

## FINAL REPORT

### Orchids and their mycorrhizal fungi at Cove Point (Calvert County, Maryland)

Melissa McCormick and Dennis Whigham,

Smithsonian Environmental Research Center, 647 Contees Wharf Rd., Edgewater, Maryland 21037.

December, 2004

#### Objectives

The objectives of this project were:

1. Conduct an inventory of fungi that form mycorrhizae with orchids at the Cove Point site.
2. Compare the fungi found at Cove Point with fungi collected from similar orchid species at the Smithsonian Environmental Research Center and other sites throughout eastern North America.
3. Conduct experiments using seed packets to determine if the distribution of appropriate orchid-fungi limits the distribution of orchids at Cove Point.
4. Initiate a monitoring project of Cove Point orchids to evaluate population dynamics.

#### Study Conclusions

**1. Inventory of fungi** – Our first goal was to locate populations of seven orchid species (*Cypripedium acaule*, *Galearis spectabilis*, *Goodyera pubescens*, *Isotria verticillata*, *Platanthera clavellata*, *Spiranthes vernalis*, *Tipularia discolor*) at Cove Point (See Fig. 1 for photos of those species we found). The species had previously been located and identified by Brent Steury and he provided us with a general map showing various locations where he had previously found plants. We visited the Cove Point site five times during 2003 and once during 2004, representing approximately 20 person-days. The main result of our searching in 2003 was frustration as we found few orchids of only two species (*Platanthera clavellata*, *Tipularia discolor*) in enough abundance to make multiple collections of roots. Because we were having difficulty in locating plants during our first collecting trip, we asked Brent to accompany us on the following visit. Brent was able to show us locations for *Galearis spectabilis* (a single plant),

*Tipularia discolor* (a few plants in one location), and *Spiranthes vernalis* (a small numbers of flowering individuals in two locations). Brent also assisted us in better interpreting the locations that he had marked on the map that he provided to us.

To date, we have not had any success in locating the population of *Isotria verticillata* that Brent had previously found. In 2004 we were able to locate 5 individuals of *Goodyera pubescens*. We also located also located ~15 plants of *Cypripedium acaule* on the property. We found separate populations of *Platanthera clavellata* on each of our first four visits to the site and found several additional populations in 2004.

We returned to Cove Point in late-October 2003 with a goal of finding *Tipularia discolor*, a species that produces new leaves in the autumn and are readily noticeable above the leaf litter. We only found, however, small numbers of *Tipularia* in two locations. We found several additional but widely scattered *T. discolor* plants during our 2004 visits. We had planned to sample *Spiranthes vernalis* in the autumn of 2003 and again in spring 2004 but we could not relocate any of the plants that Brent had shown us because the areas had been mown.

Based on our conversations with Brent and based on our experiences with many of the species that we are looking for, we believe that the orchids were more common at Cove Point in the past than they are now. The small number of individuals and species that we have found to date could be due to several factors. The area suffered two years of drought conditions and many of the species at Cove Point, especially species that only occur on the drier upland sandy soils, may have suffered from drought stress, something we have also observed at SERC. A second factor in the decline of the orchids at Cove Point may be a continuous high level of deer herbivory.

Despite additional attempts to find *Isotria* and *Galearis*, we were unable to locate any plants. *Isotria* appears very similar to *Medeola virginica*, a very common species at Cove Point, making it very difficult to locate. Brent Steury was also unable to locate the initial population. This species may have died out at Cove Point but it also may simply be that someone will stumble upon it again one day.

