Preface to the online PDF version

This online PDF version of a *Manual of Concise Descriptions of North American Ectomycorrhizae* (CDNAE) contains all 39 descriptions of ectomycorrrhizae, 20 of which were previously only available in the print version of the manual. This manual was intended to provide a primarily North American audience of ecologically-oriented researchers with illustrated descriptions, including DNA characterization, of ectomycorrhizae (EM) identified to plant and fungus species. Ectomycorrhizae of fungal species that have been described elsewhere are included, though preference and emphasis was on previously undescribed ectomycorrhizae.

The print manual was published between 1996 to 2008 as a series of 8 folios which were sent to those who initially or subsequently purchased the manual through Mycologue Publications. It's principal, Bryce Kendrick, indicated he would allow for this open access version of the manual to be prepared and circulated. A limited number of print versions of the manual are available.

In this PDF version, corrections have been made to CDE 1 as noted in the Fourth Folio errata and to CDEs, 6, 8, and 10 as noted in the Sixth Folio errata. The front matter in this version is a combination of the Table of Contents, Introduction, revised Sections 2, 3, and 10, Appendices, and Literature Cited from the Sixth Folio and sections 4, 5, 6, 7, and 8 from the Fourth Folio. Prefaces from each of the folios are also included in the front matter. Sections 6 and 7 are no longer relevant as no further submissions are being accepted for publication in the CDNAE manual.

Data for the 39 ectomycorhizae described in CDNAE are included in the Ectomycorrhizae Descriptions Database (EDD) which also includes data for 150 ectomycorrhizae described in Photoprofiles of Ectomycorrhizae (PoE) and descriptive data only for ~150 ectomycorrhizae described in Agerer's Colour Atlas of Ectomycorrhizae. Up until 2023 the British Columbia Ectomycorrhizal Research Network (BCERN) website provided access to the interlinked set of database applications and was used by regional, national, and international researchers and biologists for the identification of ectomycorrhizal fungal species. The databases represent collaborative inputs from researchers within the Canadian Forest Service (CFS), external agencies, academic institutions, and mycological societies. External granting agencies funded much of the work to conduct the research, develop the databases and website with the CFS providing a portal (https://cfs.nrcan.gc.ca/projects/111) to the BCERN website to make them publicly available.

In recent years maintenance of the applications and server had been limited and as a result functionality has been lost and access for external users to the BCERN website was cut in 2023. A new proposal was submitted in 2024 to the CFS to update and migrate the BCERN applications to a server hosting the CFS Pacific Forestry Centre Fungal Herbarium (DAVFP) online database (https://cfs.nrcan.gc.ca/herbarium/), with the new BCERN website planned to be operational early in 2025.

The editors

Table of contents

Contributors

Quentin F. Baldwin
Natural Resources & Environmental Studies,
University of Northern BC,
3333 University Way, Prince George, B.C. V2N 4Z9.
Phone: 250-960-5730. FAX: 250-960-5538.
Email: baldwinq@unbc.edu

Shannon M. Berch
B.C. Ministry of Forests,
Glyn Road Research Station,
PO Box 9536 Stn Prov Govt, Victoria, BC V8W 9C4.
Phone: 250-952-4122. FAX: 250-952-4119.
Email: Shannon.Berch@gems7.gov.bc.ca

Mary Berbee
University of British Columbia
Department of Botany
6270 University Blvd, Vancouver BC V6T 1Z4
Phone: 604 822-3780
Email: berbee@interchange.ubc.ca

Daniel M. Durall
University British Columbia, Okanagan Campus,
3333 College Way, Kelowna, B.C. V1V 1V4.
Phone: 250-762-5445 ex7549. FAX: 250-470-6005.
Email: dmdurall@okuc02.okanagan.bc.ca

Joyce Eberhart
Department of Forest Science, Oregon State University,
Corvallis, OR 97331, USA.
Phone: 541-750-7490. FAX: 541-737-1393.
Email: eberharj@fsl.orst.edu

Keith N. Egger
Natural Resources & Environmental Studies,
University of Northern BC,
3333 University Way, Prince George, B.C. V2N 4Z9.
Phone: 250-960-5860. FAX: 250-960-5538.
Email: egger@unbc.ca

S.M.K. (Michelle) Gillespie (formerly S.M.K. Harniman)
Okanagan University College, North Campus,
3333 College Way, Kelowna, B.C. V1V 1V4.
Phone: 250-762-5445 ex7585. FAX: 250-470-6005.
Email: mharnima@okcins.okanagan.bc.ca

Doug M. Goodman
Pacific Forestry Centre, Canadian Forest Service,
506 Burnside Road West, Victoria, B.C., Canada, V8Z 1M5.
Phone: 250-363-0770. FAX: 250-363-0797.
Email: dgoodman@pfc.forestry.ca

Gavin Kernaghan
Forest Genetics Laboratory,
Department of Renewable Resources,
718 General Services Bldg., University of Alberta,
Edmonton, AB T6G 2H1.
Phone: 403-492-9093 (office) or 492-4415 (lab).
FAX: 403-492-4323.
Email: gavin.kernaghan@ualberta.ca

Dan L. Luoma
Department of Forest Science, Oregon State University,
Corvallis, OR 97331, USA.
Phone: 541-750-7490. FAX: 541-737-1393.
Email: luomad@fsl.orst.edu

Hugues B. Massicotte
College of Science and Management
Natural Resources & Environmental Studies,
University of Northern BC,
3333 University Way, Prince George, B.C. V2N 4Z9.
Phone: 250-960-5813. FAX: 250-960-5538.
Email: hugues@unbc.ca

Thom E. O'Dell
USDA Forest Service, Forest Sciences Laboratory,
3200 Jefferson Way, Corvallis OR 97331 USA.
Phone: 541-750-7404. FAX: 541-737-1393.
Email: odellt@fsl.orst.edu

J.A. (Tony) Trofymow
Pacific Forestry Centre, Canadian Forest Service,
506 Burnside Rd. W., Victoria, B.C. V8Z 1M5.
Phone: 250-363-0677. Fax: 250-363-0797.
Email: ttrofymow@pfc.forestry.ca

1. Introduction

by D.M. Durall and S.M. Berch

The two sources of inspiration for this manual are the Colour atlas of ectomycorrhizae by Reinhard Agerer (1987-1995) and Identification of ectomycorrhizas by K. Ingleby, P.A. Mason, F.T. Last and L.V. Fleming (1990). To researchers trying to identify ectomycorrhizae, the most useful part of both is the illustrations, especially the colour plates that capture what a thousand words could not. But the usefulness of both publications to non-Europeans trying to identify ectomycorrhizae is limited. Identification of ectomycorrhizas by Ingleby et al. combines illustrations with descriptions thereby facilitating comparison to the researcher's ectomycorrhizae in the lab. However, only 24 ectomycorrhizae are described and there is no plan to add to this. Agerer's Colour atlas of ectomycorrhizae (1987-1995) continues to grow as new ectomycorrhizae are described, but currently the detailed taxonomic descriptions are scattered through the scientific literature and not included in the atlas. Although Agerer invites contributions by other researchers and from other regions, only one is yet included. Neither Agerer's (1987- 1995) atlas nor Ingleby et al.'s (1990) book includes characterizations of DNA. Both publications are primarily European in focus. Finally, Agerer's (1987-1995) atlas is expensive for the individual researcher. Thus we perceived the need for a North American manual of concise descriptions of ectomycorrhizae that would incorporate written descriptions with illustrations, continue to grow through contributions from various researchers, and be in a format that is affordable and easy to use in the lab. To accompany the manual we envisioned an electronic database of descriptions linked to an electronic synoptic key. This combination would be invaluable to taxonomists as well as those doing practical studies that require one to distinguish or identify ectomycorrhizae.

This manual is intended to provide a primarily North American audience of ecologically-oriented researchers with illustrated descriptions, including DNA characterization, of ectomycorrhizae identified to p lant a nd f ungus s pecies. E ctomycorrhizae involving unidentified fungi are included and can be referred to using our sequential numbering system, though we have to acknowledge that unidentified ectomycorrhizae could represent a single species of fungus or a group of similar species. Although most of the ectomycorrhizae in the first edition are unidentified, we hope that the mycobionts of these will be determined and specified in future editions. Ectomycorrhizae that have been described elsewhere are included, though we prefer to emphasize previously undescribed ectomycorrhizae. The manual will be updated as warranted and new information on published ectomycorrhizae, such as identity of the fungus, will be included.

The instructions for submission provide a straight-forward guide for contributions by anyone currently identifying ectomycorrhizae while the review of submissions by the editorial committee ensures high quality.

This manual provides colour and black and white illustrations in the style of Agerer's (1987-1995) atlas and Ingleby et al.'s (1990) book, linked to concise descriptions of distinctive characteristics, plus RFLP patterns following a standard procedure and format. Authors are invited to provide the molecular information as outlined in section 3C, but if they cannot, this service will be provided at cost. The DNA characterization serves not only to further describe ectomycorrhizae for the purpose of identification, but also to link an ectomycorrhiza with a sporocarp and thus conclusively identify the fungus.

2. How to store, clean, and photograph ectomycorrhizae and prepare voucher material

by T.E. O'Dell, H.B. Massicotte, and G. Kernaghan

Storage

For minimal disturbance and best preservation, leave ectomycorrhizal roots in the original soil sample until time allows for further processing. Soil samples containing mycorrhizae should be stored either at 2-4 °C in plastic bags (it may be necessary to add water to prevent drying) or freeze the soil samples and thaw them only once to preserve the DNA. If storing at 2-4 °C mycorrhizae are best extracted, photographed and described as soon after collection as possible. Individual tips may be frozen in distilled water for future reference with good preservation of morphology and anatomy, but possible loss of colour and reaction to chemical reagents (see *Preparation of voucher material*, p. 2.3, for details on freezing material for DNA extraction).

Cleaning

In order to observe their features, mycorrhizae must be extracted from soil and cleaned of adhering debris. These procedures are explained in detail by Agerer (1991). To minimize breakage and loss of emanating elements (e.g. cystidia, extramatrical hyphae, mycelial strands), roots must be cleaned gently under a dissection microscope. Morphological variation between mycorrhizae due to age and other factors is most easily observed if systems of ectomycorrhizal tips remain unbroken. To remove tightly adhering materials, the root system should be placed under water and cleaned with forceps, a paint brush and fine nozzled squirt bottle. More vigorous cleaning techniques often damage ectomycorrhizae, but are acceptable in some cases. The majority of loosely adhering soil or organic material can be removed by gently washing the sample under running water in a soil sieve or an elutriator (Furlan and Fortin 1975). Agitation in a 0.1M sodium pyrophosphate (or soap, Tween, or Calgon) solution may also help to disperse clay particles, but is not recommended as it may alter the ectomycorrhizae. Sonication is not advised as mantles may be damaged. Note should be made if certain ectomycorrhizal types tend to have tightly adhering soil materials as that can be a useful taxonomic character. In most cases, a few tips can be found with areas of mantle not coated with mineral soil particles. Use these areas to describe the mantle structure. Mycelial strands and emanating hyphae will be lost from some ectomycorrhizae if they are washed, in which cases it is necessary to dissect the soil carefully at 25x after it has been soaked. Cleaned mycorrhizae should be refrigerated in distilled water at 2°C and examined within 24 hours, as contamination or outgrowth of hyphae may occur rapidly.

