STUDY ON ECTOMYCORRHIZAE OF SIBERIAN PINE (*PINUS SIBIRICA*)

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ABSTRACT

The ectomycorrhizal fungal community associated with Pinus Sibirica (Siberian Pine) in a Mongolian forest was investigated in this study. The ectomycorrhizaes were isolated from roots of Siberian pine and identified as Russulia Sardonia, Rhodocollybia butyracea, Tuber borchii, Tricholloma auratum and Lactarius deliciosus. Hartig net, external hyphae and mantle structures of ectomycorrhizae were observed in Siberian pine roots.

KEY WORDS: *Pinus Sibirica*, ectomycorrhizae, Hartig net, mantle

INTRODUCTION

Siberian pine (*Pinus sibirica*) is an important tree species for the timber products in Mongolia. But there is not so much specific research for the Siberian pine. The basic material of Siberian pine was developed from the research and survey for Mongolian coniferous tree by the "Biological expedition of Mongolia and Russia".

Ectomycorrhizal fungi are functionally important in temperate forest ecosystems and play influential roles in forest community dynamics (Smith and Read 1997). Ectomycorrhizal associations lead to morphological changes and a complex development of specific structures in both plants and fungi (Martin et al. 2001). Ectomycorrhizae are characterized by 3 structural components, a mantle or fungal sheath that encloses the root, a Hartig net and extra-radical mycelia, which extend into the soil surrounding the root and are responsible for water and nutrient uptake (Smith and Read 1997). Trees with ectomycorrhizae are more tolerant of environmental and biotic stresses (Smith and Read 2008). However, the diversity and protective effects of mycorrhizae on trees have not yet been studied in Mongolia.

The aims of this study were to isolate and identify the ectomycorrrhizae on Siberian pine by morphological and anatomical structure.

MATERIALS AND METHODS

Sample collection

Root samples of Siberian pine and the rhizosphere soil were collected from East Khentii Mountain range in Mandal soum, Selenge aimag, in August 2013. Feeder roots, soil, and root samples were collected from 0 to 20 cm in depth from 8 individual plants, and stored at 5~10℃ in polyethylene bags until being analyzed.

Morphology of ectomycorrhiza

Siberian pine roots were cleaned with water in a supersonic oscillator (Upson et al. 2007). The morphology of mycorrhiza was observed under a stereomicroscope (Usuki and Narisawa 2005).

