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Review

Problems and solutions for the integration of glomalean taxonomy, systematic biology, and the study of endomycorrhizal phenomena

Joseph B. Morton

Division of Plant and Soil Sciences, P.O. Box 6057, 401 Brooks Hall, West Virginia University, Morgantown, WV 26506-6057, USA

Abstract. Complete explanations of mycorrhizal phenomena require knowledge of phylogenetic relationships among the fungal as well as the plant symbionts. Such interpretations derive from systematic analyses, which in turn depend on a strong taxonomic data base. Glomalean taxonomy is still in the formative stages of exploration and documentation of fungal diversity, but it is undermined by inattention to the biological properties of an unwieldy fungal organism and an inability or unwillingness to obtain living germ plasm for comparative analyses. Few published descriptions are accurate and the type method in its present form has failed to provide a fixed reference point for restudy. New discoveries are in danger of being redundant and inconsequential unless a high priority is placed on re-evaluation of known taxa. Systematic studies also are jeopardized by taxonomic deficiencies. Yet a phylogenetic perspective is essential to understand the fungal organism, define population and species concepts, formulate speciation and biogeographic process theories, and restructure classification to reflect evolutionary trends. A phylogeny is in place as a tool for making rigorous morphological, ontogenetic, ecological, physiological, and molecular comparisons. The International Culture Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi is a resource that can provide culturable homogeneous germ plasm for these kinds of experiments, nonliving specimens as known references for taxonomic investigations, and combinations of materials to aid in the understanding of the scope of fungal diversity. As causal connections between patterns of fungal diversification and mycorrhizal phenomena become known through all of these efforts, research results in systematics and other disciplines can be unified and incorporated into more universal theories.

Key words: Vesicular-arbuscular mycorrhizas - symbiosis - Phylogeny - Cladistics

Introduction

Taxonomy of glomalean fungi is less than 20 years old, starting with the formal Linnaean classification by Gerdemann and Trappe (1974). Systematic investigations are less than 2 years old, beginning with the phylogenetic hypothesis of Morton (1990a). This time span is very short compared to the 200+ years in which similar activities were carried out by botanists and zoologists (Stuessy 1990). Most mycorrhizologists, educated in plant and animal taxonomy, disregard this historical asymmetry and burden glomalean studies with expectations unrealistic for their present stage of scientific development. Taxonomic studies have not been able to provide explanations of the nature of fungal diversity needed by other disciplines because the fungal entities in the mycorrhizal symbiosis have not received sufficient attention. Over 190 papers on studies of mycorrhizal interactions were published before a formal classification was even recognized (Mosse 1973), and hundreds more were published before researchers had a clue of phylogenetic relationships among known taxa. Explanations of experimental results could not have been based on well-defined taxonomic or systematic conclusions because they did not exist.

Taxonomy and systematics of glomalean fungi are plagued with many of the same problems faced by biologists working on other groups. Mosse et al. (1981) outlined some of these explicitly, but they never were tackled to any substantive extent. Glomalean fungi have problems unique to their biology and organizational features, most of which are closely tied to evolution of the endomycorrhizal symbiosis and the dynamic interactions between various parts of the fungal organism and their interactions with host plants. As a result, knowledge of fungal diversity must take into consideration ecological processes, and ecology must incorporate the causal processes responsible for the taxonomic entities observed in natural environments.

In this paper, I closely examine the status of glomalean taxonomy and systematics, disclose important problem areas, and then propose some solutions. This is ac-

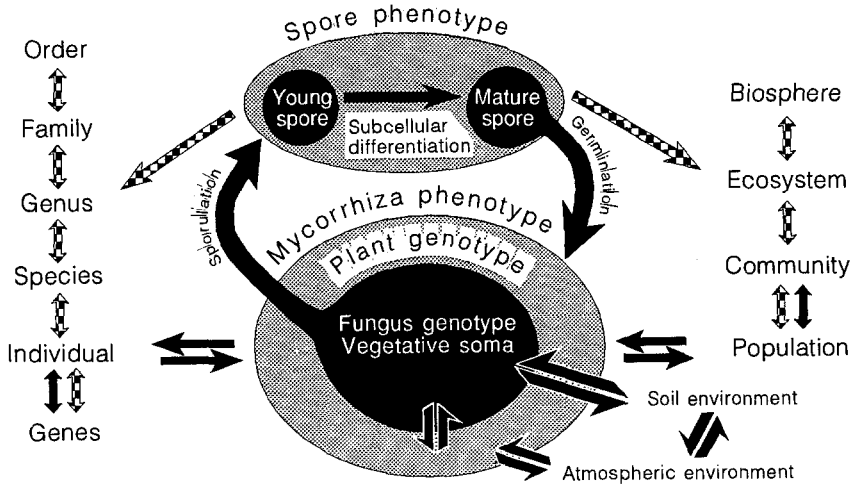


Fig. 1. Heuristic depiction of dynamics between parts of individual glomalean organisms which produce discrete phenotypes, and the levels in the genealogical and ecological hierarchies at which these phenotypes provide biologically important information. *Solid arrows* mark nonhistorical (proximal) interactions and *cross-hatched arrows* pinpoint effects of historical (phylogenetic) relationships

complished within a hierarchical framework to pinpoint levels where processes generating biotic and ecological diversity interact and where they are independently of each other. I focus on the organization and complexity of glomalean fungal organisms, how developmental properties impact on recognition and explanation of taxonomic patterns and the genealogical (evolutionary) processes, and where ecological forces impact on emergence of taxonomic diversity.

Certain terms have specific meanings in this paper. An *isolate* includes all fungal propagules obtained from a single sample (so that multiple sampling from the same site yields multiple isolates). Methodologically, an isolate is the closest entity to a fungus individual collected from the soil environment. A fungal *individual* is circumscribed as the product of a single spore. Its boundaries are definable only when propagated in containerized plants. A *population* consists of all fungal units of a species taxon in a local plant community. Its boundaries are inseparable from that of the individual; molecular markers will be needed to make distinctions. *Evolution* in asexual fungal individuals is considered to be the process by which new variation is introduced in one or more suborganismal parts in an ancestral organism, which then is passed on to descendants through semiconservative replication to form a monophyletic group. Each *monophyletic group* is the ancestor (where a unique variant arose) and all of its descendants. Effects of *historical causation* are recognized by irreversible changes (those possessing directionality independent of contemporary ecological conditions) through time and space. They can be depicted cladistically by dichotomous branch patterns indicative of common ancestry. Evolutionary trees incorporate hypotheses of direct ancestry into cladistic patterns (see Morton 1990a). Effects of *proximal causation* are changes temporarily reversible with different environmental conditions or during reproduction. They show no time directionality and thus are not phylogenetically informative (Brooks and O'Grady 1986).