Photography

Due to recent advances, digital photography is the recommended method for photographing mycorrhizal tips under both the dissecting scope and the compound microscope. If film is used, it is important that film and light source are matched to obtain natural colour. Tungsten film or the appropriate setting on a digital camera must be used for unfiltered tungsten quality light, such as that from fiber optic lighting. Tungsten quality light must pass through a blue filter if daylight film or setting is used. Anytime a tungsten quality light source is used, with or without a filter, it should be at maximum power to keep the light at its specified spectrum. Prints for preparation of plates for publication may be better quality if made directly from print film rather than via slides (transparencies),

depending on the quality of processing and films. Digital photos should be saved at least at a 600dpi setting and with minimal compression in .TIF format.

i) Dissection microscope. Whole ectomycorrhizae can be photographed with a dissecting digital photomicroscope. Microscopes designed for use with photographic systems produce the best photographs. It is important to have a digital camera/computer system that has a capacity to obtain a large depth of field to maintain focus throughout the mycorrhizal tip. It is also important to have a uniformly coloured background. A new petri plate or other container void of scratches should be used..Viewing systems under water usually produce the best results. Considerable experience may be required before high quality photographs are obtained. The use of small apertures is important for sufficient depth of field. This may necessitate long exposure times, leading to problems if the microscope is subject to vibration. Elevate the subject above the background so that only the subject is in focus. Specimens should be photographed under several centimeters of water. This prevents glare from wet specimens or the water surface, and allows emanating hyphae and mycelial strands to spread out and approximate their natural position. To hold the subject under water, use reverse forceps and a "third hand" soldering accessory. Dust and soil particles can be avoided by cleaning the subject thoroughly, using distilled water, and reducing air-currents in the laboratory. Dust or soil particles in or on the water will not appear in the photo if they are moving and exposure times are several seconds or more. To reduce the formation of bubbles, store water for 24 hours, and keep the water and subject at room temperature.

Experiment with positioning the light sources to obtain the best definition of the subject with the least glare. Fiber-optic light sources may be used. A black background allows optimal illumination and colour of the subject, except for dark subjects, which may need colourless grey backgrounds. Photographs at a variety of magnifications are needed to thoroughly document the ectomycorrhiza. Overall features of a system of ectomycorrhizal root tips are seen at 4x-7x. Details such as texture, hyphae (e.g. laticifers), cells, cystidia, and mycelial strands require at least 25x magnification, and 40x is often useful. Keep reference photos of a micrometer or ruler at several magnifications to calculate the scale of photographs.

ii) Compound microscope. Photographs taken through a compound microscope do not normally require colour. Photograph mantle layers using an oil immersion objective (100x), or a high-dry (40x) objective if the scale of the mantle pattern is too large. Distinctive features such as cystidia, clamp connections, and hyphal ornamentation should be photographed at 1000x. Lower magnification may be needed to photograph longer cystidia. Nomarski differential interference contrast microscopy is preferred to illustrate features such as internal structure of the mantle, mycelial strands and rhizomorphs, thin walled hyphae on the surface of the mantle, cystidia, ornamentation and crystals.

Use of a green filter can improve the contrast of black and white photos of blue-stained material.

Preparation of voucher material

Reference materials are voucher materials that accompany and support each description of an ectomycorrhiza, and will be retained in the Canadian Forest Service herbarium at the Pacific Forestry Centre in Victoria, B.C., Herbarium DAVFP, curator Dr. Brenda Callan. Reference materials will be held for future examination of morphology, anatomy, or chemical analysis (e.g. DNA analysis). Specimens should be typical and labeled with binomials and authorities of both the fungus and host.

i) Microscope slides. Slides of mantle peels or glancing sections of the mantle should be prepared. Squash mounts of whole ectomycorrhizae are not acceptable as voucher specimens. On each slide, mount some pieces of the mantle with the outer surface up and some with the inner surface up. Slides should be prepared so that any emanating elements are visible. Specimens are to be mounted in lactoglycerol (50% lactic acid + 50% glycerol). Seal the cover slip edges with Entellan or another permanent mounting medium. Avoid the use of nail polish as a sealant, as it may react with lactic acid. Material may be stained if necessary.

ii) Freeze-dried ectomycorrhizae. Samples should be lyophilized for future DNA analysis as follows: i) put fresh tips (with any emanating elements still attached) in vials without water, ii) if desired, the vials may be stored in liquid N2 or in a -20°C freezer, iii) freeze the tips directly in liquid N2, iv) lyophilize in a precooled lyophilizer. It is important to keep the tips frozen during the process. Store sealed samples in a frost-free freezer or desiccator. Samples should not undergo multiple freeze thaw cycles. See section 3C for details on storage of DNA.

iii) Air-dried sporocarps and spore-prints. Before drying, place the sporocarp pileus on a black and white paper to obtain a spore print. Dry specimens until brittle in a dryer with good ventilation and moderate heat. Avoid excessive heat, which can destroy both appearance and microscopic structure. Large fleshy specimens may be sliced to reduce drying time, but take care to preserve features that might have taxonomic use. For further details contact the DAVFP herbarium at the Pacific Forestry Centre, curator Dr. Brenda Callan, phone 250-363-0744.

3. Describing ectomycorrhizae

A. Describing morphology and anatomy

by D.M. Goodman, D.M. Durall, and J.A. Trofymow

This section discusses some aspects of describing ectomycorrhizae with use of the "**Checklist of Ectomycorrhizal Characters**" (section 4). The checklist is designed to be enlarged with a photocopier to fit on 8.5" by 11" sheets prior to use. For additional details see Agerer (1991) and Ingleby *et al*. (1990).

On the checklist, choices of character states are in italics. Refer to the glossary (section 5) for definitions of the character states. Indicate the applicable state or states by circling or highlighting one or more of the choices. When two choices are circled, it may be valuable to indicate under "Notes" whether the character is intermediate between the circled states, or takes on both states. There are characters that are optional or not listed in the checklist that may be very useful taxonomically, but require more time and effort to determine than those listed. Although not as reliable as some anatomical characters, morphological characters are taxonomically important.

Ensure that fresh material of mature but not senescent ectomycorrhizae is used. Note how younger and senescent ectomycorrhizae differ from mature, healthy ectomycorrhizae.

Collection information

Describe the **Location** by a nearby geographical feature. Specify **Latitude** and **Longitude** to within 30 seconds.

Ecology

Specify the biogeoclimatic zone or **Ecological Zone** according to an authority for the region of the collection. In British Columbia, give the biogeoclimatic zone, subzone and variant. Elsewhere, name (with reference to an authority) or describe the community of vegetation.

Soil Horizons within the forest floor (= organic horizon) have the following synonyms: L is the litter layer (= O_L or O_1), F is fragmented or fermentation litter (= O_F or O_2 or O_e), and H is the humus layer (= O_H or O_3 or O_a).

"**Months of Survey**" and "**Months Ectomycorrhiza Observed**" together indicate if any seasonal pattern of occurrence was observed. Recording the months that a survey (if any) was made is necessary to tell whether the ectomycorrhiza was not observed in certain months because of its rarity during those months, or rather because no survey was conducted during those months.

Identification Procedure

Zak (1971, 1973), Ingleby *et al*. (1990), and Chilvers (1968) have discussed the methods for identification of ectomycorrhizae, which are preferably used in combination to reach a positive identification:

i) Compare the unknown ectomycorrhiza with ectomycorrhizae synthesized using cultures of fungi of known identity. Be aware that even with the same isolate, natural and synthesized ectomycorrhizae may differ in morphology, due to the presence of sugars in the culture medium and difference in age of the ectomycorrhizae (Godbout and Fortin 1985).

ii) Compare cultures of fungi isolated from the unknown ectomycorrhiza with cultures of known fungi. Chu-Chou and Grace (1981a, 1981b, 1983a, 1983b) used this method successfully to identify numerous ectomycorrhizae in New Zealand conifer

plantations. It is best to use isolates from sporocarps found in the vicinity of the ectomycorrhiza rather than isolates in culture collections or herbaria. Hutchison's (1991) key separated 95 species of ectomycorrhizal fungi in pure culture on the basis of their ability to degrade carbon and nitrogen compounds, tolerance to temperature extremes and temperature preferences, polyphenol oxidase activity, staining with diazonium-blue-B and colony morphology. Besides the difficulty in culturing ectomycorrhizal fungi, there are other problems with this method. Cultures can spontaneously change from dikaryons to monokaryons after a few transfers, in which case clamp connections are lost and other cultural characters may change (Hutchison 1991); and, different fungi can look similar in culture (Agerer 1986).

iii) Compare the mycelium and mycelial strands attached to the unknown ectomycorrhiza with those attached to the base of nearby sporocarps of known identity. This was Zak's (1973) favourite method, because he found it accurate and readily applied. However, Agerer (1986) considers it insufficient in forests with many ectomycorrhizal fungi.

iv) From the unknown ectomycorrhiza, trace hyphae or strands that form a continuous connection to a sporocarp of known identity. This method may be tedious, inaccurate if not carefully done, and may be impractical if ectomycorrhizae and sporocarps are far apart (Chilvers 1968, Agerer 1986), but has been used extensively by Agerer (1986). If done "critically and patiently", Agerer (1986) considers this a good method, especially if there are mycelial strands.

v) Compare the unknown ectomycorrhiza with published descriptions of identified ectomycorrhizae, possibly with the assistance of keys. This is becoming more practical as additional detailed descriptions are published. In addition to this manual, the most useful references are Agerer (1996-1997), Agerer (1987-1996) and Ingleby *et al*. (1990).

vi) Compare RFLP data or DNA sequences from the unknown ectomycorrhiza with RFLPs or sequences from identified fungi, most often using sporocarps collected from the same location as the unknown ectomycorrhiza. Libraries of DNA information have been gathered that may allow identification to genus of some ectomycorrhizae, e.g. Gardes *et al*. (1991).

vii) Consistently finding the unknown ectomycorrhiza close to sporocarps of a known identity provides some evidence that the species fruiting might also be forming the mycorrhiza. Consistent association strengthens an identification by one or more of the above methods.