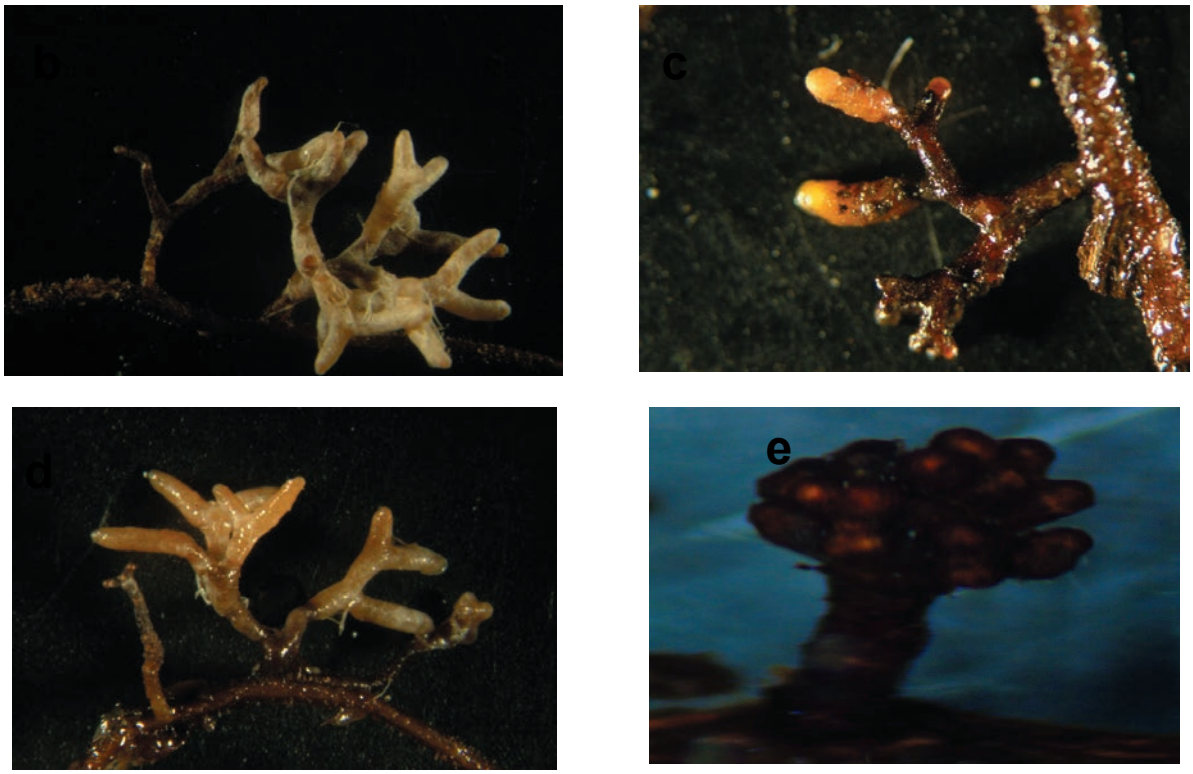
For light microscopy, pine roots were cut into small pieces, fixed overnight in formalin: acetic acid: alcohol, 5: 5: 50, v/v (FAA) then rinsed with distilled water 3 times and dehydrated in 70% ethanol and a series of concentrations (20, 35, 55, 75, and 100%) of TBA. Specimens were embedded in paraffin wax (with a melting point of 56℃). Transverse sections 10~12 μm thick were cut with a rotary microtome. Paraffin was removed with xylol, and sections were stained with safranin and Fast green (Ruzin 1999).

For the ultrastructural study, root samples were fixed with 2.5% glutaraldehyde and 4% paraformaldehyde fixative in a phosphatebuffered solution (PBS, 0.1 M, pH 7.0) for 4 h at room temperature, then rinsed with PBS 3 times, each time for 15 min, followed by serial dehydration in 30, 50, 70, 80, 95, and 100% ethanol and 100% acetone. Finally, samples were dried in a critical-point dryer using liquid carbon dioxide. Dried materials were mounted on an aluminum stub with adhesives, coated with gold, and observed with a scanning electron microscope (SEM, Hitachi S-3500N) (Brundrett et al. 1996).

Morphological anatomical characteristics of the ectomycorrhiza were observed and described with reference to the key provided by DEEMY (An Information System for Characterization and Determination of Ectomycorrhizae, homepage: http://www.deemy.de/) (Agerer 1991).

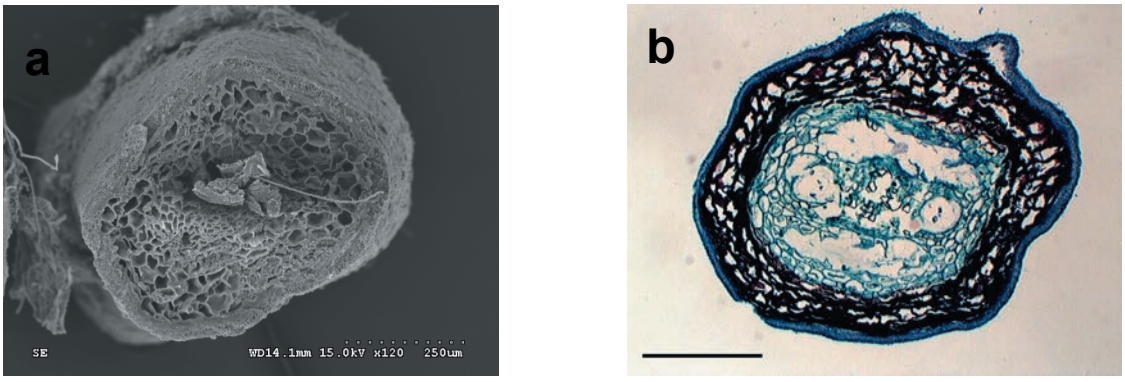
RESULTS AND DISCUSSION

At sampling sites, we found ECM associations with Siberian pine (Pic.1). Ectomycorrhizas were extracted from the surface soil horizons of Siberian pine forests. The number of mycorrhizas of each ECM fungal species was recorded using morphotyping combined with anatomical structure.We determined 5 species of the ectomycorrhizae on Siberian Pine which were *Russulia Sardonia, Rhodocollybia butyracea, Tuber borchii, Tricholloma auratum* and *Lactarius deliciosus* (Pic. 3, 5, 7, 9 and 11)**.**

