**Investigation of the Russula diversity in Battagram Khyber Pakhtunkhwa Province, Pakistan**

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The genus *Russula* Pers. is one of the most abundant and widely distributed ectomycorrhizal fungal genera (Buyck et al. 2008). However, first studies estimated around 780 species worldwide (Wang et al*.* 2015), the most recent study (Adamčík et al. 2019) estimated more than 2000 worldwide. The genus was erected by Persoon (1796) during studies of macro fungi in northern Europe. With a few exceptions from North America Bills and Miller (1984), Africa Buyck (1997), South America and cosmopolitan treatments such as Singer (1986), most work on *Russula* infra-generic classification has been accomplished in Europe based primarily on European species Sarnari (2005). Information about *Russula* diversity in Asia is quite scattered. *Russula* is represented by at least 132 species from India, most of which have been reported from the subtropical to subalpine Himalayan region and a few from tropical regions (Das et al. 2014). A total of 24 new *Russula* taxa have been described from south western China and the adjacent Himalayan mountain region during 2005-2016 (Li et al. 2016). However, there is a considerable increase of new *Russula* species described from South-East Asia in the recent years. Further, 8 new species were described from the area in 2017 by (Das et al. 2017). The total number of known *Russula* species update in China is about 180. (http://www.indexfungorum.org).

In Pakistan, the genus *Russula* is represented by only 32 species (Jabeen et al. 2017, Sarwar et al. 2019 Adamčík et al. 2019 and Ullah et al. 2019). Additionally, four new species originating from Pakistan were published in single year 2019: *R. shanglaensis* (Ullah et al. 2019), *R. quercus-floribundae* (Crous et al. 2019), *R. aurantioflava* (Adamčík et al. 2019) and *R.swatica* (Sarwar et al. 2019) species. Additionally, the recent surveys of *Russula* species (Razaq et al. 2019) showed the large diversity, which vary amongst the surveyed regions.

Battagram is a district in the Khyber Pakhtunkhwa province of Pakistan. So far, this region was not surveyed properly for diversity of macrofungi yet. It has a total land area of 1301 square kilometers. The average temperature in Battagram is 18.5 °C, while the annual precipitation averages 1218 mm. Even in the driest months, there is a lot of precipitation. November is the driest month with 28 mm of precipitation, while July, the wettest month, has an average precipitation of 229 mm. June is the hottest month of the year with an average temperature of 27.9 °C (Climate-Data.org 2016). The coldest month January has an average temperature of 7.6 °C. District Battagram is located between 34° 33′ and 34° 47′ north latitude and 72° 55′ and 73° 14′ east longitude. Nandiar Khuwar catchment ranges in altitude from 525m at Thakot to 3817m above mean sea level at Malkisar (Haq et al*.* 2010).

**Objective:**

Aim of the proposed project is to perform microscopic identification of the *Russula* collection originating from Battagram and confirm those identifications by molecular analysis. Additionally, all collections recognized as a new species will be properly described with support of multi-locus DNA analysis.

**Methods**

**Sampling and preliminary species identifications**

Our study will be based on specimens collected in the years 2019. All collections are labeled, provided with the necessary macromorphological notes and photos. All of them are putatively identified according to widely-used identification keys Sarnari (1998) and Sarnari (2005).

**Morphological descriptions**

Field characters were described following the standards for the Russula given in (Caboň et al. 2017) and (Adamčík et al. 2019), using a common template and unified terminology. For the each representative specimen, we will provide full morphological descriptions following (Adamčík et al. 2019). Additionally, importance of the morphological characters will be considered according to relevant species group and those will be further measured. Measurements of all characters will be based on 30 replications per each character and observed on at least 3 specimens, if possible.

**Material sequencing**

DNA extractions will be performed using the EZNA Fungal DNA Kit. For DNA amplification three regions will be used: (1) the ITS of the ribosomal DNA as a barcode, (2) partial mitochondrial small subunit ribosomal DNA (mtSSU), and (3) the region between domains six and seven of the nuclear gene encoding the second largest subunit of RNA polymerase II (rpb2).

**Phylogenetic analysis**

Sequences will be edited in the BioEdit version 7.2.5 Hall (2013). Intra-individual polymorphic sites having more than one signal will be marked with NC-IUPAC ambiguity codes. For the final datasets MAFFT on-line alignment software will be used. All single-locus datasets will be concatenated into one multi-loci dataset. Multi-loci dataset will be analysed using Maximum Likelihood and Bayesian inference analysed.

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