteristically found in tropical and subtropical regions of the world.

James Cavender spent much of his academic career at Ohio University and remained a resident of the state post-retirement. He collected at various localities in the state over a period of more than forty years. Not surprisingly, the dictyostelid biota of Ohio is exceeding well-known. One of the localities is of particular interest because it has yielded nineteen species, only six fewer than the total (25) known for the entire state. This unusually biodiverse locality is Butterfly Woods in the Wilds, a large international conservation center located in southeastern Ohio (Cavender & Cavender 2013). In 2006, Cavender and Vadell published *Cellular Slime Molds of Ohio* (Cavender & Vadell 2006), which provides keys, descriptions, and pen and ink drawings for the twenty-four species of dictyostelids then known from Ohio.

In 1998, coauthor Stephenson collected samples for isolation of dictyostelids throughout New Zealand, the first time any temperate forest in the Southern Hemisphere had been investigated for these organisms. Temperate forests in New Zealand are largely dominated by podocarps on the North Island and by southern beech on the South Island (Fig. 16E). Collecting sites used in this study included examples of the main forest types found in the country and extended over a range of 35º to 47º S. Thirteen species of dictyostelids were recovered. All of these occurred at low frequencies and densities. This total included a number of species (e.g., *Dictyostelium mucoroides* and *Polysphondylium violaceum*) that are common and widespread throughout the Northern Hemisphere as well as several other species that have a more restricted distribution. Among the latter were *Cavenderia faciculata*, not previously known outside of Europe, and *D. rosarium*, previously reported from only a few scattered localities in the Northern Hemisphere. Five of the species (*Cavenderia antarcticum*, *D. leptosomum*, *D. quercibrachium*, *Heterostelium anisocaule*, and *Raperostelium australe*) were described by Cavender et al. (2002). New Zealand is the most isolated land mass of its size in the world, and the assemblage of dictyostelids present appeared to reflect this isolation. Prior to this study, the only records of dictyostelids from New Zealand were three species listed with no locality data by Olive (1975) and seven species reported by Stephenson et al. (1995) on limited collecting carried out in the country in 1992 and 1995.

There have been few reports of dictyostelids from non-tropical regions of Australia. In an unpublished M.Sc. thesis, Robson (1978) reported eight different forms that were recovered from samples collected in New South Wales, the Australian Capital Territory, and Queensland. These consisted of an unknown form suggestive of *Coremiostelium polycephalum*, *Dictyostelium purpureum*, a member of the *D. mucoroides* complex, *Heterostelium pallidum*, *Polysphondylium violaceum*, *Raperostelium minutum*, a form similar to *Tieghemostelium lacteum*, and another unknown form with brown pigmentation.

When Landolt et al. (2008) carried out their survey of the dictyostelids of Australia, primary emphasis was on the tropical forests of northern Queensland, but samples also were collected in the

Northern Territory, Tasmania, Victoria, and Western Australia. Most of the isolates recovered from the 223 samples collected in the entire survey could be assigned to described species, with *Dictyostelium mucoroides*, *Heterostelium pallidum*, *Polysphondylium violaceum*, and *D. giganteum* represented by the most clones. However, as noted elsewhere in this section, this set of isolates also yielded eight species new to science. One of these was from Victoria and another was from Western Australia.

Landolt et al. (2009a) reported the results obtained from 100 samples collected in seven collecting sites in Tasmania. All seven sites were located in southern beech forests. The samples yielded 125 isolates, and the number of clones/g was 21. Four described species and two others (*Dictyostelium* sp. and *Heterostelium* sp.) that appeared to represent species new to science were represented among the 125 isolates. *Dictyostelium mucoroides* was the most abundant species and made up almost half (47%) of all clones. Other species recorded were *Heterostelium pallidum*, *D. giganteum*, and *Cavenderia aureostipes*.

The large number of new species of dictyostelids recorded in the study mentioned above strongly suggests that additional species new to science await discovery in Australia. Regions of the country such as the Victorian Alps in New South Wales (Fig. 16F) and the southwestern portion of Western Australia (Fig. 16G) are largely covered by well-developed and relatively little disturbed forests in which suitable habitats for dictyostelids surely exist.

The temperate forest ecosystem type encompasses numerous different forest expressions that differ with respect to physiognomy, floristics, and climate. The forests of the Mediterranean Basin are among the most floristically diverse although also among the forests with the longest disturbance history by humans. Romeralo & Lado (2006) carried out the first extensive survey for dictyostelids in the region during 2003 and 2004. Collecting sites ranged in elevation from 10 to 1,700 meters and particular focus was on evergreen forests with oak present. Two hundred samples collected in Spain and Portugal were processed for dictyostelids. These samples yielded 200 isolates representing 14 different species, one of which (*Dictyostelium firmibasis*) was recorded for the first time from Europe. *Dictyostelium mucoroides* was the single most abundant species, with other commonly isolated dictyostelids (*D. giganteum*, *Raperostelium minutum*, *D. sphaerocephalum*, and *Polysphondylium violaceum*) also present. Prior to their study, only six species of dictyostelids were known from the two countries.

The first study of the dictyostelids associated with the temperate forests of southern South America was carried out in 2005. Forty-seven samples for isolation of dictyostelids were collected from five different provinces and six national parks in Patagonia and Tierra del Fuego, Argentina. Primary focus was directed towards southern beech forests (Fig. 16H), but samples also were collected in valdivian temperate rain forests and patches of forests containing such conifers as *Araucaria*. The 47 samples yielded 1,079 clones, and the mean number of clones/g was 383. One sample from Tierra del Fuego yielded 8,650 clones/g, which was one of the largest totals ever recorded. *Dictyostelium mucoroides* was the overwhelming dominant, and this species made up 83.5% of all clones. *Dictyostelium sphaerocephalum* and *Polysphondylium violaceum* were next in importance. Seven of the species recorded (*D. austroandium*, *D. chordatum*, *Cavenderia fasciculoideum*, *D. gargantuum*, *D. leptosomopsis*, *D. valdivianum*, and *Polysphondylium patagonicum*) were new to science and described by Vadell et al. (2011). As a result of this study, the authors concluded that the assemblages of dictyostelids associated with temperate forests of southern South America are more similar to those associated with forests in New Zealand than the assemblages present in temperate forests of North America.

China and eastern North America (more specifically the eastern United States both occur in the north temperate zone and are characterized by a cover of vegetation, at least over large portions of each country, that was derived from the Arcto-Tertiary Geoflora that once extended across higher latitudes of the Northern Hemisphere. The occurrence of representatives of some of the same genera of vascular plants in the two countries (i.e., "Grayan disjunction" referred to earlier in this paper) has long been noted. Liu et al. (2020) compared the assemblages of dictyostelids known from China and the United States. At the time this paper was prepared, 55 taxa (54 species and one variety) were

known from China, which was slightly higher than the total of 50 taxa (46 species and four varieties) known from the United States. Twenty-seven species were shared in common.

Temperate deciduous forests (Fig. 16I) which extend from about 23º26′ to 53°33′ N in China are characterized by the highest biodiversity of dictyostelids. Thirty-eight species have been reported from these forests. Among the most common and widespread of these are *Cavenderia aureostipes*, *Dictyostelium mucoroides*, *D. purpureum*, *Heterostelium candidum*, *H. pallidum*, and *Polysphondylium violaceum*. Thirty species of dictyostelids have been reported from temperate mixed forests, 19 species from temperate coniferous forests, 11 species from shrub communities, seven species from grasslands, seven species from economic crop fields, three species from bamboo forests, three species from alpine tundra, two species from alpine birch forests, two species from alpine grasslands, and two species from areas of grassland within forests (Liu et al. 2020, Li et al. 2021, Wei et al. 2021, Zhu et al. 2021, Zou et al. 2022, Zhang et al. 2023a).

In summary, the first studies of the distribution patterns of dictyostelids were carried out in temperate forests of North America, but subsequent studies have encompassed other regions of the world where this ecosystem type exists. Temperate forests in the Northern Hemisphere are better known and more extensive than those of the Southern Hemisphere. However, the more limited studies of the latter that have been carried out suggest that the assemblage of dictyostelids present probably contains an appreciable number of species not yet known to science.

6.4 Studies of dictyostelids in boreal forest and tundra ecosystem

Boreal forests occupy the northern portions of North America, Europe, and Asia. Many of the same genera of trees, although usually not the same species, occur throughout this entire ecosystem. Trees are mostly conifers but some broadleaf trees (e.g., birch and aspen) are also present. The boreal forest has a subalpine expression that is typically found at higher elevations but sometimes extends down into lowland areas just south of the true boreal forest (Fig. 17A). Tundra occurs north of boreal forests and temperate forests or sometimes grasslands are found to the south. The tundra-boreal forest ecotone is often rather indistinct, with portions of the southernmost tundra interspaced with areas with trees present. There is considerable variation in the climatic conditions associated with boreal forests, with a major factor being the latitude at which they occur. At the very highest latitudes, conifers prevail, but as latitude decreases, the proportion of broadleaf trees tends to increase, and some forests classified as boreal are actually mixed conifer/broadleaf forest (Fig. 17B). All this has to be considered when reviewing reports of dictyostelids from this ecosystem type.

Cavender (1972) carried out the first study of dictyostelids of boreal forests when he investigated the distribution and occurrence of these organisms in eastern Canada. Four species (*Dictyostelium mucoroides, Raperostelium minutum, Heterostelium pallidum*, and *P. violaceum*) were found to be common in the soils of spruce-fir forest soils, with *D. mucoroides* and *R. minutum* as the two main dominants. Numbers of clones were exceedingly high (up to ca 20,000 clones/g) for some microsites (presumably with very high densities of bacteria) on the forest floor. Other species recorded were *D. discoideum, Tieghemostelium lacteum*, and *Acytostelium leptosomum*.

Later, Cavender (1978) extended his studies to Alaska, collecting samples for isolation of dictyostelids in the boreal forests of the southeastern coastal region, interior Alaska, and in the Alaska Range. *Dictyostelium mucoroides* was the overwhelming dominant and was recorded for every collecting site. Other species recorded from at least one site were *D. giganteum*, *D. sphaerocephalum*, and *Tieghemostelium lacteum*. The samples also yielded a number of isolates that could not be identified and one species (*D. septentrionale*) new to science.

Frøyen & Langvad (1984a, 1984b) provided data on the distribution and occurrence of dictyostelids in Norway (50º to 70º N). Seventy-seven samples for isolation of dictyostelids were collected in from four different vegetation zones that ranged from mixed deciduous to alpine. Eleven different species were recorded, but three of these could not be identified. *Dictyostelium mucoroides* was the dominant species throughout, with *Polysphondylium violaceum* and *Cavenderia aureostipes* less abundant overall but dominant or subdominant in some collecting sties. *Dictyostelium fasciculatum*, *Raperostelium minutum*, *Polysphondylium pallidum* and an unidentified *Dictyostelium*

were ubiquitous but never dominant. The authors noted differences in abundance for some species that seemed to be related to increasing latitude or the associated changes in vegetation.

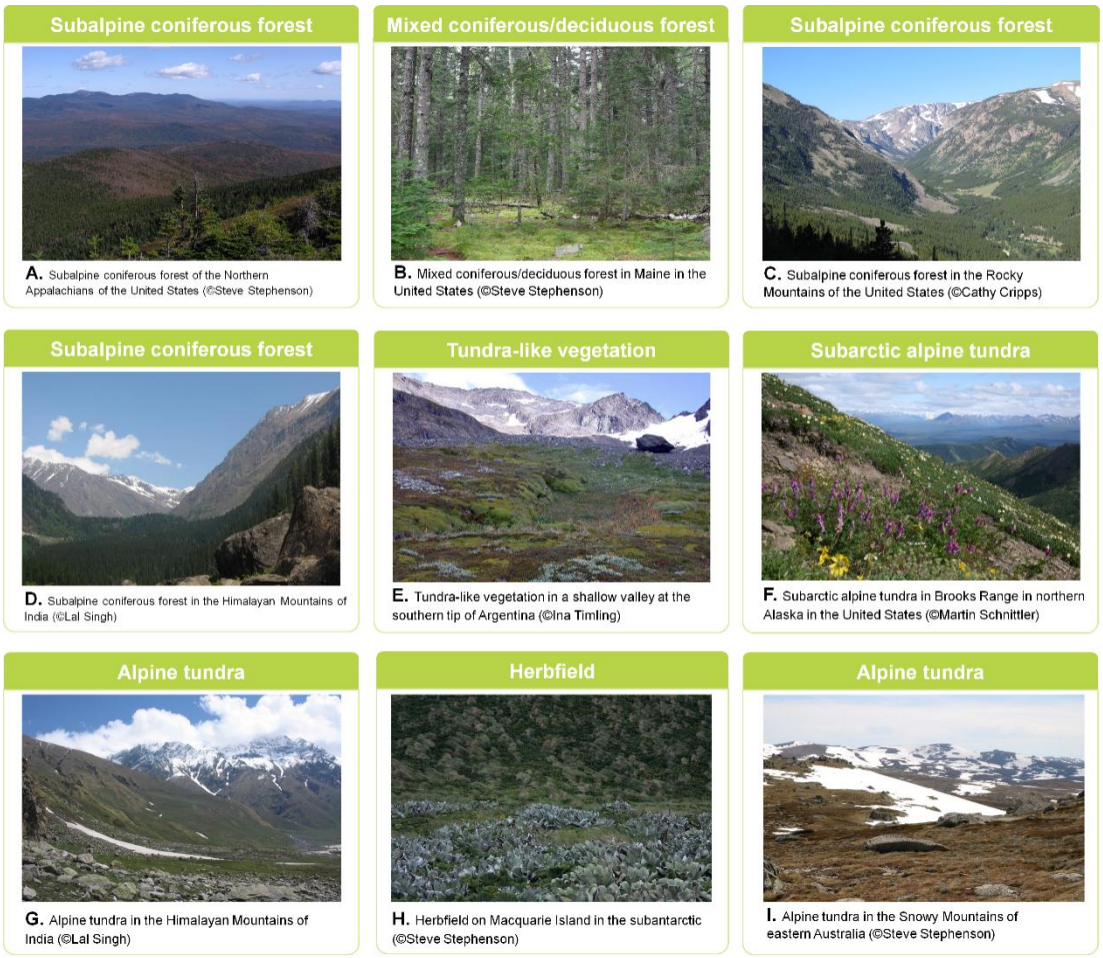


Figure 17 – The habitat types in boreal forest and tundra ecosystems of dictyostelids, including subalpine coniferous forest (A), mixed coniferous/deciduous forest (B), subalpine coniferous forests (C), subalpine coniferous forest (D), tundra-like vegetation (E), subarctic alpine tundra (F), Alpine tundra (G), herbfield (H), and alpine tundra (I).

Kawabe (1995) obtained 77 samples of forest soil from collecting sites located between 56º and 68º N (Lappland, Dalarna, Uppland, Skåne) in Sweden. The collecting sites included four different types of forests (oak/beech, pine, birch, and spruce). Only those forest types located at the highest latitudes sampled would be considered as falling completely within the boreal forest zone. The samples yielded a total of 2,739 clones, and numbers of clones/g ranged between 13 to 118. Only four species of dictyostelids were recovered. *Dictyostelium mucoroides* (considered *senu lato* in this study) was present in collecting sites, including the spruce forest type, where it was the only species isolated. *Dictyostelium purpureum*, *Polysphondylium violaceum*, and *Heterostelium pallidum* occurred much less frequently.

Romeralo et al. (2010a) described two new species of dictyostelids from coniferous forests in Alaska, one (*Dictyostelium ammophilum*) from the Kobuk Valley National Park (67º N) and the other (*Heterostelium boreale*) from the Caribou-Poker Creek Research Watershed (65º N). Both were ultimately recognized as species new to science on the basis of SSU rDNA sequence data. Other species recorded from this study were *D. mucoroides* and *D. sphaerocephalum* from the first sampling site along with *Raperostelium minutum* and *D. mucoroides* from the second sampling site (Overking et al. 1999, Landolt, unpub. data).