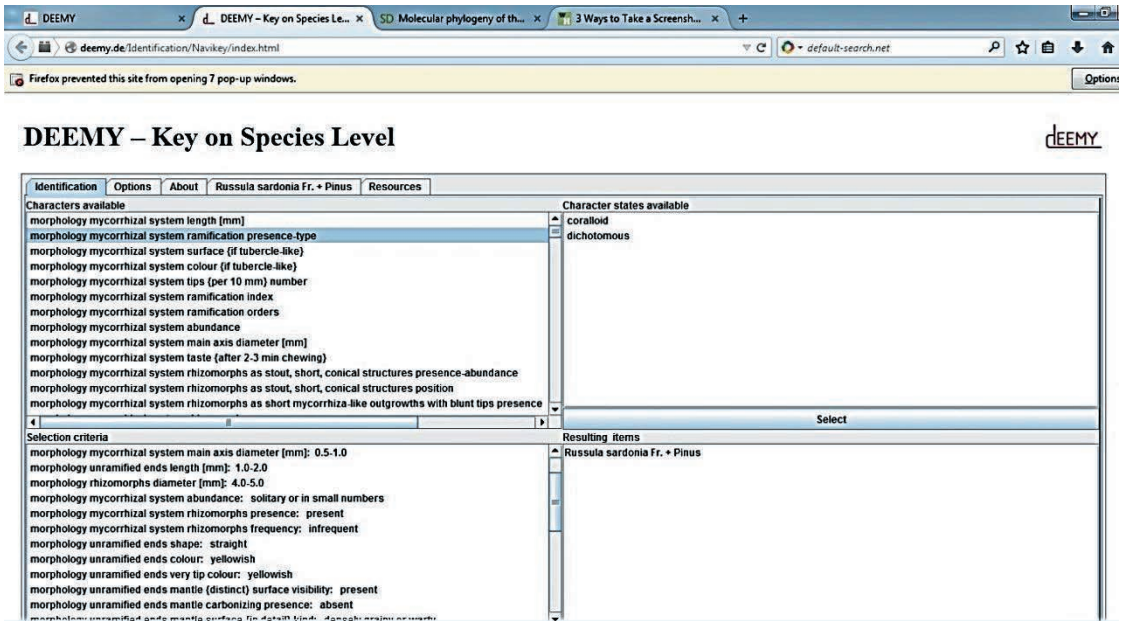


Picture 1. Morphological structures of ectomycorrhizaes of Siberian pine
a. *Russulia Sardonia Fr. + Pin;* **b.** *Rhodocollybia butyracea + Pin;* **c.** *Tuber borchii Vitt + Pin;* **d.** *Tricholloma auratum (Paul: Fr.) + Pin;* **e.** *Lactarius deliciosus L.: Fr. + Pin.*

