Developing an online database of descriptions of ectomycorrhizae

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Abstract

Ectomycorrhizae are the mutually beneficial symbioses of fungi and fine roots, and are responsible for the uptake of nutrients that support tree growth. Many species of fungi form ectomycorrhizae with conifers; most are Basidiomycetes or Ascomycetes that form large mushrooms found in forests. It is, therefore, important to investigate how forest management practices affect the health, growth patterns, physiological function, and taxonomic diversity of ectomycorrhizae. Identification of ectomycorrhizae is fundamental to such research.

To date, several hundred detailed descriptions of ectomycorrhizae have been published in books, journal articles, and on a compact disk database called *DEEMY* (*DEtermination of EctoMYcorrhizae*), but hundreds more are unavailable in researchers' private databases. The *Database of Descriptions of Ectomycorrhizae* (*DDE*) web site aims to be a comprehensive tool for the identification of ectomycorrhizae by bringing together as many published and unpublished descriptions as possible. A search function, available to all users, has been programmed to act as an electronic synoptic key. The *DDE* system also allows qualified researchers to add their unpublished descriptions to the database, and to update these descriptions as needed. The *DDE* database currently contains 338 descriptions; 318 from *DEEMY* and 20 from *A Manual of Concise Descriptions of Ectomycorrhizae* (*CDE*). Entry of descriptions and search profiles is done using a web form with all the characters in the *CDE* checklist and links to the *CDE* illustrated glossary.

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Introduction

As natural habitats are destroyed or altered, species are becoming extinct at an unprecedented rate (Ehrlich and Ehrlich 1981). The work of describing the species that exist on the planet, however, has just begun. This is especially true for fungi and bacteria (Bull et al. 1992). Moreover, humanity has a limited understanding of the functions of most species. Biological diversity is valuable in many ways (Kellert 1986), including its contribution to ecosystem stability (Elton 1958; Naeem et al. 1995; Tilman and Downing 1994; Tilman et al. 1996). To best conserve and use species in managed ecosystems, they need to be collected and described.

Mycorrhizae are mutually beneficial symbioses of fungi and fine roots of plants (Smith and Read 1997). They are essential interfaces between plants and soil and are responsible for uptake of the nutrients that trees use to grow and develop. Hyphae and mycelium of mycorrhizal fungi spread through soil, acting as extensions of root systems. Ectomycorrhizae, the predominant type of mycorrhiza of temperate forest ecosystems, are characterized by a well-developed fungal mantle or sheath surrounding the plant root, and a network of hyphae (the Hartig-net) that penetrate between, but not inside, root cells. Ectomycorrhizae of conifers are formed by hundreds of species of fungi, mostly Basidiomycetes and Ascomycetes, and include most of the fungi that form large mushrooms in our forests. Individual ectomycorrhizal fungi often associate with more than one plant at a time, thus forming nutritional links between trees (e.g. Simard et al. 1997b), and most probably between trees and shrubs (e.g. Amaranthus and Perry 1989). Besides their nutritional role, ectomycorrhizal fungi benefit their hosts by protecting them from pathogens, toxins, and desiccation (Smith and Read 1997). It is, therefore, important to investigate how forest management practices affect the health, growth patterns, physiological function, and taxonomic diversity of ectomycorrhizal fungi.

To date, applications of scientific knowledge of ectomycorrhizae in forest management have been limited. Where soils have had insufficient amounts of ectomycorrhizal fungi, such as in some plantations in the tropics, mine spoils, or old agricultural fields, reforestation has been made possible or has been enhanced by planting seedlings in symbiosis with specific ectomycorrhizal fungi (Trofymow and Van den Driessche 1991). Reforestation of conifers may also be enhanced by maintaining alternative hosts of ectomycorrhizal fungi such as *Arbutus* or *Arctostaphylos* (e.g. Horton et al. 1999).