Hierarchies relevant to mycorrhizal phenomenology

A glomalean fungus lacks individuality in the traditional sense of organisms with distinct somatic boundaries (e.g. vertebrates, invertebrates, plants unable to multiply clonally). It consists of many repeating parts that are interconnected but only loosely integrated. Open-ended growth prevents delimitation of somatic boundaries. The vegetative soma consists of a hyphal network which interacts with a host plant and forms a functional mycorrhizal association. The interactions between host and fungus are manifested in the mycorrhizal phenotype (Fig. 1). Spores are produced asynchronously from the vegetative thallus and are multinucleate single cells. The spore phenotype is the result of developmental processes completely different from those in the vegetative thallus, so that it is autonomous in form and function. Each phenotype (spore and mycorrhizal) of the fungal organism provides different information in elucidation of mechanisms causing organization of the genealogical hierarchy (expressed in the traditional levels of the formal Linnaean system), and the ecological hierarchy of Vrba and Eldredge (1984) (Fig. 1).

All somatic portions of the fungal organism concerned with forming and maintaining an obligatory physiological mutualism in plants include the filamentous hyphal network, arbuscules, vesicles, and auxiliary cells. These parts originate either from the germ tube of a spore or from a totipotent hyphal fragment. The soil environment establishes the initial conditions for mycorrhiza establishment and acts directly on the hyphal thallus during premycorrhiza formation and on extraradical hyphae during later establishment and growth phases (Brundrett 1991). The plant, as a higher level of organization in this dual association, determines the boundary conditions within which the fungus is able to grow, reproduce, and evolve. Thus, suborganismal parts of the fungal thallus in a mycorrhiza respond directly to plant factors but only indirectly to environmental variables through the host filter. These dynamic interactions are likely to produce transitory phenotypes, since responsiveness to changing environmental factors determines the ability of the fungal thallus to survive

and reproduce as an organism alone or within a population. Unless phenotypic responses are stable in descendant populations, they are phylogenetically uninformative.

Spores are morphologically specialized cells which do not contribute to or support activities in mycorrhizal development and host-fungus interactions. Their function is to sequester the genetic information system of the fungal organism, disperse this information to new habitats, and initiate new individuals spatially separated from the parent organism. Since spores are produced from somatic hyphae, their growth is influenced directly by the physiology of the fungus in the mycorrhiza and indirectly by extrinsic environmental factors filtered through the parent hyphae. Thus, rate or quantity of sporulation is a function of the mycorrhizal phenotype rather than of the spores themselves (Fig. 1). The most important property of spores is that many of their component morphological structures are stable under almost any set of environmental conditions. Fidelity in replication is an indication of historical causation and thus is important taxonomically (and phylogenetically).

Autonomy of spore and mycorrhizal phenotypes prohibits a smooth transition from nontaxonomic entities (organisms and populations) to taxonomic entities (species and higher taxa). The latter determine categories of Linnaean hierarchical classifications. The former are important in understanding the cause and effect relationships that led to the existence of present-day diversity.

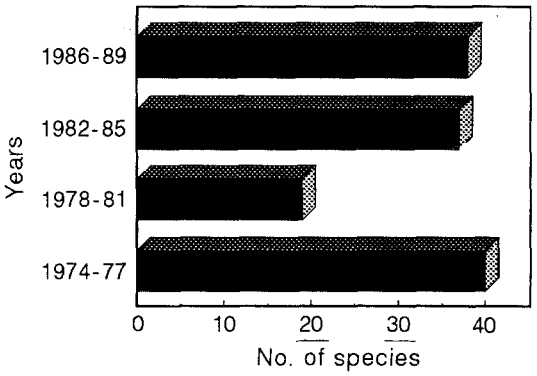


Fig. 2. The number of glomalean species described between 1974 and 1989

Fig. 3. General locations where new species described between 1974 and 1989 were discovered. *Dots* do not mark actual sites, but only countries or regions of a country; some represent regions where two or more new species were found

Glomalean taxonomy

The first classification cataloguing diversity of zygomycetous endomycorrhizal fungi (Gerdemann and Trappe 1974) was based on intuitive estimates of similarity in spore phenotypes (Fig. 1). Such an approach was unavoidable at that time because the range of diversity was virtually unknown. Historically informative characters of the vegetative portion of the fungal thallus were ignored, however, so that taxonomic conclusions were divorced completely from causal explanations of the organism as an evolving entity. Methods to obtain classifications based on phylogeny reconstruction were available (e.g. Hennig 1979), but the taxonomic data base was too limited for any robust analysis.

Approximately 150 species (Schenck and Pérez 1990) have been described based on morphological features of spores. The increase in number of new species descriptions was fairly steady between 1974 and 1989, except for the period 1978–1981 (Fig. 2). This trend is phenomenal considering that fewer than 10 individuals were active at any one time (Morton 1988). The current view of low diversity levels compared to other mycorrhizal groups (Brundrett 1991) has two causes. The first is the nature of diversification in glomalean organisms. Only so many structural characters can arise during differentiation of unicellular spores. The second is a sampling bias (Fig. 3). Many regions of the world remain unexplored as of this writing. Complications besetting taxonomic studies are concerned with how this diversity is recognized so that it accurately mirrors discontinuities due to real biological processes. They are confronted in this section by the sequence in which new species achieve status as named entities.

Exploration and discovery

Exploration for mycorrhizal fungi in different habitats does not result in the instantaneous gratification many botanists and zoologists enjoy, because the organisms are microscopic and below ground. Discovery also is dependent on technically simple, but time-consuming and labor-intensive, laboratory operations to extract spores. The presence of spore propagules in rhizosphere soil samples is unpredictable, even when all plants are mycorrhizal (McGee 1989). When participants of a workshop collected samples from plants in a dry tropical forest in Chamela, Mexico, no intact identifiable field-collected spores were obtained. A later visit by others to the same area bore opposite results (M. Allen, personal communication). Patchy distribution of fungal propagules (and mycorrhizas) in roots, soils, and plant communities; activities of other organisms transporting or consuming these propagules; and local and seasonal environmental fluxes affecting numerical abundance of propagules (Allen 1991; Brundrett 1991; Walker et al.

1982) hinder efforts to obtain consistent and repeatable results.

The only ways to compensate for all of this variability are to collect multiple samples at different times of the year *and* to set up greenhouse pot cultures that might induce spore production by weak or nonsporulating colonizers. The latter procedure does not guarantee success because pot or greenhouse conditions also act as modifiers of fungal colonization and sporulation patterns. For example, only spores of *Glomus diaphanum* Morton & Walker and *G. tenue* (Greenall) Hall were recovered directly from a partially reclaimed minesoil in West Virginia over a 6-year period (soil no. 6 in Morton 1985). When rhizosphere samples were cultured on sudan grass [*Sorghum sudanense* (Piper) Staph.] in a higher pH growth medium, *G. tenue* was lost completely, *G. diaphanum* persisted, and spores of a previously undetected isolate of *G. etunicatum* Becker & Gerd. appeared consistently.