Morphology of Ectomycorrhizal System (Dissection Microscope)

Examine tips in water. **Dimensions** of ectomycorrhizae, i.e. the length of the system and the length and width of tips, are affected by physical properties of the substrate in which the ectomycorrhizae have grown, and by the host species. Given a similar substrate and host, however, some fungi have been found to form ectomycorrhizae of different dimensions than others. These three characters are most important when unusual or distinctive, in which cases it may be worthwhile to take more time or care in their measurement. Always determine the mean and range. For example, the length of systems may be 12 (1-20) mm, indicating a mean length of 12 mm, a minimum of 1 mm, and a maximum of 20 mm. If necessary give some information on the nature of the distribution of sizes; eg. "occasional systems with large tips, 800 µm", or, "all systems with variable sized tips, 400 µm - 800 µm". To measure with the dissection microscope, a grid in an eyepiece can be calibrated at several magnifications by examining a stage micrometer and a ruler.

To classify the **Texture** of ectomycorrhizae, at least 25x magnification, and sometimes 40x, is needed. Although "reticulate" is included by Agerer (1987-1995)

as a texture, he notes that this is an appearance taken on by smooth ectomycorrhizae that have laticifers. We have omitted "reticulate" because it is an apparent texture only. Finely grainy textures intergrade with smooth matte textures. Texture may appear different depending on magnification and age of ectomycorrhizae, in which case notes are recommended.

Note if the host is partially visible through the mantle.

To describe **colour**, use combinations of the following basic set of colours: Black (BK), Blue (BL), Brown (BR), Green (GR), Grey (GY), Orange (OR), Pink (PK), Purple (PU), Red (RD), White (WH), and Yellow (YE), modified if necessary with dark (DK) or light (LT) to indicate intensity of colour. Avoid more ambiguous terms such as cream, tan, teal, lime, and avocado. Thus LT-BR-YE refers to a yellow colour that is brownish and light in intensity (pale). For observations of colour, use the appropriate blue filter to obtain daylight quality illumination.

Chemical Reactions. Reactions of ectomycorrhizae or parts of ectomycorrhizae to reagents can be recorded in six different places in the checklist. Only the tests with KOH and Melzer's are required. Test whole ectomycorrhizae, mantle scrapings, specialized cells in the mantle, strands, emanating hyphae, and cystidia. The tests are performed by placing a few drops of each reagent on an ectomycorrhiza for 2-5 minutes, then rinsing. It may also be useful to mount specimens directly in KOH or Melzer's and observe immediately without rinsing.

Anatomy of Mantle in Plan View (Compound Microscope)

Microscopy Technique. To observe mantles, scrape or peel the mantle off the host and mount in water with and without staining, and in lactic acid. Mantles that are both very thin and prosenchymatous may need to be examined in glancing sections; i.e. a section that crosses the mantle but is at a very narrow angle to the longitudinal axis of the root. To see natural colours and features that dissolve in acid, observe the mantle in water without staining. To more clearly see delicate hyphae, use a stain such as toluidine blue or cotton blue, or use Nomarski differential interference contrast microscopy. Use lactic acid to give a clearer image than water. Observe at 400x and 1000x. Although most observations are made at 1000x, some patterns of cellular or hyphal arrangement are best observed at 400x. Specialized cells may be more clearly seen after application of stains or reagents. The content of laticiferous hyphae varies from tip to tip of an ectomycorrhiza. Young laticiferous hyphae have contents that appear granular, oily, or otherwise not clear.

Describing mantle layers. By focusing up and down, one can gain a three dimensional mental image of the mantle structure. A clearer image of subsurface layers of the mantle may be obtained using Nomarski microscopy. The arrangement and shape of cells or hyphae in the innermost and outermost layer of the mantle (mantle **Type**) according to the classes of Ingleby *et al*. (1990) are required characters. Fully describe any distinct layers between the inner and outer layers of the mantle, using the same characters as required for the inner and outer layers. If microscopy shows that there are no distinct "middle" layers, then record this fact on the checklist.

Prosenchymatous mantles are also known as plectenchymatous, and synenchymatous is synonymous with pseudoparenchymatous. Additional information may be provided by specifying a correspondence to one of the mantle types of Agerer (1991), which are designated by the letters A through Q. The anatomy of some mantle layers may not closely fit any of the pictures of either Ingleby *et al*. (1990) or Agerer (1991). To choose the closest fit in these cases, it may be necessary to consider the definition of the mantle types. For example, many ectomycorrhizae have an inner mantle with groups of parallel hyphae uncommonly branched, but with little to no inter-hyphal spaces. According to

the glossary, this pattern fits best in the category "net synenchyma", although atypical. The key features in this example are elongation of the cells, and paucity of inter-hyphal spaces. In such cases, describe how the anatomy differs from the typical pattern.

Many ectomycorrhizae have mantles varying in type, or mantles that are covered in places with superficial layers of a different type. More than one choice may be circled on the checklist, and notes may be made of superficial layers or variation.

Cell Lengths and Widths. Measurements of cell lengths and widths in the mantle are problematic in that it is not always clear what distances to measure. If such problems could lead to serious discrepancies, make a note of what distances were measured. For triangular cells, the length is defined as the longest side, and the width as smallest height. For irregularly shaped cells, the length is the longest dimension, and the width the average or most common size at right angle to the length. Although somewhat imprecise, these measurements quantify visually noticeable differences in size and shape. Notes may be used to give information on distinctive cell shapes that are not adequately described by the dimensions and the mantle type. If irregularly shaped cells are encountered for which the above definitions of length and width are not appropriate, make note of the alternative used.

Hyphae. Whether in the mantle, in mycelial strands, or emanating from the mantle, a similar set of characters is used to describe hyphae. If, at first, septa are not visible due to ornamentation, keep looking. Usually a few smooth or sparsely ornamented hyphae can be found. Note if septa are not visible due to hyphal ornamentation. Note also if ornamentation is an integral part of the cell wall.

Anatomy of Mycelial Strands in Plan View (Compound Microscope)

If strands are differentiated with a central core, or highly differentiated, they may be called rhizomorphs. Observe squashed strands in water or lactic acid, and in 15% KOH or 50% H_2SO_4, which will loosen tissues and make individual hyphae easier to see. Cross-sections may occasionally be useful.

Cystidia (Compound Microscope)

Use the term setae for thick-walled cystidia, usually darkly pigmented. The character "**Frequency of Clamped Septa**" refers to the proportion of cystidia with one or more septa clamped.

Chlamydospores

For an extensive list and explanation of spore shapes and surface textures, see Hawksworth *et al*. (1995).

Autofluorescence of Whole Tips

Autofluorescence is especially useful for *Cortinarius* (Ammirati 1972, Agerer 1987-1995).

DNA Description

See sections 3B and 3C. If you are submitting a description to this manual, then you may either characterize DNA yourself or provide frozen material and have the characterization done for you at a price designed to cover costs (see section 6, Procedures for submitting descriptions).

Additional Characters

Features of ectomycorrhizae in longitudinal section are optional. Several methods may give suitable sections for examination of the Hartig net, intracellular elements, and measurement of the thickness of the mantle. Without embedding, tips can be sliced using a hand-held syringe needle as a scalpel. Thinner hand-held sections can be made rapidly with a razor blade by shaving a semi-frozen drop of water containing an ectomycorrhiza or mycelial strand. The drop of water can be maintained at an appropriate hardness on a cryotome. Tips may also be embedded in other materials and sectioned with a microtome.

Enzymes, NMR data, and ecological information such as alternative hosts may also be described in this section.

Distinguishing Features

Describe those features that allow the ectomycorrhiza to be distinguished from others, focusing on easily determined and less variable characters. It may be useful to indicate how this ectomycorrhiza differs from others similar in appearance. Some characters are more consistent and reliable for taxonomy, and can be ranked accordingly. Ranking is important i) when only a quick description can be made, ii) in construction of efficient keys, and iii) in judging what differences and similarities are most significant when comparing an ectomycorrhiza with a published description. Agerer (1987-1995) puts more emphasis on colour and texture than Ingleby *et al*. (1990). Morphology may be specific to ecological, environmental, or geographical conditions. When choosing distinguishing features, use both morphological features that are readily ascertained, and anatomical features that are consistent.

References

Refer to any literature describing the same or similar ectomycorrhizae, ecological zones, or communities of vegetation. Also refer to the publication that you used to identify the sporocarp linked to the ectomycorrhiza.

B. Identifying ectomycorrhizae using molecular techniques

by K.N. Egger, M. Berbee, G. Kernaghan, T.E. O'Dell, and Q. F. Baldwin

Although the process of identifying ectomycorrhizal fungi through morphological characterization has been refined over several decades, there are limitations to this approach. Ectomycorrhizae can express variable morphological traits on different hosts and under different environmental conditions, which complicates identification. Also, ectomycorrhizal roots can be colonized by more than one fungal symbiont, which may change morphological appearance. Characterization of ectomycorrhizal fungi based upon cultured isolates is an alternative approach, however some ectomycorrhizal fungi cannot be cultured, or are poor competitors with more robust mycorrhizal or non-mycorrhizal root fungi. These inherent difficulties may lead to an inaccurate estimate of mycorrhizal fungal diversity.

Molecular analysis eliminates many of the problems involved with morphological characterization and culturing (Egger 1995). A change in the appearance of a particular fungal species from one host or environment to another is generally the result of differential gene expression, and does not usually constitute a genetic alteration. Thus, environmental factors are largely transparent at the molecular level, making species identification more precise. Also, the ability of molecular techniques to detect multiple symbionts on roots facilitates studies of competition or cooperation among ectomycorrhizal fungi.

Methods for DNA analysis of ectomycorrhizae are helpful in: 1) identifying species (or sometimes higher taxa) from a sample with one or more fungal species; and 2) discriminating between genotypes within a species. This chapter emphasizes molecular identification of single taxa. However, related methods that apply to characterizing all of the fungi in diverse samples are developing rapidly (e.g. Bastiasa et al. 2007; O'Brien et al. 2005), as are approaches to discriminating genotypes and recognizing fungal individuals (e.g. Kretzer et al. 2005, Hadrys et al. 1992; Leesa and Applebaum 1993).

The basic procedure for identifying taxa is to extract DNA from sporocarps, mycorrhizae or cultured mycelia, and then use the polymerase chain reaction (PCR) with primers selected to amplify a specific portion of that DNA. Once a high concentration of the targeted segment has been obtained, it can then be characterised, usually by direct assessment of the nucleotide sequence although restriction fragment length polymorphism (RFLP) analysis is also possible. While RFLP can be useful for screening isolates, it only detects a small amount of the variation present. While this may be sufficient for distinguishing taxa and identifying isolates by comparison to fruit bodies, it is not adequate for determining phylogenetic affinities of unknown isolates. More information can be obtained by DNA sequencing

The most commonly used region for identification of mycorrhizal fungi is a segment of the nuclear-encoded ribosomal DNA (rDNA) unit that is approximately 700-1200 base pairs in length. The region includes Internal Transcribed Spacer (ITS) regions of the rDNA as well as the eukaryotic D1 and D2 divergent domains near the 5' end of the large subunit (28S) gene (Hibbett and Vilgalys 1993). Thanks to the low levels of selective pressure that act on them, the ITS regions are often variable enough to distinguish among closely-related species (e.g. Froslev et al. 2007). The 28S region is more highly conserved as it does encode an RNA that becomes an essential physical part of the large subunit of a ribosome. The 28S is useful in broad scale surveys of ectomycorrhizae, when generic rather than species-level identification is required.