Additional descriptions and identifications of ectomycorrhizae are needed to improve our ability to monitor changes in ectomycorrhizal fungal communities, and to relate these changes to management practices or naturally occurring changes in

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the forest environment. Ectomycorrhizal descriptions also allow us to study the roles of individual ectomycorrhizal fungal species. Descriptions require detailed microscopic examination of morphology and anatomy. To determine the identity of the fungal partner, an ectomycorrhiza must be "linked" to a sporocarp (spore-producing structure) of known identity. Ectomycorrhizal fungi form sexual sporocarps in various forms (most commonly mushrooms or truffles). Linkages can be made by observing physical connections by mycelial strands, by close physical association and similarity of anatomical features, or by obtaining identical DNA fingerprints. Several publications have presented detailed descriptions of ectomycorrhizae, including high-quality colour photographs (most obtain a resolution of about 3◁ :m at a magnification of 40X (Ingleby et al. 1990; Agerer 1987-1998; Goodman et al. 1996; Agerer 1996-1998; Agerer and Rambold 1998). Many other descriptions of varying levels of detail have been published in journal articles. As the techniques for describing ectomycorrhizae have become better known, ecological studies have increasingly generated descriptions of considerable detail. However, many of these remain unpublished because they are not sufficiently detailed, or because the studies' goals are to generate ecological rather than taxonomic data. There is a need, then, for a comprehensive database to facilitate the collection and comparison of ectomycorrhizal descriptions.

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Project Background

International cooperation to develop standards for describing ectomycorrhizae began in 1993 at the 9th North American Conference on Mycorrhizae in Guelph, Ontario at a workshop organized by S.M. Berch. A poll by D.M. Durall of 200 researchers from the international community indicated a general view that 1) a concise, standard set of morphological and molecular characters be published and used to describe ectomycorrhizae, and 2) a common system for storing morphological and molecular data on ectomycorrhizae be developed. The first of these goals was fulfilled by *A Manual of Concise Descriptions of North American Ectomycorrhizae* (*CDE*) (Goodman et al. 1996-1998), which was initiated by researchers in western North America in 1995 at a workshop organized by J.A. Trofymow at Mesatchie Lake, British Columbia. The goal of an international database of ectomycorrhizal remains to be realized.

Over the past five years, researchers in several regions in Canada and North America have been studying the ecological roles and diversity of ectomycorrhizal fungi in forests (e.g. Goodman and Trofymow 1998a, b; Amaranthus and Perry 1994; Simard et al. 1997a, b; Baxter et al. 1999; Hagerman et al. 1999; Jones et al. 1997; Kranabetter et al. 1999; Kranabetter and Wylie 1998). Much of the research has occurred in British Columbia as part of Forest Resource Development Agreements (FRDA) and Forest Renewal British Columbia (FRBC) funded projects. In the course of this work, ectomycorrhizae formed by several hundred fungal species have been characterized, but few descriptions have been published. To be published in the *CDE* manual or other peer-reviewed journals, a description must include high-quality photomicrographs; a complete set of morphological, anatomical, chemical, and molecular characters; and properly prepared voucher specimens. In their present form, most descriptions from the ecological studies do not satisfy these criteria and hence will remain unpublished.

Presentations of the *CDE* manual at meetings of the International Congress of Mycorrhiza in 1996, Soil Ecology Society in 1997, Ecology of Northern Forest Soils conference in 1998, and at the British Columbia Ectomycorrhizal Research Network (BCERN) meeting in 1998, met with support for the ongoing effort. It was also agreed at the meetings that an online database was needed to share the existing, unpublished descriptions and serve as the basis for a future online publication of descriptions. In 1996, the BCERN laid the foundation for such a database by adopting the standard *CDE* checklist of morphological and molecular features, and creating a first version of an electronic form for entering database records. Further information on BCERN and the online version of the *CDE* manual is also available (*www.pfc.cfs.nrcan.gc.ca/ecosystem/ ectoweb/default.html*).