Laboratory procedures used to collect spores may also bias knowledge of fungal organisms present in a soil sample. Most species taxa in the first classification were sporocarpic (Gerdemann and Trappe 1974), in part because they were more easily detected in soil sievings. As other extraction procedures were introduced (Daniels and Skipper 1982), more nonsporocarpic species were discovered. Additional biases were introduced with these methods owing to differences in: (1) washing procedures to remove spores on or in roots, (2) sizes of sieve openings used to capture spores, (3) and centrifugation procedures. Standardization of methodology is difficult because biotic and abiotic components in the mycorrhizal symbiosis can differ so much from one plant community to another. Modifications often are necessary to meet each set of circumstances. Investigators must conduct preliminary experiments to optimize sampling of all organisms at their sites.

Separation of discrete organisms from samples

Spores are the most retrievable part of the fungal organism because each can be manipulated as a discrete object. However, most samples collected from the field usually contain spores of different species taxa. Molina et al. (1978) recovered an average of two to five species from *Festuca* plants in the western United States. Even in extreme soil conditions, species mixtures are common (see Morton 1985). If the investigator cannot recognize spores of a single species taxon, then serious complications ensue. Spores must be recovered under a dissecting microscope, but the characters observable at this level often overlap among different species and even among different genera (e.g. *Gigaspora* and *Scutellospora*).

Lack of experience is the most fundamental and pervasive problem in recognizing spores of glomalean fungi from all other biotic and abiotic entities in soil extracts. Any taxonomist can relate tales of slides (and even type specimens) consisting of rounded and smooth soil crystalline deposits, nematode and insect eggs, sclerotia, algal cells, and other structures mistaken for spores. Even when spores are recognized, their taxonomic characters are assumed to be intact and unmodified. In reality, most spores are either parasitized, deteriorated, or modified in some way to cause misinterpretation of character properties or their occurrence. In a West Virginia soil, for example, spores of an *Acaulospora* and a *Scutellospora* species from the field were indistinguishable because they lacked diagnostic hyphal attachments and overlapped in color, size, and shape.

The only direct solution to these problems is to propagate infectious propagules in field soils in greenhouse "trap cultures". The procedure is simple: rhizosphere soil is chopped up, mixed with a coarse sand, seeded with a host plant of the same or related species as that at the source location, and left to grow for 3–4 months. Despite the long wait between sampling and analysis, significant benefits accrue. Spores are: (1) consistent in appearance with most or all diagnostic features intact with hyphal attachments present, (2) of similar age and physiological condition for direct manipulation in experimental regimes, (3) abundant enough to produce replicate cultures from multi-spore or single-spore inocula, and (4) in a condition which permits direct comparison of properties in spores from the original field site.

Recognition of species taxa

Taxonomic decisions at the species level require observations of broken spores with the light microscope, because many characters, especially those in Acaulosporaceae and Gigasporaceae, are subcellular (Morton and Benny 1990). The magnitude of errors is inversely proportional to the quality of microscope optics and the ability of the observer to delineate and interpret a bewildering array of minute structures.

Most subcellular structures of spores have been interpreted as various types of walls based on the adult phenotype. Walker (1983) was the first to propose terms for putative wall types, and other terms were added as novel forms were discovered (Morton 1988). Structures within the spore are the most difficult to discern because they are colorless (unless immersed in Melzer's or other histochemical reagents) and they exhibit varying degrees of flexibility. All tend to wrinkle, fold, break in different places, overlap, clump, or become outstretched when pressure is applied to spores. Some walls become separated easily, others remain attached irrespective of manipulations. Delineation of individual structures in this jumbled agglomeration can be formidable even for someone with years of experience. Interpretation problems include recognizing stable differences as opposed to minor variation and translating properties of a three-dimensional object from two-dimensional observations. Decisions will remain subjective until characters are objectified according to their individuality and origin (see glomalean systematics, below).

The assumption that field-collected spores possess intact all the informative characters for taxonomic decisions at the species level is erroneous and must be abandoned. All structural components of spores are suscepti-

ble to alteration or deterioration by a wide range of biotic or abiotic agents in the soil environment (see Morton 1988 for some examples). When spores of different *Scutellospora* species from sand dunes (which often appear healthy) were compared with their pot-cultured counterparts, few spores in the former were free of modifications to inner flexible walls (J. B. Morton and R. E. Koske, unpublished work). Comparisons between field-collected and pot-culture-collected spores then became a necessity to characterize their properties and produce an accurate species diagnosis or description.

Collection of spores from pot cultures are essential for identification or characterization of *Glomus* spores. Many species-level characters are outer components of spores exposed directly to the soil environment (Morton and Benny 1990). They are ephemeral and, therefore, absent in field-collected specimens. Only in pot cultures do these outer spore wall components remain partially or completely intact, especially when spores are collected 75–90 days after the start of the culture (J. B. Morton, unpublished work).

Acquiring the knowledge needed to recognize and interpret subcellular spore structure is not easy for any mycorrhizologist. Few quality reference materials have been published, and species descriptions are too inadequately documented or inaccurate (see below) to be of instructional benefit. Hands-on experience is difficult to obtain except through workshops or short courses. These are being organized on a regular basis in conjunction with scientific meetings and conferences, but they suffer from being too abbreviated. A set of 60 slides, along with a manual explaining characters visualized in photographed spores, was published by Morton (1989). These photographs have some inherent limitations in that they portray ideal rather than the more commonly encountered "messy" specimens, they represent only one plane of view, and they fail to show the range of variation in characters with different preparatory methods. New approaches are being taken through the International Culture Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM), which will be discussed later in this paper.

Spores collected from trap cultures can be used to identify and describe species taxa, but they are problematical if the investigator has difficulties in recognizing and grouping similar phenotypes. Moreover, these cultures often contain other organisms (particularly eggs and larvae of nematodes and small soil insects) which jeopardize their long-term viability. Single-isolate cultures are the optimal source of propagules for characterizing a species taxon, whether it is new or already described. The obvious taxonomic value is validation of mycorrhizal status and the capability to study modules of glomalean organisms at the same time (Fig. 1). Moreover, direct comparisons of mycorrhizal ontogeny and spore differentiation among different isolates of the same and related species becomes possible. Both approaches eliminate many variables that confuse and hinder interpretations accompanying extraction of field-collected spores.