An important consideration in choice of primers for PCR amplification is their level of specificity. Primers from highly conserved regions ("universal" primers) will amplify across a wide range of organisms, and are thus only appropriate for pure mycelial cultures

or sporocarps in order to avoid spurious amplification of nonfungal or contaminant fungal sequences. Amplification of DNA directly from the fungal component of ectomycorrhizae is made possible by primers which target only fungal DNA or the DNA of certain groups of fungi, thereby avoiding the amplification of plant DNA (Egger 1995, Gardes and Bruns 1993). While no primer combination is perfect in all situations, fungal-specific ITS1f (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) and ITS4b (5'-CAGGAGACTTGTACACGGTCCAG-3'), designed to amplify only basidiomycetes, as well as the universal primers ITS1 and ITS4 (Gardes and Bruns 1993) have been used with wide success in mycorrhizal studies. Other commonly amplified rDNA regions, and primer sequences have been developed (Egger 1995) including ITS1f and TW13 (5'-GGTCCGTGTTTCAAGACG-3'), <http://plantbio.berkeley.edu/~bruns/tour/primers.html> as well as NSI1 and NLC2, which are useful primer sets for both basidiomycetes and ascomycetes (Martin and Rygiewicz, 2005).

When the ITS regions are not variable enough to distinguish among species of interest, a range of alternative loci have proven useful including genes coding for proteins such as beta-tubulin and the elongation factor 1-alpha. In the nuclear ribosomal repeat regions, the intergenic spacer region (IGS) has provided variation helpful in species recognition (Anderson and Stasovski 1992). Mitochondrial rDNA has also been used for molecular identification (Gardes et al. 1991, Bruns and Gardes 1993). Some commonly amplified rDNA regions, and primer sequences, are given in Egger (1995)

For screening studies or comparisons of sporocarps with associated ectomycorrhizae RFLP techniques are an inexpensive alternative (Egger 1995). The ITS region of the rDNA is sufficiently variable that species-specific RFLP patterns can be easily distinguished using only a few restriction enzymes. This is partly due to the low selection constraints on this region, but also to the fact that insertion/deletion events are common and are easily detected by gel electrophoresis. The enzymes AluI (AGCT) and HinfI (GANTC) are routinely used for restriction analysis, but may be supplemented by RsaI (GTAC) and/or HhaI (GCGC) or its isoschizomer CfoI (GCGC) if additional resolution is desired. These four enzymes exhibit no overlap in their restriction sites, which ensures that all mutations detected by restriction digests are independent.

C. Protocols for analysis of DNA from mycorrhizal roots

by Q. F. Baldwin, K.N. Egger, and M. Berbee

Following are some protocols used in our laboratory for molecular analysis of mycorrhizal roots.

DNA Extraction

DNA may be extracted from fresh, freeze dried, or frozen root tips (see section 2, p. 2.3, Preparation of voucher material, for methods of freeze-drying). Frozen tips should not be subjected to numerous freeze-thaw cycles since the DNA may be subjected to shearing, and degradation from cellular nucleases. Many laboratories use kits for DNA extraction such as the DNeasy Plant Minikit (Qiagen Inc. Canada, Mississauga, Ontario), which are convenient but expensive. A simple alternative to a kit is outlined here.

Nucleic acids can be isolated from the tips (2-3 mm) of mycorrhizal roots using a modification of the miniprep protocol of Zolan and Pukkila (1986). Freeze root tips (we freeze at -80°C) for 10-15 minutes, then crush with cold micropestles (Mandel Scientific) before adding extraction buffer. Grinding of the tissue is continued after the addition of 350 µl of a 2x CTAB buffer (1.4 M NaCl, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 2% CTAB, 0.2% beta-mercaptoethanol). The buffer-suspended tissue is then incubated in a 60°C water bath for approximately 45 minutes. After incubation 350 µl of chloroform:isoamyl alcohol (24:1) is added to the tissue and mixed by vortexing briefly. The mixture is then centrifuged (13,000 rpm) for 10 minutes at room temperature. The aqueous phase is removed and transferred to a new centrifuge tube. DNA precipitation is accomplished with the addition of an equal volume of cold isopropanol; the tube is inverted frequently to assist in mixing and precipitation. Pellet the DNA with another 10 minute centrifugation (nucleic acid concentrations are minute, so you may not see a pellet). Remove most of the supernatant, then wash the DNA pellet with ice-cold 70% ethanol followed by a 3 minute centrifugation at room temperature. Repeat with a second ethanol wash. Remove excess ethanol via vacuum centrifugation and re-suspend the DNA in 50 µl of 8mM NaOH. There are recently developed techniques that are potentially more rapid and efficient for extracting DNA that the reader can also consider

DNA amplification

Approximately 3.0 µl of the genomic DNA is used as a template for the amplification of a portion of the ribosomal DNA using the polymerase chain reaction (PCR). A typical, successful 10-25 µl PCR reaction produces enough DNA for several sequencing reactions. For the purposes of restriction fragment length polymorphism (RFLP) analysis, 30 µl reaction volumes are sufficient. The PCR mixture contains 1-2 units of Taq DNA polymerase (Taq polymerase licensed for PCR is available from several manufacturers), one-tenth volume of 10x reaction buffer (Note: it is important to use the buffer provided by the manufacturer with the enzyme), 2 mM $MgCl_2$, 50mM each of dATP, dCTP, dGTP, and dTTP, and 0.8 mM of each oligonucleotide primer. The parameters used for amplification depend on the model of the DNA thermocycler being used. In a thermocycler such as an Applied Biosystems GenAmp System 9700, we have used the following parameters: 94°C for 10 sec, 55°C for 20 sec, and an extension step at 72°C for 30 sec initially but increased by 1 sec with each successive cycle. Maximum ramp times are used. This cycle is repeated 35 times with an initial denaturing step of 2 min at 94°C and a final extension step of 7 min at 72°C. These temperatures may vary slightly with different thermocycles

DNA sequencing

Protocols for sequencing depend on the kind of sequencing machine to be used at the electrophoresis stage and so a first step in the sequencing process is to consult the applicable technical bulletins provided by the sequencing machine's manufacturer. In general, in preparation for DNA sequencing, the PCR product is purified to separate it from the primers and buffers used in the initial PCR amplification. This can be accomplished using precipitation or a variety of kits. Applied Biosystems' BigDye Terminator Cycle Sequencing kits are widely used for sequencing reactions. Cycle sequencing requires only a small amount of purified PCR product, a primer, and a mixture of chemical components, including an enzyme and nucleotides that are supplied together in a commercial premix. From 0.1 µl to 4 µl of PCR product will usually provide the ~20 ng of DNA currently recommended for a standard cycle sequencing reaction. Like a PCR reaction, a cycle sequencing reaction takes place in a thermocycler. Afterwards, the sequencing reaction products are purified to separate them from unincorporated primers and nucleotides, and the sequence is analyzed by electrophoresis. Many researchers perform sequencing reactions themselves while others find it more convenient to send PCR product to one of the several commercial sequencing laboratories that perform all the steps for a moderate fee.

Sequence data are not included in the descriptions of ectomycorrhizae in section 10 and are generally submitted to central databases such as GenBank, available on the web. If DNA sequence data is available the region sequenced is noted under "Other Features, DNA", and the database accession number(s) recorded under the Notes field. Sequence data are not included in the descriptions of ectomycorrhizae in section 10 and are generally submitted to central databases such as GenBank or UNITE, available on the web.

RFLP analysis

In cases where target species can be distinguished by their restriction sites, RFLPs can be an economical aid to species detection. To select the restriction enzymes to use for species identification, sequence data can be a convenient starting place because expected RFLP patterns can be predicted from the sequence. Trial-and-error, using different enzymes with PCR-amplified DNA also works. For descriptions to be included in this manual use primers ITS1 and NL6Bmun (Egger 1995) for amplification for RFLP analysis. Digest approximately 1 µg of amplified DNA with the restriction endonucleases such as AluI, HinfI, and RsaI (Pharmacia Biotech) for 3-5 hours at 37°C using the buffer provided by the manufacturer. After digestion load the sample on a 2.5% agarose gel (after digestion, we use 1.5% Nusieve agarose (Mandel Scientific) mixed with 1.0% regular agarose) and separate fragments by electrophoresis in 0.5x Tris-borate buffer (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA). Ethidium bromide or SYBR green staining (either by incorporating in the gel or by staining after electrophoresis) and UV light can be used to visualize the bands. Images can be recorded by photography. We use a Gel Print 2000i photodocumentation system (BioPhotonics Corp.) to capture a digital image, which is then analyzed and entered in a database using RFLPscan software (Scanalytics).

The section of DNA amplified by the ITS1 and the NL6Bmun primers is cut by the enzymes AluI, HinfI, and RsaI. The lengths of the resulting fragments, expressed in number of base pairs, are listed under "Other Features, DNA", for each ectomycorrhiza described in section 10.