Database Concept and Development

In January 1999, work began on the preparation of the web-based database program. The existing electronic entry form was converted to an online version, with sufficient fields for a complete and detailed description of morphological, anatomical, chemical, and molecular (restriction fragment length polymorphisms (RFLPs) and DNA sequences) features of each ectomycorrhiza. We have designed an online database — *Database of Descriptions of Ectomycorrhizae* (*DDE*) — that has a search function to identify or compare ectomycorrhizae. The search function acts as a synoptic key, but with additional features that allows any number of characters to be combined, and gives the user control over the listing of descriptions that match or do not match specific characters.

We plan to allow researchers to contribute their descriptions directly. The same form that is used to enter a search profile can also be used to enter descriptions to the *DDE,* thus allowing the database to conveniently serve as a central and up-to-date collection of descriptions made worldwide. The form for online data entry includes all of the characteristics used for *CDE* descriptions, as well as fields for notes on other characteristics or other character states.

The *DDE* uses standard hypertext markup language (HTML) elements like those used for other worldwide-web searchable databases. The *DDE* "Search Results" page lists the descriptions of ectomycorrhizae, and the number of characters of each that match the search profile. Program languages and concepts used in the development of *DDE* follow upon previous work done by one of us (A.J. Trofymow) in the design of online databases for tree diseases (Thomson et al. 1998,
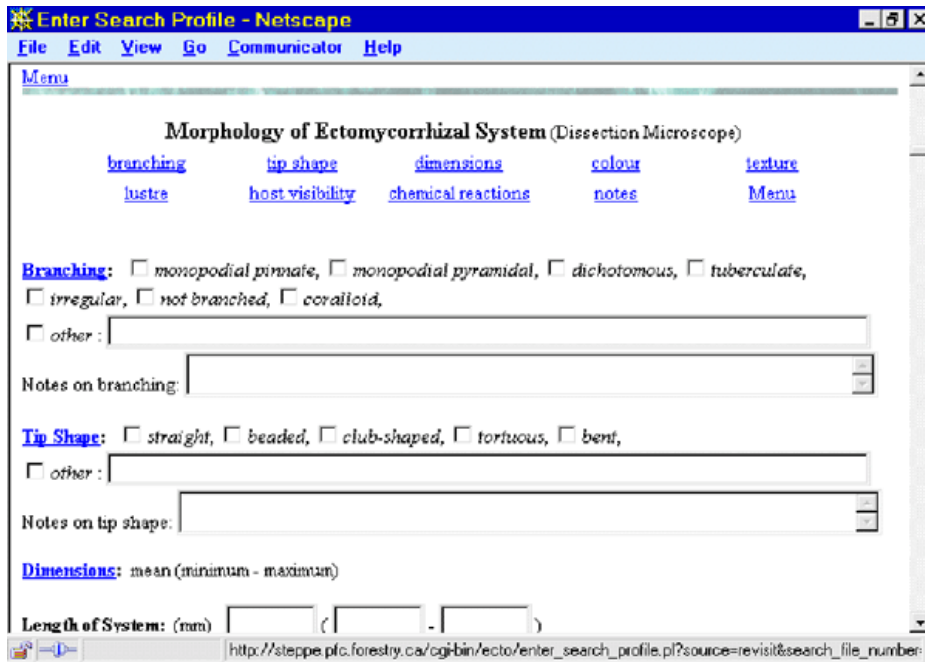
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Figure 1. Example of a section of the data entry form: Morphology of the Ectomycorrhizal System.

www.pfc.cfs.nrcan.gc.ca/health/td_web/) and for forestry researchers (*www.pfc.cfs.nrcan.gc.ca/cfrd/*). Programs written in the *Practical Extraction and Report Language* (PERL) (Wall et al. 1996) process user input, write to and create files and web pages, and perform the database searches. All pages contain only HTML. Data files, including the database, are DOS text files. Personal computers and *Netscape Communicator 4.7* were used for system development.

