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A survey of *Russula* species (Fungi, Basidiomycota, Agaricomycetes) at the Boston Harbor Islands National Park area, Massachusetts

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How many fungi are really out there? Entire groups of fungi remain uncharacterized and understudied (Blackwell 2011). Of the estimated 5.1 million species of fungi, only 100,000 species are described and more importantly there are groups for which even the most basic phylogenetic work is lacking. It is a fact that recent molecular innovations are changing our traditional views on Basidiomycota. Generally, however, taxonomic implications of molecular data should only be put in place after careful examination of specimens and after a thorough search for morphological and/or ecological evidence and confirmation (e.g. Verbeken & Nuytinck 2013).

As for the order Russulales, the large genera *Russula* Pers. and *Lactarius* Pers. (milk caps) are very different from other mushrooms in the class Agaricomycetes and form their own order. Recent molecular insights show that these two genera should be considered in a "new generic landscape." For example, the genus *Multifurca* Buyck & Hofstetter includes some "old" *Russula* and *Lactarius* species (Buyck et al. 2008).

Main features for mushrooms in the genus *Russula* are a white stem, white gills, and an often brightly colored cap (or pileus). The context of these mushrooms is typically very brittle. Ecologically, mushrooms in the genus *Russula* are important as ectomycorrhizal fungi. Ectomycorrhizal hyphal networks help in water and nutrient uptake, protection against pathogens, and enzyme production. In return, the plant symbiont provides carbohydrates to the fungus. High ectomycorrhizal diversity is important in the healthy performance of a woodland (e.g. Horton et al. 2013). Second, studies of mycophagy have shown that *Russula* mushrooms serve as ephemeral substrates as food or microhabitat. They are frequently visited by flies (Diptera: Drosophilidae, Sphaeroceridae, Phoridae, Anthomyiidae, Lauxaniidae), wasps (Hymenoptera: Figitidae, Braconidae, Ichneumonidae), and rove beetles (Coleoptera: Staphylinidae) (Hanley & Goodrich 1995, Wertheim et al. 2000).

Since December 2012, a fungal inventory was conducted at the Boston Harbor Islands National Park Area (BHI). Four sites were targeted: Grape Island, Peddocks Island, Thompson Island, and World's End peninsula. Calf Island, Great Brewster Island, Slate Island, and Webb Memorial State Park were occasionally visited for sampling. This documentation resulted in over 900 collections, of which 313 have been identified to species level, accounting for 172 species (Haelewaters et al. in prep.). For identification, we extracted DNA from rice grain-sized pieces of fruiting bodies and sequenced the internal transcribed spacer (ITS) region of the ribosomal DNA. This ITS region has been suggested as the "barcoding region" for fungi (Schoch et al. 2012) because it is easy to amplify and able to separate intraspecific from interspecific taxa. In addition, for some collections, (micro-) morphological characters were used to determine the species.

The goals of this project are to 1) provide detailed data on species of the genus *Russula* (Fungi, Basidiomycota, Agaricomycetes) at the Boston Harbor Islands National Park area

(BHI); 2) record information about their distribution, habitat, and dates of fruiting; and 3) provide documentation for these species via voucher specimens that will be deposited and available for study at the Farlow Herbarium, Harvard University.

Four field sites, representing most of the park's diversity, have been sampled: World's End, Grape Island, Peddocks Islands, and Thompson Island. The above-ground ephemeral fruiting bodies were collected in brown paper bags. Specimens were assigned a BHI-F collection number, and associated information was noted, including the date, specific locality on the island/peninsula, GPS coordinates (when available), substrate, and surrounding habitat notes. Photographs were taken on site with 1 cm-grid paper as background. Names were tentatively assigned to specimens after initial morphological examination. Collections were preserved by dehydration (Presto Dehydro: 7-9 hours at 35 °C), packaged, labeled, and deposited at the Farlow Herbarium at Harvard University (Cambridge, MA).

DNA from rice-grain sized parts of gills was isolated using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA). The ITS region was amplified and sequenced. The ITS region was recently selected as the fungal barcode at the 97% similarity threshold level (Schoch et al. 2012) and underpins the molecular work for the North American Mycoflora (Bruns 2011). Primers used were ITS1f and ITS4. PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen) and subsequently sequenced making use of the sequencing facility at Harvard University (FAS Center for Systems Biology). Generated sequences were assembled, trimmed, and edited in Sequencher v4.10.1 (Gene Codes Corporation, Ann Arbor, MI). Sequences were identified by comparison to GenBank's nonredundant sequence database using BLAST; a boundary of 97-99% sequence similarity with >80% query coverage was used to name a species as correctly as possible via the ITS.