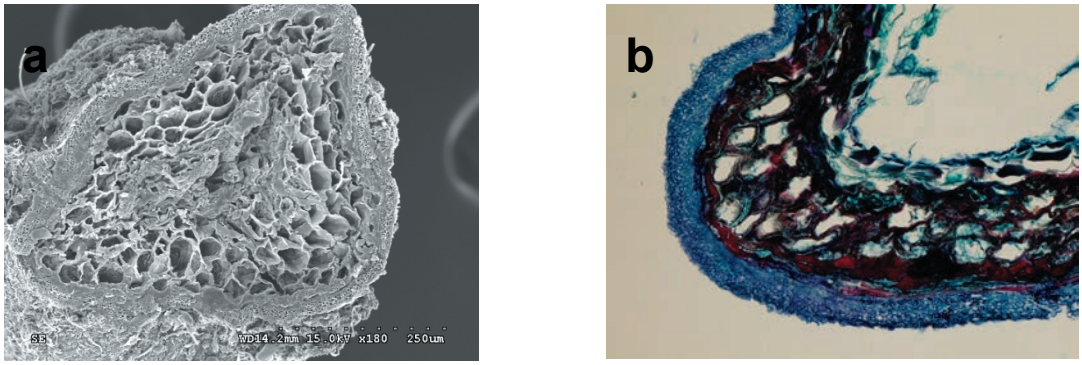
ECM morphotypes were orange and yellow colored (Pic.1). Mycorrhizal root tips were dichotomous coralloid and long, and the dimensions of the last-order axis tips ranged 0.3~0.9 mm in length (Pic.1a-e). A cross-section of pine root tips revealed ectomycorrhizal features with a thick mantle and distinct Hartig net surrounding the epidermal and cortical cell layers (Pic. 2, 4, 6, 8). Morphological aspects of the ectomycorrhizal roots were studied in a number of plant-fungus combinations and recently reviewed by Smith and Read (1997). Thorough characterization of ectomycorrhizal species by detailed morphological and anatomical descriptions (Miller et al. 1991; Burenjargal and Lee 2012) and identification of their fungal partners (Agerer 1991) is a prerequisite for recognizing mycorrhizal diversity in ecosystems and for comparison of data obtained by other researchers.



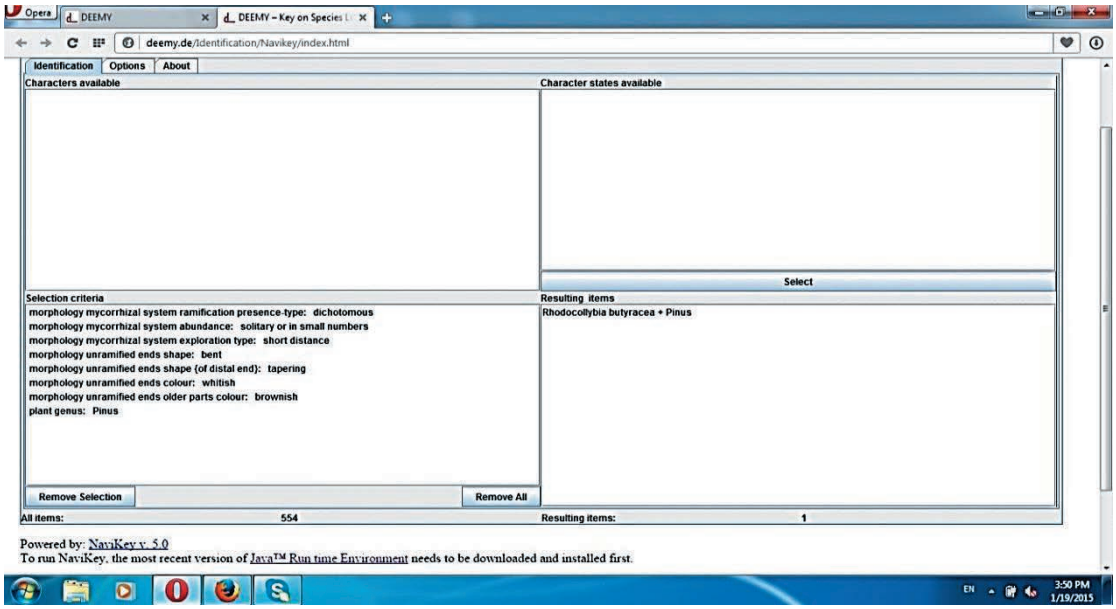
Picture 2. Anatomical structure of *Russulia Sardonia Fr. + Pin*