Database Product

At present, the *DDE* contains 338 descriptions from two sources. Twenty descriptions were taken from *CDE* (Goodman et al. 1996-1998) and 318 from *DEEMY* (Agerer and Rambold 1998). The *DEEMY* product is a database on a compact disk that is searchable and contains colour photographs. (It may be purchased from the authors.) Each *DEEMY* description is a composite description of a single fungal species on one or more host species within a single genus. *DEEMY* descriptions were converted from the *DEEMY* to *CDE* database format by a PERL program, translating fields and terminology. The program used a conversion table and a series of conditional statements to handle special cases where more than one *DEEMY* field determines the content of a single *DDE* field. Unlike *DEEMY*, the *DDE* will include descriptions that have not been

published elsewhere, and has been designed to safely utilize less detailed descriptions.

The *DDE* system allows users to search the database and download descriptions listed on the "Search Results" page. For *CDE* descriptions, links are provided to photographs; we did not receive permission to use the photographs in *DEEMY*.   In addition, researchers will be able to modify the database by adding, copying, editing, and deleting descriptions. In order to reduce the workload that would result from proofreading the expected number of descriptions, it will be necessary to standardize data quality from the user. Therefore, access to the modification options will be controlled through the use of a username and password, which will be made available to those, who in the judgement of the *CDE* editorial board, are able to accurately describe the most important characteristics of ectomycorrhizae. Where possible, we intend to include images for descriptions submitted directly to *DDE*.

The data entry form used to enter search profiles and to enter or edit descriptions contains 1921 features. Sections of the form allow entry of information about collection, identification, and ecology; morphology of the ectomycorrhizal system (Figure 1); morphology of mycelial strands and emanating hyphae; anatomy of the mantle, including up to four mantle layers; anatomy of mycelial
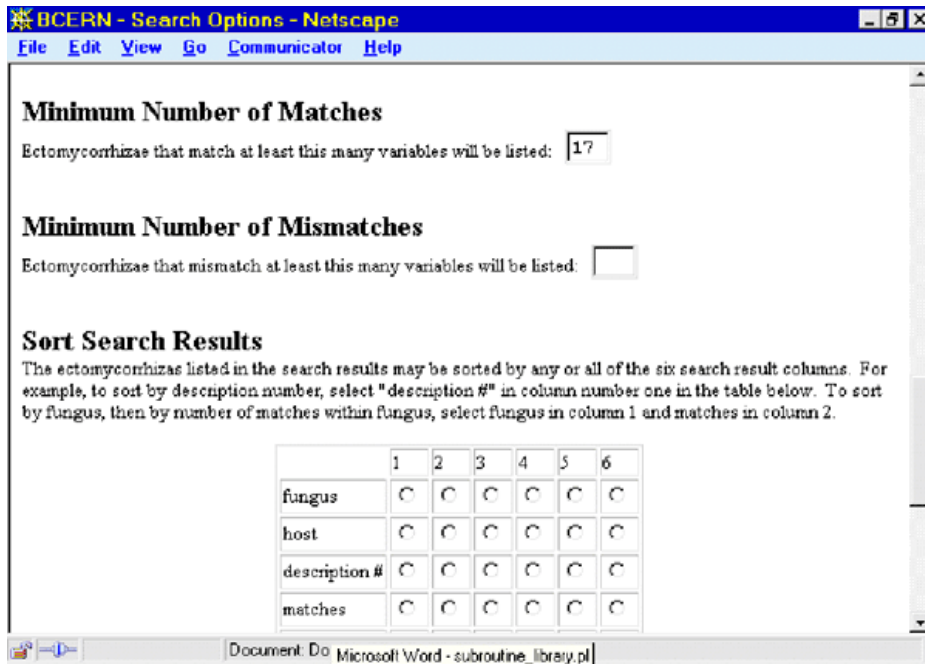
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Figure 2. Specifying the search options.

strands, emanating hyphae, cystidia, sclerotia and microsclerotia; autofluorescence of whole ectomycorrhizae; DNA RFLPs; and other distinguishing features. The number of data entries in descriptions ranges from 39 to 253 per description. Most contain 100–150 data entries. Many of the fungal characters in the form have a list of checkboxes indicating the possible character states; for example, there are six patterns of arrangement of cells or hyphae in a mantle layer. Any number of checkboxes may be checked, (note fields are available to give information on the relative frequencies of character states) or on intermediate or unlisted states. Links to the illustrated glossary of the *CDE* manual describe many of the character states. Standards for a minimum set of characters required for inclusion of a description in the *DDE* have not yet been established. At present only the search function is installed on the web site.