Perrigo et al. (2013) sampled for dictyostelids in high latitude (59º to 67º N) in northern Sweden. Collecting sites ranged in elevation from 20 to 470 m and encompassed a number of different boreal forest types, including those consisting of various admixtures of spruce, birch, and pine. Nine species of dictyostelids were recorded, including two species (*Dictyostelium barbibulus* and *Polysphondylium fuscans*) described as new to science. Only three of the nine species (*Raperostelium minutum*, *D. mucoroides*, and *P. fuscans*) were previously known from Sweden. The relatively low number of species recorded provided additional evidence of the pattern of decreasing biodiversity of dictyostelids with increasing latitude. This study was one of the first to use SSU rDNA sequences for accurate species identification of dictyostelids.

The subalpine expression of the boreal forest occurs on the higher peaks and ridges of the mountain ranges that extend southward into the temperate zone or in a few instances even into the tropics. When Cavender carried out a survey of the dictyostelids associated with the vegetation types that occur in the Rocky Mountains (Cavender 1983), he included some sampling sites in subalpine spruce-fir forests. of the Rocky Mountains (Fig. 17C).

Soil populations of dictyostelids were found to be generally smaller and less diverse in soils of Rocky Mountain forests than in eastern deciduous forests. Dictyostelids were found at all elevations and habitats sampled, including desert scrub, pinyon-juniper, ponderosa pine, spruce-fir, and tundra. Of these habitats, all of which exist along an elevational gradient, the subalpine spruce-fir zone, the most humid of the forest zones in this relatively dry region, had the largest and most diverse populations. Almost all sites were dominated by *Dictyostelium mucoroides* and *D. sphaerocephalum*. Since Rocky Mountain soils are exposed to severe environmental fluctuations these two species appear to be the most tolerant of the dictyostelids, particularly with respect to the stresses associated with widely varying conditions of moisture and temperature. *Dictyostelium septentrionalis* was the only rare species discovered.

In his survey of the distribution and occurrence of dictyostelids in India, two of his collecting sites were in subalpine spruce-fir forests in the Himalayan Mountains (2,700 m, 31º N). Some extensive areas of this type of forest occur in northwestern India (Fig. 17D). Four different species (*Dictyostelium mucoroides*, *Heterostelium pallidum*, *D. giganteum*, and *D. purpureum*) were recovered from these samples, and the mean number of clones/g was 252 (Cavender & Lakhanpal 1986).

Spruce-fir forests are limited to a few of very highest summits and ridges in the Central and Southern Appalachians. Stephenson & Landolt (1987) investigated the dictyostelids associated with this geographically restricted forest type. They collected samples from localities in Virginia, West Virginia, North Carolina, and the Great Smoky Mountains National Park. Eight species were recovered, but only *Polysphondylium violaceum* and *Dictyostelium discoideum* occurred in all four localities. Numbers of clones were very low (19 to 67), with the highest number recorded from a spruce-fir forest located at 2,030 m.

Those examples of boreal forests that have been investigated have yielded relatively few species of dictyostelids, which suggests that the biodiversity of these organisms is low in this type of ecosystem. However, there are vast stretches of northern North America and Asia where studies of dictyostelids have yet to be carried out. The same is true, albeit to a lesser extent, to the subalpine expression of boreal forests that occupies higher summits and ridges of mountains in the temperate and (more rarely) tropical zones.

As mentioned earlier in this paper, it could be argued that “true” lowland tundra does not exist in the Southern Hemisphere. The most tundra-like vegetation (Fig. 17E) occurs in the shallow valleys of the Alps at the very tip of southern Argentina (55º S). However, alpine tundra does exist at several places in the higher mountains of the Southern Hemisphere.

There have been relatively few studies of dictyostelids at the very highest latitudes in the Northern Hemisphere where tundra exists. Cavender (1978) reported two species (*Dictyostelium mucoroides* and *D. sphaerocephalum* from wet tundra in extreme northern Alaska (71º N). The samples from which they were isolated yielded 410 clones/g. One additional species (*D. gigiantum*)

was isolated from samples collected from subarctic alpine tundra (63º N) in the Alaska Range. Similar areas of subarctic alpine tundra are found in the Brooks Range in northern Alaska (Fig. 17F).

In a later study carried out in the Rocky Mountains of the western United States, Cavender (1983) recovered only three species viz. *Dictyostelium mucoroides*, *D. sphaerocephalum*, and *D. giganteum*. Numbers of clones were appreciably lower (mean = 180 clones/g) than the values (mean = 885 clones/g) he had recorded for alpine tundra sites in Alaska (Cavender 1978).

Stephenson et al. (1991) collected samples from nine study sites in areas of subarctic alpine tundra or arctic tundra in central and northern Alaska. Only the same two species reported by Cavender were recovered. Both were present in six of the study sites, only *D. mucoroides* at one study site, and two study sites yielded no dictyostelids. The mean number of clones/g was 118, but three of the four arctic tundra study sites yielded >200 clones/g.

Landolt et al. (1992a) investigated the distribution patterns of dictyostelids in the Kantishna Hills region (63º N) of Denali National Park and Preserve in Alaska. The predominant vegetation of the Kantishna Hills consists of subarctic alpine tundra on the higher peaks and ridges and low shrub communities on lower slopes. Samples were collected from seven areas of natural vegetation and also from seven plots being used in a streamside vegetation restoration project. The mean value of clones/g was much higher for the natural vegetation sites (430) than for the restoration sites (87). *Dictyosteloum mucoroides* and *D. sphaerocephalum* were the overwhelming dominants, but four other species (*Cavenderia aureostipes*, *Raperostelium minutum*, *D. giganteum*, and *Heterostelium pallidum* were recorded.

Stephenson et al. (1997) collected samples for isolation of dictyostelids from five sites in extreme western Alaska (>65º N latitude), including the Pribilof Islands, and seven sites Russian Far East (>59º N latitude) in 1994 and 1995. The collecting sites included tundra (including both wet and dry types) along with areas dominated by low shrubs, alder thickets, and larch woodlands. The sites in eastern Russia yielded five species while the sites studied in western Alaska yielded only two species. All of the species recorded for eastern Russia were new records for this region of the world, and one (*Polysphondylium violaceum*) had not been reported previously from Alaskan tundra.

Dictyostelium mucoroides and *D. sphaerocephalum* are clearly the dominant dictyostelids in both alpine and subarctic alpine tundra, based on the relatively limited numbers of studies that have been carried out. For example, Hagiwara (1982, 1990) recorded these two species in the alpine zone of the Himalayas of Nepal and also (Hagiwara 1992b) from alpine soils in Pakistan. However, *D. mucoroides* and *D. sphaerocephalum* appeared to be absent from some high-elevation localities, since Hagiwara (1982) and Kanda & Sato (1982, 1985) did not record these or any other species of dictyostelids from alpine areas in Japan. Another species of dictyostelid that has been reported from alpine tundra is *Dictyostelium aureocephalum* from the Himalayan Mountains of Nepal and Pakistan (Hagiwara 1990, 1992b). Kawabe (1993) listed *D. purpureum*, *P. violaceum*, *Heterostelium pallidum*, and *D. mucoroides* from the subalpine-alpine zone on Mount Fuji. In what appears to be the only effort to isolate dictyostelids from soil samples collected from the alpine zone of a mountain in the tropics, Landolt failed to recover any dictyostelids from samples collected by Stephenson at an elevation of approximately 4,000 m on Mount Kenya in East Africa (Cavender et al. 2010).

In their survey of the dictyostelids of India, Cavender & Lakhanpal (1986) did not collect any samples from alpine tundra. However, extensive areas of alpine tundra can be found throughout the Himalayan Mountains **(**Fig. 17G).

Although not necessarily associated with tundra vegetation, note should be made of the study by Liu et al. (2019c), who collected samples of isolation of dictyostelids at elevations >2,000 m on the Qinghi-Tibet Plateau in western China. The species recovered included two species (*Raperostelium minimum* and *D. multiforme*) new to science, but the more remarkable result of this project was to document the occurrence of dictyostelids at very high elevations. Five species were recorded from samples collected at elevations of 2,000 to 3,000 m, four species from samples collected at elevations of 3,000 to 4,000 m, and four species from samples collected at elevations of 4,000 to 5,000 m.

In April of 1995, coauthor Stephenson isolated *Dictyostelium mucoroides* from a mixed herbfield community on subantarctic Macquarie Island (54º S). Macquarie Island is an isolated oceanic island located 640 km from the nearest other island and 1,000 km from Tasmania. The predominant vegetation at lower elevations on the island consists mostly of low-growing vascular plants (Fig. 17H), and only few different types of communities are generally recognized, with herbfield the most widespread. *Dictyostelium mucoroides* was recorded from eight of sixteen collecting sites. Numbers of clones were invariably low (<50 clones/g), but one sample yielded 135 clones/g. In general, dictyostelids were more abundant in the layer of litter occurring above the soil than in the soil/litter interface zone.

In addition to the samples collected specifically for isolation of dictyostelids, single clones of *Dictyostelium mucoroides* were recorded from each of two moist chamber cultures prepared for myxomycetes. One culture was prepared with samples of rabbit (*Oryctolagus cuniculus*) dung and the other with moist leaf litter.

Six years later, Stephenson sent a series of samples collected from subantarctic Campbell Island (52º S) to Landolt, who processed the samples for dictyostelids. *Dictyostelium mucoroides* was isolated from four different samples (Landolt & Stephenson 2024). The vegetation of Campbell Island is similar to that of Macquarie Island, with the major difference being the presence of some woody plants the former and their complete absence on the latter.

Somewhat tundra-like vegetation occurs in a few alpine areas in the mountains of eastern Australia, including on the upper slopes surrounding Mt. Kosciuszko, the highest mountain in on the entire continent (Fig. 17I). The same situation is true in New Zealand, where tundra-like vegetation can be found in the Southern Alps, on the three volcanic peaks in the center of the North Island, and on Mt. Taranaki in the southeastern part of North Island. However, the vegetation bears little resemblance to the vegetation associated with typical alpine tundra vegetation in the Northern Hemisphere. Moreover, some of the tundra-like alpine areas found in the Southern Hemisphere are better characterized as fell fields with large rocks often present.

Other than those deserts where extremely severe conditions of high temperature and low availability of moisture exist, tundra is the ecosystem consistently characterized by the lowest biodiversity of dictyostelids. However, there are a number of different types of tundra, and in some of these there are microsites favorable enough to allow the occurrence of dictyostelids other than the two species (*Dictyostelium mucoroides* and *D. sphaerocephalum*) that are most associated with tundra.

7. Molecular studies of dictyostelids and the current system of classification

Dictyostelids are now recognized as model organisms for biomedical and human research, and they are also ideal organisms for studying fundamental processes in cell and developmental biology (Eichinger et al. 1999, Firtel & Meili 2000, Romeralo et al. 2013a, Mathavarajah et al. 2021). Moreover, dictyostelids also can serve as models for cell-autonomous defenses, human diseases, and social evolution (Hagele et al. 2000, Cosson et al. 2002, Steinert & Heuner 2005, Alibaud et al. 2008, Annesley et al. 2014, Dunn et al. 2018). Species numbers depend on the taxonomic concept used, and taxonomy is the study of identifying, grouping, and naming of organisms, based on similarities in such things as structure, origin, and their established natural relationships (Stephenson & Rojas 2017).

In this paper, we provide a historical review of dictyostelid classification from morphological-based approaches to those based on molecular biology. Traditionally, dictyostelids have been assigned to three genera viz. *Dictyostelium*, *Polysphondylium*, and *Acytostelium*. This changed when data obtained from modern molecular methods were used to develop a new system of classification. Dictyostelids were divided into four groups by molecular phylogeny with parallel small subunit ribosomal RNA and α-tubulin data sets (Schaap et al. 2006). Later study of Romeralo et al. (2011a) had expanded the four major groups. With deepening research, twelve genera—the old three ones *Acytostelium*, *Dictyostelium*, and *Polysphondylium* and nine new genera *Cavenderia*,

Coremiostelium, *Hagiwaraea*, *Heterostelium*, *Raperostelium*, *Rostrostelium*, *Speleostelium*, *Synstelium*, *Tieghemostelium* were finally confirmed in 2018 (Sheikh et al. 2018).

7.1 Molecular studies from one species sequencing to the new classification

As compared to studies of their morphology, molecular studies of dictyostelids had a lag time of nearly a century. Molecular phylogeny (Fig. 18) of dictyostelids had its beginning in 1965 but with a focus on only one species—*Dictyostelium discoideum* (Olsen & Sogin 1982). The complete nucleotide sequence of the gene for the *D. discoideum* small-subunit ribosomal RNA was determined for the first time in 1983 (McCarroll et al. 1983). Phylogenetic relationships of *D. discoideum* (then considered to be a member of the Protista) and the other major eukaryotic kingdoms (Plantae, Animalia, and Fungi) were explored in 1985 (Hasegawa et al. 1985). The molecular phylogenetic trees of cytochrome b and SSU rRNA suggested that *D. discoideum* is closer to green plants than to animals and fungi in 1995 (Angata et al. 1995). The complete genome sequence of *D. discoideum* was published in 2005 (Eichinger et al. 2005).

During the past few decades, molecular phylogeny has indicated that the traditional morphological taxonomy used for dictyostelids was flawed (Swanson et al. 2002, Sheikh et al. 2015, Singh et al. 2016). Schaap separated dictyostelids into four groups on the basis of molecular phylogeny using parallel small subunit ribosomal RNA (SSU) and alpha-tubulin data sets (Schaap et al. 2006). The nucleotide sequences of the ribosomal internal transcribed spacers DNA (ITS rDNA) and the 5.8S ribosomal DNA gene were shown to be able to distinguish some dictyostelids in 2007 (Romeralo et al. 2007a). Due to the generally slowly evolving nature of SSU rDNA, only SSU rDNA sequences fail to resolve the majority of branches within the four groups, and phylogenetic analyses of ITS rDNA sequences combined with SSU rDNA sequences were shown to be effective in 2010 (Romeralo et al. 2010b). Later, follow-up studies revealed a total of eight well-supported major groups, thus extending the findings of Romeralo et al. (2011a). With the increasingly larger body of data available, Sheikh et al. (2018) combined molecular and morphological data and proposed a major realignment of dictyostelids from the classic four genera, two families, and one order that can distinguished morphologically (on the basis of differences in sorophore structure and branching pattern) to 12 genera, four families and two orders on the basis of unique 18S rRNA sequence signatures.

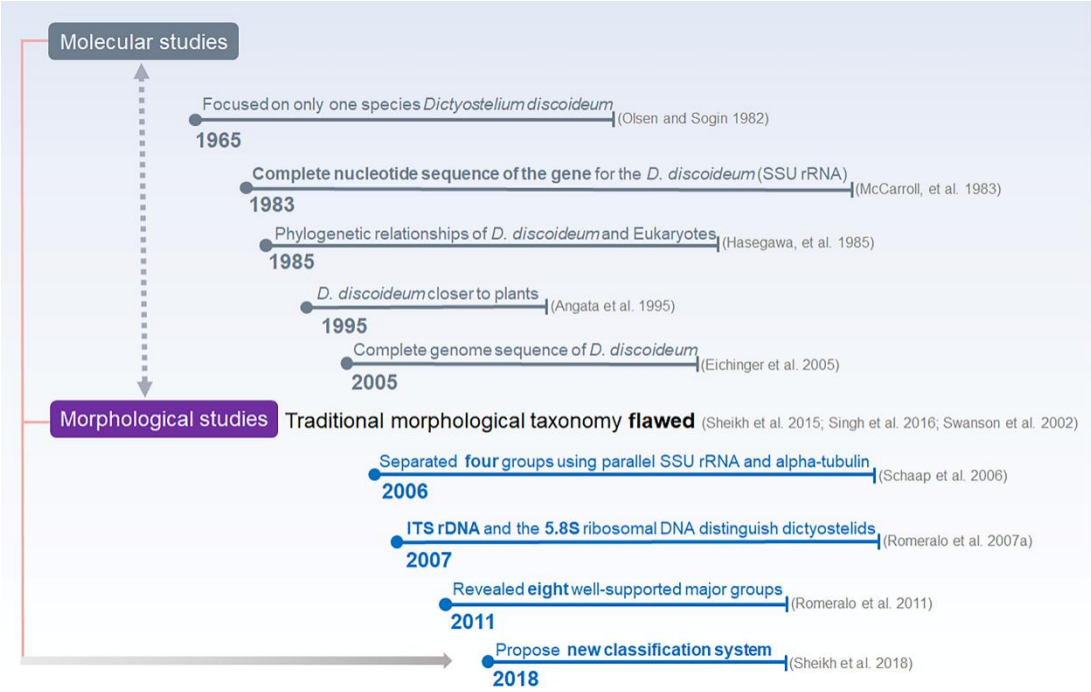


Figure 18 – An overview of molecular studies of dictyostelids.