Table 1 shows the results of our sampling to date. We sampled 20 individuals of *Platanthera* in five separate areas. The plants were returned to SERC and we found fungi in 13 plants. Isolated fungi have grown in culture for three individuals and we have saved the fungi from the others (those indicated as no growth in Table 1). We were able to amplify and sequence fungal sequences from 7 *P. clavellata* plants. All fungi from these plants belonged to the genus *Tulasnella* but belonged to several distantly related clades (groups) within the genus. This indicates that these plants can associate with a wide variety of fungi, at least as adults. In two instances (Isolates 516 and 609) we were able to successfully isolate and grow three fungi. For plant 516, two of the fungi that are in culture appear very similar and one (516-3) is different. This makes very clear that *P. clavellata* plants can associate

with multiple fungi, something that was not previously known. All three fungi from plant 609 are similar in culture. We attempted sequencing of fungi from the remaining 13 plants (in some cases, multiple times) but they yielded poor quality sequences, most likely due to the presence of multiple fungi. We have rarely found such high rates of association with multiple fungi and have not found it in any other species of *Platanthera*. Identification of all fungi associated with these plants will require DNA cloning, something we are currently not set up to do, but may be in the future. Due to the low success in sequencing fungi directly from *P. clavellata* roots we were unable to clearly determine whether plants from different populations associated with different fungi but it was clear that plants within a single population associated with multiple different fungi. Fungi 604, 607, 609, and 610 all came from a single population but span three distantly related groups of *Tulasnella*.

We removed roots from 5 individuals of *Tipularia discolor* growing in two different locations. Roots of two plants (626 and 627) did not contain any fungi and fungi removed from plants 624 and 625 have not grown. We successfully sequenced DNA from 2 *T. discolor* plants (623 and 624). These fungi both belonged to the genus *Tulasnella*. We attempted sequencing from the remaining 3 plants but they yielded poor quality sequences, most likely due to the presence of multiple fungi (as we have found in other *T. discolor* plants). As for *P. clavellata*, resolution of all fungi associated with these plants will require DNA cloning.

We sampled a single root from one individual of *Goodyera pubescens*. We obtained 2 isolates from this plant, both of which are alive and growing. DNA analysis revealed that the fungus from this plant was typical of the *Goodyera* that we have previously sampled from throughout its range. We also removed roots from three individuals of *Cypripedium acaule* but fungi from this species are well known to be unculturable (although they are not yet identified) so we did not attempt to culture them. We did extract and amplify DNA but it was very clear from gels run on the PCR product that multiple fungi were present. As for *P. clavellata* and *T. discolor*, the presence of multiple fungi means that identification of these fungi will require DNA cloning.

**2. Comparison of Cove Point with fungi isolated from the same species in other locations** – We found that the fungus obtained from *Goodyera* at Cove Point was very similar to those we have obtained at SERC and elsewhere (see Fig. 2), a currently undescribed, but likely saprotrophic, species of *Tulasnella* (see Fig. 3). Fungi obtained from Cove Point *T. discolor* fall into two groups of *Tulasnella*, one that we have previously isolated from *T. discolor* and one that we have not previously found in orchids. The fungi in both these groups are likely ectomycorrhizal fungi (see Fig. 3), which depend on the presence of ectomycorrhizal trees (mostly oak, beech, hickory, and pine in this area) for their nutrition.

Fungi from *P. clavellata* at Cove Point belonged to four groups of *Tulasnella*. One of these groups included 600 and 604 and is made up of saprotrophic fungi that are closely related to those isolated from *G. pubescens*. Fungi within this group have also been found in *P. clavellata* in Virginia and West Virginia. Two other groups included 607 + 610 and 609 and are likely ectomycorrhizal fungi. These are closely related to fungi from *T. discolor* at other locations but have not previously been found in *P. clavellata*. An additional fungus, 516-3, is not closely related to any other fungi we have obtained and is of unknown ecology. *P. clavellata* associated with a diversity of fungi at each of the sites we sampled, suggesting that presence of adult plants is most likely limited by conditions, not by the presence of appropriate fungi. The same impression might be obtained from *T. discolor* adults, which also associate with a wide range of fungi. However, germination of *T. discolor* seeds depends on both the appropriate environmental conditions (decomposing wood of an appropriate stage; Rasmussen and Whigham 1998) and presence of a very specific group of fungi (McCormick et al. 2004). The same might be true for *P. clavellata* but we were unable to conduct the seed packet experiments we would need to determine this (see below).

