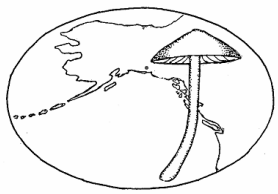
Pacific Northwest Fungi



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Russula crassotunicata identified as host for *Dendrocollybia racemosa*

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Abstract: *Russula crassotunicata* was identified as a host species for the mycosaprobic basidiomycete *Dendrocollybia racemosa.* Sclerotia of the latter species were harvested from the gills of sporocarps known to be *R. crassotunicata* and isolated in pure culture. DNA sequences of the ribosomal ITS1-5.8S-ITS2 region of these sclerotia were used to identify and phylogenetically place the species as *D. racemosa.* This paper represents the first report of a confirmed host for *D. racemosa.*

Key Words: *Dendrocollybia racemosa, Russula crassotunicata, Collybia,* mycoparasite, mycosaprobe, sclerotia.

Introduction: *Russula crassotunicata* Singer is an ectomycorrhizal basidiomycete distributed in the western part of North America from northern California to British Columbia, and east in the boreal forests of Canada as far as the northern Great Lakes region (Singer, 1957). *R. crassotunicata* produces large, fleshy basidiocarps characterized by an unusually thick, rubbery pellicle, which can be easily separated from the pileal context (Fig. 1A). In the Pacific Northwest, we have observed *R. crassotunicata* to be common under conifers and is often locally abundant to prolific.

At our field site, a low-elevation *Sphagnum* bog southeast of Seattle, Washington, *Russula crassotunicata* (identified using Thiers 1997), actively fruits from late June through October (Fig. 1B). However, the basidiocarps are slow to decay and are present almost year-round in various states of decomposition. The basidiocarps of *R. crassotunicata* provide a substrate for many mycosaprobic organisms, including *Collybia tuberosa* (Bull.: Fr.) Kummer, a mycosaprobic basidiomycete that is widely distributed and common in the northern latitudes. *C. tuberosa* is frequently collected at our field site, and its characteristic reddish brown, ellipsoid sclerotia are often observed growing on the rotten basidiocarps of *R. crassotunicata*. The presence of these distinctive sclerotia facilitates the quick identification of *C. tuberosa,* even when *C. tuberosa* basidiocarps are not present. In our survey, we frequently checked the underside of rotten *R. crassotunicata* basidiocarps for the presence of *C. tuberosa* sclerotia. On several occasions, along with sclerotia characteristic of *C. tuberosa,* we observed blackish, irregularly globose sclerotia (Fig. 1C) growing between the gills of decaying *R. crassotunicata* sporocarps. Because these sclerotia were clearly morphologically different than those of *C. tuberosa*, we decided to investigate them further.

Materials and Methods

Field collections Since August 2003, the authors have made weekly collections along one of three transects at our field site for a survey of the macrofungi of Shadow Lake Bog, in Maple Valley, Washington. The area, a lacustrine bog that was formed by glacial recession at the end of the Pleistocene, is dominated by *Tsuga heterophylla, Rhododendron groenlandicum*, *Kalmia microphylla, Sphagnum,* and other moss species. In February 2004 we collected several decomposing *R. crassotunicata* fruiting bodies that had two types of sclerotia located within the decaying gills. The specimens were transported back to the lab and photographed.

Cultures The black, globose sclerotia were removed from the *R. crassotunicata* gills and gently agitated in a 2% hypochlorite solution for 15 minutes to achieve surface sterilization. The sclerotia were then triple-rinsed in DI water and plated on various growing media. The most abundant hyphal growth was observed on 33% V8 agar containing 0.5% ampicillin, tetracycline, and streptomycin. Cultures were incubated for one month in ambient temperature and light conditions.

DNA Extractions DNA was extracted using the FastDNA® Kit and the FastPrep® FP120 Bead Beater (Qbiogene, Inc., CA). Approximately 0.1g of mycelium was scraped from the surface colonies growing on a V8 agar media and placed in lysing tubes containing 1 ml of CLS-Y buffer. The mycelium was disrupted at a setting of 5.0 for 20 seconds and DNA purified according to the FastDNA kit instructions.