Nomenclatorial problems

Most named species taxa in Glomales have been circumscribed strictly according to properties of the type specimen, in part because no other conceptual basis has existed to delineate species boundaries. Type specimens serve to provide a physical link between the name and the organism being described. They are not meant to delimit species boundaries because that requires knowledge of intraspecific variability. One crucial assumption of the type method is that characters in collected specimens do not change through time. Internal flexible wall components of fungal spores, however, are not stable except in a frozen or lyophilized state. Most glomalean type specimens have been stored in vials or mounted on glass slides surrounded by a chemical medium containing phenol, lactic acid, formalin, sodium azide or combinations of these. Chemical interactions have gradually modified subcellular structures to the point where some wall types are unrecognizable in relation to live spores (Morton 1988). In this respect, the type method has failed.

Other less universal problems with type specimens include: (1) collecting spores of two species mistakenly thought to be one, (2) mixing dead or microbially degraded spores with healthy ones, and (3) including propagules of other fungi mistaken for spores. The tendency to equate microbially degraded and chemically altered spores as representative of a species in a healthy state is pervasive, but cannot be judged from published accounts without living propagules to provide legitimate comparisons.

The type method demands a specimen, but fortunately it can range from a physical part of the organism to an illustration or even a photograph. The current procedure of storing spores in formalin or other chemical should be maintained to obtain an overall view of the spore population being described even though subcellular structure will likely be modified. The minimum requirement for the type specimen, however, must be something that is an unchangeable reference point – and only a color photograph or transparency of a specimen showing the diagnostic features of the species taxon meets that requirement. Unfortunately, even these materials begin to deteriorate after 20–40 years.

Species descriptions

At least 90% of the descriptions published to date are inaccurate or incomplete to varying degrees, including many redescribed taxa. Many named species which never have been proven mycorrhizal arguably do not even belong in Glomales when character convergence is taken into account (Morton 1990a). Errors in species descriptions are not easy to detect by reviewers or readers unless they have live specimens to reference and compare. Some specimens seem to be completely inaccessible (e.g., those described by Ferrer and Herrera 1981). At the same time, study of type specimens is devalued by structural instability or possible noncorrespondence to live fungal propagules (see above).

A number of common errors are committed in describing a taxon. First, new structures or new properties of existing structures are missed if other more obvious diagnostic features are present. For example, subcellular wall structure of *Acaulospora bireticulata* was not noted because of the striking ornamentation pattern of the laminate spore wall (Rothwell and Trappe 1979); paired "coriaceous walls" in *Scutellospora erythropa* were mistakenly interpreted as an inner laminated wall (Koske and Walker 1984). Often, very thin "membranous walls" were overlooked because they were inseparable from other slightly thicker "membranous walls" in field-collected spores (e.g., *S. coralloides*, *S. fulgida*, *S. gregaria*, *S. persica*, *S. verrucosa*; J. B. Morton and R. E. Koske in preparation). Second, properties of individual characters are not understood and distinct characters are treated as one or vice versa. Blaszkowski (1988) compares new *Acaulospora* species relative to known species with completely different characters and even describes a *Glomus* which suspiciously has *Acaulospora*-like features. Third, structures in preserved or parasitized spores are interpreted as representing those in live or healthy characters. Walker and Hall (1991) acknowledge the distortions of preserved material, but still redescribe characters of a species that probably do not correspond to healthy spores in nature. Fourth, features in a few spores collected from a heterogeneous field site are generalized as archetypal of a whole species. Examples include *G. botryoides* (Rothwell and Victor 1984) and *A. bireticulata* (Rothwell and Trappe 1979).

Inadequate knowledge of the origin and nature of characters and their role in defining a species taxon accounts for many of the problems mentioned above. A host of other conditions plague observational studies, such as inexperience and the desire to publish at the expense of caution and more thorough comparative study. An insidious problem has been propagation of a fungal organism in greenhouse pot cultures, but then storing collected spores in a chemical environment prior to taxonomic study. This practice is not acknowledged in descriptions and thus leaves the impression only fresh spores were examined. As isolates of more species are obtained in culture, such discrepancies will be exposed.

Weak standards reflect poorly on taxonomy as a science. More rigorous criteria are needed to group and rank organisms as members of a species taxon. In addition, future descriptions must include clear photographs showing *all* pertinent characters. The microscopic properties of spore characters and their changeability in any sort of preserved medium demands this practice. All disciplines reliant on these descriptions would benefit, because visual pointers would correspond to the narrative.

International Culture Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi

A living culture collection of glomalean fungi from all parts of the world was started by Norman Schenck at the University of Florida in 1985, with funding from the National Science Foundation. The INVAM collection was transferred to West Virginia University, where funding continues through 1995. It came into being because of an urgent need to preserve fungal germ plasm. Experimental organisms were being lost at an alarming rate, mostly because cultures could not be maintained or stored without contaminant build-up or mortality. One of the major accomplishments of the Florida group was to develop and implement cryopreservation techniques for longer storage (Douds and Schenck 1990), although the rate of propagule die-off with time still needs to be evaluated. INVAM now is capable of short- and long-term storage of trap or monocultures of almost any fungal isolate. Since this service is available to all scientists, loss of experimental isolates so clearly needed to repeat or validate published studies no longer is excusable. For systematic studies, organisms of species taxa may be propagated further, partitioned into cultures originating from single-spore inoculations, or cryopreserved to preserve phenotypic and physiological properties.

Every culture started and harvested in the collection is accompanied by voucher specimens to record taxon identification and to monitor phenotypic variation and occurrence of contaminants. Vouchers include whole and broken spores permanently mounted in polyvinyl alcohol - lactic acid - glycerin (PVLG) and PVLG + Melzer's reagent (see Morton 1988 for chemical components), color slide and print photographs of mounted spores, and whole spores preserved in 0.05% sodium azide. All specimens and appended materials are accessible to scientists interested in developing their own taxonomic expertise. A listing of all cultures and voucher materials will be published in catalog form near the end of 1992 to inform the scientific community of available resources.

INVAM offers a number of service functions designed to promote identification and preservation of glomalean germ plasm. Individuals or populations of discrete species taxa are propagated from almost any source. Experimental cultures mailed to INVAM may be cryopreserved, propagated further, or reisolated from mixtures. Vials of fresh or preserved spores of single taxa extracted from cultures also have been provided (e.g. Simon et al. 1992). Information services include: (1) a biennial newsletter reporting all facets of culture development, handling and storage operations, and issues of taxonomy and systematics, (2) local, institution, or society-arranged workshops emphasizing hands-on experience toward the culture, identification, and systematic analysis of known fungal diversity, and (3) customized kits containing voucher materials of different species to help scientists develop their own expertise in recognition and identification.