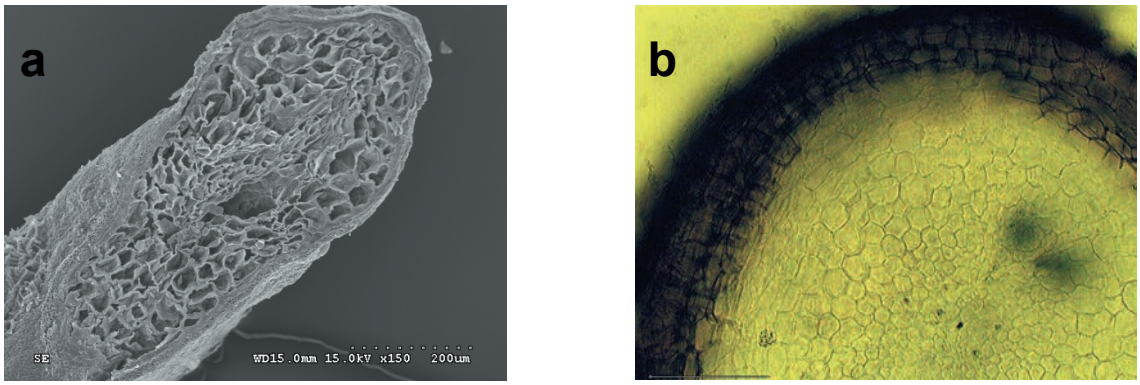
Picture 3. Determination of *Russulia Sardonia Fr. + Pin* – using anatomical and morphological structure by DEEMY.DE



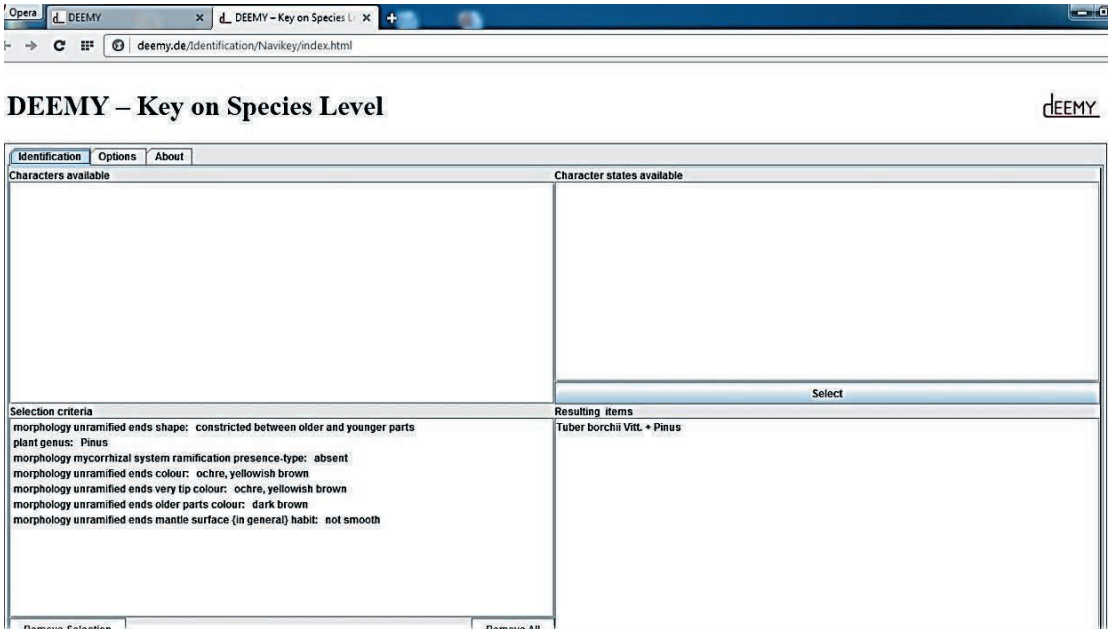
Picture 4. Anatomical structure of *Rhodocollybia butyracea + Pin*