From that time, this new classification has been recognized, with the genus *Cavenderia* in the family Cavenderiaceae; the genera *Acytostelium*, *Rostrostelium* and *Heterostelium* in the family Acytosteliaceae; the families Cavenderiaceae and Acytosteliaceae in the order Acytosteliales; the genera *Tieghemostelium*, *Hagiwaraea*, *Raperostelium* and *Speleostelium* in the family Raperosteliaceae; the genera *Dictyostelium* and *Polysphondylium* in the family Dictyosteliaceae; the families Raperosteliaceae and Dictyosteliaceae together with the problematic genus *Coremiostelium* in the order Dictyosteliales; and the orders Acytosteliales and Dictyosteliales along with the genus *Synstelium* in the class Dictyosteliomycetes or the subclass Dictyostelia (Sheikh et al. 2018, Adl et al. 2019, Leontyev et al. 2019). These data provided a new insight into the taxonomy of dictyostelids and, as a result of this new classification, two families, nine genera, and 92 new combinations were recognized at the level of species and variety. However, this new classification is also a framework for the dictyostelids at species level and affirms the phylogenetic positions of dictyostelids in the eukaryotes on the basis of this analysis (Adl et al. 2019, Leontyev et al. 2019, Wijayawardene et al. 2020). These positions also have been confirmed by cladistic analysis of morphological characters and a molecular phylogeny based on alpha-tubulin and alpha-tubulin + SSU rRNA, ITS + 5.8S rRNA, ITS + SSU rRNA genes (Swanson et al. 2002, Schaap et al. 2006, Romeralo et al. 2007a, 2011a, 2013b, Sheikh et al. 2015, Singh et al. 2016, Rukseree et al. 2018). It should be noted that a core-phylogeny of six proteins (Agl, AmdA, PurD, PurL, RpaA, and SmdA) has been analyzed to construct a more robust phylogeny than SSU (Schilde et al. 2019).

7.2 Challenges for the phylogeny of dictyostelids

The small subunit ribosomal DNA (SSU rDNA) is widely used for taxonomy-based analyses (Medlin et al. 1988, Pace 1997, Steenkamp et al. 2006, Wang et al. 2017). The SSU rDNA gene encodes for the structural RNA component of the small ribosomal subunit conserved in all dictyostelids, and its sequence has been used to assign taxonomic relationships for several decades (Baldauf et al. 2000, Schaap et al. 2006, Rukseree et al. 2018, Sheikh et al. 2018, Zou et al. 2022). There are numerous SSU sequences of dictyostelids available for identification, including some different sequences with a same species name that occupy different positions on the phylogenetic tree (Raper 1984, Hagiwara 1989, Schaap et al. 2006). Some sequences are from species complexes that have been delimited by methodology and phylogeny in earlier studies (Romeralo et al. 2007a, Kawakami & Hagiwara 2008, Romeralo et al. 2010b) and some were divergence sequences pointed out by later identification (Hagiwara 1989). Others were species that had previously been considered synonymous with another species but later have been re-identified; however, these sequences are listed under the older name on the GenBank database. For example, "GenBank sequence AM168104.1" should be *Heterostelium album* PN500 instead of *Heterostelium pallidum* PN500 (Schaap et al. 2006) because the isolate PN500 was later reidentified as *H. album* (Kawakami & Hagiwara 2008) and the sequence AM168104.1 was also used as a sequence of *H. album* in a classic paper (Sheikh et al. 2018).

In contemporary bioinformatic approaches, each position in an alignment is considered a single taxonomic character (Leontyev et al. 2021). Even relatively conserved sequences among morphologically similar species should have obviously different SSU rDNA sequences on a phylogenetic tree. However, SSU sequences of dictyostelids are sometimes not enough to clearly distinguish species in some genera such as *Dictyostelium* (Zou et al. 2022).

On the other hand, some sequences other than SSU have been used in phylogenetic analyses of dictyostelid taxonomy, including ITS and the ATPase subunit 1 gene of the mitochondrial genome (*atp1*) (Romeralo et al. 2007b, Liu et al. 2019c, Zou et al. 2022). However, ITS and *atp1* research has been limited with the relevant gene database still rather complete. Consequently, these two genes cannot be widely used for all species of dictyostelids. As a result, picking the best sequences for constructing a phylogenetic tree will not always yield a universal consensus (Sheikh et al. 2018).

Herein, a new trait tree (Fig. 19) of dictyostelid characters was constructed on the basis of the new classification with 12 genera formally proposed by (Sheikh et al. 2018). Previously, 12 genera

of dictyostelids could be distinguished only by molecular means. This trait tree could be helpful in identifying dictyostelids to the genera level by morphological data. In this tree, features are divided into three categories in which polar granules, spore shape, aggregation, and stalk architecture are considered to show the relationships among the 12 genera with respect to morphology. Thus, 12 genera in new classification can be clearly distinguished on the basis of morphology.

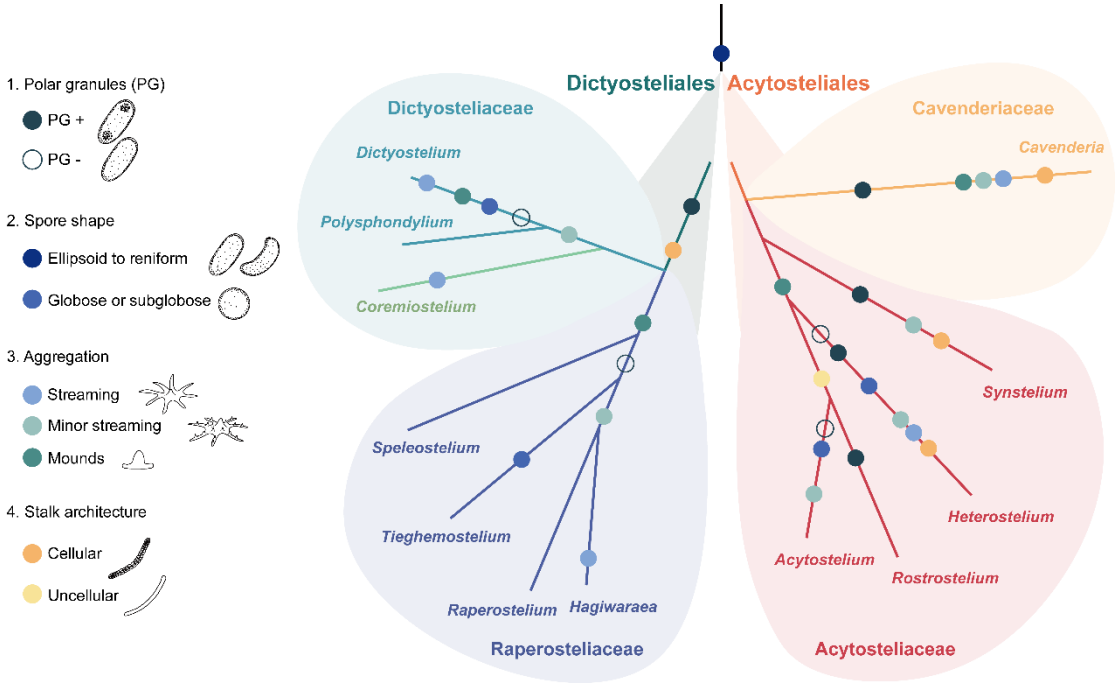


Figure 19 – Phylogenetic tree of genera of dictyostelids based on morphology and molecular systems.

8. Chemical compounds from dictyostelids

In 1999, dictyostelids were chosen by the National Institute of Health (NIH, United States) as a new non-mammalian model organism for biomedical research. In 2011, Escalante Ricardo assembled the research carried out in numerous labs as the basis for proposing dictyostelids as a model for human pathology (Maniak 2011). These organisms are known to mediate important biological functions and have been increasingly involved in studies of cell physiology and efforts to develop a better understanding of many human diseases, especially in oncology (Pearce et al. 2019). Among the organisms currently known to science, the dictyostelid *Dictyostelium discoideum* is considered to be an excellent model organism for the study of cell and developmental biology because of its unicellular and multicellular life cycle (Kikuchi et al. 2008, Nakajima-Shimada et al. 2013) and the similarities in structure of its cells and mammalian cells, which makes it possible to study orthologs of cancer-associated genes in both phases (Martin-Gonzalez et al. 2021, Mathavarajah et al. 2021).

Both the identification and taxonomy of dictyostelids have traditionally been based on morphological characters of the sorocarp, although those of a number of species often appear to be almost indistinguishable (Schaap et al. 2006). Sheikh et al. (2018) proposed a new classification based on monophyletic entities with consistent and strong molecular phylogenetic support. In this classification, dictyostelids were placed into two orders, four families, and twelve genera. Using the latest classification system as the taxonomic framework, the research progress that has been made on the chemical components and pharmacological effects of 31 species belonging to seven genera of dictyostelids is presented below (Fig. 20).

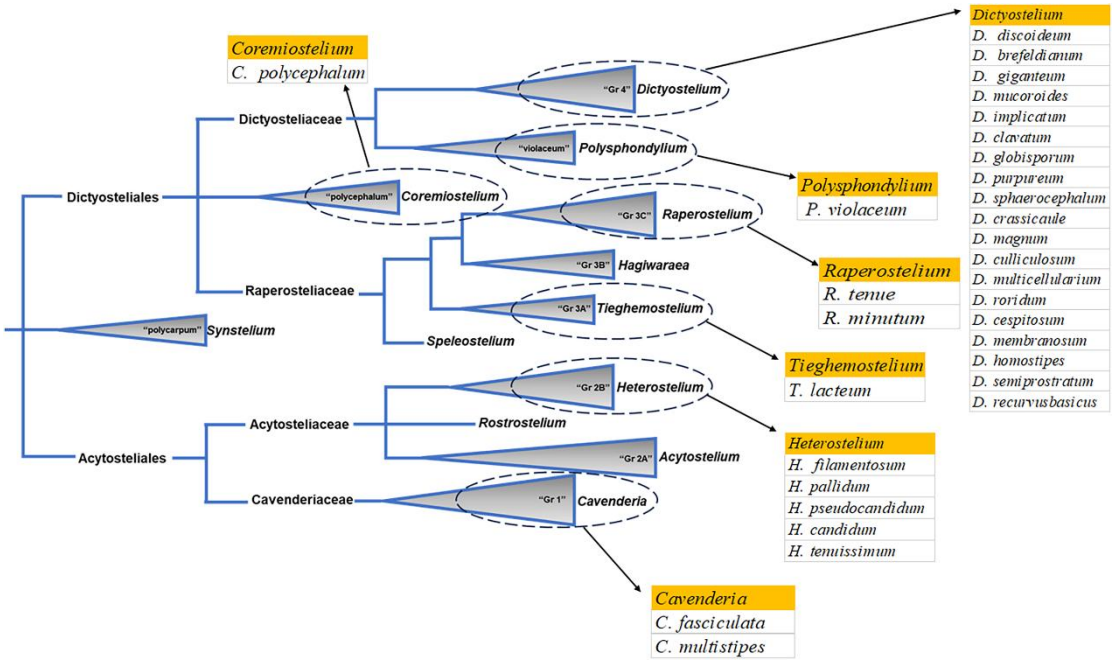


Figure 20 – Summary of dictyostelid species from which compounds have been isolated. Note: This figure also depicts the phylogenetic relationship among species of dictyostelids, as informed by the new classification system proposed by Sheikh et al. (2018).

8.1 Metabolites of dictyostelids

Dictyostelids represent a taxon consisting of almost 200 described species. Among the compounds that have isolated from the approximately 31 species that have been investigated are the differentiation-inducing factor and its derivatives (Kikuchi et al. 2008, Kubohara et al. 2013, Kuwayama & Kubohara 2016), chlorobenzofuran (Kikuchi et al. 2013), acrasin (Barkley 1969, de Wit & Konijn 1983), fatty acids and phosphoric acid compounds (Saito & Ochiai 1996, 1998, 1999), enzymes (Freeze & Etchison 1984, John & Stephen 1986), saccharides (Ceccarini & Filosa 1965, Mereyala et al. 2004), and volatiles (Wei et al. 2021) (Fig. 21). These compounds have important biological activities and pharmacological effects such as inhibiting the proliferation, migration, and metastasis of osteosarcoma cells, inhibiting the proliferation of breast cancer cells (Totsuka et al. 2019), and treating leukemia (Kubohara et al. 1993). **Chemical compounds from members of the genus *Dictyostelium*:** *Dictyostelium* is one of the largest genera in the dictyostelids, and the appreciable number of species isolated in the past reflects the number of compounds that have been isolated. More than 100 compounds have been isolated from 19 species representing the genus *Dictyostelium*. These are mainly aromatic compounds containing benzene rings, fatty acids, and lipids that have related structures, as well as small amounts of acrasin, saccharides, and enzymes (Table 1).

The fact that the model organism *D. discoideum* has been used for the study of eukaryotic cell functions (e.g., signal transduction, cell motility, chemotaxis, autophagy, and death) over the past 80 years (Bretschneider et al. 2016, Mesquita et al. 2017, Dominguez-Martin et al. 2018, Stuelten et al. 2018) undoubtedly accounts for the high proportion of compounds attributed to this species. Differentiation-inducing factors (DIFs) were identified in *D. discoideum* as morphogens required for stalk cell differentiation (Morris et al. 1987). DIF-1, DIF-2, and DIF-3 (Fig. 22) are lipophilic signal molecules (chlorinated alkylphenones) in *D. discoideum* (Gokan et al. 2005). DIF-1 has been identified as 1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl) hexan-1-one, which is the most active DIF, so that DIF-1 at nanomolar levels dose-dependently induces stalk-cell differentiation *in vitro*. DIF-3 [1-(3-chloro-2,6-dihydroxy-4-methoxy-phenyl) hexan-1-one] is the initial product in the process of DIF-1 breakdown and is much less active in inducing stalk-cell differentiation (Morris et al. 1988, Gokan et al. 2005). The order of the stalk-cell differentiation-inducing activity in

Dictyostelium discoideum in vitro is DIF-1 > DIF-2 >> DIF-3 (Kubohara & Kikuchi 2019). *Dictyostelium discoideum* has been found to contain a high percentage of unsaturated fatty acids (Davidoff & Korn 1963a), and several of these are unique dienoic acids that do not contain the methylene-interrupted double bonds present in most natural polyenoic fatty acids. An aggregate-less mutant of *D. discoideum* has been demonstrated to synthesize unsaturated fatty acids efficiently from exogenous, labeled precursor fatty acids (Davidoff & Korn 1963b). The molecule 4-methyl-5-pentylbenzene-1,3-diol (MPBD) (Fig. 23) is a secondary metabolite product of SteelyA polyketide synthase, which regulates cell aggregation and spore maturation in *D. discoideum* (Murata et al. 2016, Kondo et al. 2019). The two 3,6-anhydrosugars, furanodictine A and furanodictine B (Fig. 24). were isolated from a methanol extract of the multicellular sorocarp of *D. discoideum*. Their relative structures were elucidated by spectral means, and the absolute configurations were confirmed by asymmetric syntheses of furanodictine $A_{(1)}$ and $B_{(2)}$ (Kikuchi et al. 2001). Kikuchi (2007) isolated the derivatives of amino sugars dictyoglucosamine A and dictyoglucosamine B again (Fig. 24).