The genus *Russula* is composed of 750 species (Kirk et al. 2001). 334 species have been reported from the United States (Buyck 2007). Unfortunately, many taxa are poorly known, due to 1) short, incomplete diagnoses of taxa, 2) the lack of extensive studies of the genus *Russula* in North America, and 3) the lack of molecular data (Looney 2014). Because of their interrelationships with woody plants and mycophagous fauna, *Russula* species are important components of woodland ecosystems.

To date, 40 collections of *Russula* have been sampled at BHI, of which 11 are identified to species level, good for 7 species (Table 1). Interestingly, the ITS sequences of a majority of these *Russula* collections are <97% similar to any *Russula* sequence available in NCBI GenBank. This means that we could be dealing with undescribed species or species that have been described but never sequenced. However, through both legacy taxonomic assignment and common misidentification, many sequences from North America are mislabeled as European species. Further study is necessary for definitive identification of BHI collections for which the ITS sequence is <97% similar to any *Russula* sequence, including comparison with types and multi-locus sequencing for phylogenetic placement.

Once complete, this project will provide information on *Russula* mushrooms in woodlands and forests of BHI that will help 1) NPS and ecologists in dealing with these ecologically important fungi (management) and 2) mycologists/taxonomists in a better comprehension of the diversity of this genus.

Table 1: Confirmed species of *Russula* at the Boston Harbor Islands, with number of collections found per field site.

<i>Species</i>	Grape Island	Thompson Island	World's End
<i>Russula mariae</i> Peck		(1)	(2)
<i>Russula modesta</i> Peck		(1)	(1)
<i>Russula mutabilis</i> Murrill		(1)	
<i>Russula pectinatoides</i> Peck			(1)
<i>Russula</i> aff. <i>subsulphurea</i> Murrill		(1)	(1)
<i>Russula ventricosipes</i> Peck	(1)		
<i>Russula vesicatoria</i> Murrill		(1)	

REFERENCES

Blackwell M. 2011. The fungi: 1, 2, 3 ... 5.1 million species? *American Journal of Botany* 98 (3): 426-438.

Bruns T. 2011. Working toward a North American Mycobiota for macrofungi – what's stopping us? *Inoculum* 62: 1-3.

Buyck B. 2007. A new initiative towards the study of *Russula* in the eastern USA. *Pagine di Micologia* 27: 81-86.

Buyck B, Hofstetter V, Eberhardt U, Verbeken A, Kauff F. 2008. Walking the thin line between *Russula* and *Lactarius*: the dilemma of *Russula* sect. *Ochricompectae*. *Fungal Diversity* 28: 15- 40.

Haelewaters D, Kappler LA, Dirks AC, Mitchell JK, Buyck B, Pfister DH. A Preliminary Checklist of Fungi at the Boston Harbor Islands. *Northeastern Naturalist*, *in preparation*.

Hanley RS, Goodrich MA. 1995. Review of mycophagy, host relationships and behavior in the New World Oxyporinae (Coleoptera: Staphylinidae). *The Coleopterists Bulletin* 49 (3): 267-280.

Horton BM, Glen M, Davidson NJ, Ratkowsky D, Close DC, Wardlaw TJ, Mohammed C. 2013. Temperate eucalypt forest decline is linked to altered ectomycorrhizal communities mediated by soil chemistry. *Forest Ecology and Management* 302: 329-337.

Kirk P, Cannon P, David J, Stalpers J, eds. 2001. *Dictionary of the Fungi*. 9th ed. Wallingford: CAB International.

Looney BP. 2014. Molecular annotation of type specimens of *Russula* species described by W.A. Murrill from the southeast United States. *Mycotaxon* 129 (2): 255-268.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* 109 (16): 6241-6246.

Verbeken A, Nuytinck J. 2013. Not every milkcap is a *Lactarius*. *Scripta Botanica Belgica* 51: 162-168.

Wertheim B, Sevenster JG, Eijs IEM, Van Alphen JJM. 2000. Species diversity in a mycophagous insect community: the case of spatial aggregation vs. resource partitioning. *Journal of Animal Ecology* 69 (2): 335-351.