**3. Seed packet experiments** – We employ seed packets to conduct germination tests and also to obtain protocorms (small individuals between the stage when a seed germinates and a seedling appears above ground) that can be sampled for fungi. In 2003, we only encountered one *Platanthera* that had fruits and two individuals of *Tipularia*. We saw several flowering individuals of *Spiranthes* when Brent showed us the locations of the plants in the late-spring. When we returned in the autumn to collect seeds, however, the inflorescences were completely gone due to mowing. We believe that it is not appropriate to collect fruits and seeds when there are so few reproductive individuals. In 2004, we visited Cove Point during *Platanthera* and *Tipularia* flowering times but had little luck locating flowering plants. Failure to locate seeds prevented us from conducting a seed packet study.

**4. Population monitoring** - We have not initiated any monitoring activities because of the general lack of success in locating populations of the seven orchid species. If our initial impressions are correct, however, it is important that a strategy be developed to monitor and, if necessary, protect the orchids at Cove Point. We are interested and willing to assist in this effort but we believe that it first requires a discussion with individuals who are responsible for the site. If deer browsing is resulting in a depletion of understory herbs and a failure of tree regeneration, those responsible for the conservation areas need to determine if they will be proactive in maintaining and restoring species at the site. Given the sensitivity of the site, a strategy that is likely to work will involve protecting plants by excluding deer. If time allows and it is acceptable to the Cove Point Natural Heritage Trust, we

would like to institute a population monitoring program for *P. clavellata* at Cove Point. We now know where several populations are and these populations occur in a relatively large range of conditions. It would be very interesting to monitor changes in populations growing in different environments over time. If we are successful in locating flowering plants, which we are hopeful will occur after this year's more favorable climatic conditions, we would also like to incorporate seed packets into this study.

### **References**

McCormick, M.K., D.F. Whigham, J.P. O'Neill. 2004. Mycorrhizal diversity in photosynthetic terrestrial orchids. *New Phytologist* **163**: 425-438.

Rasmussen, H.N., and D.F. Whigham. 1998. Importance of woody debris in seed germination of *Tipularia discolor* (Orchidaceae). *American Journal of Botany* **85**(6): 829-834.

| Isolate | Orchid species                | Status of fungus | Source of fungus | Where collected        | State | Isolation date |
|---------|-------------------------------|------------------|------------------|------------------------|-------|----------------|
| 516-1   | <i>Platanthera clavellata</i> | alive            | adult            | Cove Point, MD         | MD    | 6Jun03         |
| 516-2   | <i>Platanthera clavellata</i> | alive            | adult            | Cove Point, MD         | MD    | 6Jun03         |
| 516-3   | <i>Platanthera clavellata</i> | alive            | adult            | Cove Point, MD         | MD    | 6Jun03         |
| 597-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 1 | MD    | 26Sep03        |