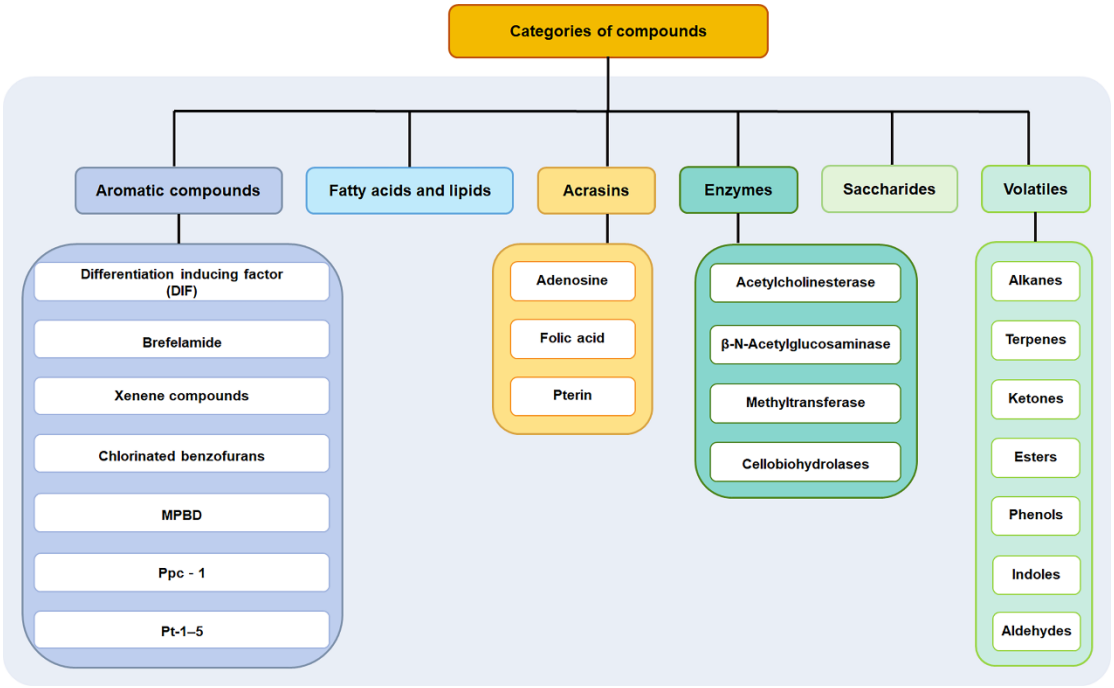


Figure 21 – Categories of compounds obtained from dictyostelids.

The cellobiohydrolases Cel7A (DdiCel7A) was isolated from *Dictyostelium discoideum* and consists of a catalytic domain and does not exhibit a carbohydrate-binding module (CBM) (Hobdey et al. 2016). The nonlysosomal endo-*β*-*N*-acetylglucosaminidase (EC 3.2.1.96) was found in vegetative cells of *D. discoideum.* This hydrolyzes the di-*N*-acetylchitobiosyl linkage found in asparagine-linked oligosaccharides (Freeze & Etchison 1984). Cyclic AMP (cAMP) is a well-known extracellular signal molecule essential for dictyostelid development (Gerisch et al. 1975). It has a strong effect on the aggregation of vegetative amoebae and induction of stalk cell differentiation (Bonner 1970, Bonner et al. 1970).

A new aromatic amide brefelamide (Fig. 25) has been isolated from methanol extracts of the sorocarps of *Dictyostelium brefeldianum* and *D. giganteum*. Its structure was determined by spectral methods, including EIMS and ^{1}H and ^{13}C NMR (Kikuchi et al. 2005a). In addition, An (2015) obtained a total of 40 fatty acid signatures by measuring the phospholipid fatty acids profile of *D. giganteum*. The main fatty acid signatures were 18:1 w9c, 16:00, 17:0 cyclo, 15:0 iso, 17:0 ANTEISO, 15:0 ANTEISO, 16:1 w6c/16:1 w7c, and 14:0 3OH/16:1 iso I, with a total content of 72.8%.

Kikuchi et al. (2006) isolated a novel aromatic compound, 4-methyl-5-n-pentylbenzene-1,3-diol (MPBD) (Fig. 23) from the sorocarps of *D. mucoroides* and assessed the *in vitro* antiproliferative activities of MPBD, FDs, and DGs in human leukemia K562 and HL-60 cells. An (2015) obtained a total of 24 fatty acid signatures from *D. mucoroides*, and the main fatty acid signatures were 18:1

w9c, 16:00, 17:0 cyclo, 18:00, 14:00, 18:1 w6c/18:1 w7c, 16:1 w6c/16:1 w7c, 14:0 3OH/16:1 iso I, with a total content of 68.25%.

Hobdey et al. (2016) extracted the cellobiohydrolase DpuCel7A from *Dictyostelium purpureum* Cel7A. This compound, composed of a catalytic region and a carbohydrate, plays a significant role in the degradation process of eukaryotic cell walls. Motohashi et al. (2012) utilized genome data of *D. purpureum* to identify a des-methyl-DIF-1 methyltransferase named DpdmtA.

A total of 10 fatty acid signatures have been identified for *Dictyostelium implicatum*, and the main fatty acid signatures were 16:00, 14:00, 17:0 cyclo, with a total content of 67.8%. *Dictyostelium clavatum* produced a total of 16 fatty acid signatures, and the main fatty acid signatures were 16:00, 14:00,17:0 cyclo, 15:0 iso, 12:00, with a total content of 55.4%. The unique fatty acid signature was 17:1ANTEISOw9c. *Dictyostelium globosisporum* was found to have a total of 18 fatty acid signatures, and the main fatty acid signatures were 16:00, 10:03OH, 17:00 cyclo, 12:00, with a total content of 64.4%. The characteristic fatty acid signature was 10:0 (An 2012). *Dictyostelium sphaerocephalum*, *D. crassicaule*, *D. magnum*, *D. culliculosum*, *D. multicellularium*, *D. roridum*, *D. cespitosum*, *D. membranosum*, *D. homostipes*, *D. semiprostratum*, and *D. recurvusbasicus* had a total of 24 main fatty acid signatures. Among the most common and abundant fatty acid signatures were 14:00, 16:00, 18:00, 17:0 cyclo, 15:1 w6c, 16:1 w6c|16:1 w7c, 14:0 3OH/16:1 iso I, 18:1 w9c, 18:1 w6c/18:1 w7c, with a content ranging from 58% to 79.74%. The three fatty acids with the highest content were 14:00, 16:00 and 18:00 (An 2015).

Table 1 – Chemical compounds obtained from members of the genus *Dictyostelium*.

Dictyostelid	Chemical compounds	References
Dictyostelium discoideum	**Differentiation inducing factors (DIF)** DIF-1, DIF-2, DIF-3, Differanisole A (DA), DIF-1(+1), DIF-1(3M), Et-DIF-1, Bu-DIF-1, DIF-3(+1), DIF-3(3M), Et-DIF-3, Bu-DIF-3, DIF-3(-1), TH-DIF-1, TM-DIF-1 (Fig. 22) **MPBD** 4-Methyl-5-pentylbenzene-1,3-diol (MPBD) (Fig. 23) **Fatty acids** Methyl β,γ-tetradecenoate, Methyl α,β-tetradecenoate, Methyl palmitoleate, Methyl *cis*- and *trans*-β,γ-hexadecenoa, Methyl trans-α,β-hexadecenoate, Methyl β,γ-octadecenoate, Methyl α,β-octadecenoate, Methyl β-hydroxymyristate, Methyl β-hydroxypalmitate, Methyl β-hydroxystearate, Acetate-1-C^{14}, Hexanoate-1-C^{14}, Octanoate-1-C^{14}, Pelargonate-1-C^{14}, Decanoate-1-C^{14}, Laurate-1-C^{14}, Myristate-1-C^{14},	(Davidoff & Korn 1963a, b, 1964, Bonner 1970, Freeze & Etchison 1984; Kikuchi et al. 2001, 2005a, 2006, 2007, 2008, Kanai et al. 2003, Gokan et al. 2005, Kubohara et al. 2008, 2013, 2015, 2021, An 2012, 2015, Du et al. 2013, Nakajima-Shimada et al. 2013, Nguyen et al. 2015, Oladimeji et al. 2015, Dubois et al. 2016, Hobdey et al. 2016, Kawaharada et al. 2016, Kuwayama & Kubohara 2016, Murata et al. 2016, Arioka et al. 2017, Yamada et al. 2017, Furukawa et al. 2019, Kondo et al. 2019, Seto-Tetsuo et al. 2021, Inoue et al. 2023, Kuwayama et al. 2023)

Dictyostelid	Chemical compounds	References
	Palmitate-1- C^{14}, Margarate-1-C^{14}, Stearate-1-C^{14}, Stearate-C^{14}, 16:00, 14:00, 17:0 cyclo, 12:00, 20:0, 16:0 3OH, 19:00, 14:00, 18:1 2OH, 15:1 iso G, 18:1 w9c	
	Phospholipids	
	Heptanoic Pelargonic, Hexanoic Octanoic Pelargonic Decanoic, Heptanoic Octanoic Pelargonic, Heptanoic, Octanoic, Heptanoic Pelargonic, Azelaic Pimelic, Undecanedioic Azelaic Suberic Pimelic, Succinic Glutaric, Adipic Succinic Glutaric	
	Lipid Proteins	
	LdpA, Net4	
	Saccharides	
	Furanodictine A, Furanodictine B, Dictyoglucosamine A, Dictyoglucosamine B (Fig. 24)	
	Cellobiohydrolases	
	Cel7A (DdiCel7A)	
	Acetylglucosaminase	
	Endo-*β*-*N*-acetylglucosaminidase	
	Acrasin	
	Adenosine	
	Cyclic AMP	
D. brefeldianum	**Brefelamide** (Fig. 25)	(Bai et al. 2019)
D. giganteum	**Brefelamide** (Fig. 25)	(An 2015, Bai et al. 2019)
	Fatty acids	
	18:1 w9c, 16:00, 17:0 cyclo, 15:0 iso, 17:0 ANTEISO, 15:0 ANTEISO, 16:1 w6c/16:1 w7c, 14:0 3OH/16:1 iso I, 13:0 iso 3OH	
D. mucoroides	**MPBD**	(An 2015, Kondo et al. 2019)
	4-Methyl-5-pentylbenzene-1,3-diol (MPBD) (Fig. 23)	
	Fatty acids	

Dictyostelid	Chemical compounds	References
	18:1 w9c, 16:00, 17:0 cyclo, 18:00, 14:00, 18:1 w6c|18:1 w7c, 16:1 w6c/16:1 w7c, 14:0 3OH/16:1 iso I, 10:00, 17:00, 16:0 ANTEISO	
D. implicatum	**Fatty acids** 16:00, 14:00, 17:0 cyclo, 19:0 cyclo w8c	(An 2012)
D. clavatum	**Fatty acids** 16:00, 14:00, 17:0 cyclo, 15:0 iso, 12:00, 17:1 ANTEISOw9c, 15:0 iso 3OH, 18:00, 16:0 iso, 18:1 w9c	(An 2012, 2015)
D. globisporum	**Fatty acids** 16:00, 10:03OH, 17:0 cyclo, 12:00, 10:00, 18:1 2OH, 17:00, 17:1 w7c, 19:00	(An 2012, 2015)
D. purpureum	**Methyltransferase** Des-methyl-DIF-1(Dp dmtA) **Cellobiohydrolases** Cel7A (DpuCel7A)	(Motohashi et al. 2012, Hobdey et al. 2016)
D. sphaerocephalum	**Fatty acids** 18:1 w9c, 16:00, 17:0 cyclo, 18:00, 14:00, 16:1 w6c|16:1 w7c, 14:0 3OH/16:1 iso I	(An 2015)
D. crassicaule	**Fatty acids** 18:1 w9c, 16:00, 17:0 cyclo, 18:00, 15:1 w6c, 14:00, 18:1 w6c|18:1 w7c, 16:1 w6c/16:1 w7c,14:0 3OH/16:1 iso I, 18: 2OH, 13:0 iso 3OH, 11:0 iso	(An 2015)
D. magnum	**Fatty acids** 18:1 w9c, 16:00, 17:0 cyclo, 18:00, 15:1 w6c, 14:00, 18:0 ANTE/18:2 w6, 9c	(An 2015)
D. culliculosum	**Fatty acids** 10:0 3OH, 18:1 w9c, 16:00, 17:0 cyclo, 18:00, 15:1 w6c, 14:00, 15:0 iso, 15:0 ANTEISO,	(An 2015)

Dictyostelid	Chemical compounds	References
	18:1 w6c|18:1 w7c, 16:1 w6c/16:1 w7c, 16:0 2OH, 20: 4 w6,9,12,15c	
D. multicellularium	**Fatty acids** 18:1 w9c, 16:00, 17:0 cyclo, 18:00, 14:00, 18:1 w6c|18:1 w7c, 12:0 3OH, 16:1 w9c	(An 2015)
D. roridum	**Fatty acids** 18:1 w9c, 16:00, 12:0 2OH, 12:0 3OH, 18:00, 18:1 w6c|18:1 w7c, 16:1 w6c/16:1 w7c	(An 2015)
D. cespitosum	**Fatty acids** 16:00, 12:0 2OH, 17:0 cyclo, 12:0 3OH, 18:00, 15:1 w6c, 14:00, 18:1 w6c|18:1 w7c, 16:1 w6c/16:1 w7c, 14:0 3OH/16:1 iso I	(An 2015)
D. membranosum	**Fatty acids** 18:1 w9c, 16:00, 18:00, 14:00, 18:1 w6c|18:1 w7c, 16:1 w6c/16:1 w7c	(An 2015)
D. homostipes	**Fatty acids** 18:1 w9c, 16:00, 17:0 cyclo, 16:1 w6c/16:1 w7c, 14:0 3OH/16:1 iso I, 19:0 cyclo w8c, 11:0 iso 3OH，13:0 2OH, 18:1 w9c	(An 2015)
D. semiprostratum	**Fatty acids** 18:1 w9c, 16:00, 14:00, 18:00, 18:1 w6c|18:1 w7c, 16:1 w6c/16:1 w7c, 14:0 3OH/16:1 iso I	(An 2015)
D. recurvusbasicus	**Fatty acids** 16:00, 18:00, 14:00, 18:1 w6c|18:1 w7c, 16:1 w6c/16:1 w7c	(An 2015)

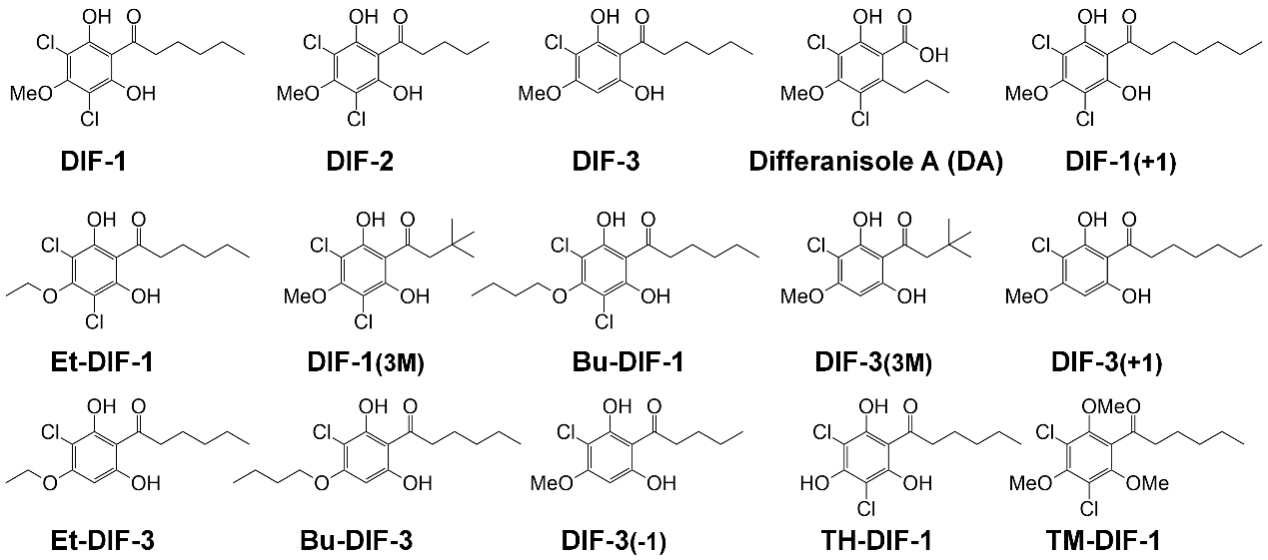


Figure 22 – Structures of DIF and its derivative (Kubohara & Kikuchi 2019).