PCR and DNA Sequencing Amplification using PCR was accomplished using 20 µl reaction volumes consisting of 20 ng genomic DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2% Triton X-100, dNTPs and 200 µl each, 0.5 units of *Taq* DNA polymerase (Promega), and 0.125 µM

primers. The ITS1-5.8s-ITS2 region of rDNA was amplified with ITS1F (5' TTGGTCATTTAGAGGAAGTAA 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers (White et al., 1990). PCR's were initiated with a 93 °C dwell for 2 min, followed by 39 cycles of the following: 93°C denature for 15 sec, 60°C for 1.5 min, 72° C for 1.5 min. Amplified DNA fragments were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide, and checked under ultraviolet light to confirm a single product. PCR products were cut out of agarose gels and centrifuged through glass wool at 6000 rpm for 4 min. PCR products were sequenced by Northwoods DNA Inc. (http://www.nwdna.com) using ITS1F and ITS4 primers (White et al., 1990).

Phylogenetic Trees Sequences of the ITS1-5.8S-ITS2 region were analyzed and compared using Sequencher (Gene Codes Corporation, USA). A BLAST search was performed to identify similar sequences in GenBank. Phylogenetic trees were constructed with the rDNA sequences we generated and GenBank sequences of related taxa. Along with our sequence, eighteen accessions representing 7 species were selected from the GenBank sequence database (http://evolve.zoo.ox.ac.uk/software.html?id=seal) based on previously published phylogenetic trees (http://mor.clarku.edu, Hughes, et al. 2001). Accession numbers are listed in Table 1. *Tricholoma atroviolaceum* Smith was included to serve as an outgroup and to root the trees. The sequences were aligned using ClustalW (http://www.ebi.ac.uk/clustalw) and visually checked using Se-Al v2.0a11 (http://evolve.zoo.ox.ac.uk/software.html?id=seal). Any alignment errors observed among sequences were corrected manually. Maximum parsimony trees were generated using PAUP* v4.0. A branch and bound search method using PAUP* default parameters was used to search for trees, with MAXTREES set to 1000. Branching order significance was assessed by bootstrapping with 1000 replicates.

Results: A GenBank nucleotide BLAST search with our sequences using the default NCBI parameters for BLAST returned an exact match for *Dendrocollybia racemosa* with 100% sequence similarity (708/708 identities). Maximum parsimony analyses performed using the branch and bound search method resulted in 36 equally parsimonious trees. A consensus tree with 1000 bootstrap replicates is represented in Figure 2.

Discussion: *Dendrocollybia racemosa* (Pers. : Fr) R. H. Petersen & Redhead has been collected from Northern California to British Columbia. It is less common than the more widely distributed species, *C. tuberosa*. Both species are often collected from badly decayed basidiocarps, in a condition that makes identification of the exact host taxon impossible. Some authors (Hughes et al.. 2001, Aurora 1986) have suggested members of the genera *Lactarius* and *Russula* as probable hosts. To our knowledge, there have been no published reports definitively identifying the host(s) of these mycosaprobic fungi. The degree of host specificity of these species is still unclear, and *R. crassotunicata* may represent one of several possible hosts.

We were able to identify the host fungus of *Dendrocollybia racemosa* as *Russula. crassotunicata* with confidence and without the need for molecular analysis of the decayed sporocarps due to the unique nature of our survey, which included weekly trips to our field site for a period of over two years. During this time, we collected sporocarps and phenological data along three transects. For many of the fleshier and more persistent species, these weekly visits allowed us to observe the progression of individual sporocarps from button stage through maturity and into decomposition. Thus we were able to definitively identify individual *R. crassotunicata* sporocarps in the field before they began to decay, and return to the same individuals to check for the presence of sclerotia once decomposition had progressed to an

advanced state. Based on this familiarity with individual *R. crassotunicata* sporocarps, we are confident that the sclerotia identified here through DNA analysis were in fact harvested from a fruiting body of *R. crassotunicata*.