Most scientists working with glomalean organisms have not recognized the necessity to prepare and lodge voucher specimens. Unequivocably, any study without such materials immediately is robbed of empirical content because taxonomy has not advanced enough for any decision of identity to be truly definitive (see discussions above). INVAM offers the opportunity to photographically record vouchers, type specimens, or other

materials depending on the research goals of individual scientists. This practice not only benefits the investigator, but it improves documentation of the scope of fungal diversity. Vouchers of any type may be deposited with INVAM, and with it the opportunity to compare experimental isolates against known species taxa in the collection. This service would be most valuable for scientists publishing experimental results using organisms of equivocal identity. For example, although Siqueira et al. (1991) experimented with an unresolved *Glomus* species, living germ plasm with complementary vouchers were assigned an INVAM number to establish a trail of continuity to support future work on that isolate.

Glomalean systematics

Systematic investigations go beyond the process of discovery and description to explain the origin and nature of taxonomic diversity. Reconstruction of evolutionary history (phylogeny) becomes essential, and this involves methods that are not intuitive. Still, cladistic methods are enjoying widespread acceptance because of their potential to organize patterns of diversity in ordered hierarchical patterns with powerful taxonomic implications (Stuessy 1990). Greater attention to empiricism in evaluating different data sets (e.g., molecular restriction fragment length polymorphism (RFLP) and sequence data, developmental patterns of morphological characters) are making phylogeny reconstruction a more robust measure of historical relationships among living organisms. The important point to keep in mind is that each group of organisms has its own set of biological properties that determines the types of variation (manifested in observable discontinuities) and the causal processes responsible for that variation.

From this perspective, glomalean fungi have unique biological properties that most certainly impacted on their evolution: (1) they are obligate symbionts of ancient origin (Pirozynski and Dalpé 1989); (2) they have persisted for over 250 million years despite perceived negative attributes of asexual reproductive strategies (Maynard Smith 1989); (3) species-level properties in spore phenotypes have remained stable through geologic time (Morton 1990b); and (4) population-level properties in mycorrhizal phenotypes exhibit the plasticity necessary to track shifts in environmental conditions (Brundrett 1991). Recent explanations to account for these properties have ignored historical relationships (see contributions in Margulis and Fester 1991). These workers cannot be faulted because no phylogeny existed at the time of writing.

The first attempt at a phylogenetic tree (Morton 1990a) was morphology based, and even it was weak because of a sparse and inaccurate taxonomic data base (see Glomalean taxonomy, above). However, it had some redeeming benefit by incorporating historically informative morphological characters of both spore and mycorrhizal phenotypes of glomalean organisms into a more natural classification (Morton and Benny 1990). It also did away with the assumption that all diversity among endomycorrhizal fungi was random and lacked evolutionary boundaries in relation to nonmycorrhizal fungi with similar superficial characters (Fig. 4). Many challenges remain, and they focus on empirical tests to revise hypothesized phylogenetic relationships using as many different criteria as possible.

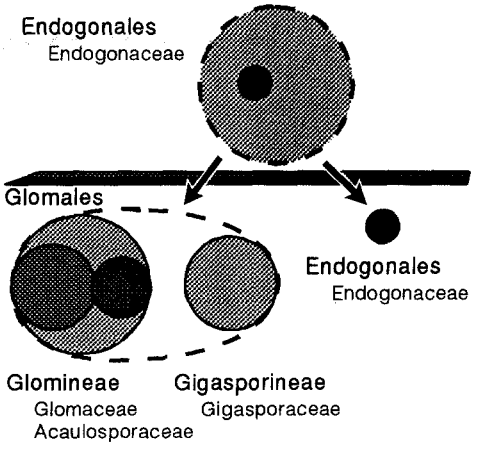


Fig. 4. Reappraisal of phylogenetically significant groupings of endomycorrhizal fungi with transition from the classification of Gerdemann and Trappe (1974) to that of Morton and Benny (1990)

Nature of glomalean species

The fundamental task of a systematist is to rank organisms or populations into a species so that they may be recognized by a formal name in a Linnaean classification and studied to understand the mechanisms responsible for their existence. The paradigm species has been defined biologically according to discontinuities in the capability to interbreed (Mayr 1963). In reality, this concept is applicable only to those groups of organisms which exhibit correspondence between breeding strategy, morphological properties, and ecological niches (Mishler and Brandon 1987). No such correspondence is found among glomalean organisms. Not only are they mostly asexual, but morphological properties of spores are not correlated with functional properties of the mycorrhiza and, by extension, their niche requirements. Uncoupling of taxonomic and functional characters can be traced to autonomy between spore and mycorrhizal phenotypes (Fig. 1). Yet species still are recognized as discrete and stable taxonomic entities according to their spore phenotypes. The extent to which characters in the somatic module of the fungal organism may delimit species depends on distinguishing historical (persistent) from nonhistorical (temporary) characters at all levels of organization.

The first place to start is in documenting properties of individual organisms. Many mycorrhizologists have erroneously equated morphological conservatism in mycorrhizal structures with absence of fungal variation. Even though arbuscule and hyphal structures of the

thallus are conserved in general form (Friese and Allen 1991) as a result of developmental controls, there still is room for expression of morphological variability in organization of the fungal thallus (hyphal architecture, arbuscule branching, area/volume ratios, etc.) and in physiological variability (Smith and Gianinazzi-Pearson 1988). The problem is not that this variation exists, but that it is difficult to measure empirically. Hyphal networks are, by their very nature, diffuse and without discrete boundaries. Physiological properties of the fungus are impossible to separate from host dynamics because the organisms cannot be cultured axenically. This problem does not detract from experimental results showing that isolates of a species differ in their symbiotic effectiveness or that isolates of different species may produce equivalent mycorrhizal phenotypes (reviewed in Brundrett 1991).

Asexuality is not likely to be a hindrance to diversification in populations of a glomalean species, for several reasons. First, fungal organisms may acclimate to different environmental conditions through regulatory rather than genetic changes. Second, considerable genetic variability may arise in somatic portions of the fungal individual if mutation rates are high. This variation is heritable because almost all cells of the organism are totipotent and capable of initiating new individuals (Buss 1983). Last, expected accumulations of detrimental mutations due to lack of recombination (Maynard Smith 1989) is negated by the potential to unload nuclei by partitioning (e.g., in spores, auxiliary cells, vesicles) or by severance of unwanted parts of the organism.

Morphologies of spore or mycorrhizal phenotypes are of no help in discerning populations of glomalean organisms. Other data sets are needed. Variable regions of rDNA may reveal measures of organismal and population interrelationships, either through RFLP or sequence analysis. Random amplification of polymorphic DNA (RAPD) using polymerase chain reaction methods most assuredly will prove useful, once experimental difficulties are worked out (Bruns et al. 1991). Protein-based comparisons also could be valuable, but not at the taxonomic levels studied to date. Monoclonal antibodies specific only against isolates of the fungus *Glomus occultum* Walker have been produced (Wright et al. 1987), but equivalent antibodies for other species are proving more elusive to obtain probably because most immunogenic molecules are not historically conserved. Isoenzyme studies have inferred species-level stability (Sen and Hepper 1986), but the experimental design problems and taxonomic ambiguities make the conclusions equivocal. They also are labile characters, and thus are more appropriate in resolving intra- and interpopulational variation, as recently suggested by comparisons among *Glomus* isolates (Rosendahl 1989).