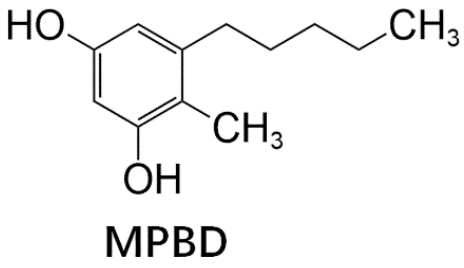


Figure 23 – Structures of MPBD (Kondo et al. 2019).

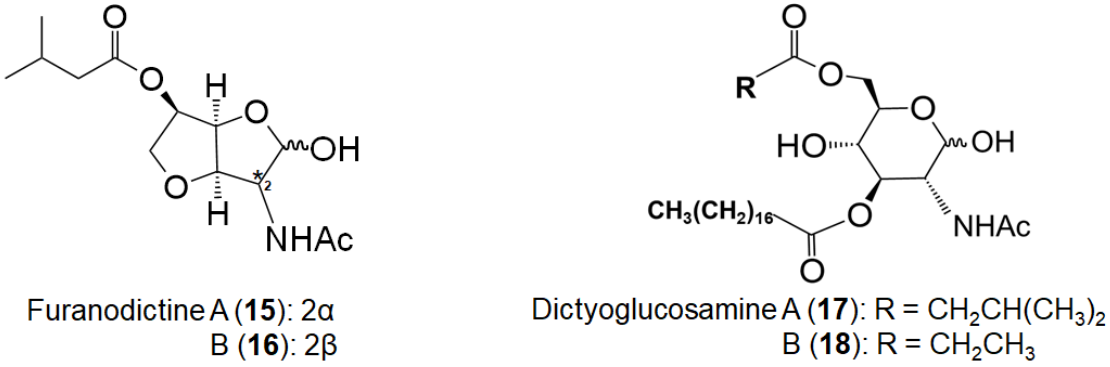


Figure 24 – Structures of furanodictines and dictyoglucosamines (Kikuchi 2007).

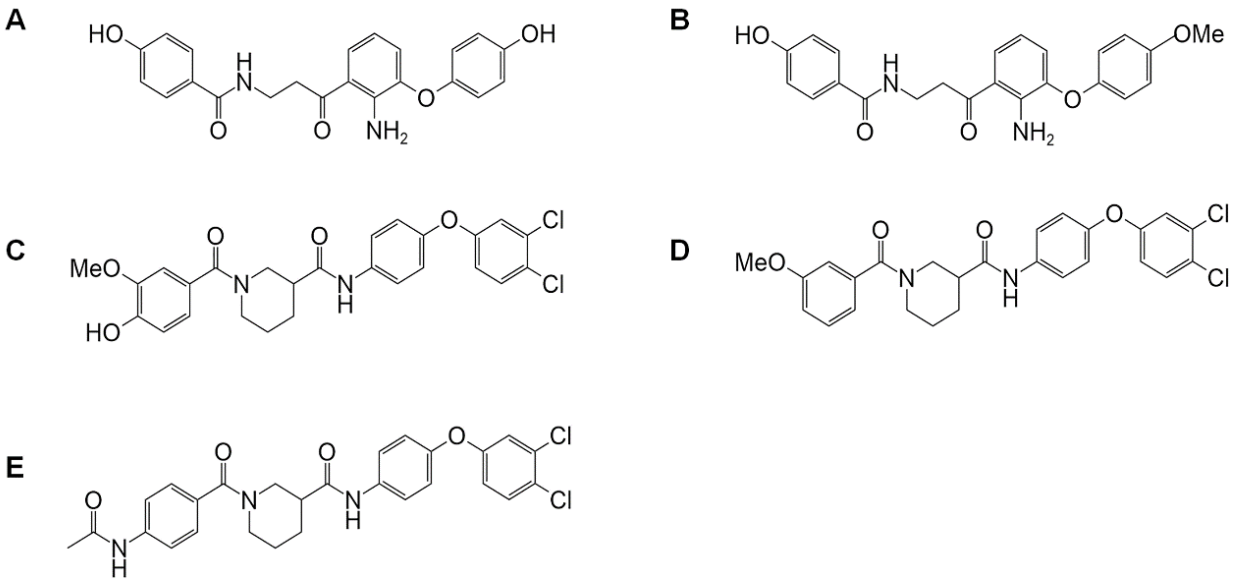


Figure 25 – Structures of Brefelamide (A) and the synthesized brefelamide derivatives (B-E) (Bai et al. 2019).

Chemical compounds from members of the genus *Polysphondylium*: at present, more than 15 compounds have been isolated from *Polysphondylium violaceum*. These compounds are mainly volatile components but also include a small number of enzymes (Table 2).

Wei et al. (2021) identified and analyzed 15 volatile components from sorocarps of *P. violaceum* by phase microextraction gas chromatography/mass spectrometry, accounting for 25% of the total components, with a total relative content of 32.8%. These compounds included alkanes (n=10, Dodecane,4-methyl, Tetradecane, Hexadecane,2,6,10,14-tetramethyl, Heptadecane, Pentadecane, Hexadecane, Octacosane, Hexadecane, 2-methyl, Heptacosane, Octadecane) (Fig. 26A), terpenes (n=1, alpha-farnesene) (Fig. 26B), ketones [(n=1), 5,9-undecadien-2-one,6,10-dimethyl] (Fig. 26C), esters (n=2, Dimethyl phthalate, Diethyl phthalate) (Fig. 26D), and phenols [n=1, Phenol,2,4-bis(1,1-dimethylethyl)] (Fig. 26E). *Polysphondylium violaceum* was found to synthesize a plasma membrane-bound acetylcholinesterase located on the surface of growing amoebae, which suggested that an acetylcholine-based sensory system might control *P. violaceum* differentiation during growth and early stages of development (John & Stephen 1986).

Table 2 – Chemical compounds from members of the genus *Polysphondylium*.

Dictyostelid	Chemical compounds	References
Polysphondylium violaceum	**Alkanes** Dodecane,4-methyl, Tetradecane, Hexadecane,2,6,10,14-tetramethyl, Heptadecane, Pentadecane, Hexadecane, Octacosane, Hexadecane,2-methyl, Heptacosane, Octadecane (Fig. 26A) **Terpenes** alpha-farnesene (Fig. 26B) **Ketones** 5,9-undecadien-2-one,6,10-dimethyl (Fig.	(John & Stephen 1986, Wei et al. 2021)

	26C)
	Esters
	Dimethyl phthalate, Diethyl phthalate (Fig. 26D)
	Phenols
	Phenol,2,4-bis(1,1-dimethylethyl) (Fig. 26E)
	Acetylcholinesterase

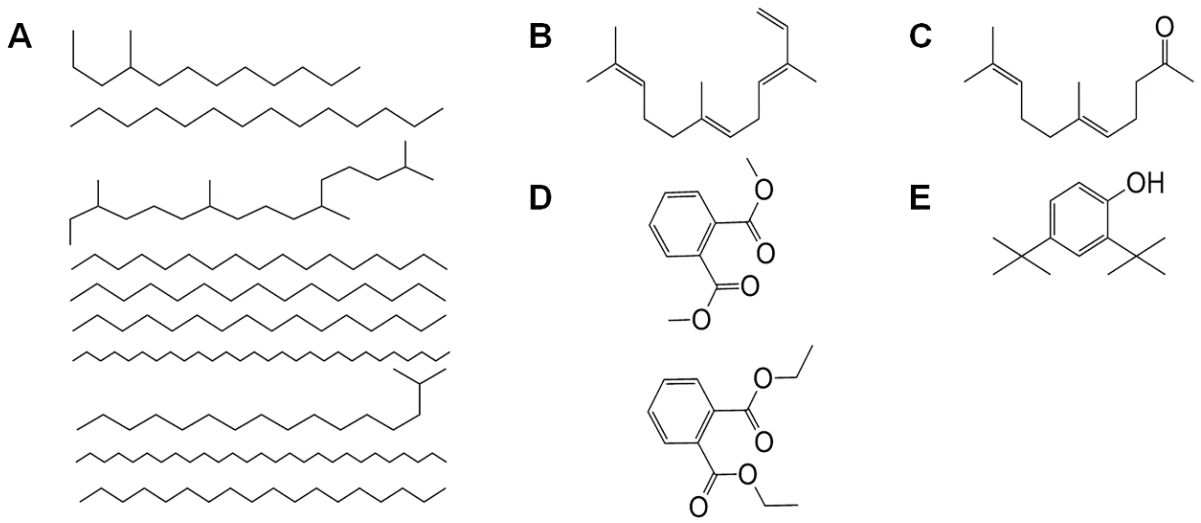


Figure 26 – Chemical compound structures of volatile components (A) Alkanes, (B) Terpenes, (C) Ketones (D) Esters, and (E) Phenols from *Polysphondylium violaceum* (Wei et al. 2021).

Chemical compounds from members of the genus *Coremiostelium*: in the new classification (Sheikh et al. 2018), the "polycephalum" complex (*Dictyostelium polycephalum*) is treated as *Coremiostelium* gen. nov. (not assigned to a family), and species in this genus are relatively rare. However, four xenene compounds have been isolated from *Coremiostelium polycephalum* (*Dictyostelium polycephalum*) (Table 3).

Kikuchi et al. (2012) isolated four compounds from *D. polycephalum*. These were Dictyobiphenyl A, Dictyobiphenyl B, Dictyoterphenyl A, and Dictyoterphenyl B (Fig. 27). Their structures were determined through chemical reverse synthesis; Dictyoterphenyl A was a nitrogen-containing triphenyl compound with selective anti-malignant cell proliferation activity.

Table 3 – Chemical compounds from members of the genus *Coremiostelium*.

Dictyostelid	Chemical compounds	References
Coremiostelium polycephalum	**Xenene compounds** Dictyobiphenyl A, Dictyobiphenyl B, Dictyoterphenyl A, Dictyoterphenyl B (Fig. 27)	(Singh 1947b, Kikuchi et al. 2012)

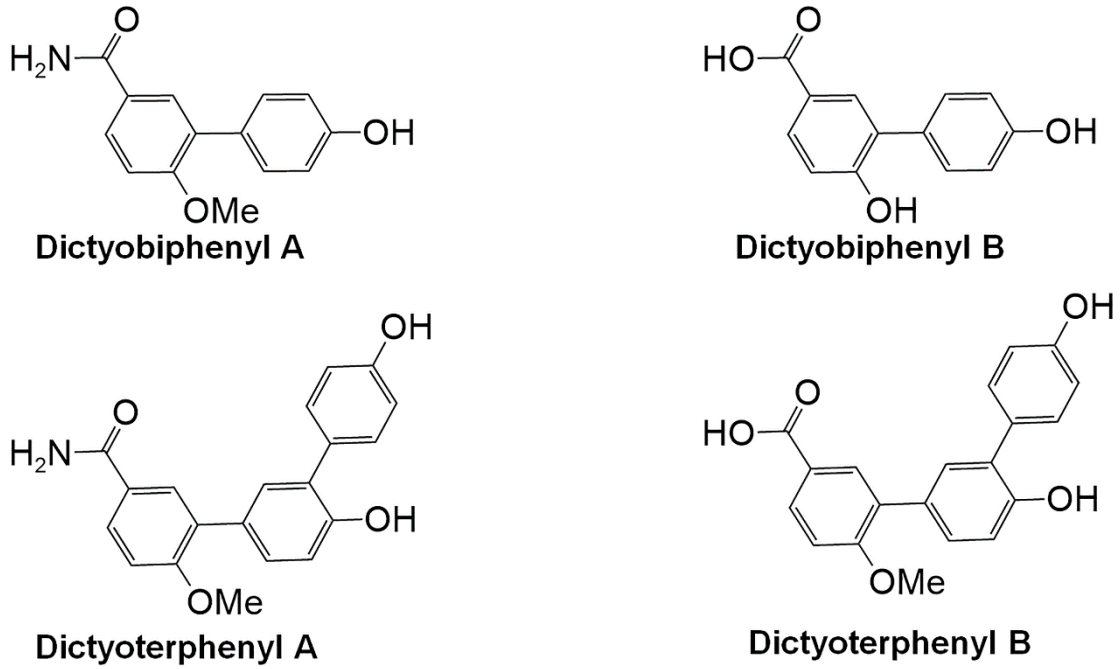


Figure 27 – Structures of Xenene compounds (Kikuchi et al. 2012).

Chemical compounds from members of the genus *Raperostelium*: more than 28 compounds have been isolated from two species representing the genus *Raperostelium*. These are mainly fatty acids, as well as small amounts of acrasin (Table 4).

Raperostelium tenue has yielded a total of 28 fatty acid signatures, and the main fatty acid signatures were 16:00, 14:00, 17:0 cyclo, 17:0 iso 3OH, and 15:0 iso, with a total content of 58.03%. Among these, the compound 17:0 iso 3OH had the highest content among the fatty acids from this species, and the compound 17:1 w8c was unique fatty acid signatures of this species with low content (An 2015).

The acrasin was identified as a folic acid C_9-N_{10} splitting enzyme (Fig. 28), which was isolated and purified from aggregating cells of *Raperostelium minutum*, based on its chromatographic properties and biological activity of the acrasin and folate derivatives. The compound was species specific and more active in the aggregative stage than in the vegetative stage (de Wit & Konijn 1983).

Table 4 – Chemical compounds from members of the genus *Raperostelium*.

Dictyostelid	Chemical compounds	References
Raperostelium tenue	**Fatty acids** 16:00, 14:00, 17:0 cyclo, 17:0 iso 3OH, 15:0 iso, 17:0 iso, 17:00, 17:1 w8c	(An 2012)
R. minutum	**Acrasin** **Folic acid** C_9-N_{10} splitting enzyme (Fig. 28)	(de Wit & Konijn 1983)

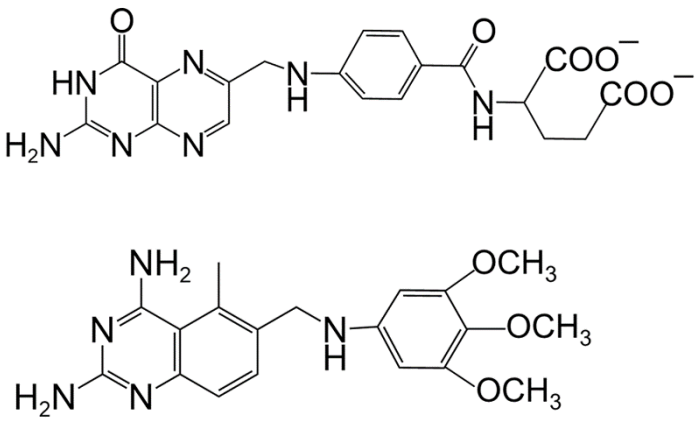


Figure 28 – Structures of Folic acid and derivatives (de Wit & Konijn 1983).

Chemical compounds from members of the genus *Tieghemostelium*: at present, only one compound has been isolated from the genus *Tieghemostelium* (Table 5). This acrasin is species specific and attracts cell aggregation at very low concentrations (0.1-0.01 AM) (Bonner 1970). In addition to the most extensively studied acrasin (cyclic AMP) in the model organism *D. discoideum*, a pterin acrasinase (Fig. 29) was isolated form *Tieghemostelium lacteum* which converts pterin into 2-deamino-2-hydroxypterin (lumazin) (Van Haastert et al. 1982).

Table 5 – Chemical compounds from members of the genus *Tieghemostelium*.

Dictyostelid	Chemical compounds	Reference
Tieghemostelium lacteum	**Acrasin** **Pterin** 2-deamino-2-hydroxypterin (lumazin) (Fig. 29)	(Van Haastert et al. 1982)

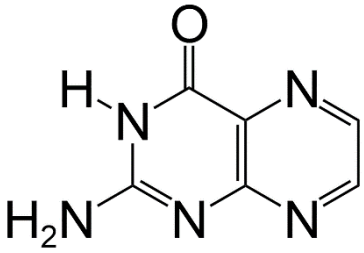


Figure 29 – Structures of Pterin (Van Haastert et al. 1982).

Chemical compounds from members of the genus *Heterostelium*: more than 20 compounds have been isolated from five species in the genus *Heterostelium*. These are aromatic compounds such as chlorinated benzofurans, Ppc-1, and Pt-1–5 (1–5), as well as fatty acids (Table 6).