4. Checklist of ectomycorrhizal characters

Fungal Species: _____ **Host Species:** _____
Collection Number:_____ **Date Collected:** _____ **Date Described:** _____
Location: _____ **Latitude:** _____ **Longitude:**_____
Collected by: _____ **Described by:** _____ **Identified by:** _____
Photography by: _____ **DNA Analysis by:** _____

Ecology

Stand Age (yrs): _____ Ecological Zone: _____ Elevation (m): _____
Silvicultural Practice: *secondary growth forest, old-growth forest, container, bareroot nursery, other*_____
Substrate: *decaying wood, decaying plant litter, mineral soil, nursery mix, other* Soil Horizon: L F H A B C
*Frequency of Mycorrhizal Type: *rare, common* Number of Root Tips Examined (roughly):_____
Months of Survey *J F M A M J J A S O N D*
Months Ectomycorrhiza Observed: *J F M A M J J A S O N D*

Identification Procedure

synthesis, pure culture, mycelium/strand comparison, physical link, published description, DNA, association

Morphology of Ectomycorrhizal System (Dissection Microscope)

Branching: *monopodial pinnate, monopodial pyramidal, dichotomous, irregular, coralloid, tuberculate, not branched, other* _____ _____
Tip Shape: *straight, beaded, club-shaped, tortuous, bent, other* _____
Length of System (mm):_____(_____-_____) Length of Tips (median) (µm):_____(_____-_____)
Width of Tips (40x) (mm):_____(_____-_____) *Width of Main Axis (40x) (µm):_____(_____-_____)
*Width of Tip Base (40x) (µm):_____(_____-_____) *Width of Tip Apex (40x) (µm):_____(_____-_____)
Colour: Young tips:_____ Older tips:_____ Apices: _____ _____
Texture: *smooth, finely grainy, coarsely grainy, felty, velvety, warty, woolly, cottony, stringy, short spiny, long spiny, other* _____
Lustre: *matte, shiny, reflective, other*_____ Visibility of Host Through Mantle: *yes, no*
Chemical Reactions of Whole Ectomycorrhizae: KOH: *none, colour*_____ Melzer's: *none, colour*_____
*Sulfovanillin: *none, colour* _____ *Toluidine Blue: *none, colour* _____
Notes: _____
_____
_____

Morphology of Mycelial Strands (Dissection Microscope)

Attachment: *restricted point, flat angle, hyphal fans* Frequency: *none, rare, common* Colour:_____
Surface (20x): *smooth, hairy, other*_____ Shape in Cross-section (20-40x): *flat, round, other*_____
Branching Frequency (4-16x) *none, rare, common* Diameter (µm):_____(_____-_____)
Notes: _____

Morphology of Emanating Hyphae (Dissection Microscope)

Frequency: *none, rare, common* Shape: *straight, curved, tortuous, other*_____ Colour:_____
Notes: _____

Anatomy of Mantle in Plan View (Compound Microscope)

Thickness: *thin, medium, thick* *Type (Agerer) (A-Q):_____ Presence of Hartig Net: *yes, no*
Chemical Reactions of mantle scrapings: KOH: *none, colour*_____ Melzer's: *none, colour*_____
*Sulfovanillin: *none, colour* _____ *Toluidine Blue: *none, colour*_____
Specialized Cells: Type: *laticifers, oleiferous cells, other*_____ Frequency: *none, rare, common*
Width (µm):_____(_____-_____) Length (µm):_____(_____-_____) Colour:_____
Contents: *clear, granular, oil-like bodies*
Chemical Reactions of Specialized Cells: KOH: *none, colour*_____ Melzer's: *none, colour*_____
*Sulfovanillin: *none, colour*_____ *Toluidine Blue: *none, colour*_____

Character	**Outer layer**	**Middle layer 1**	**Middle layer 2**	**Inner layer**
Mantle Type:	*felt prosenchyma, net prosenchyma, net synenchyma, interlocking irregular syn., non-interlocking irregular syn., regular syn.*	*none felt prosenchyma, net prosenchyma, net synenchyma, interlocking irregular syn., non-interlocking irregular syn., regular syn.*	*none felt prosenchyma, net prosenchyma, net synenchyma, interlocking irregular syn., non-interlocking irregular syn., regular syn.*	*felt prosenchyma, net prosenchyma, net synenchyma, interlocking irregular syn., non-interlocking irregular syn., regular syn.*
Matrix materials?	*yes, no*	*yes, no*	*yes, no*	*yes, no*
Description of Matrix materials	_____ _____	_____ _____	_____ _____	_____ _____

Cell Width (µm)	_____(____-____)	_____(____-____)	_____(____-____)	_____(____-____)
Cell Length (µm)	_____(____-____)	_____(____-____)	_____(____-____)	_____(____-____)
Cell Colour	_____(____-____)	_____(____-____)	_____(____-____)	_____(____-____)
Ornamentation	*none, crystalline verrucose, globular*	*none, crystalline verrucose, globular*	*none, crystalline verrucose, globular*	*none, crystalline verrucose, globular*
Cell Contents	*clear, granular, oil-like bodies*	*clear, granular, oil-like bodies*	*clear, granular, oil-like bodies*	*clear, granular, oil-like bodies*
Septa	*none, rare, common*	*none, rare, common*	*none, rare, common*	*none, rare, common*
Clamps	*none, rare, common*	*none, rare, common*	*none, rare, common*	*none, rare, common*
Hyphal Junctions	*none, rare, common*	*none, rare, common*	*none, rare, common*	*none, rare, common*
**Hyphal Junction∠*	*30° 45° 60° 90° 120°*	*30° 45° 60° 90° 120°*	*30° 45° 60° 90° 120°*	*30° 45° 60° 90° 120°*
Enlarged Junctions	*none, rare, common*	*none, rare, common*	*none, rare, common*	*none, rare, common*
Anastomoses	*none, rare, common*	*none, rare, common*	*none, rare, common*	*none, rare, common*
Anastomoses Type	*contact, H-shaped, clamps, no clamps*	*contact, H-shaped, clamps, no clamps*	*contact, H-shaped, clamps, no clamps*	*contact, H-shaped, clamps, no clamps*

*Notes:*_____

_____

_____

_____

Anatomy of Mycelial Strands in Plan View (Compound Microscope)

Type: *loose-undifferentiated, smooth-undifferentiated, slightly differentiated, differentiated-random hyphae, differentiated-central core, highly differentiated*

Hyphae (most common type): Width (µm):____(____-____) *Cell Length (µm):____(____-____) Colour:____

Ornamentation: *none, crystalline, verrucose, globular, other* *Ornamentation Frequency: *none, rare, common*

*Ornamentation Size: *small, medium, large* Contents: *clear, granular, oil-like bodies, other*

Frequency of Septa: *none, rare, common* Frequency of Clamped Septa: *none, rare, common*

Hyphal Junction Frequency: *none, rare, common* *Hyphal Junction Angle:∠ *30° 45° 60° 90° 120°*

Enlarged Junction Frequency: none, rare, common *Anastomoses Frequency: none, rare, common*

Anastomoses Type: *contact without clamp, contact with clamp, H-shaped without clamp, H-shaped with clamp*

Chemical Reactions of Strands: KOH: *none, colour*_____ Melzer's: *none, colour* _____

*Sulfovanillin: *none, colour* _____ *Toluidine Blue: *none, colour* _____

Notes: _____

_____

_____

_____

Emanating Hyphae (Compound Microscope)

Frequency: *none, rare, common* Width (µm):_____(____-____) *Cell Length (µm):_____(____-____)

Colour:_____ Shape:_____

Ornamentation: *none, crystalline, verrucose, globular, other* *Ornamentation Frequency: *none, rare, common*

*Ornamentation Size: *small, medium, large* Contents: *clear, granular, oil-like bodies, other*

Frequency of Septa: *none, rare, common* Frequency of Clamped Septa: *none, rare, common*

Hyphal Junction Frequency: *none, rare, common* *Hyphal Junction Angle:∠ *30° 45° 60° 90° 120°*

Enlarged Junction Frequency: none, rare, common *Anastomoses Frequency: none, rare, common*

Anastomoses Type: *contact without clamp, contact with clamp, H-shaped without clamp, H-shaped with clamp*

Notes: _____

_____

_____

Cystidia (Compound Microscope)

Frequency: *none, rare, common* Type:_____ Length (µm):_____(_____-_____)

Apex Width (µm):_____(_____-_____) Medial Width (µm):_____(_____-_____)

Basal Width (µm):_____(_____-_____) Wall Thickness (µm):_____(_____-_____)

Colour:_____ Ornamentation: *none, crystalline, verrucose, globular, other*_____

*Ornamentation Frequency: *none, rare, common* *Ornamentation Size: *small, medium, large*

Contents: *clear, granular, oil-like bodies, other*_____

Chemical Reactions of Cystidia: KOH: *none, colour*_____ Melzer's: *none, colour* _____

*Sulfovanillin: *none, colour* _____ *Toluidine Blue: *none, colour* _____

Frequency of Septa: *none, rare, common* Frequency of Clamped Septa: *none, rare, common*

Location of Clamps:_____

Notes: _____

_____

_____

_____

_____

Sclerotia and Microsclerotia

Frequency: *none, rare, common* Diameter (µm):____(____-____) Colour:_____
Shape:_____ Texture:_____
Notes: _____

Chlamydospores

Frequency: *none, rare, common*
Shape: *globose, subglobose, broadly ellipsoidal, ellipsoidal, oval, fusiform, filiform, acerose, cylindrical, reniform, allantoid, lunate, falcate, ovoid, pyriform, clavate, doliform, other* _____
Width (µm):____(____-____) Length (µm):____(____-____) Colour: _____
Surface: *smooth, verrucose, reticulate, striate, echinulate (spiny), warted, tuberculate, other*_____
Hyphal Attachment: *terminal, intercalary, detached* Wall Thickness (µm):____(____-____)
Notes: _____

***Autofluorescence of Whole Tips**

254 nm: *none, colour*_____ 366 nm: *none, colour*_____
Notes: _____

DNA Description

RFLP: Region Targeted: ITS *Primers used: ITS1/NL6Bmun*
Enzyme: AluI *Fragment Lengths:*_____
Enzyme: HinfI *Fragment Lengths:*_____
Enzyme: RsaI *Fragment Lengths:*_____
*Notes:*_____
*RFLP: Region Targeted:*_____ *Primers used:*_____
__
*Enzyme:*_____ *Fragment Lengths:*_____
*Enzyme:*_____ *Fragment Lengths:*_____
*Enzyme:*_____ *Fragment Lengths:*_____
*Enzyme:*_____ *Fragment Lengths:*_____
*Notes:*_____
Sequence Available?: yes, no *Region sequenced:*_____
Notes: _____

Additional characters (e.g. additional chemical tests, Hartig net in cross section)

Distinguishing Features

References

* Note that determination of characters preceded by an asterisk is optional.

5. Glossary

compiled by D.M. Durall, S.M.K. Harniman, S.M. Berch, and D.M. Goodman

This glossary is designed to help you fill in the checklist of ectomycorrhizal characters (section 4). Definitions and diagrams have been adapted from the *Colour atlas of ectomycorrhizae* (Agerer 1987-1994) with the permission of Dr. R. Agerer and Einhorn-Verlag. Types of mantle structure are taken from *Identification of ectomycorrhizas* (Ingleby *et al*. 1990) with the permission of Dr. K. Ingleby.

Identification Procedure

- **synthesis**: comparison of the unknown ectomycorrhiza with ectomycorrhizae synthesized using cultures of known fungi
- **pure culture**: comparison of cultures of fungi isolated from the unknown ectomycorrhiza with cultures of known fungi
- **mycelium/strand comparison**: comparison of the mycelium and mycelial strands attached to the unknown ectomycorrhiza with those attached to the bases of nearby sporocarps of known identity
- **physical link**: tracing of mycelial strands that form a continuous physical connection from the unknown ectomycorrhiza to a sporocarp of known identity
- **published descriptions**: comparison of the unknown ectomycorrhiza with published descriptions of identified ectomycorrhizae, possibly with the assistance of keys
- **DNA**: comparison of RFLP data or DNA sequences from the unknown ectomycorrhiza with RFLPs or sequences from identified fungi, most often using sporocarps collected from the same location as the unknown ectomycorrhiza
- **association**: consistently finding the unknown ectomycorrhiza close to sporocarps of a known identity

Morphology of Ectomycorrhizal System (Dissection Microscope)

An ectomycorrhizal system has a continuous mantle and is usually branched.