Most properties of the mycorrhizal phenotype, except for the general morphogenetic properties of arbuscules and the hyphal network, are too changeable with time and under different environmental conditions to be useful phylogenetic characters. This is not unexpected, since flexibility in organization of the hyphal network and partitioning of resources within that network optimizes responsiveness to environmental fluxes within the boundaries of developmental constraints. Invariant properties of the mycorrhizal phenotype may be found, but they are likely to be of taxonomic value only at the level of the suborder, family, or genus.

Information on the emergence of species cannot be obtained from the mycorrhizal phenotype. Speciation is the product of new innovations incorporated into successive events in spore differentiation (Morton and Benny 1990; Morton et al. 1992). Invariance in structural elements of spores must be attributed to constraints in spore developmental programs rather than on cohesiveness resulting from interactive processes. Developmental constraints are attributed to epigenetic factors such that each stage in spore ontogenesis is controlled by the position and properties of previous stages (Gould 1977; Løvtrup 1978). In glomalean organisms, most morphogenetic differentiation occurs in spores, so they become the center of attention in understanding the relationships between ontogenesis and phylogenesis. Morton et al. (1992) conceptualized a glomalean species as "the smallest assemblage of reproductively isolated individuals or populations diagnosed by epigenetic morphological or organizational properties of fungal spores that specify a unique genealogical origin based on the criterion of monophyly". This definition stresses genealogy because diversification in spore morphological properties is ordered in such a way as to define historical ancestor-descendant relationships among fungal populations (Morton 1990a). All populations sharing the same spore developmental (epigenetic) sequence are united in the same species because they are related by common ancestry.

The definition of glomalean species also emphasizes monophyly and unique genealogical origin. Spore morphological characters are simple enough in form and composition that it often is difficult to establish whether similarities are homologous (of common origin) or convergent (of independent origin). The distinction between homology and convergence is of critical importance, because only the former defines a monophyletic group united by the bonds of recent common ancestry and only the criterion of monophyly will distinguish real (as opposed to arbitrary) glomalean species. It follows that observations of similarity in form or function are not sufficient evidence of homology. A separate test showing common ancestry also is required, and this usually involves cladistic analysis (Stuessy 1990; Wiley 1981). Phylogeny reconstruction demands one important bit of information on each character: a decision as to whether a character is ancestral or derived. Only derived characters specify monophyletic groups because they pinpoint the node where descendants diverge from the ancestral phenotype (Hennig 1979). Decisions of homology and evolutionary directionality (polarity in cladistic terminology) are problematical, and thus are discussed at greater length as separate topics.

Character homology

As mentioned above, assessment of character homology demands tests using two separate criteria: (1) measures

of similarity, and (2) a phylogeny establishing that a character passing the test of similarity arose only once (unique origin) (Patterson 1982). Morphological characters are more definitive than those at the molecular level to measure similarity because a greater array of comparative tests are available (Mishler et al. 1988). Molecular data can pass only one test: positional correspondence such as that found in nucleotide sequences (Moritz and Hillis 1990). Morphological data sets can include the following measures of similarity: (1) correspondence in morphology of a structure in different taxa, (2) correspondence in form and position of adjacent or associated characters, and (3) correspondence in developmental pattern, where temporal and spatial sequences in the origin and differentiation of a character are the same in compared taxa. Molecular comparisons among glomalean taxa have only just begun and so little information is available on their robustness. Morphological characters have been measured using only correspondence in form and function, without regard for associated structures or developmental properties. Fortunately, spore characters, especially subcellular structural elements, meet essential standards to carry out all of these tests (Wagner 1989): they are unique, they can be individualized, and they are conserved in descendent populations.

Several examples follow to show how morphological correspondence alone is a poor indicator of homology. First, *Endogone* species and *Glomus* species were placed in the same family because of similarity in sporocarp formation (Gerdemann and Trappe 1974). A more rigorous assessment reveals that component spores are sexual zygospores in the former and asexual "chlamydospores" in the latter. No similarity exists except superficially in spore arrangement. Second, the germination shield in Acaulosporaceae (Morton and Benny 1990) in many ways resembles the shield in *Scutellospora* (Walker and Sanders 1986). This structure is not homologous in the two groups because they are synthesized between completely different internal flexible walls (see Morton 1990a). Third, spores of *G. tubaeforme* Tandy and other *Glomus* species resemble each other in formation and structure (Tandy 1975). Associated features, however, are quite different: *G. tubaeforme* can grow axenically and forms putative ectomycorrhizas (Warcup 1985) while other *Glomus* species are obligate symbionts and form endomycorrhizas. Taken together, these data indicate that *G. tubaeforme* does not belong in the genus *Glomus*.

Developmental patterns provide dynamic indicators of shared character evolution and a test of homology (Kluge and Strauss 1985). For example, the conclusion that arbuscular (Gigasporineae) and vesicular-arbuscular (Glomineae) fungi share a common ancestor (Morton 1990a) is likely to be incorrect because of the complete lack of any similar ontogenetic stages in differentiation of spores (M. Franke and J. B. Morton, in preparation). The hypothesis that historical constraints on arbuscule formation disguise independent origin can be tested by more detailed comparisons of mycorrhizal ontogenesis of selected species. Molecular data will be most informative in resolving phylogenetic relationships at the order and suborder levels. Simon et al. (1992) have begun work on regions of the nuclear gene encoding 18S ribosomal RNA to define the degree of genetic divergence between these major groups and to discover other taxa that might be related and serve as outgroups in cladistic analyses.

The same tests apply at the level of species when spore differentiation patterns of different species are compared. The spore wall terminology invented by Walker (1983) was intended only to optimize recognition of dissimilarities in subcellular structure. Unfortunately, these terms have been erroneously translated by many (including Morton 1990a) to reflect the results of evolutionary processes. In reality, wall characters as they currently are described are not independently formed. They originate and are synthesized in discrete pairs, with each pair of separate origin (Morton and Benny 1990). When these pairs treated as single characters (reported preliminarily in Morton et al. 1992; Fig. 5, this paper), they specify homologies defining unique speciation events.