Once a search profile or description has been entered in the form, submission of the form creates a textual description of the data entered, and options are given to continue or to revise the data. Each search criterion is compared with each description in the database or in the most recent search results list, resulting in a match, mismatch or null (no data). Text fields match if the search value is a subset of the description value. Multiple text search values may be given for a single field (separated by a "/"), generating a match if any is a subset of the description value. Numeric search values may be a single number or an interval specified using a ">", "<", or "–". When a single number is entered, a match occurs according to preset tolerances, either in absolute or relative terms. For example, restriction fragment lengths match if their difference is less than 6 base pairs. A checkbox is mismatched if not checked and one of the other boxes in the same group of character states is checked. Similarly, a checkbox comparison is declared a null if none of the checkboxes in the group are checked. It is possible to search the database using the number of data in descriptions, the name of the person who submitted the description, and whether or not the description is published elsewhere. This gives the user some control over the amount of detail or precision of the descriptions in the search results list.

By default, the most recent search results list is searched, and only those descriptions that match the entire search criteria are listed, sorted by fungus, host, and description number. A "Search Options" page allows the user to override these defaults as follows: descriptions may be listed if they match, mismatch, or have no data for specified search criteria, or if they match or mismatch a minimum number of criteria. The search results
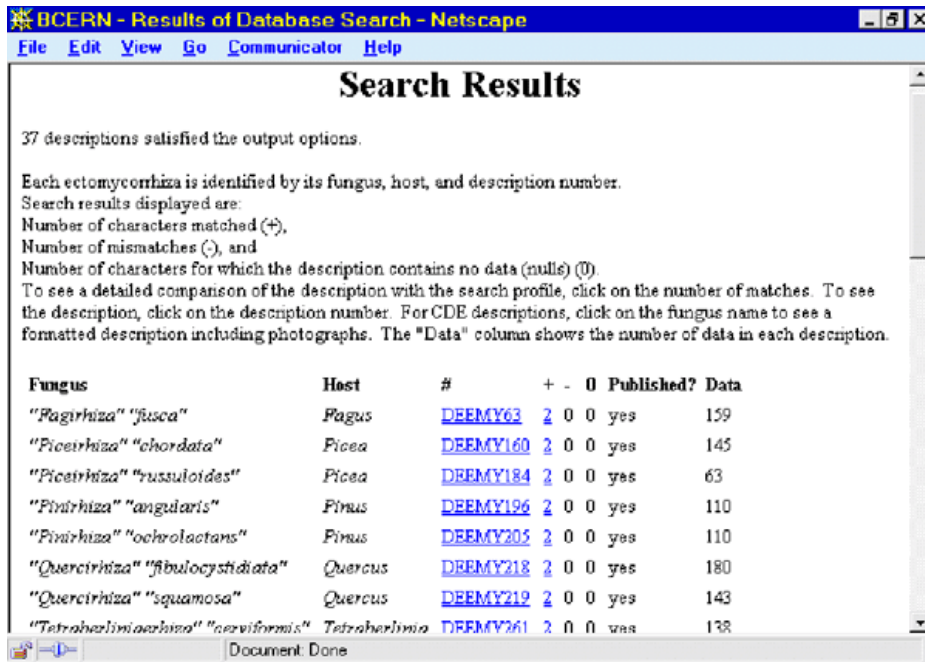
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File   Edit   View   Go   Communicator   Help

Search Results

37 descriptions satisfied the output options.