| 598-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 1 | MD    | 26Sep03        |
| 599-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 1 | MD    | 26Sep03        |
| 600-1   | <i>Platanthera clavellata</i> | alive            | adult            | Cove Point, location 1 | MD    | 26Sep03        |
| 601-1   | <i>Platanthera clavellata</i> | no pelotons      | adult            | Cove Point, location 2 | MD    | 27Sep03        |
| 602-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 2 | MD    | 28Sep03        |
| 603-1   | <i>Platanthera clavellata</i> | no pelotons      | adult            | Cove Point, location 2 | MD    | 28Sep03        |
| 604-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 3 | MD    | 28Sep03        |
| 605-1   | <i>Platanthera clavellata</i> | no pelotons      | adult            | Cove Point, location 3 | MD    | 28Sep03        |
| 606-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 3 | MD    | 28Sep03        |
| 607-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 3 | MD    | 29Sep03        |
| 608-1   | <i>Platanthera clavellata</i> | no pelotons      | adult            | Cove Point, location 3 | MD    | 29Sep03        |
| 609-1   | <i>Platanthera clavellata</i> | alive            | adult            | Cove Point, location 3 | MD    | 29Sep03        |
| 609-2   | <i>Platanthera clavellata</i> | alive            | adult            | Cove Point, location 3 | MD    | 29Sep03        |
| 609-3   | <i>Platanthera clavellata</i> | alive            | adult            | Cove Point, location 3 | MD    | 29Sep03        |
| 610-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 3 | MD    | 29Sep03        |
| 611-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 3 | MD    | 30Sep03        |
| 612-1   | <i>Platanthera clavellata</i> | no growth        | adult            | Cove Point, location 3 | MD    | 30Sep03        |
| 613-1   | <i>Platanthera clavellata</i> | no pelotons      | adult            | Cove Point, location 3 | MD    | 30Sep03        |
| 634     | <i>Cypripedium acaule</i>     | NA               | adult            | Cove Point             | MD    | 10May04        |
| 635     | <i>Cypripedium acaule</i>     | NA               | adult            | Cove Point             | MD    | 10May04        |
| 636     | <i>Cypripedium acaule</i>     | NA               | adult            | Cove Point             | MD    | 10May04        |
| 623-1   | <i>Tipularia discolor</i>     | no growth        | adult            | Cove Point             | MD    | 17Nov03        |
| 624-1   | <i>Tipularia discolor</i>     | no growth        | adult            | Cove Point             | MD    | 17Nov03        |
| 625-1   | <i>Tipularia discolor</i>     | alive            | adult            | Cove Point             | MD    | 17Nov03        |
| 625-2   | <i>Tipularia discolor</i>     | alive            | adult            | Cove Point             | MD    | 17Nov03        |
| 625-3   | <i>Tipularia discolor</i>     | alive            | adult            | Cove Point             | MD    | 17Nov03        |
| 626-1   | <i>Tipularia discolor</i>     | no pelotons      | adult            | Cove Point             | MD    | 17Nov03        |
| 627-1   | <i>Tipularia discolor</i>     | no pelotons      | adult            | Cove Point             | MD    | 17Nov03        |
| 633-1   | <i>Goodyera pubescens</i>     | alive            | adult            | Cove Point             | MD    | 10May04        |