Comparative studies by Morton (1985) showed that spore morphological characters were highly stable in different host-soil environments. Other observations of the same spore phenotype in different geographic locations and in soil versus root organ cultures (Morton 1990b) corroborate these findings. The invariance associated with subcellular structural components in spores is traceable to their pattern of formation during ontogenesis. Each spatially discrete structure is synthesized as an independent entity that marks a new stage in a linear sequence of events. The sequence is causally linked because a new stage forms only if the preceding stage is completed successfully. It is this strict organization which constitutes the epigenetic program of the spore and buffers the differentiation process against destabilizing effects of external environmental forces or internal physiological fluxes.

More variable regions of the versatile rDNA molecule (Bruns et al. 1991) may identify matching nucleotide sequences at the species level. However, any work along these lines must be complemented by greater resolution of developmental patterns to compare relationships with those generated by morphological patterns. Molecular data offer the most promise in the study of relationships among *Glomus* and *Gigaspora* species because spore differentiation patterns do not contribute as much to diversity in these groups (Morton 1988).

Character polarity

Outgroup and ontogenetic comparisons are important tools to separate ancestral from derived characters, because both specify new evolutionary events that result in new taxonomic entities (Rieppel 1988). The problem with outgroups is that they are not always recognizable, as is the case in Glomales (Morton 1990a). Glomineae is not a suitable outgroup for Gigasporineae because all morphological characters except the arbuscule are decisively nonhomologous according to the criteria dis-

cussed above. Worse, no other group of fungi has been found with characters similar enough to test for homology. Rapid morphological evolution within and among fungal groups may be obscuring these relationships. If so, then only nonmorphological data sets such as sequence analysis of conserved regions of rDNA (e.g., Bruns et al. 1991; Simon et al. 1992) will root each clade. Glomineae also does not have a narrowly defined outgroup based on morphological or developmental characters. The evolutionary directionality of characters describing mode of spore formation in *Glomus* (Morton 1990a) may be completely reversed and still not conflict with some members of the highly generalized outgroup, Zygomycetes. The early fossil record does resolve this problem because both ancestral and derived characters at the root and tip of either phylogeny are represented (Pirozynski and Dalpé 1989). Ontogenetic tests are not possible because most of the sporocarpic species have not been established successfully in culture (Morton 1988).

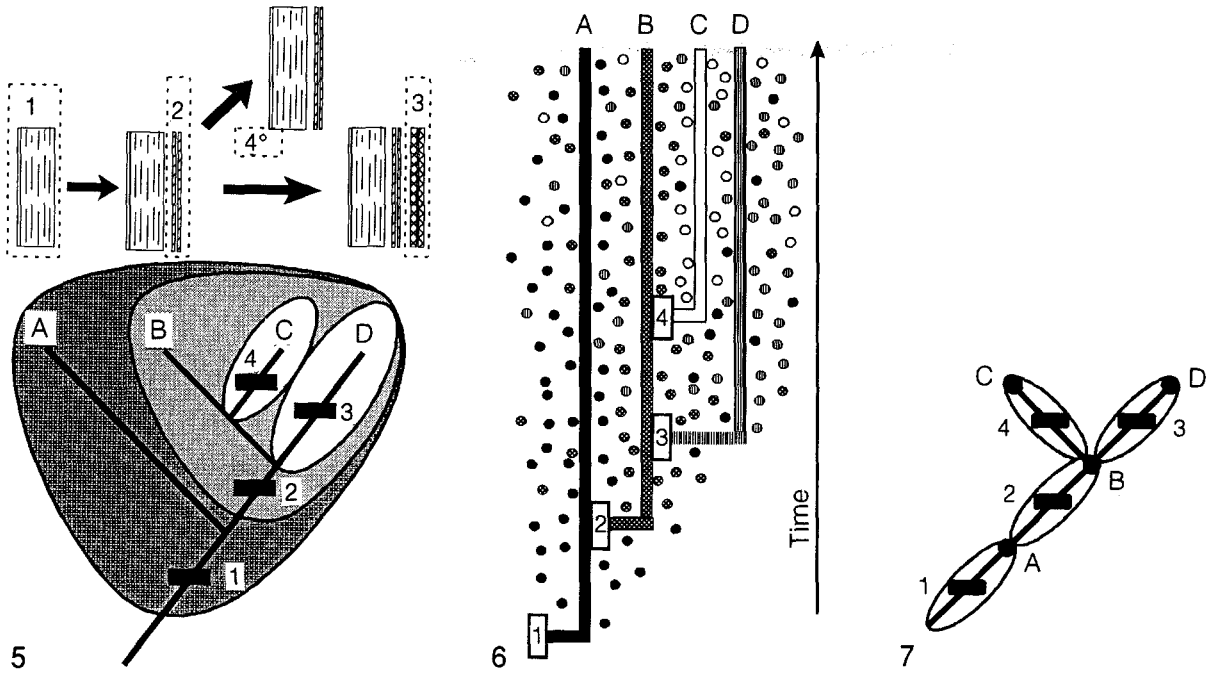
Isolates of species culturable in pots or in root explants provide ideal material for ontogenetic comparisons. Ontogeny is most useful in assigning polarity when new characters are added terminally in a developmental program. In these instances, ontogeny often recapitulates phylogeny (Gould 1977). Evidence is accumulating to indicate that related species share identical stages in spore differentiation until they diverge (Morton et al. 1992). Homology is easily established (see above), after which polarity is ascertained using three taxon comparisons (to show that two species are more closely related to each other than to a third). Two criteria are utilized (Kluge and Strauss 1985). The first is temporal pattern: the stage which forms first in a sequence is ancestral to a stage formed later. The second is generality in distribution among the three taxa: the stage common to all taxa is ancestral to stages confined to one or two taxa. In Fig. 5, for example, the adult stage of *Gigaspora margarita* in homologous with juvenile stages of all *Scutellospora* species tested. It satisfies both criteria: it is found in spores of all taxa being compared and it is an early stage in spore ontogenesis in the *Scutellospora* species. The paired "membranous" walls in the *Scutellospora* species is ancestral to the paired "coriaceous" walls of *S. calospora* because the former is synthesized first in the sequence and is more generally distributed. The latter is derived because it is the last structure to form and it is found only in one taxon (*S. calospora*).

The problem of living ancestors

Gaps between species and higher taxonomic groups are not just the result of large or rapid changes in form and function, but also extinction of ancestral groups (Levinton 1988). Fungi are problematical in theories of ancestry for several important reasons. First, they are not confined by spatial (or even temporal) boundaries. All mycorrhizas in a plant community may become extinct, but the fungal organisms survive if hyphal fragments or spores are able to start new individuals at different locations. Totipotency in all parts of the organism is a powerful mechanism for longevity. Second, developmental homeostasis in spore characters that define species taxa provides no information on what is occurring at the level of the fungal individual or population. When spores are extracted from a site, it is not known from this data alone whether the "parent" organism/population is viable or extinct (although this is testable by setting up trap cultures using root or hyphal inoculum). Similarly, the absence of spores does not indicate absence of the organism/population, but only that sporulation is suppressed (this conclusion also is conditionally testable with trap cultures). The evolutionary result is the opposite of that found in groups where whole organisms evolve as cohesive units. The ancestor of a glomalean species is more likely to be a subset of the parent organism. A new innovation in a spore cell would be conserved in descendent individuals of that spore, but the vegetative thallus would continue to undergo change during acclimatization to new habitats. The ancestral cell(s) could become extinct without notice as long as the ancestral spore phenotype persisted. Whole populations could become extinct in time, but species longevity would not be affected if the spore phenotype persists in at least one part of one surviving organism.