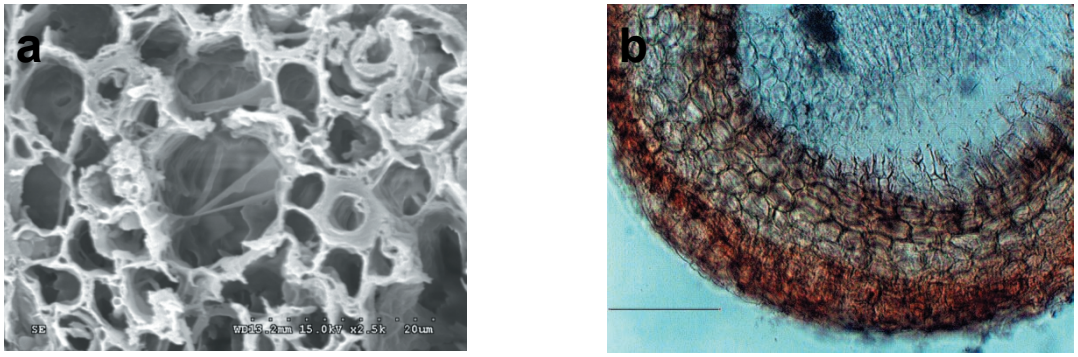
Picture 5. Determination of *Rhodocollybia butyracea + Pin* – using anatomical and morphological structure by DEEMY.DE



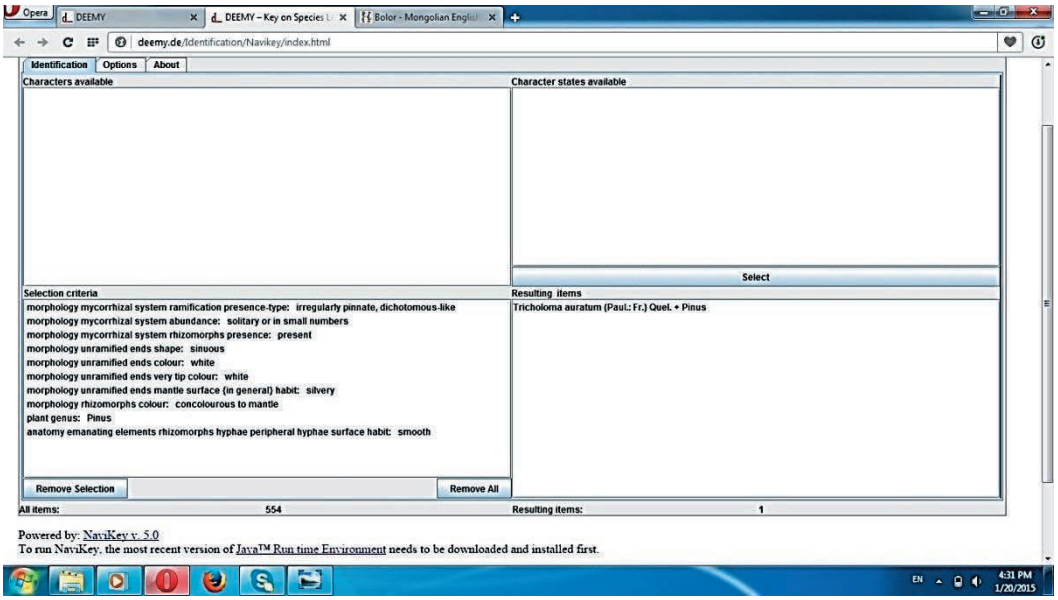
Picture 6. Anatomical structure of *Tuber borchii Vitt + Pin*



Picture 7. Determination of *Tuber borchii Vitt + Pin* using anatomical and morphological structure by DEEMY.DE