Branching

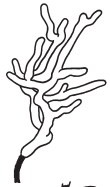
monopodial pinnate: a mycorrhizal system with an axis from which branches originate that are shorter than the axis and lie more or less in one plane

monopodial pyramidal: like monopodial pinnate, but the branches lie in 3 or more planes

dichotomous: the root meristem divides in two branches that grow to similar lengths and may divide and grow repeatedly

coralloid: very densely branched, resembling coral

tuberculate: very densely branched and enveloped by a dense mat of hyphae (the rind); shaped like a small potato; individual tips are seen if the rind is removed or the tubercle is cut



irregular: without a main axis or regular dichotomies

not branched: simple, unbranched mycorrhizae

Tip shape

A tip of an ectomycorrhiza is a distal unbranched section (see Dimensions—Length of tips, b, below). Axes are parts of ectomycorrhizal systems other than tips.

straight: linear, smooth sided

beaded: like a string of beads

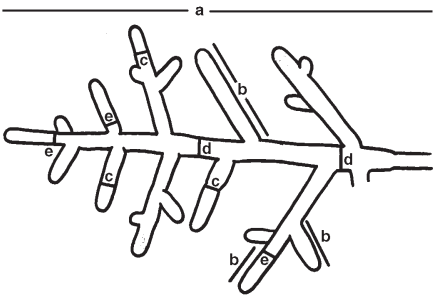
club-shaped: wider at apices than at bases

tortuous: tips bent or curved several times, most tips not in one plane

bent: curved or kinked, each tip lies in one plane

Dimensions

Dimensions are given as means and range, e.g. 450 (300-750) µm.



a = **Length of System** (mm)

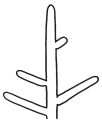
b = **Length of Tips** (µm)

c = **Width of Tips** (µm)

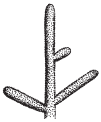
d = **Width of main Axis** (µm)

e = **Width of tip Base** (µm)

Texture



smooth: with little or no texture and few or no emanating hyphae



grainy: with small papillae or grains, like fine or medium grained sandpaper

felty: with appressed hyphae or hyphal strands, like coarsely felted wool

velvety: like velvet cloth, caused by short emanating hyphae

warty: with tubercles or warts, like coarse sandpaper

woolly: with copious thick emanating hyphae and hyphal strands

cottony: with copious thin emanating hyphae, individual hyphae not distinct

stringy: with small hyphal strands on surface

spiny: with conspicuous stiff cystidia that are either **short** (< 1/4 of tip diam.) or **long**

Lustre

matte: surface of ectomycorrhiza is neither shiny nor reflective
shiny: lustrous, especially if obliquely lit
reflective: Light is reflected by many air bubbles trapped in surface hyphae, often in a mantle of loosely woven hyphae, gleaming or brilliant. Reflectivity is lost when the trapped air is replaced by water during storage, by various chemicals, or by touching.

Chemical Reactions

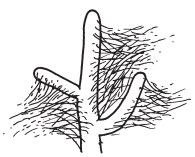
KOH: A general test that may cause several colour changes. Colour reaction to 15% KOH may be throughout the tissue or localized. Normal negative reaction is a bleaching or light yellowing.
Melzer's: Amyloid: blue or dark blue reaction. Dextrinoid: purple or reddish-brown reaction (Baral 1987). Normal negative reaction is a light yellowish-brown staining.
Sulfovanillin: Laticifers may stain blue, red or almost black, reacts with *Lactarius* and *Russula*. Normal negative reaction is a light pink staining.
Toluidine Blue: Stains most fungi. A positive reaction of hyphal walls may be purple, blue or pink.
PDAB: A positive reaction is purplish-red, e.g. with *Tricholoma* (Ovrebo 1980)
$FeCl_2$: A positive reaction is black or greenish-blue, e.g. with *Boletinus* and *Chroogomphus* (Singer 1986)

Morphology of Mycelial Strands (Dissection Microscope)

Attachment

restricted point: strands of densely woven or packed hyphae grow more or less perpendicularly from the mycorrhizae

flat angle: strands with loosely woven proximal ends grow along the surface of the mantle then grow off in a flat angle



hyphal fans: emanating hyphae from large area of mycorrhiza surface aggregate to form loose mycelial strands

Anatomy of Mantle in Plan View (Compound Microscope)

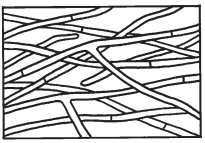
Specialized cells

laticifers: hyphae usually wider than surrounding hyphae and usually with opaque granular cytoplasm (latex). Latex is milky or colourless and may have typical colours, as for fruitbodies.

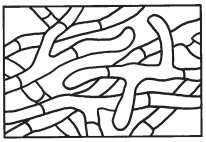
oleiferous cells (or hyphae): larger than surrounding cells (or hyphae), filled with an oily substance that gains contrast in 15% KOH, not reacting to sulfovanillin

laticiferous cells: cells that contain latex (but are not obviously elongated)

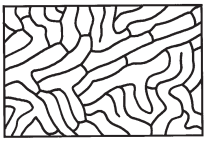
Mantle Type



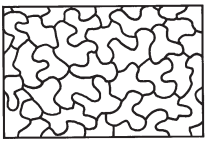
felt prosenchyma:
loose, not organized; abundant interhyphal spaces; cells distinctly elongated; hyphae similar to emanating hyphae



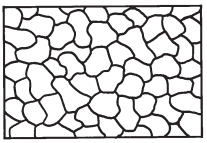
net prosenchyma:
loosely organized; interhyphal spaces; cells distinctly elongated; hyphae wider, shorter celled, and more branched than those emanating



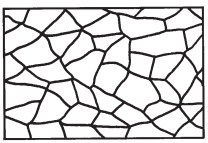
net synenchyma:
compact, without obvious interhyphal spaces; cells elongated



interlocking irregular synenchyma:
compact, no obvious interhyphal spaces; cells not distinctly elongated; like a jigsaw puzzle



non-interlocking irregular synenchyma:
compact, no obvious interhyphal spaces; cells not distinctly elongated, cells generally rounded



regular synenchyma:
compact, no obvious interhyphal spaces; cells with straight sided walls

Matrix materials: amorphous material between cells or hyphae

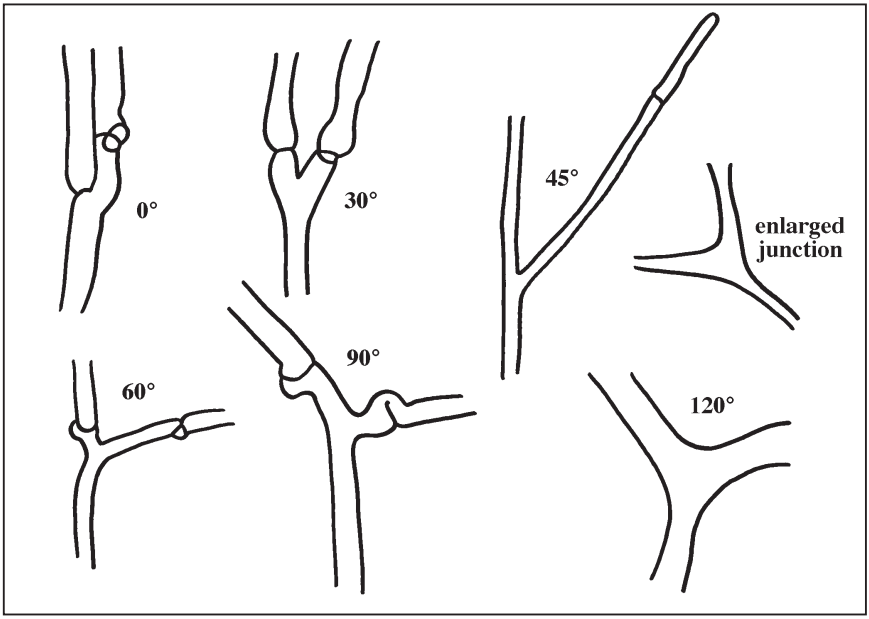
Ornamentation: surface texture of the outer hyphal or cell wall, due to attached particles or droplets, or due to outgrowths of wall. Size of ornamentation is defined relative to the hyphal diameter: small < 20%, medium 20-50%, large >50%

crystalline: sharp-edged, crystal-like particles
verrucose: wart-like particles or outgrowths
globular: round bodies or droplets

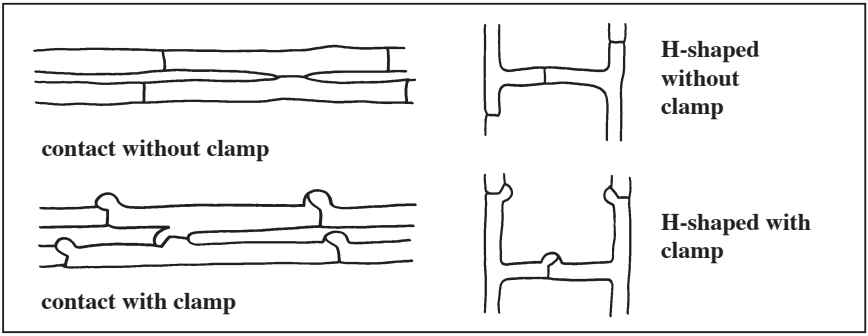
Cell or hyphal Contents (without regard to colour)

clear: transparent
granular: containing small particles
oil-like bodies: containing globular highly refractive bodies

Hyphal Junction: point of branching of hyphae



Hyphal Anastomosis: point of fusion of hyphae or two parts of the same hypha



Anatomy of Mycelial Strands in Plan View (Compound Microscope)

Type

loose-undifferentiated: hyphae loosely woven and of same diameter, with hyphae emanating from surface

smooth-undifferentiated: compact, with few interhyphal spaces, hyphae of same diameter

slightly differentiated: compact, central hyphae slightly wider than others and may have trumpet-like swellings at septa

differentiated-random hyphae: with some very wide hyphae that appear to be randomly distributed and have some enlarged septal pores

differentiated-central core: wide hyphae form a central core, septa complete

highly differentiated: wide hyphae form a central core, septa often partially to completely dissolved

Cystidia

bristle-like, awl-shaped

bristle-like, apical lobes

capitate

thin walled, slightly acuminate

acanthocystidium

bottle-shaped with finger-like outgrowths

bottle-shaped, straight neck

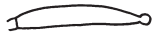
bottle-shaped, bent neck

ramified

sickle-shaped, with thick walls

fusiform

globular



flask-shaped, with apical knobs

lateral with tapering, capitate branch

Additional Characters

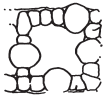
Hartig Net


common and widely distributed: with typical palmetti and single hyphal rows


coarse, with broad and infrequently ramified lobes


common, with lobed or ramified haustoria

common, with small globular haustoria



beaded with globular thickenings

6. Procedures for submitting descriptions to this manual

by D.M. Goodman, S.M. Berch, and J.A. Trofymow

Criteria for selection of ectomycorrhizae for inclusion in this manual

We are most interested in common or distinctive ectomycorrhizae, or ectomycorrhizae for which ecological information is available. Identified ectomycorrhizae of which detailed descriptions have not been published are preferred. Descriptions of identified ectomycorrhizae (species of host and of fungus known) are preferred because a single species of fungus may form ectomycorrhizae variable in morphology, and, conversely, several species of fungi may form morphologically and anatomically indistinguishable ectomycorrhizae. The identity of the host species must be known. Descriptions of ectomycorrhizae need to be based on numerous samples and extensive study in order to represent the most common or typical morphology and anatomy. Ensure each sample has been identified. Describe the techniques used to describe the ectomycorrhiza if they differ from those outlined in section 3.