Russula crassotunicata is abundant and common at our collection site and fruits in massive quantities for five months out of the year, followed by a long, persistent decaying period. These slowly decaying sporocarps provide a nearly year-round substrate for mycosaprobic species. *R. crassotunicata* was the primary substrate for most of our *C. tuberosa* sporocarps, and many of these collections were made from *R. crassotunicata* fruiting bodies in early stages of decay that still allowed identification in the field. *Dendrocollybia racemosa* sporocarps have been collected on only one occasion over the course of our survey (October 2004), and while the host species was likely *R. crassotunicata,* we were unable to identify the specimen definitively due to its advanced state of decomposition. Based on our observations in this and other field sites, *C. tuberosa* may produce sporocarps on less decayed substrates, while *D. racemosa* has been observed to only produce them on much more heavily decayed substrates (Allen, pers. obs). Since sclerotia of both species have been observed at the same time on *R. crassotunicata*, it is possible that *D. racemosa* requires a longer incubation period or more substrate preparation for sporocarp production than *C. tuberosa*.

Dendrocollybia racemosa was recently removed by Hughes et al. (2001) from the genus *Collybia* based on ITS evidence that suggests its phylogenetic placement outside the *Collybia* clade; the placement of our *D. racemosa* sequence (Fig. 2) is consistent with this conclusion. The *Collybia* clade includes the other mycosaprobic species; *Collybia tuberosa* (Bull.:Fr.) Kummer, *C. cookei* (Bres.) Arnold, and *C. cirrhata* (Pers.) Quélet. In addition to molecular differences, *D. racemosa* is easily distinguished morphologically by its formation of short conidia-producing outgrowths along the length of its stipe (Fig. 1D). It can also be distinguished by its sclerotia, which are blackish and irregularly globose. In comparison, *C. tuberosa* forms reddish brown, ellipsoid sclerotia, *C. cookei* forms ochraceous, irregularly shaped sclerotia, and *C. cirrhata* does not produce sclerotia.

Dendrocollybia racemosa is listed as a threatened Survey and Manage species by the Oregon Natural History Information Center (http://oregonstate.edu/ornhic/index.html) but its actual rarity is unclear. The small size and ephemeral nature of the fruiting bodies may be implicated in the dearth of *D. racemosa* collections deposited in herbaria. Definitive identification of host species could be useful for assessing the current status of *D. racemosa* populations and for the development of future conservation and management strategies.

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Table 1. NCBI Accession numbers of species used in phylogenetic analysis. The sequence generated in this study is shown in boldface.

GenBank Accession No.	**Species**
AF357063	*Clitocybe nebularis*
AF274380	*Collybia cirrhata*
AF274381	*Collybia cirrhata*
AF274382	*Collybia cirrhata*
AF065120	*Collybia cookei*
AF065122	*Dendrocollybia racemosa*
AF065123	*Collybia cookei*
AF274374	Dendrocollybia *racemosa*
AF274375	*Dendrocollybia racemosa*
AF274383	*Collybia cookiei*
DQ644556	***Dendrocollybia racemosa***
AF065121	*Collybia tuberosa*
AF065124	*Collybia tuberosa*
AF274376	*Collybia tuberosa*
AF274377	*Collybia tuberosa*
AF274378	*Collybia tuberosa*
AF274379	*Collybia tuberosa*
AY265850	*Hypsizygus ulmarius*
AY750166	*Tricholoma atroviolaceum*

Figure 1. 1A: Rubbery pellicle of *Russula crassotunicata* peeling away from the pileal context; 1B: *R. crassotunicata* at Shadow Lake Bog; 1C: Black, irregular globose sclerotia of *Dendrocollybia racemosa* in gills of decomposing *R. crassotunicata*; 1D: *D. racemosa* (photo by Sara Clark).