Morton (1990a) did not fully appreciate these factors and erroneously depicted species and even a complete genus (*Gigaspora*) as "whole" living ancestors on a phylogenetic tree. It was not the actual ancestors that survived through geologic time. Rather, it was descendants forming spores of the same ancestral phenotype conserved faithfully as a result of constraints on introduction of variation in the process of spore differentiation.

The co-occurrence of taxa with ancestral and descendent spore phenotypes is a serious problem for phylogenetic analysis because the former groups are *paraphyletic* (each taxon includes the ancestor but not *all* descendants). This problem is exemplified by the analysis summarized in Figs. 5–7. Taxa A–D are organized cladistically in Fig. 5 based on patterns of spore differentiation discussed earlier. Only taxa C and D are truly monophyletic because they are closed lineages that include the ancestor and all its descendants. Taxa A and B are paraphyletic because they have no unique characters of their own and they exclude descendants C and D. While plant and animal groups are paraphyletic because of large gaps in overall similarity or adaptative uniqueness (de Quieroz 1988), glomalean species are paraphyletic for strictly genealogical reasons (persistence of the ancestral phenotype). Thus, glomalean organisms are *grouped* according to the criterion of monophyly wherein they all share the same historically unique innovation. The are *ranked* together as a species because they represent the smallest group sharing the same homology. Using the terminology of Griffiths (1974), these segments depict *time-extended* taxonomic entities based on homeostasis and longevity of spore phenotypes (Fig. 6). Genetically, physiologically, and biogeographically independent fungal organisms functioning in mycorrhizas (large dots in Fig. 6) are recognized as part of a species



Figs. 5–7. Phylogenetic relationships based on cladistic patterns, a phylogenetic tree, and pattern of diversification in modules of glomalean organisms (modified from Morton et al. 1992). **Fig. 5.** Phylogeny of *Gigaspora margarita* (*A*), *Scutellospora fulgida* (*B*), *S. verrucosa* (*C*) and a putative *S. calospora* (*D*) reconstructed cladistically from homologous spore wall characters 1–4 (enclosed in *dashed rectangles*). See Morton (1988) for explanation of murographs. Adult spore wall organization does not match published descriptions; they are being redescribed (J. B. Morton and R. E. Koske, in preparation). **Fig. 6.** Pattern of descendant species lineages (*A–D*) (*unbroken lines*) diverging from ancestral ones with addition or modification of spore characters (1–4) (Fig. 5). Spatially and genetically isolated fungal organisms participating in functional mycorrhizas (*dots*) are unified in a species when they produce the same spore phenotype (*fill pattern in dots*). **Fig. 7.** Cladogram converted to a phylogenetic tree, with each *encircled segment* representing evolution of an extant species (*A–D*)

lineage only by commonality in the spore phenotype (fill pattern in dots). When a cladistic analysis is converted to a phylogenetic tree (Fig. 7), each segment represents a genealogically-derived species taxon diagnosable by one or more developmentally constrained homologies in spores (characters 1–4). Species A and B appear to be ancestral in this phylogeny, but in fact they contain organisms sharing only the spore phenotype of the actual ancestor.

Implications of systematic relationships to mycorrhizologists

Much of the published literature on mycorrhizal phenomena treats species taxa as the units participating in the symbiosis. Clearly, this stems from an erroneous preconception that these fungi are in some ways like other more cohesive organisms (most animals and some plants). However, the mechanisms imparting cohesiveness are the product of constraints on variation during spore differentiation rather than interactive processes. Glomalean species, then, are no different from higher taxonomic groups. All are delimited by historical and developmentally constrained structures, from spore formative characters in genera and families to somatic characters in suborders (Morton and Benny 1990). All are equivalent as historical entities (Wiley 1980), differing only in the generality of character distributions that define each taxonomic group.

Historical characters are relevant in ecological interactions *only* when they have *present-day* functional significance. Characters at the family level and above associated with the fungal thallus are of obvious importance because they are active in specifying the broad niche within which glomalean organisms are able to grow and reproduce. However, the shift in diversifying evolution from the vegetative soma to spore formation and differentiation also signals a shift from functional properties of the mycorrhizal phenotype to completely different properties of the spore phenotype. The functional value of subcellular structures in spores is unknown, if any even exist. It is easy to come up with untestable explanations. Morton (1990b) speculated that additional internal "spore walls" may enhance survival by resisting hyperparasitism, but there is no gradient in survivability that correlates with the gradient in increased spore complexity. The important point is that taxonomic characters of spores are *completely uncoupled* from those associated with the mycorrhiza. Species are not the units to explain mycorrhizal processes. They are the result of evolutionary processes in just one suborganismal cell lineage, the spore.

The participatory units in present-day mycorrhizal associations are individual organisms, either alone or aggregated in populations. They are the units which must be response to changing environmental conditions.

They are the only units subject to the sorting process of natural selection. Clearly, this nontaxonomic diversity is important to an understanding of mycorrhizal processes and must be recognized, just as it is among other groups of organisms that undergo populational changes not extending to species. This can be accomplished quite easily with any code or numerical designation, as long as it is consistent. A species name is important if the researcher wants the information on the isolate to be compared to other isolates related by descent. The practice of generalizing properties of an experimental isolate to all members of the same species without the proper comparative evidence cannot continue. It only serves to obscure the extent of nontaxonomic diversity that exists in nature and gives the false impression that species share common properties because of interactive processes.

The value of taxonomic properties of spore lineages produced by glomalean organisms must not be underestimated. They are very important in understanding the relationship between epigenetic processes at the organismal level and genetic processes at the molecular level, dispersal versus vicariance patterns of species distribution, and paleontological relationships. From an operational perspective, spores signify single individuals that can be propagated and manipulated. Spore characters insure that the organism at the beginning of an experiment is the same at the end.

The limitations of the current taxonomy and systematics are an inevitable condition of their young age. Researchers must compensate by making extra effort to carefully document experiment isolates. Taxonomists, conversely, must take a more experimental approach and work harder to incorporate research results from all disciplines. Only then can systematic theories be formulated that contribute to knowledge of evolutionary processes within the boundary constraints of an obligate mutualistic symbiosis.

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