Each ectomycorrhiza is identified by its fungus, host, and description number.
Search results displayed are:
Number of characters matched (+),
Number of mismatches (-), and
Number of characters for which the description contains no data (nulls) (0).
To see a detailed comparison of the description with the search profile, click on the number of matches. To see the description, click on the description number. For CDE descriptions, click on the fungus name to see a formatted description including photographs. The "Data" column shows the number of data in each description.

Fungus	Host	#	+	-	0	Published?	Data
"Fagirhiza" "fusca"	Fagus	DEEMY63	2	0	0	yes	159
"Piceirhiza" "chordata"	Picea	DEEMY160	2	0	0	yes	145
"Piceirhiza" "russuloides"	Picea	DEEMY184	2	0	0	yes	63
"Pinirhiza" "angularis"	Pinus	DEEMY196	2	0	0	yes	110
"Pinirhiza" "ochrolactans"	Pinus	DEEMY205	2	0	0	yes	110
"Quercirhiza" "fibulocystidiata"	Quercus	DEEMY218	2	0	0	yes	180
"Quercirhiza" "squamosa"	Quercus	DEEMY219	2	0	0	yes	143
"Tetraheliniaerhiza" "cerviformis"	Tetraheliniaa	DEEMY261	2	0	0	yes	138

Document: Done

Figure 3. Search results page, showing description numbers as links.

list may be sorted by any combination of fungus, host, description number, number of matches, number of mismatches, or number of nulls (Figure 2). For a new search, the user may choose to search the entire database instead of the most recent results list. On the "Search Results" page (Figure 3) description numbers are linked to descriptions, and fungus names are linked, if possible, to descriptions on other web sites, such as the *CDE* online manual[1].   A detailed comparison of search criteria with description values is available by clicking on the number of matches, showing how a description matched, mismatched, or had no data for each of the search criteria.

Future Work

At present, only the search function for *DDE* is installed on the web site (*www.pfc.cfs.nrcan.gc.ca/biodiversity/ecto/main_menu.htm*). Installation of the tools to add, edit, copy, and delete descriptions is planned for mid 2000. To avoid excessive impact on the other applications, a limit has been placed on search run-time. The product of the number of search criteria and number of descriptions to be searched is limited to 1200. Thus, an initial search of the entire database of 340 descriptions is limited to three or fewer search criteria, and if the list of ectomycorrhizae of interest is reduced to 100, then 12 search criteria may be used. To improve search speed, we plan to convert the system to use a commercial database query language and compiled programs. This will allow us to accept numerous contributions from researchers worldwide.

We estimate that the size of the description database may eventually grow to greater than 5000 descriptions. This implies a total database size of 750 thousand to 1.25 million records. The most robust and flexible design for a database of this size would be based on commercial relational

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[1] In the example shown in Figure 3 the names shown are for mycorrhizae described by Agerer as published in *DEEMY* (Agerer and Rambold 1998) and are not true binomial names of species, but "pseudobinomials" of unidentified ectomycorrhizae, indicating the genus of the host and a characteristic of the mycorrhiza. These names are similar in concept to binomials for lichens or fungal anamorphs. If the mycobiont of an unidentified ectomycorrhizae is identified, the pseudobinomial will no longer be used, except to refer to publications that have previously used it. The fungal species name will be used when known.

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database management system (RDBMS). In light of the wide use of the Oracle RDBMS, it is proposed to re-engineer the prototype in Oracle, while retaining for the most part, the existing look-and-feel of the web interface. This approach would guarantee a database platform that can be scaled-up to meet future growth, and integrate with other data holdings (e.g. the mycorrhizal DNA sequence database being developed by Egger at UNBC).

Researchers from BCERN, and elsewhere (L. Jonsson, Swedish University of Agricultural Sciences; T. Bruns, University of California Berkley; R. Molina, Oregon State University), have supported the *DDE* initiative and many have offered to contribute descriptions. In collaboration with John Dighton of Rutgers University, a proposal has been made to the National Science Foundation (NSF) to prepare descriptions of ectomycorrhizae on pine in New Jersey for the *DDE*. As it expands, it is hoped that *DDE* will serve the growing need for identification of ectomycorrhizae in forest research.

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