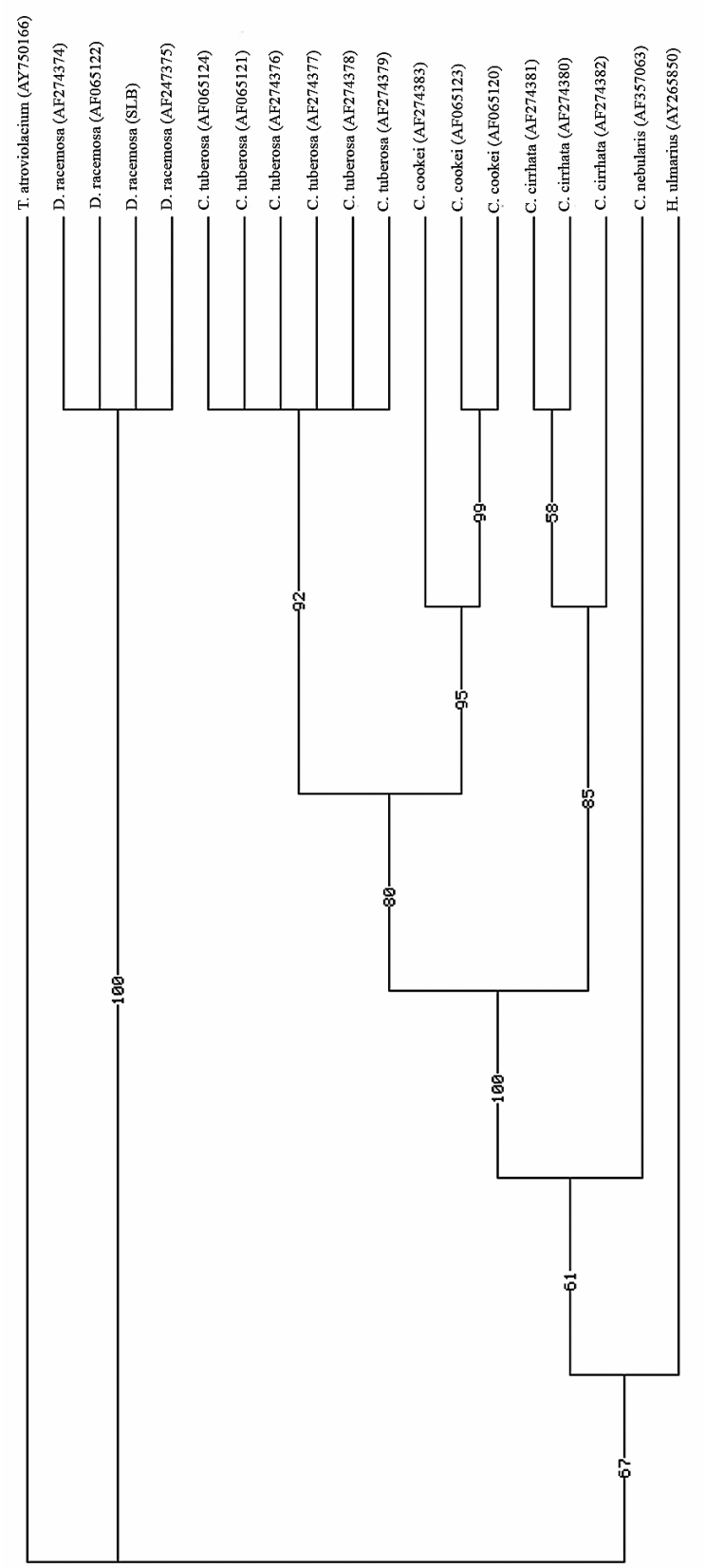

Figure 2. A branch and bound search using maximum parsimony criteria found 36 equally parsimonious trees. A consensus tree is shown here. Numbers on the branches represent node support for 1,000 bootstrap replicates.