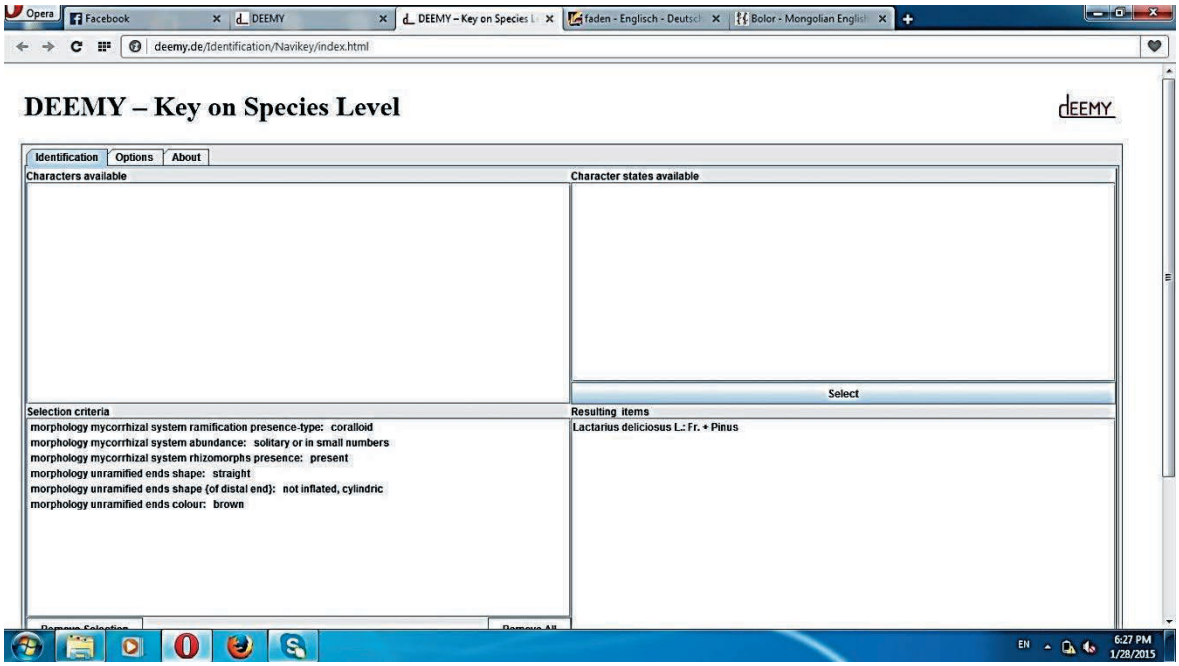


Picture 8. Anatomical structure of *Tricholloma auratum (Paul: Fr.) + Pin*



Picture 9. Determination of *Tricholloma auratum (Paul: Fr.) + Pin* using anatomical and morphological structure by DEEMY.DE

Picture 10. Morphological structure of *Lactarius deliciosus L.: Fr. + Pin*



Picture 11. Determination of *Lactarius deliciosus L.: Fr. + Pin* using anatomical and morphological structure by DEEMY.DE

CONCLUSION

1. In this study, ectomycorrhizaes were sampled from roots of Siberian pine and identified *Russulia Sardonia Fr. + Pin* (figure 1a), *Rhodocollybia butyracea + Pin* (figure 1b), *Tuber borchii Vitt + Pin* (figure 1c), *Tricholloma auratum (Paul: Fr.) + Pin* (figure 1d) *Lactarius deliciosus L.: Fr. + Pin* (figure 1f)].
2. Morphological and anatomical experiments revealed that Hartig net, external hyphae and mantle structure of ectomycorrhizaes were observed in cortical cells of Siberian pine roots.

REFERENCES

1. Agerer, R. 1991. Characterization of ectomycorrhiza. In *Techniques for the study of mycorrhiza*, 25-73. London: Academic,
2. Brundrett MC., Bougher N., Dell B., Grove T., Malajczuk N. 1996. *Working with mycorrhizas in Forestry and Agriculture.* Australian Centre for International Agricultural Research Monograph.
3. Burenjargal O, Ming-Jen Lee. 2012. *Pinus sylvestris* can form ectomycorrhiza with *Phialocephala fortinii*. Taiwan Journal of Forest Science 27(3):265-81.
4. Miller SL, Koo CD, and Molina R. 1991 Characterization of red alder ectomycorrhizae: a preface to monitoring belowground ecological responses. Canadian Journal of Botany 69:516-31.
5. Read SE., Smith DJ. 1997. *Mycorrhizal symbiosis*. London: Academic Press
6. Trappe, J. M. 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. *Ecophysiology of VA Mycorrhizal Plants, G. R. Safir (EDS)* (CRC Press)
7. Usuki F, Narisawa K. 2005. Formation of structures resembling ericoid mycorrhizas by the root endophytic fungus *Heteroconium chaetospora* within roots of *Rhododendron obtusum* var. *kaempferi*. Mycorrhiza 15:61-4.