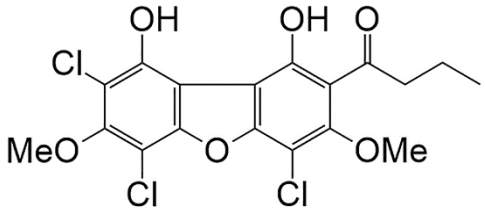
Kikuchi et al. (2013) obtained the two chlorinated benzofurans (Fig. 30) Pf-1 (4) [1-(4,6,8-trichloro-1,9-dihydroxy-3,7-dime-thoxy-2-dibenzofuranyl) butanone] and Pf-2 (5) [1-(4,6,8-trichloro-9-hydroxy-1,3,7-trimethoxy-2-dibenzofuranyl) butanone] from a methanol extract from sorocarps of *Heterostelium filamentosum*. These were found to display multiple biological activities, including stalk cell differentiation-inducing and inhibitory effects on cell proliferation in mammalian cells and gene expression in *Drosophila melanogaster*.

Six prenylated and geranylated aromatic compounds named Ppc-1 (Fig. 31) and Pt-1–5 (1–5) (Fig. 32) were isolated from the methanol extract of sorocarps of *Heterostelium pseudocandidum* and *Heterostelium tenuissimum*. The structures of these compounds were determined by spectral analysis, and compound Pt-5 displayed glucose consumption-promotive activity on 3T3-L1 cells (Kikuchi et al. 2010).

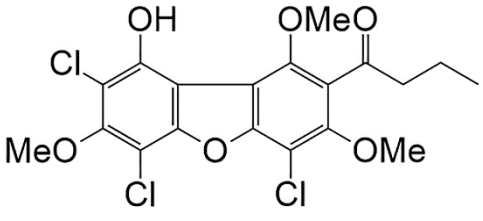
Saito & Ochiai (1998) obtained fatty acids that were from vegetative cells of *Heterostelium pallidum*. These included C-17 and C-19 cyclopropane fatty acids and also straight-chain, saturated fatty acids. An (2012) obtained a total of 31 fatty acid signatures from *Heterostelium candidum*, and the fatty acid signatures were 10:0 3OH, 12:1 3OH, 16:00, 17:0 iso 3OH, with a total content of 48.03%.

Table 6 – Chemical compounds from members of the genus *Heterostelium*.

Dictyostelid	Chemical compounds	References
Heterostelium filamentosum	**Chlorinated benzofurans** **Pf-1(4)** 1-(4,6,8-trichloro-1,9-dihydroxy-3,7-dime-thoxy-2-dibenzofuranyl) butanone **Pf-2 (5)** 1-(4,6,8-trichloro-9-hydroxy-1,3,7-trimethoxy-2-dibenzofuranyl) butanone (Fig. 30)	(Kikuchi et al. 2013)
H. pallidum	**Fatty acids** 9,10-methylene 5-hexadecenoic acid, 11,12-methylene 5-octadecenoic acid	(Saito & Ochiai 1998, An 2012)
H. pseudocandidum	**Ppc-1** 3-methylbut-2-eny1-4-methoxy-8-[(3-methylbut-2-enyl)oxy] quinoline-2-carboxylate (Fig. 31)	(Kikuchi et al. 2010, Azelmat et al. 2015)
H. tenuissimum	**Pt-1–5 (1–5)** (Fig. 32)	(Kikuchi et al. 2010)
H. candidum	**Fatty acids** 10:0 3OH, 12:1 3OH, 16:00, 17:0 iso 3OH, 12:1 3OH, 10:0 2OH, 17: 0 ANTESO	(An 2012)

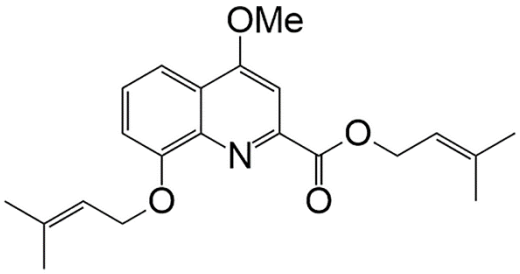


Pf-1 (4)



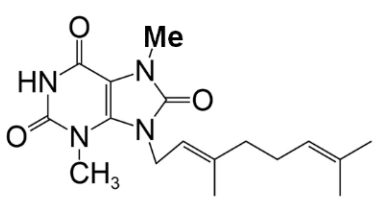
Pf-2 (5)

Figure 30 – Structures of Pf-1 (4) and Pf-2 (5) (Kikuchi et al. 2013).

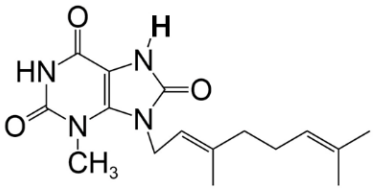


Ppc-1

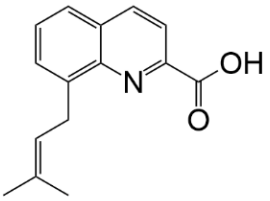
Figure 31 – Structures of Ppc-1 (Kikuchi et al. 2010).



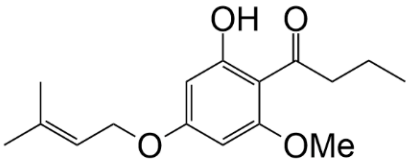
Pt-1 (1)



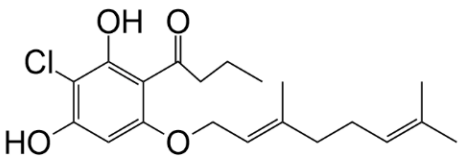
Pt-2 (2)



Pt-3 (3)



Pt-4 (4)



Pt-5 (5)

Figure 32 – Structures of Pt-1–5 (1–5) (Kikuchi et al. 2010).

Chemical compounds from members of the genus *Cavenderia*: more than 30 compounds have been isolated from two species in the genus *Cavenderia*. These are fatty acids and volatile components that have related structures (Table 7).

Wei et al. (2021) identified 27 volatile components from the sorocarps of *Cavenderia fasciculata* by phase microextraction gas chromatography/mass spectrometry. These accounted for 36% of the total components, with a total relative content of 38.51%. These compounds included hydrocarbon (n=19, Undecane,2-methyl, Undecane,2,4-dimethyl, Undecane,2,6-dimethyl, Undecane,4,8-dimethyl, Dodecane,4,6-dimethyl, Dodecane,4-methyl, Tridecane, Tridecane,2-methyl, Tetradecane, Dodecane, Heptadecane, Pentadecane, Pentadecane, 3-methyl, Hexadecane, Heptadecane,2,6-dimethyl, Octadecane, Hentriacontane, Hexadecane,2,6,10,14-tetramethyl, Cyclohexadecane (Fig. 33A), aldehydes (n=3, Nonanal, Decanal, Benzaldehyde,3, 5-dimethyl (Fig. 33B), indoles (n=1, Indole) (Fig. 33C), terpenes (n=1, alpha-farnesene) (Fig. 33D), ketones [(n=1), 5,9-undecadien-2-one,6,10-dimethyl] (Fig. 33E), esters (n=1, Dimethyl phthalate) (Fig. 33F), and phenols [n=1, Phenol,2,4-bis(1,1-dimethylethyl)] (Fig. 33G).

A total of 27 fatty acid signatures were obtained from *Cavenderia multistipes*, and the main fatty acid signatures were 16:00, 18:00, 14:00, 18:1 w6c|18:1 w7c, 18:1 w9c, and 17:0 cyclo, with a total content of 66.86% (An 2015).

Table 7 – Chemical compounds from members of the genus *Cavenderia*.

Dictyostelid	Chemical compounds	References
Cavenderia fasciculata	**Hydrocarbons** Undecane,2-methyl, Undecane,2,4-dimethyl, Undecane,2,6-dimethyl, Undecane,4,8-dimethyl, Dodecane,4,6-dimethyl, Dodecane,4-methyl, Tridecane, Tridecane,2-methyl, Tetradecane, Dodecane, Heptadecane, Pentadecane, Pentadecane, 3-methyl, Hexadecane, Heptadecane,2,6-dimethyl, Octadecane, Hentriacontane, Hexadecane,2,6,10,14-tetramethyl, Cyclohexadecane **Aldehydes** Nonanal, Decanal, Benzaldehyde,3, 5-dimethyl **Indoles** Indole **Terpenes** alpha-farnesene **Ketones** 5,9-undecadien-2-one,6,10-dimethyl **Esters** Dimethyl phthalate **Phenols** Phenol,2,4-bis(1,1-dimethylethyl) (Fig. 33)	(Wei et al. 2021)
C. multistipes	**Fatty acids** 16:00, 18:00, 14:00, 18:1 w6c|18:1 w7c, 18:1 w9c, 17:0 cyclo	(An 2015)

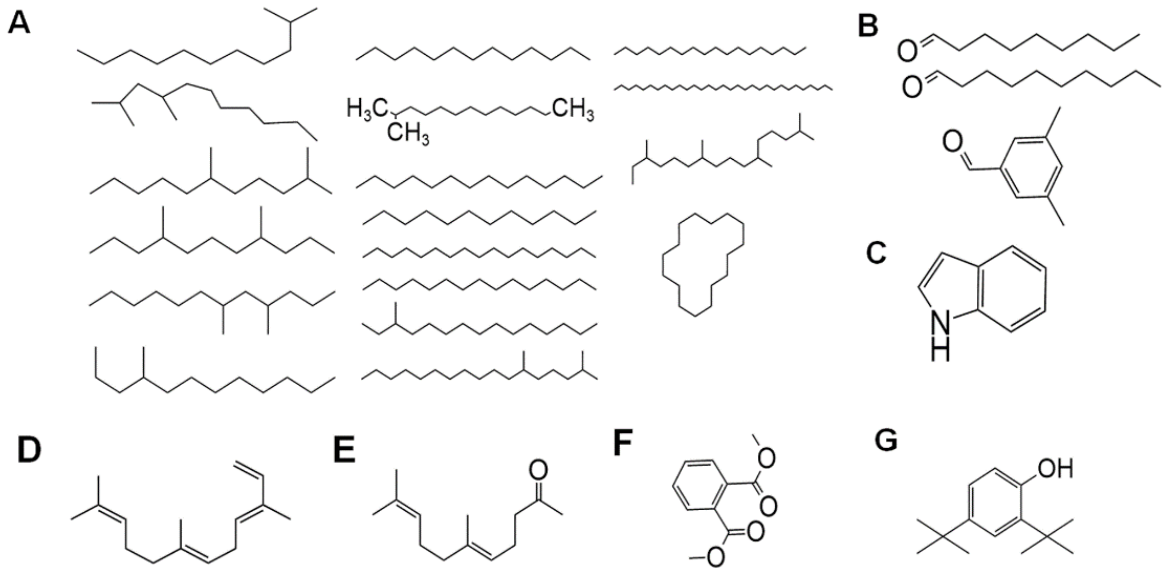


Figure 33 – The chemical compounds structures of volatile components (A) Hydrocarbon, (B) Aldehydes, (C) Indoles, (D) Terpenes, (E) Ketones, (F) Esters, and (G) Phenols from *Cavenderia fasciculata* (Wei et al. 2021).

8.2 Pharmacology of metabolites of dictyostelids

Antitumor activity: DIFs are considered anti-tumor agents because they inhibit proliferation of mammalian tumor cells *in vitro*, including leukemia (Shimizu et al. 2004, Gokan et al. 2005), cervical (Takahashi-Yanaga et al. 2003), gastric (Kanai et al. 2003), and colon (Kubokura et al. 2015) tumor cells. Activity of different DIFs to inhibit the proliferation of mammalian tumor cells contrasts to the activity demonstrated for differentiation of *D. discoideum* stalk cells; the most potent anti-tumor agent is DIF-3 (Takahashi-Yanaga et al. 2003, Gokan et al. 2005). Moreover, DIF-1and DIF-3 inhibit tumor growth in mice *in vivo*. DIF-3(+1) strongly inhibited both cyclin D1 promoter activity and proliferation of MCF-7 and T47D breast cancer cells stably over expressing PAK1 in response to prolactin, estrogen, epidermal growth factor and heregulin (Oladimeji et al. 2015).

Brefelamide is an aromatic amide isolated from *Dictyostelium* that has a potent inhibitory growth effect measured by MTT assay in 1321N1 human astrocytoma cells (Honma et al. 2009). Brefelamide inhibited survival of 1321N1 cells via multiple effects including suppression of HGF receptor expression and HGF secretion and inhibition of ERK and AKT phosphorylation (Honma et al. 2018). Pf-1 (4) and Pf-2 (5) displayed inhibitory activities on cell proliferation in mammalian cells (Kikuchi et al. 2013).

Immunomodulatory activity: DIF derivatives can be developed as new drugs to activate IL-2 (interleukin-2) production and thus stimulate the immune system (Takahashi et al. 2011). Some DIF derivatives, including TH-DIF-1, TM-DIF-1, and Bu-DIF-3, significantly inhibit ConA-induced IL-2 production at low doses (e.g., 5 μM), while other DIF derivatives, including DIF-$1_{(+1)}$ and DIF-$3_{(3M)}$, significantly promote IL-2 production in human Jurkat T cells, which is a model cell line suitable for the study of T lymphocytes (Takahashi et al. 2009, 2011).

Glucose uptake-promoting activity: DIF-1 and its derivatives may have therapeutic potential for the treatment of obesity and diabetes (especially of insulin-resistant diabetes). DIF-1 promoted glucose uptake in a dose dependent manner at 5–20 μM, without affecting the cell morphology and number of 3T3-L1 fibroblasts and RGM-1 cells, as well as 3T3-L1 adipocytes (Omata et al. 2007). According to a chemical structure-activity relationship analysis, some DIF derivatives such as DIF-1 and DIF-$1_{(3M)}$ can increase glucose uptake two to three times in 3T3-L1 fibroblasts cultured in vitro (Kubohara et al. 2008).

Chemotaxis activity: low concentrations (1–5 μM) of DIF-1 promoted granulocyte

differentiation in human HL-60 leukemia cells induced by retinoic acid *in vitro* but does not affect monocyte differentiation of HL-60 cells induced by vitamin (Kubohara 1997). DIFs 1 and 2 function as inducers of stalk-cell differentiation and as modulators of chemotactic cell movement in *D. discoideum*.

The polyketide MPBD (4-methyl-5-pentylbenzene-1, 3-diol) is a signaling molecule that regulates *D. discoideum* development, regulates chemotactic cell aggregation, and induces spore maturation in the sorocarp stage (Narita et al. 2017, Kondo et al. 2019). Cell aggregation in *D. discoideum* also is mediated by chemotaxis to cyclic AMP (Van Haastert et al. 1982).

Acrasin is species specific and attracts cells at very low concentrations (0.1–0.01 AM) (Van Haastert et al. 1982). During the aggregation stage of *D. discoideum*, a chemotactic agent or acrasin is produced, which is cyclic AMP (cyclic-3',5'-adenosine monophosphate). This acrasin is clearly produced only in large quantities during the aggregation stage, during which cells become particularly sensitive to it (Bonner et al. 1970).

9. Dictyostelids and their symbiotic bacteria

Dictyostelids are members of a large and ancient group of protists that share the capability of producing pseudopods that can extend or retract. These pseudopods allow them to move, feed primarily on bacteria, and digest these through phagocytosis (Fig. 34). This group of protists makes up a monophyletic taxon in the phylum Amoebozoa (also called free-living amoebae) (Raper 1984, Fiore-Donno et al. 2010, Romeralo et al. 2012). The free-living amoebae, the metabolically active stage the life cycle, feed on bacteria and multiply by binary fission (Urushihara & Muramoto 2006, Bozzaro et al. 2019). Dictyostelids produce unusual multicellular fruiting bodies (sorocarps) in their life cycle, are primarily isolated from the plant-soil interface and humus layer in natural environments worldwide, and play an integral role as components of trophic food webs and nutrient cycles (Cavender 1978, Stephenson & Rajguru 2010, Cavender et al. 2021, Zou et al. 2022).