Submission and review process

1. To avoid duplication, tell the editor the species of fungus and plant you wish to describe. Contact Dr. J.A. Trofymow or Dr. D.M. Goodman (addresses on page ix).
2. The editor will send you a package confirming the species, informing you of the cost for the DNA characterization service, and providing other relevant information including the format for submissions.
3. Prepare the description in the requested format, including DNA characterization if possible, and submit together with the needed voucher materials by June. Inform the editor if you are unable to characterize the DNA, and will be using the DNA service.
4. The editors will check the submission for completeness and format and, if acceptable, it will be reviewed by the editors and by one other expert. During an August workshop, the review committee will evaluate the submitted descriptions, reviewers comments, and voucher materials.
5. If the description is accepted, reference material will be accessioned into the herbarium DAVFP, and you will be asked to submit samples for DNA analysis if you haven't provided RFLP data. Minor changes indicated by the reviewers will be made by the editors and the description sent to you for approval. Final revisions must be made and the document returned to the editors by October, in time for publication in December.
6. If the reviewers indicate that there are major revisions needed, the submission will be returned to you. Descriptions requiring major revision will have to be resubmitted for at least editorial review. In some cases, it may be necessary to resubmit the description for complete review in the following year.

Materials to be submitted

1. A **colour plate** of 2-8 photos and 0-3 line drawings, for a total of ≤ 8 figures. Include both a low magnification (≅ 10x) photo of the overall morphology of the ectomycorrhizal system, and a high magnification (≅ 40x) photo showing the texture of the mantle surface. Submit either a photographic plate or, preferably, a high-resolution digital image file (TIFF, JPEG, or PICT). If submitting a digital image, also send a high quality proof, which will be used to judge the image quality, and to adjust colour if necessary.

2. Three 100% (no enlargement or reduction) **colour photocopies of the colour plate** (for reviewers), with labelling as described below. (How to prepare colour and black and white plates).

3. A **black and white plate** of 2-8 photos and 0-3 line drawings, for a total of ≤ 8 figures. Show the most important anatomical details, eg. mantle, hyphae, mycelial strands, cystidia. Most photos will be taken at high power (1000x) although some may be taken at 400-500x. Submit either a hard copy or, preferably, a high resolution digital image file (TIFF, JPEG, or PICT).

4. Three 100% (no enlargement or reduction) **photocopies of the black and white plate**, with labelling as described below.

5. The **text describing the ectomycorrhiza**, as a file in a specified format (contact the editor), and one printed copy.

6. A copy of the completed **checklist**. This will be used in case there is any doubt or confusion about the text, and will be used to enter data into a database used to construct synoptic keys.

7. **Reference material**:
   i) two semi-permanent **microscope slide mounts** of peels or glancing sections of the mantle in lactoglycerol (see page 2.3)
   ii) ≥ 25 tips of **freeze-dried ectomycorrhizae** in a single vial
   iii) (where applicable) **air-dried sporocarps** that have been linked to the ectomycorrhiza.

   Reference materials will be accessioned into the herbarium DAVFP of the Pacific Forestry Centre of the Canadian Forest Service.

8. **Frozen ectomycorrhizae** for DNA analysis. After a description is accepted for publication the author will be given instructions as to how and where to send these. **Do not send with items 1-7**. RFLP information will be included in the text of the description. Authors may either submit frozen ectomycorrhizae for DNA analysis, or do the DNA analysis themselves as detailed in section 3.C and submit RFLP data.

How to prepare colour and black and white plates

i) **colour plate of morphology (dissection microscope)**. At least two photos are required. Photograph at 4x-7x to illustrate features of a system of ectomycorrhizal root tips, and at ≥ 25x (preferably both 25x and 40x) to show details such as texture, hyphae (e.g. laticifers), cells, cystidia, and mycelial strands. Put the lower magnification photos before the higher magnification photos. Final magnification on the published plates will be about 10x for photos taken at 4x-7x, and about 40x for photos taken 25x. A black

background is required except that a colourless grey background may be used for dark subjects. Photos will not be accepted for publication if they contain bubbles, out-of-focus roots, or reflections.

ii) **black and white plate of anatomy (compound microscope)**. At least two photos are required. Photos of the outer mantle and inner mantle will normally be included. Photos of emanating hyphae, mycelial strands and cystidia should also be included if these features are present.

iii) **both plates:** The following instructions apply to both the colour and black and white plates, and to both photos and line drawings. Line drawings (optional) are not to occupy more than 50% of the area of the plate. If you do not submit a digital image, mount figures on paper, not card (to allow scanning with a drum scanner). Ensure that each figure is filled with useful information, i.e. that areas of background only, or of subject that is out of focus, are not excessive. Place individual figures directly against each other, i.e., without space between the figures. A thin white line will be added between all figures electronically after the plate is scanned. Do not put any labeling (letters, numbers, scale bars, arrows, etc.) on the plate. These will be added electronically. The plate must be 175 mm high by 114 mm wide, no larger or smaller, and have no blank spaces within this area (see page 6.4). On the photocopies number each figure according to its position in the plate, starting in the upper left and proceeding as words are read on this page, left to right and top to bottom. Number figures in the black and white plate x+1, x+2, ..., where x is the number of figures in the colour plate. Try to order the figures as the features they illustrate are ordered in the written description. On or next to each figure on the photocopy indicate the scale, either as a magnification, or by giving the length in mm of a scale bar. For colour figures of morphology use scale bars that represent 100, 200, 500, 1000, or 2000 µm. For black and white figures of anatomy use scale bars that represent 10, 20, 50, or 100 µm. Any arrows to appear on the figures also need to be positioned on the photocopies. In the word processor file containing the text of the description, provide a short title for each figure, e.g. 1. Mycelial strand, 2. Emanating hyphae, 3. Outer mantle.

How to prepare the text of a description

Characters are described in an order similar to that of the checklist, using the same terminology. Contact the editor to obtain a word processor file containing the standard style and formatting, and use the most recently published descriptions as a guide. If you include characters indicated as optional, or characters not on the checklist, place them under existing headings, rather than creating new headings.

Title. Specify the order and family of the fungus according to Hawksworth *et al*. (1995).

Anatomy of the mantle. For clarity, describe the mantle cells in addition to stating the type of mantle, e.g., "a regular synenchyma with triangular to quadrangular cells". To describe more than two layers, use CDE13 as a guide.

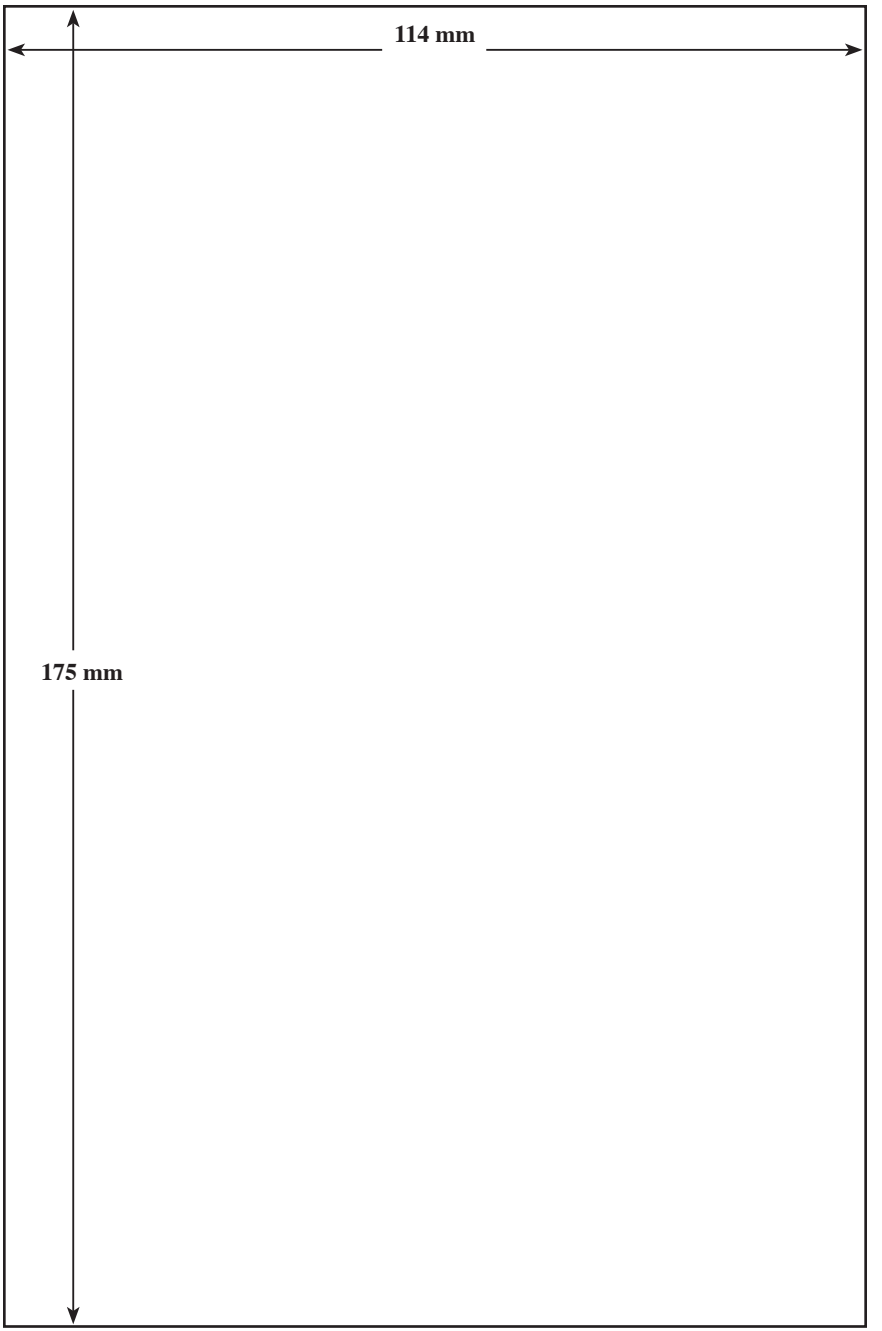
Ecology. List any other hosts on which you know that the fungus forms similar ectomycorrhizae.