Table 1. Status of fungi collected from Cove Point orchids in the spring, summer, and autumn of 2004. Column 1 is the SERC designation for the fungus collected from the orchid. Column 2 lists the orchid from which the fungus came. Columns 2 and 4 indicate the condition of the fungus as of December 2003 and the source of the fungus (i.e. whether it was collected from an adult plant or a protocorm).

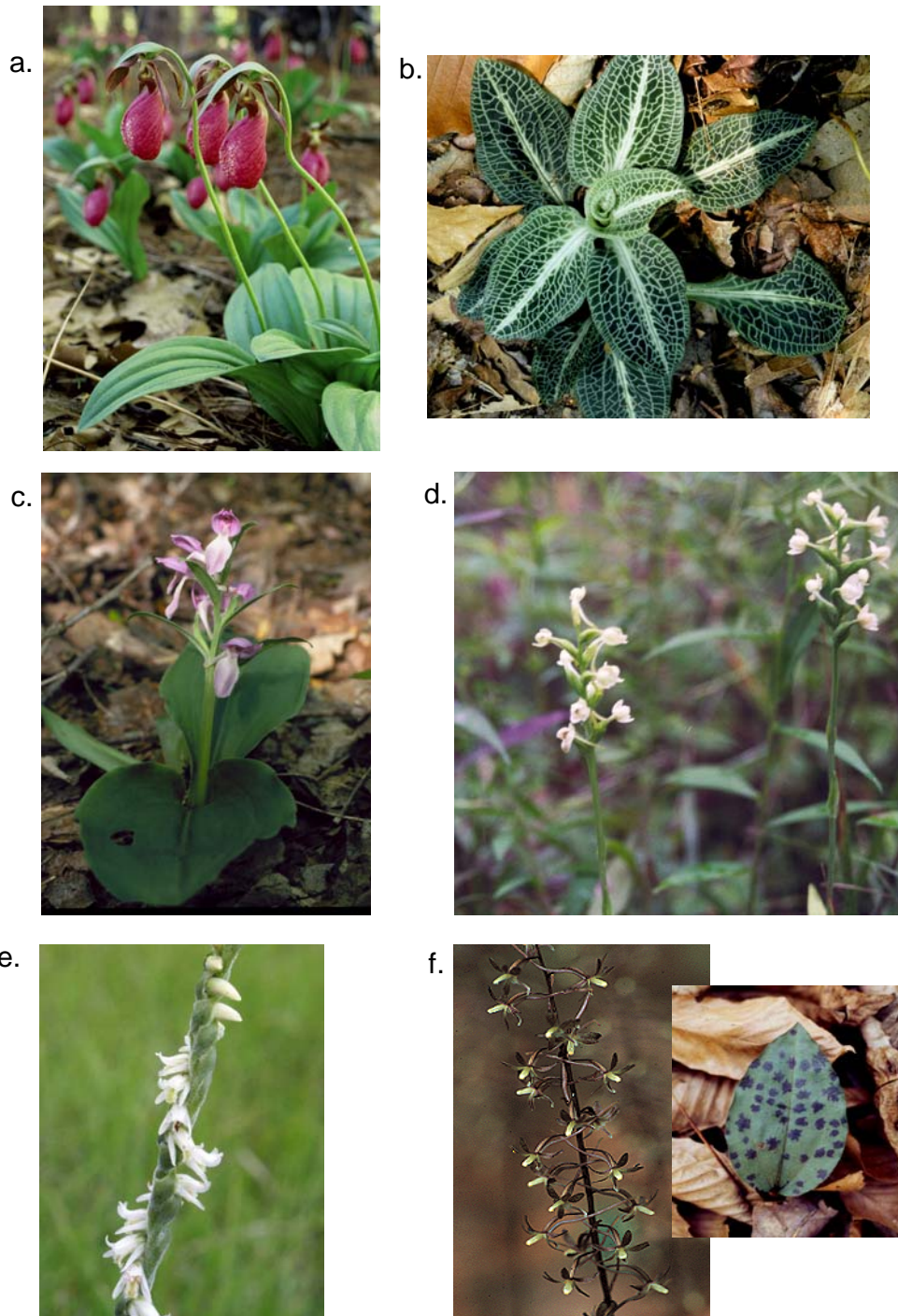


Figure 1: Orchids found at Cove Point. Approximately 15 individuals of **a.** *Cypripedium acaule* were found. Five plants of **b.** *Goodyera pubescens* were found. Only one plant of **c.** *Galearis spectabilis* was found. The most abundant orchid was **d.** *Platanthera clavellata*. A few plants of **e.** *Spiranthes vernalis* (photo property of Paul L. Redfearn Jr., Southwest Missouri State Univ. Herbarium) were found but mowing prevented subsequent sampling. A few widely scattered **e.** *Tipularia discolor* plants were found. Photos a and c are property of Melissa McCormick. Photos b, d, and f are property of The Smithsonian Institution.