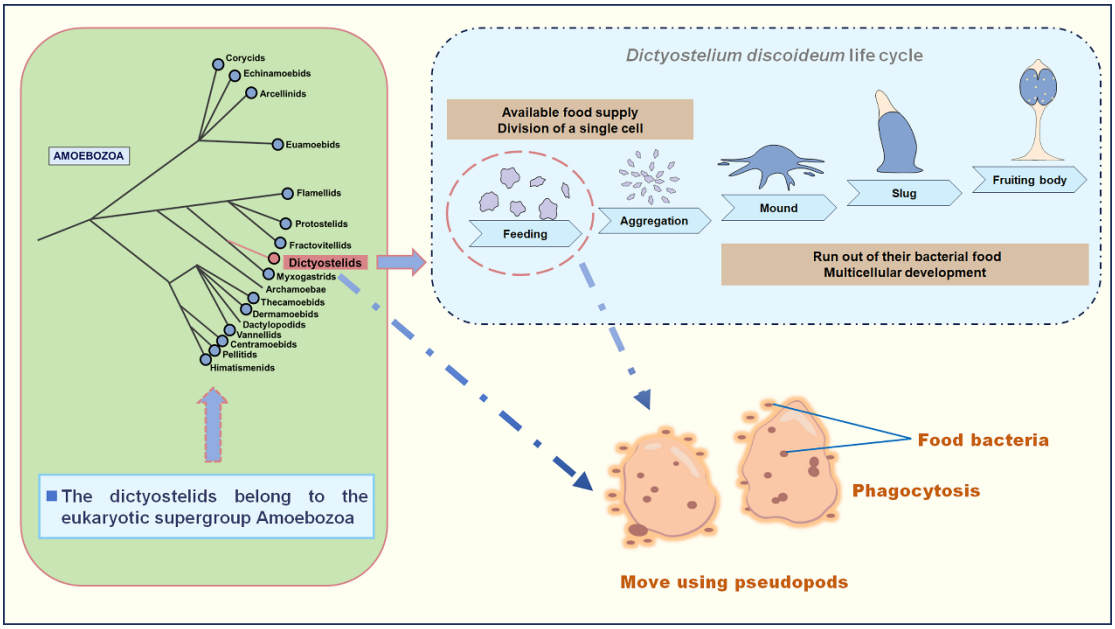


Figure 34 – Schematic diagram showing the predation of bacteria by the model organism *Dictyostelium discoideum*. Single-celled amoeboid cells feed upon bacteria, grow, and multiply when there is a food supply available. However, when food bacteria become depleted, they undergo multicellular development and form fruiting bodies (sorocarps) bearing asexual spores (Sanders et al. 2017, Mather et al. 2023).

The free-living amoebae of dictyostelids feed on bacteria by phagocytosis and digestion occurs

within phagolysosomes. However, some microorganisms have evolved to become resistant to protists. These organisms are not internalized and are able to survive, grow, and exit free-living amoebae after internalization. These amoeba-resistant microorganisms are referred to as the amoeba-resistant bacteria (ARB) (Greub & Raoult 2004).

Biologists have long appreciated the roles that a vast range of organisms play when they team up with microbes, including such examples as microbial symbioses in particular groups such as marine organisms (Apprill 2020), insects (Salem & Kaltenpoth 2022), and nematodes (Kaur et al. 2021), whether in shared ecosystems or intimate symbioses, is fundamentally altering our understanding of biology. However, due to the fact that the distribution patterns exhibited by protistan communities are largely unknown, this limits our understanding of their symbioses with microorganisms (Foissner 2006). Herein, we review the origin of the interactions between eukaryotes and natural bacteria in the single-celled bacterivorous amoeboid dictyostelids. In addition, we conclude this paper with a synthesis section aimed at examining general patterns on the ecology and evolution of amoebae-bacteria symbioses.

9.1 Bacteria and the origin of dictyostelids

Understanding how associations among bacteria and dictyostelids first evolved may reveal the foundations of ecological rules that govern such interactions today. Bacteria (the earliest prokaryotes appeared about 3.5 billion years ago) and amoebae (the earliest eukaryotic organisms to appear approximately 1.85 billion years ago) are among the earliest life forms to appear on the Earth (Porter et al. 2003, Glansdorff et al. 2008) (Fig. 35). Since German mycologist Oskar Brefeld isolated and described the first species of dictyostelid (*Dictyostelium mucoroides*) in 1869 (Brefeld 1869), this type of organism has now been known for more than 150 years.

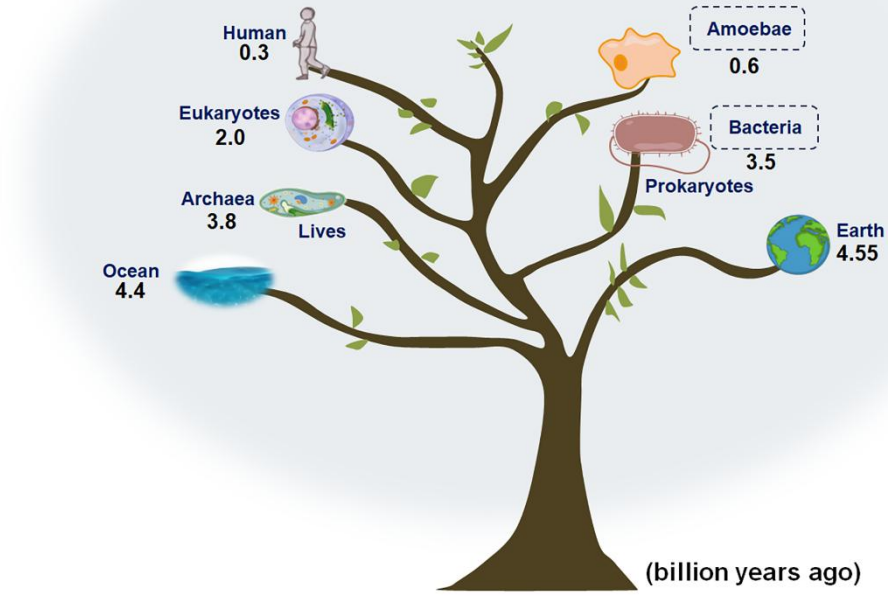


Figure 35 – Evolutionary timeline of amoebae and bacteria.

Historically, as a model eukaryotic organism, the social amoeba *D. discoideum* inhabits forest soils (Raper 1935). Kenneth Raper first reported dictyostelids as a predator of soil bacteria in 1937. His research indicated that the NC-4 clone of *D. discoideum*, isolated in the forests of North Carolina, could feed and develop on both gram negative and gram positive non-pathogenic bacteria (Raper

1937). Later, Raper and Smith also tested the growth of NC-4 against species of pathogenic bacteria that also may occur in the soil, indicating that *D. discoideum* has a wide range of prey bacteria (Raper & Smith 1939). As research on this subject continued to expand, the astonishing discovery was made that dictyostelids engage in a farming symbiosis in which they (as "farmers") stably associate with bacteria partners throughout the *D. discoideum* life cycle (Brock et al. 2011, DiSalvo et al. 2015) (Fig. 36A).

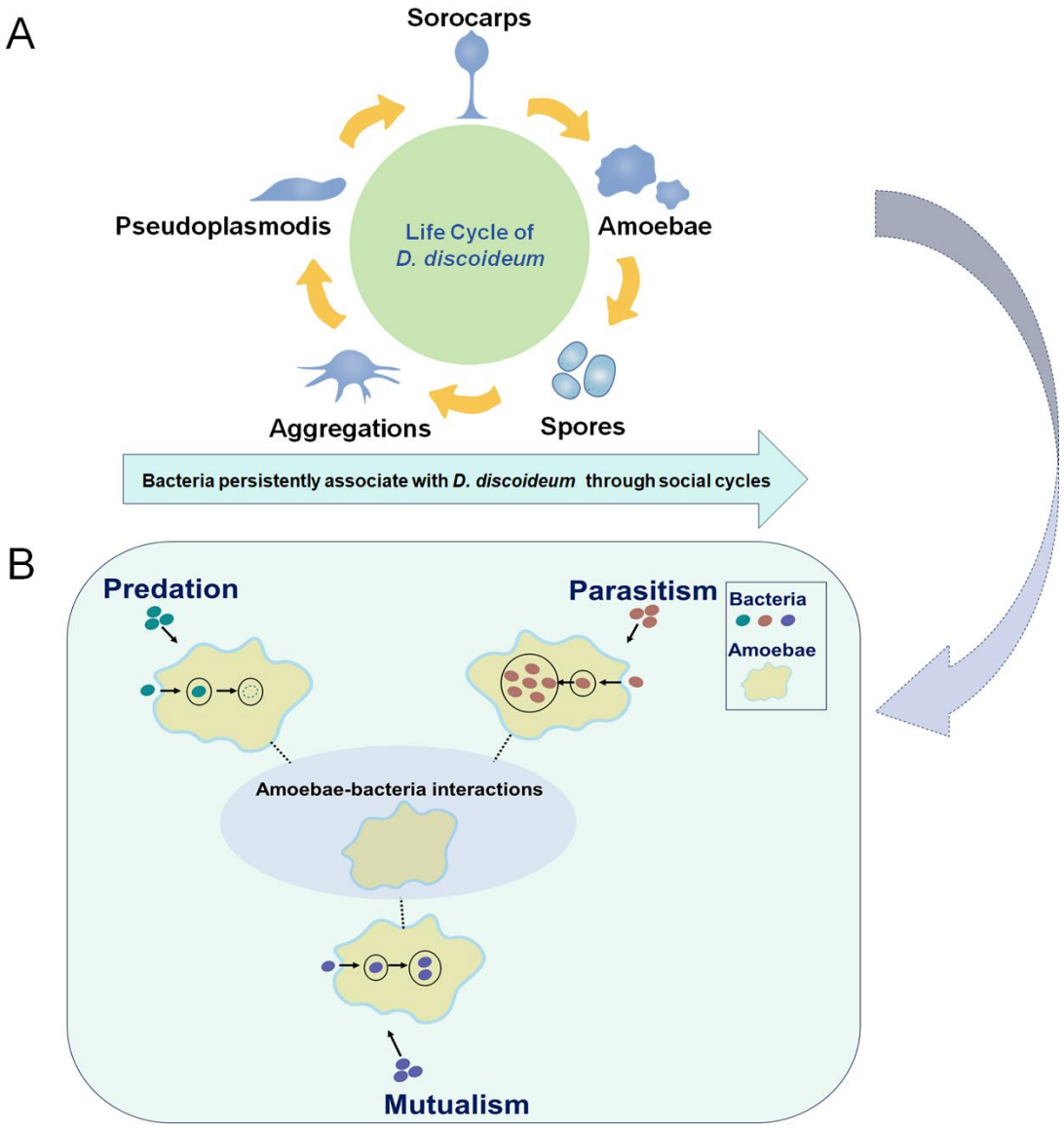


Figure 36 –Bacteria associated with the amoebae of *D. discoideum* throughout the entire life cycle (A) and a diagram of amoebae-bacteria interactions (B).

9.2 The interactions between dictyostelids and bacteria

Recently, the interactions between amoebae and bacteria have gradually become a hot research topic. Although dictyostelid amoebae have evolved a series of mechanisms to track, prey, kill, and digest bacteria (Dunn et al. 2018, Rashidi & Ostrowski 2019), they still form complex interactions with a large number of bacteria in the environment, which range across the whole spectrum from predation to symbiosis (Greub & Raoult 2004, Shi et al. 2021) (Fig. 36B). Amoebae-bacteria interactions contribute to studies of predation, symbiosis, pathogenesis, and human health (Khan 2003).

9.3 Predation

In natural microbial ecosystems, predation is an important relationship that amoebae have with bacteria. Amoebal predation is a significant factor affecting the structure of microbial communities and thus the patterns of natural microbial nutrient cycling; the latter has attracted increasing attention for this relationship alone (Saleem et al. 2013, Inoue et al. 2022).

Amoebae are very effective bacteria killers, and recognition of food bacteria is the first step for a successful hunt (Cosson & Lima 2014). They track bacteria through chemotaxis and kill them using phagocytosis. Phagocytosis is the process in which cells ingest or engulf other cells or particles, and amoebae can engulf and kill most bacteria. After engulfment, the phagosome undergoes maturation, making it a highly acidic, degradative, and oxidative compartment (Russell et al. 2009). Amoebae use various strategies to kill bacteria. Phagosome acidification is the first step, in which V-ATPase plays a central role (Marshansky & Futai 2008). In addition, the phagosome also contains proteases (Journet et al. 1999), hydrolases (Müller et al. 2005), certain metals (German et al. 2013, Hao et al. 2016), and ROS (reactive oxygen species) (Lardy et al. 2005), which combine to kill and break down bacteria (German et al. 2013, Cosson & Lima 2014). With all of these bacterial-control strategies, amoebae can very effectively kill and digest bacteria.

Thus far, the selective predation mechanism of amoebae on bacteria remains a problem that needs to be elucidated, as it relates to selective community regulation and ecological function. Nasser et al. (2013) conducted transcriptional profiling of *D. discoideum* responses to different bacteria and found significant gene expression differences between gram positive and gram negative species, and genes gp130 and AlyL were expressed only when preying on gram positive species. Rashidi & Ostrowski (2019) demonstrated through chemotaxis experiments that *D. discoideum* exhibits more preferential migration towards gram negative species, also supported by the study conducted by Yulo & dela Cruz (2012). Shu et al. (2021) found that *D. discoideum* can readily discriminate and sense the different bacteria during the dormant period, producing more spores on gram negative and non-motile bacteria. Zhang et al. (2023b) cultured actinomycetes (gram positive) from 19 species of dictyostelids (38 strains), and just three symbiotic actinomycetes were isolated. These studies have indicated that dictyostelids seem to be more inclined to prey on gram negative bacteria.

9.4 Parasitism

Among the numerous amoeba resistant bacteria, some have evolved the ability to infect or even kill their amoeba hosts. These are referred to as amoeba pathogenic bacteria (Strassmann & Shu 2017). The social amoeba *D. discoideum* is a well-established haploid model that has been used in several host-pathogen studies (Solomon & Isberg 2000, Solomon et al. 2000, Hasselbring et al. 2011, Steinert 2011). Pathogens include *Legionella* and *Mycobacterium*, with the most widespread among them being *Legionella pneumophila*, the gram negative bacterium that causes Legionnaires' disease.

Although the amoebae of *D. discoideum* are viewed as primitive, actively phagocytosing eukaryotic cells, they are also highly homologous to animal immune cells in an evolutionary sense (Bruhn & Leippe 2001). Amoebae can provide a replicative niche and can likely act as a reservoir for these pathogens in the environment while the cystic form can protect the internalized bacteria (Paquet & Charette 2016). These amoeba pathogenic bacteria are often pathogenic to animals and even humans. This means they can easily infect and cause diseases, thereby affecting human health. The long-term interactions between amoebae and bacteria have likely selected for bacterial virulence traits, leading towards an adaptation towards an intracellular lifestyle, and some amoeba resistant bacteria have acquired the ability to infect mammals (Tosetti et al. 2014).

9.5 Symbiosis

The fruiting body (sorocarp) of a dictyostelid consists of a spherical sorus containing spores and a stalk that holds it, which facilitates spore dispersal in *D. discoideum*. For decades, social amoebae were thought to be free of bacteria in their fruiting bodies (multicellular stage) (Raper 1937). However, it has rather surprising been shown that at least one-third of the lineages of wild *D.*

discoideum carry bacteria both intracellularly and extracellularly in their fruiting bodies, and these "farmers" have stable interactions with different bacterial partners that serve as both food and weapons (Brock et al. 2011, Stallforth et al. 2013).

Subsequent research has shown that the trait of carrying and sowing bacteria is induced by a symbiotic bacterium called *Burkholderia* that has been isolated from colonized *D. discoideum* nonfarmers and infectiously endows them with farmer-like characteristics (DiSalvo et al. 2015). *Burkholderia* belongs to the β-Proteobacteria, which are widely distributed in the environment, are abundant in soil, and form symbiotic relationships with many plants, fungi, and invertebrates (Kikuchi et al. 2005b, Garcia et al. 2014, Stopnisek et al. 2016). In their symbiotic relationships, dictyostelids provide a carrier for the growth and transmission of *Burkholderia*, while *Burkholderia* provides advantages for the growth, toxin resistance, and intraspecies competition of dictyostelids (Brock et al. 2013, 2016, DiSalvo et al. 2015, Shu et al. 2018a).