References. Format as in the Canadian Journal of Forest Research or as in this manual.

Requirements for reference materials

Microscope slides are to show both the outer and inner surfaces of the mantle, and are to be prepared as described in section 2. All reference materials need to be labelled with the species of fungus and host. In addition to the reference materials, which are typical and on which the description has been based, voucher materials may also be submitted to document variation in the ectomycorrhiza. Be sure to distinguish reference materials from other voucher materials.

Size of plates



7. How to cite a description from this manual

Cite an individual description as follows:

Goodman, D.M. and Trofymow, J.A. 1996. *Piloderma fallax* (Libert) Stalpers + *Pseudotsuga menziesii* (Mirb.) Franco, CDE1. *In* Concise Descriptions of North American Ectomycorrhizae. *Edited by* D.M. Goodman, D.M. Durall, J.A. Trofymow, and S.M. Berch. Mycologue Publications, and Canada-B.C. Forest Resource Development Agreement, Canadian Forest Service, Victoria, B.C. pp. CDE1.1-CDE1.4.

8. Appendices

A. List of fungi

	Description
Amphinema byssoides-like	CDE6
Boletus cf. *ferrugineus*	CDE33
Cantharellus formosus Corner	CDE21
Cenococcum geophilum Fr.	CDE10
Clavulina cf. *cristata*	CDE34
Cortinarius cf. *glaucopus*	CDE35
Cortinarius sp.	CDE24
Craterellus tubaeformis (Fr.) Quélet	CDE23
Hebeloma sp.	CDE36
Hysterangium_separabile	CDE25
Lactarius rubrilacteus Hesler & Smith	CDE15
Lactarius scrobiculatus (Fr.) Fr.	CDE11
Leccinum cf. *scabrum*	CDE26
Piloderma fallax (Libert) Stalpers	CDE1
Rhizopogon vinicolor-like	CDE7
Russula cf. *brevipes*	CDE37
Russula delica Fr.	CDE16
Russula densifolia (Secr.) Gillet	CDE22
Russula cf. *nigricans*	CDE38
Russula nigricans (Bull.) Fr.	CDE17
Russula cf. *occidentalis*	CDE39
Russula-like	CDE13
Russula sp.	CDE27
Sebacinaceae sp.	CDE28
Suillus caerulescens A.H. Smith & Thiers	CDE19
Thelephora terrestris-like	CDE20
Tomentella cf. lapida	CDE29
Tomentella-like	CDE2
Tomentella sp. (UBCODM1)	CDE30
*Tomentella sp. (*UBCODTM3)	CDE31
Tomentella sp (UBCODTS1)	CDE32
Tricholoma magnivelare (Peck) Redhead	CDE18
Truncocolumella citrina Zeller	CDE9
unidentified	CDE3-5,8,12,14

B. List of plants

	Description
Betula papyrifera Marsh.	CDE20,24,26,28,31,32
Betula platyphylla Sukatchev var. *japonica* Hara	CDE16,17
Picea engelmannii (Parry) Engelm.	CDE6,10
Pinus contorta var. *latifolia* Engelm.	CDE18
Pseudotsuga menziesii (Mirb.) Franco	CDE1-5,7-9,15,19,22,23,25,27,29,30
Pseudotsuga menziesii (Mirb.) Franco	CDE33-39
Tsuga heterophylla (Raf.) Sarg.	CDE11-14,21,23

C. List of authors

	Description
S.M. Berch	CDE11
R.E. Countess	CDE21
C. Chplyk	CDE19
J. Dennis	CDE34, 35
D.M. Durall	CDE6,10,20, 24-33
J. Eberhart	CDE9,15,22,23
D.M. Goodman	CDE1-5,7,8,12-14,19,21
S.M.K. Gillespie	CDE20
S.M. Hagerman	CDE20
S.M.K. Harniman	CDE6,10,20
M. Hoch	CDE19
G. Kernaghan	CDE11
S. Kurucz	CDE19
C.K. Lefevre	CDE18
D.L. Luoma	CDE9,15,22,23
W.R. Müller	CDE18
R. Outerbridge	CDE34-39
M.J. Trappe	CDE23
J.A. Trofymow	CDE1, 37, 38
B. Tweig	CDE 24-33
A. Yamada	CDE16,17

9. Literature cited

Agerer, R. 1986. Studies on ectomycorrhizae II: Introducing remarks on characterization and identification. Mycotaxon **26**: 473-492.

Agerer, R. (*Editor*) 1987-1995. *Colour atlas of ectomycorrhizae*. Einhorn-Verlag Eduard Dietenberger GmbH Schwäbisch Gmünd, Munich.

Agerer, R. 1991. Characterization of ectomycorrhiza. *In* Techniques for the study of mycorrhiza. *Edited by* J.R. Norris, D.J. Read, and A.K. Varma. Academic Press. London. pp. 25-73.

Agerer, R. (*Editor*) 1996-1997. Descriptions of ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger GmbH Schwäbisch Gmünd, Munich.

Ammirati, J. 1972. The section *Dermocybe of Cortinarius* in North America. Ph.D. dissertation. University of Michigan.

Anderson, J., and Stasovski, E. 1992. Molecular phylogeny of Northern Hemisphere species of *Armillaria*. Mycologia **84**: 505-516.

Baral, H.O. 1987. Lugol's solution/IKI versus Melzer's reagent: hemiamyloidity, a universal feature of the ascus wall. Mycotaxon **29**: 399-450.

Bastiasa BA, Anderson IC, Xuc Z, Cairney JWG, 2007. RNA- and DNA-based profiling of soil fungal communities in a native Australian eucalypt forest and adjacent Pinus elliotti plantation. Soil Biology and Biochemistry 39: 3108-3114.

Bruns, T.D., and Gardes, M. 1993. Molecular tools for the identification of ectomycorrhizal fungi - taxon specific oligonucleotide probes for suilloid fungi. Mol. Ecol. **2**: 233-242.

Chilvers, G.A. 1968. Some distinctive types of eucalypt mycorrhizae. Aust. J. Bot. **16**: 49-70.

Chu-Chou, M., and Grace, L.J. 1981a. Mycorrhizal fungi of *Pseudotsuga menziesii* in the North Island of New Zealand. Soil Biol. Biochem. **13**: 247-249.

Chu-Chou, M., and Grace, L.J. 1981b. *Tuber* sp., a mycorrhizal fungus of Douglas fir in New Zealand. Trans. Br. Mycol. Soc. **77**: 652-653.

Chu-Chou, M., and Grace, L.J. 1983a. Characterization and identification of mycorrhizae of radiata pine in New Zealand. Aust. For. Res. **13**: 121-132.

Chu-Chou, M., and Grace, L.J. 1983b. Characterization and identification of mycorrhizas of Douglas fir (*Pseudotsuga menziesii*). Eur. J. For. Pathol. **13**: 251-260.

Cullings, K.W. 1992. Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. Mol. Ecol. **1**: 233-240.

Egger, K.N. 1995. Molecular analysis of ectomycorrhizal fungal communities. Can. J. Bot. **73**: S1415-S1422.

Froslev TG, Jeppesen TS, Laessoe T, Kjoller R, 2007. Molecular phylogenetics and delimitation of species in Cortinarius section Calochroi (Basidiomycota, Agaricales) in Europe. Molecular Phylogenetics and Evolution. 44: 217-227.

Furlan, V., and Fortin, J.A. 1975. A flotation-bubbling system for collecting Endogonaceae spores from sieved soil. Nat. Can. (Que). **102**: 663-667.

Gardes, M., and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. **2**: 113-118.

Gardes, M., White, T.J., Fortin, J.A., Bruns, T.D., and Taylor, J.W. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Can. J. Bot. **69**: 180-190.

Godbout, C., and Fortin, J.A. 1985. Synthesized ectomycorrhizae of aspen: fungal genus level of structural characterization. Can. J. Bot. **63** : 252-262.

Hadrys, H., Balick, M., and Shierwater, B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology **1**: 55- 63.

Hawksworth, D.L., Kirk, P.M., Sutton, B.C., and Pegler, D.N. (*Editors*). 1995. Ainsworth and Bisby's dictionary of the fungi, eighth edition. International Mycological Institute, CAB International, Wallingford, UK.

Hibbett, D.S., and Vilgalys, R. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. Syst. Bot. **18**: 409-433.

Hutchison, L.J. 1991. Description and identification of cultures of ectomycorrhizal fungi found in North America. Mycotaxon **152**: 387-504.

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of ectomycorrhizas, Institute of Terrestrial Ecology (ITE) research publication no. 5. (112 pp.) HMSO, London.

Kretzer AM, Dunham S, Molina R, Spatafora JW, 2005. Patterns of vegetative growth and gene flow in Rhizopogon vinicolor and R. vesiculosus (Boletales, Basidiomycota) Molecular Ecology 14:2259-2268.

Leesa, E.P., and Applebaum, G. 1993. Screening techniques for detecting allelic variation in DNA sequences. Mol. Ecol. **2** : 119-129.

Martin KJ and Rygiewicz. 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC microbiology 5(1):28.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo J-M, Vilgalys R, 2005. Fungal community analysis by large-scale sequencing of environmental samples. Applied and Environmental Microbiology 71: 5544-5550.

Ovrebo, C.L. 1980. A taxonomic study of the genus *Tricholoma* (Agaricales) in the great lakes region, Ph.D. dissertation. University of Toronto. Toronto, Ontario. 308 pp.

Singer, R. 1986. The Agaricales in modern taxonomy. (981 pp.) Koeltz Scientific Books, Koenigstein, Germany.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press. New York.

Zak, B. 1971. Characterization and identification of Douglas-fir mycorrhizae. *In* Mycorrhizae—proceedings of the first North American Conference on Mycorrhizae, 1969 Apr, Washington, D.C., *Edited by* Hacskaylo, E. U.S. Government Printing Office. pp. 38-53.

Zak, B. 1973. Classification of ectomycorrhizae. *In* Ectomycorrhizae, their ecology and physiology. *Edited by* G.C. Marks and T.T. Kozlowski. Academic Press. New York. pp. 43-78.

Zolan, M.E., and Pukkila, P.J. 1986. Inheritance of DNA methylation in Coprinus cinereus. Mol. and Cell. Biol. **6**: 195-200.