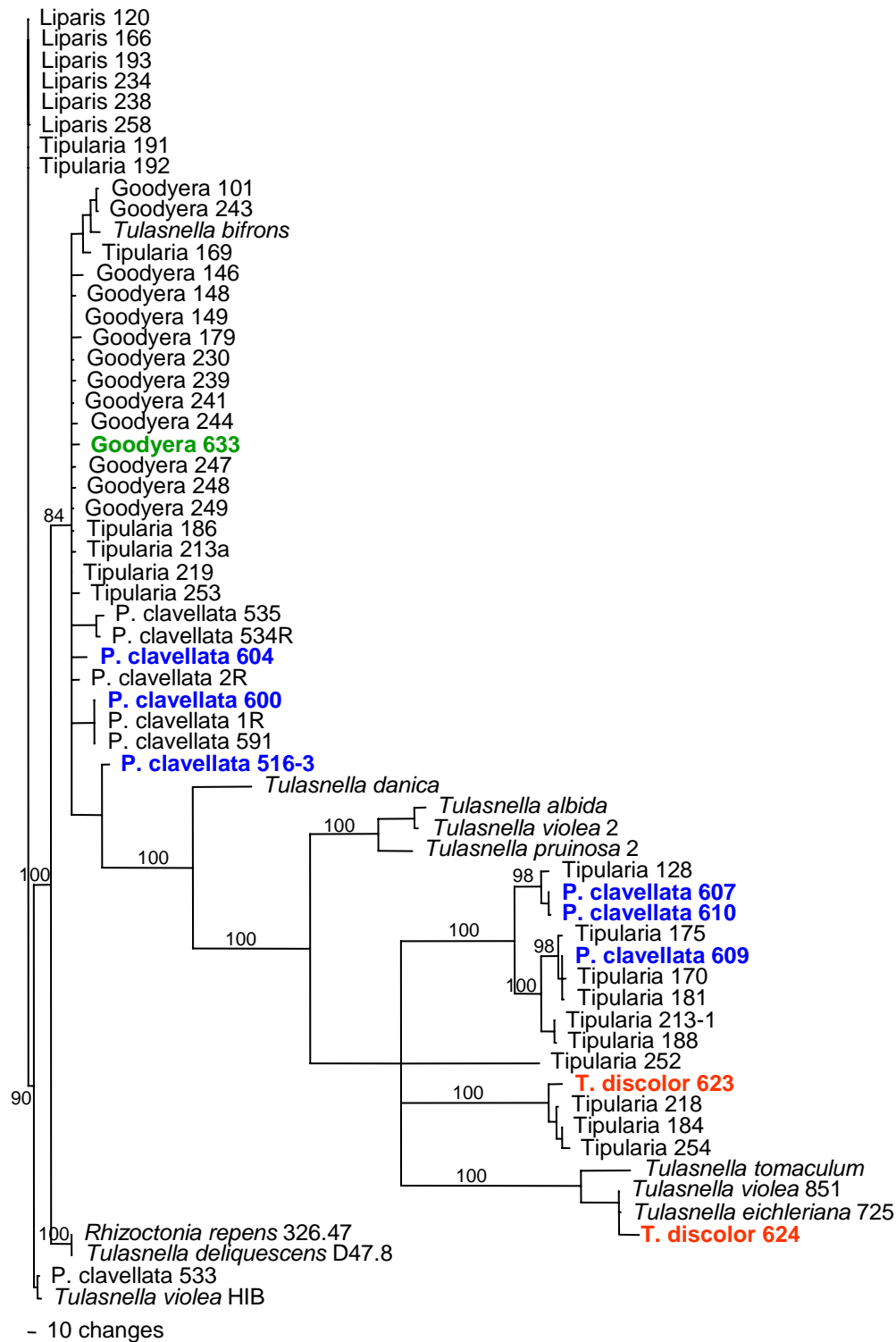


Figure 2: Phylogenetic tree generated from nrITS sequences showing relationships between fungi sampled from orchids at Cove Point and those previously sampled from those species at other sites. Sequences of fungi from some other orchid species are included for reference. Samples from Cove Point orchids are shown in bold and in color. Sequences of reference *Tulasnella* fungi (McCormick et al. 2004) are also included.



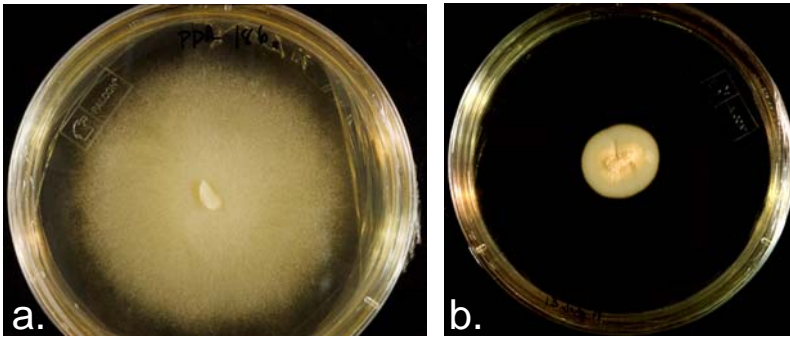


Figure 3: Typical fungi isolated from orchids. Fungus **a.** is typical of saprotrophic fungi isolated from *Goodyera pubescens*. Fungus is shown after 2 weeks of growth on potato dextrose agar growing medium. Fungus **b.** is typical of ectomycorrhizal fungi isolated from *Tipularia discolor*. Fungus is shown after 7 weeks of growth on potato dextrose agar growing medium.