9.6 Diversity of symbiotic bacteria

Dictyostelids can selectively prey on bacteria and have a symbiotic relationship with them, while occupying a special ecological niche. It should be noted that it has been demonstrated that there are a large number of symbiotic bacterial resources (Liu et al. 2019a). In fact, to describe a particular microbe's physiology and growth characteristics, including to reliably classify its pathogenicity, antibiotic susceptibility and serological testing, it needs to be grown in laboratory culture (Lloyd et al. 2018).

As is well known, the majority (>99%) of all microorganisms on our planet cannot be cultured (Kaeberlein et al. 2002, Epstein 2013). However, the application of high-throughput technologies is accelerating progress in understanding the diversity of bacteria and in developing a system-level understanding of model bacterial organisms (van Opijnen & Camilli 2013, Franzosa et al. 2015).

The symbiotic bacteria associated with dictyostelids have attracted the interest of many researchers. In this paper, our purpose is to provide an overview of the known diversity of symbiotic bacteria (Table 8) with respect to the associations that exist with phylogenetically and morphologically diverse species of dictyostelids (Fig. 37). These data are based on combining cultural microbiology and sequence-based approaches, with an emphasis on the host specific amoeba microbiomes. The bacteria of amoeba-associated dictyostelids are primarily distributed in the phyla Proteobacteria, Bacteroidetes, Actinobacteria, Chlamydiae, Firmicutes, and Acidobacteria (Brock et al. 2018, Liu et al. 2019a, Sallinger et al. 2021, Zhang et al. 2023b). Within these taxa, based on results obtained with the pure culture method, Brock et al. (2018) isolated a total of 174 bacterial isolates belonging to the Proteobacteria, Actinobacteria, Bacteriodetes, and Firmicutes from more than 250 samples of wild *D. discoideum* fruiting bodies, with one to six species of bacteria per fruiting body sorus in one-third of the tested wild *D. discoideum* obtained from soil and deer feces at the Mountain Lake Biological Station in Virginia in the United States. Also, Zhang et al. (2023b) isolated 55 strains of actinomycetes from 38 strains of 19 species of dictyostelids in Guizhou, Gansu, and Jilin provinces in China by using an actinomycete selective isolation medium (modified Gao′s No. 1). These 55 strains represented two orders, three families, and three genera. These studies also showed that environmental factors have more influence than the species of dictyostelid on the symbiont species of symbiotic bacteria present. Zhang et al. (2025) identified diverse bacterial symbionts in dictyostelids from Chinese soils, revealing their ecological significance and potential impacts on soil functions and public health.

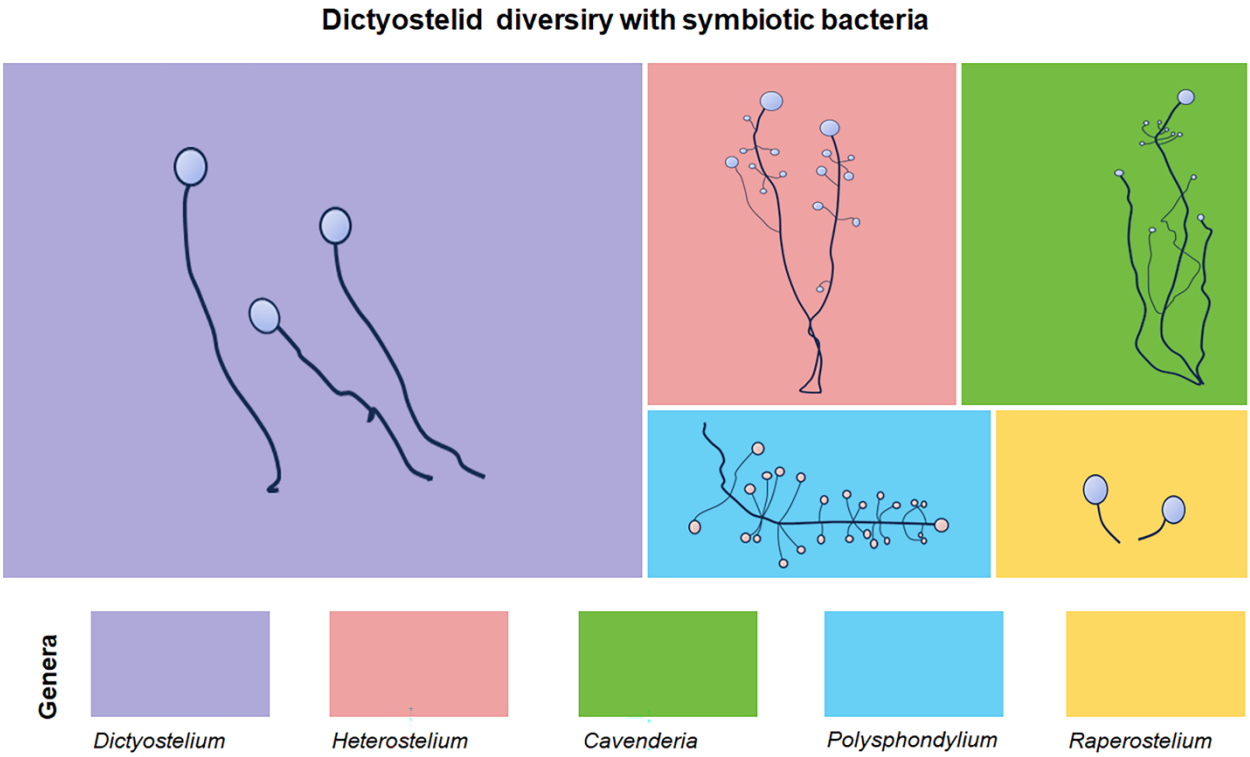


Figure 37 – The diversity of dictyostelids, as indicated by nuclear small subunit (18S) rDNA sequences, in the new classification Sheikh et al. (2018). The known genera of dictyostelids with symbiotic bacteria are labeled in color, and the area of the square represents the number of species of dictyostelids associated with symbiotic bacteria. Detailed information on the associated species of bacteria is shown in Table 8.

Table 8 – Bacteria associated with dictyostelids, based on pure culture and 16S rRNA amplicons.

Genus	Species	Symbiotic bacteria	References
Dictyostelium	*D. discoideum**	*Achromabacter*, *Ancylobacter*, *Chryseobacterium*, *Agrobacterium/Rhizobium/Beijerinckia*, *Brucella/Ochrobactrum*, *Buttiauxella/Lelliottia/Kluyvera*, *Comamonas*, *Shigella/Brenneria*, *Mycobacterium*, *Flavobacterium*, *Kaistia*, *Microbacterium*, *Oxalicibacterium*, *Raoultella*, *Shinella*, *Bacillus*, *Sphingobacterium*, *Stenotrophomonas*,	(Brock et al. 2018) (Brock et al. 2011, 2018) (DiSalvo et al. 2015), (Shu et al. 2018a, 2018b), (Garcia et al. 2019), (Haselkorn et al. 2019) (Brock et al. 2011) (Hagedorn et al. 2009), (Gerstenmaier et al. 2015) (Brock et al. 2020) (Inglis et al. 2018) (Taylor-Mulneix et al. 2017)

Genus	Species	Symbiotic bacteria	References
		Nocardia, *Variovorax*, *Williamsia*, *Paenibacillus*, *Pandorea*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Rahnella*, *Staphylococcus*, *Escherichia*, *Burkholderia*, *Klebsiella*, *Paraburkholderia*, *Bordetella*	
	*D. giganteum****	*Chlamydiales*, *Variovorax*, *Pantoea*, *Escherichia*, *Shigella*	(Sallinger et al. 2021) (Zhang et al. 2025)
	*D. purpureum****	*Chitinophagaceae*, *Sphingomonadaceae*, *Microbacterium*, *Stenotrophomonas*, *Agrobacterium*	(Sallinger et al. 2021) (Zhang et al. 2023b, 2025)
	*D. firmibasis**	*Microbacterium*, *Pseudomonas*	(Zhang et al. 2023b, 2025)
	*D. sphaerocephalum**	*Microbacterium*, *Rhodococcus*, *Brevibacterium*, *Enterobacter*, *Sphingobacterium*, *Bacillus*, *Agrobacterium*, *Stenotrophomonas*, *Pseudomonas*, *Variovorax*	(Zhang et al. 2023b, 2025)
	*D. mucoroides**	*Microbacterium*, *Brevibacterium*, *Enterobacter*, *Flavobacterium*, *Bacillus*, *Stenotrophomonas*, *Achromobacter*, *Chryseobacterium*, *Pseudomonas*	(Zhang et al. 2023b, 2025)
	*D. macrocephalum**	*Microbacterium*, *Stenotrophomonas*, *Enterobacter*	(Zhang et al. 2023b, 2025)
	*D. brefeldianum**	*Microbacterium*	(Zhang et al. 2023b)

Genus	Species	Symbiotic bacteria	References
	*D. multiforme**	*Agrobacterium*	(Zhang et al. 2025)
	Dictyostelium sp.***	*Microbacterium*, *Caulobacteraceae*, *Pseudomonas*, *Achromobacter*, *Escherichia*, *Flavobacterium*, *Burkholderia*	(Zhang et al. 2023b, 2025) (Sallinger et al. 2021)
Polysphondylium	*P. violaceum****	*Microbacterium*, *Sphingomonas*, *Pseudomonas*, *Brucella*, *Pedobacter*, *Escherichia*, *Lysinibacillus*	(Zhang et al. 2023b, 2025) (Sallinger et al. 2021)
	Polysphondylium sp.*	*Microbacterium*, *Achromobacter*, *Pseudomonas*, *Enterobacter*, *Escherichia*	(Zhang et al. 2023b, 2025)
Raperostelium	*Raperostelium* sp.*	*Microbacterium*, *Serratia*, *Chryseobacterium*, *Stenotrophomonas*	(Zhang et al. 2023b, 2025)
Heterostelium	*H. colligatum***	*Rhodococcus*, *Propionibacterium*, *Staphylococcus*, *Advenella*, *Streptococcus*, *Vulcaniibacterium*	(Liu et al. 2019a)
	*H. pallidum**	*Microbacterium*, *Bacillus*, *Enterobacter*, *Escherichia*	(Zhang et al. 2023b, 2025)
	*H. tenuissimum**	*Microbacterium*, *Enterobacter*, *Pandoraea*	(Zhang et al. 2023b, 2025)
	Heterostelium sp.**	*Paenibacillaceae*, *Xanthobacteraceae*, *Burkholderiaceae*	(Sallinger et al. 2021)
Cavenderia	*C. microspora**	*Microbacterium*	(Zhang et al. 2023b)
	*C. fasciculata**	*Rhodococcus*	(Zhang et al. 2023b)
	*C. delicata**	*Bacillus*, *Pseudomonas*	(Zhang et al. 2025)
	Cavenderia sp.*	*Pseudomonas*, *Microbacterium*,	(Zhang et al. 2025)

Genus	Species	Symbiotic bacteria	References
		Rhodococcus, *Escherichia*	

*Refers to species based on pure culture.
**Refers to species based on 16S rRNA amplicons.
***Refers to species based on pure culture and 16S rRNA amplicons.

9.7 Methodological approaches for studying symbiotic bacteria in dictyostelids

The isolation and characterization of symbiotic bacteria associated with dictyostelids involve a multi-step process combining culturing, molecular techniques, and bioinformatics. First, dictyostelid fruiting bodies are isolated from diverse soil habitats (Brock et al. 2018; Liu et al. 2019a; Zhang et al. 2025). To minimize contamination, spores are isolated using sterile techniques and plated on selective media such as SM/5 agar (Brock et al. 2011) or non-nutrient water agar supplemented with *E. coli* as a food source (Zhang et al. 2025). For microbiome characterization, DNA is extracted directly from pooled sori or fruiting bodies using commercial kits (e.g., Qiagen DNeasy PowerSoil Pro Kit), with modifications such as extended vortexing to lyse amoebal spores (Sallinger et al. 2021). To avoid amplification of host mitochondrial DNA, primer pairs targeting the V3–V4 region (e.g., 341F-806R) are preferred for 16S rRNA amplicon sequencing (Liu et al. 2019a).

For culturable symbionts, bacterial colonies are purified from spore suspensions streaked on media like SM/5 agar (Brock et al. 2011, 2018), followed by Sanger sequencing of 16S rRNA using universal primers (27F/1492R) and phylogenetic analysis (Zhang et al. 2025). Taxonomic classification is performed via BLAST against reference databases (e.g., NCBI), with potential novel species identified at <98.65% sequence similarity (Zhang et al. 2025). Bioinformatics pipelines such as QIIME2 are employed for denoising, diversity metrics (alpha/beta-diversity), and functional prediction (e.g., FAPROTAX) (Sallinger et al. 2021; Zhang et al. 2025).

Challenges include contamination control, low biomass in amoeba samples, and distinguishing transient vs. stable symbionts. Integrating culturing with metagenomics is recommended to capture both cultivable and unculturable taxa. Standardized metadata collection (e.g., soil pH, moisture) ensures reproducibility and facilitates ecological correlations. These methods collectively enable robust exploration of host specificity, environmental drivers, and functional roles in dictyostelid-bacteria symbioses.

Conclusions

Dictyostelids, also known as cellular slime molds, represent a fascinating yet underappreciated group of microorganisms in terrestrial ecosystems. Their widespread occurrence, from high latitudes of the Arctic and subarctic to the tropics, underscores their adaptability and resilience across diverse environments. The intricate relationships they form with other organisms, particularly during their life cycle as they transition from individual amoebae to multicellular fruiting bodies, offer insights into the evolution of multicellularity and cooperation. Furthermore, the diverse range of compounds produced by dictyostelids, including secondary metabolites with potential pharmaceutical applications, highlights their untapped potential as a source of novel bioactive molecules.

Through this paper, we have tried to provide a comprehensive overview of dictyostelids, encompassing their ecology, diversity, interactions with other organisms, isolation techniques, historical perspectives, and classification. The detailed description of species assemblages associated with various ecosystems underscores the importance of studying these organisms in their natural habitats to fully comprehend their ecological roles and evolutionary adaptations.

Challenges

1. Lack of awareness and research funding: despite their ecological and biotechnological significance, dictyostelids receive limited attention and funding compared to other groups of microbes. This hampers in-depth research efforts, especially in understanding their full diversity and

potential applications. Notably, this is especially the case for smaller countries where researchers face major challenges in securing resources and recognition for their work.

2. Identification and classification challenges: accurate identification and classification of dictyostelids can be challenging due to their complex life cycles, morphological plasticity, and high species diversity. This limits our understanding of their distribution patterns and ecological roles.

3. Isolation and cultivation difficulties: isolating and culturing dictyostelids in the laboratory can be technically challenging, particularly for species that are rare or have specific environmental requirements. This constraint limits experimental studies and bioprospecting efforts.

4. Ecosystem disturbances: anthropogenic factors such as deforestation, pollution, and climate change can disrupt dictyostelid habitats, affecting their population dynamics and potentially impacting their ecological functions.

Opportunities

1. Biotechnological applications: given the unique compounds produced by dictyostelids, there is an immense potential for developing novel drugs, bioactive molecules, and biopesticides. Investigating their metabolic pathways and biosynthetic capabilities can lead to significant biotechnological advancements.

2. Ecological insights: studying dictyostelids in their natural habitats can provide valuable insights into the mechanisms of microbial cooperation, speciation, and adaptation to extreme environments. This knowledge can used to enhance conservation efforts and to develop effective ecosystem management strategies.

3. Education and public awareness: raising awareness about dictyostelids and their ecological importance among the general public, educators, and policymakers can foster support for research funding and conservation initiatives. This includes highlighting the contributions being made by scientists in smaller countries where research on dictyostelids is ongoing.

4. Multidisciplinary collaborations: collaborations among ecologists, microbiologists, biochemists, and biotechnologists can accelerate research progress by leveraging their complementary expertise and resources. This approach can lead to innovative solutions for addressing challenges related to dictyostelid identification, cultivation, and bioprospecting. In addition, fostering international collaborations, particularly with researchers in smaller countries, can enhance the global perspective and diversity of research efforts.

In summary, dictyostelids represent a promising yet underexplored frontier in microbial research. Addressing the challenges and harnessing the opportunities associated with these organisms holds the potential to significantly advance our understanding of microbial ecology, evolution, and biotechnology.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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