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# A king amongst dwarfs: *Boletus edulis* forms ectomycorrhiza with dwarf willow in the Swiss Alps

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## Abstract

The ectomycorrhizal fungus *Boletus edulis*, commonly known as king bolete, Steinpilz, porcini or cep, is one of the most popular edible mushrooms in Europe, North America and Asia. To produce fruiting bodies, it usually relies on the symbiotic association with deciduous or coniferous trees. Here, we report on an exceptional finding of *B. edulis* at an altitude of 2440 m a.s.l. in the Swiss Alps and document for the first time its ectomycorrhizal association with *Salix herbacea* (dwarf willow) based on molecular markers and microscopic observations.

**Keywords** Ectomycorrhiza · Host shift · *Boletus edulis* · *Salix herbacea* · Swiss Alps

## Introduction

Mycorrhizae, the association of fungi with plant roots, are one of the most common and wide-spread symbioses (Allen 1991). They are considered especially important in ecosystems where nutrients are scarce and environmental conditions are extreme, such as alpine habitats (Peintner and Kuhner 2010). All ectomycorrhizal fungi are strictly dependent on their host plants to produce fruiting bodies, and many plants rely on their fungal partner to thrive. However, different species display different degrees of specialization (den Bakker et al. 2004). *Salix herbacea* L. (Salicaceae), a common woody plant of alpine habitats, is known to form ectomycorrhizal symbioses with numerous species of basidiomycetes (Mühlmann and Peintner 2008). It is an arctic-alpine dwarf shrub that mostly spreads below-ground with only leaves visible and typically occurs on calcium poor soils with long snow covering at altitudes of up to 3350 m a.s.l. (Landolt 2003). This host supports many larger genera of gilled mushrooms (Agaricales) such as *Cortinarius*, *Inocybe*, *Russula* and *Lactarius*, however, is rarely associated with boletes (members of the Boletales), (Graf 1994; Mühlmann and Peintner 2008).

*Boletus edulis* Bull. sensu stricto (Boletales) is an ectomycorrhizal fungus occurring throughout the Northern Hemisphere. Together with *B. aereus* Bull., *B. pinophilus* Pilat et Dermek and *B. aestivalis* Fr. it forms the *Boletus edulis* species complex (*B. edulis* sensu lato). Different species can be distinguished based on morphological characters and variability in the ITS sequence, however, neither of these methods are informative to delimitate geographical clusters within species (Leonardi et al. 2005). Fruiting bodies of *B. edulis* sensu stricto are held in the highest regard as one of the most popular edible mushrooms in the world. This species usually grows in deciduous or coniferous forests at montane and subalpine levels and is considered to be associated with a phylogenetically broad range of hosts, although it primarily forms symbiosis with members of the Pinaceae, Fagaceae and Betulaceae (Hall et al. 1998). Consequently, findings of *B. edulis* above tree line are unexpected and reports in databases are rare (Gross et al. 2019). Previous work has suggested that at this altitude mycorrhizal associations may be formed with dwarf shrubs such as *Arctostaphylos uva-ursi* (Krpata et al. 2007). In this study, we give an account of *B. edulis* at an altitude of 2440 m a.s.l., the highest documented finding in the Alps, and confirm its ectomycorrhizal association with *S. herbacea*.

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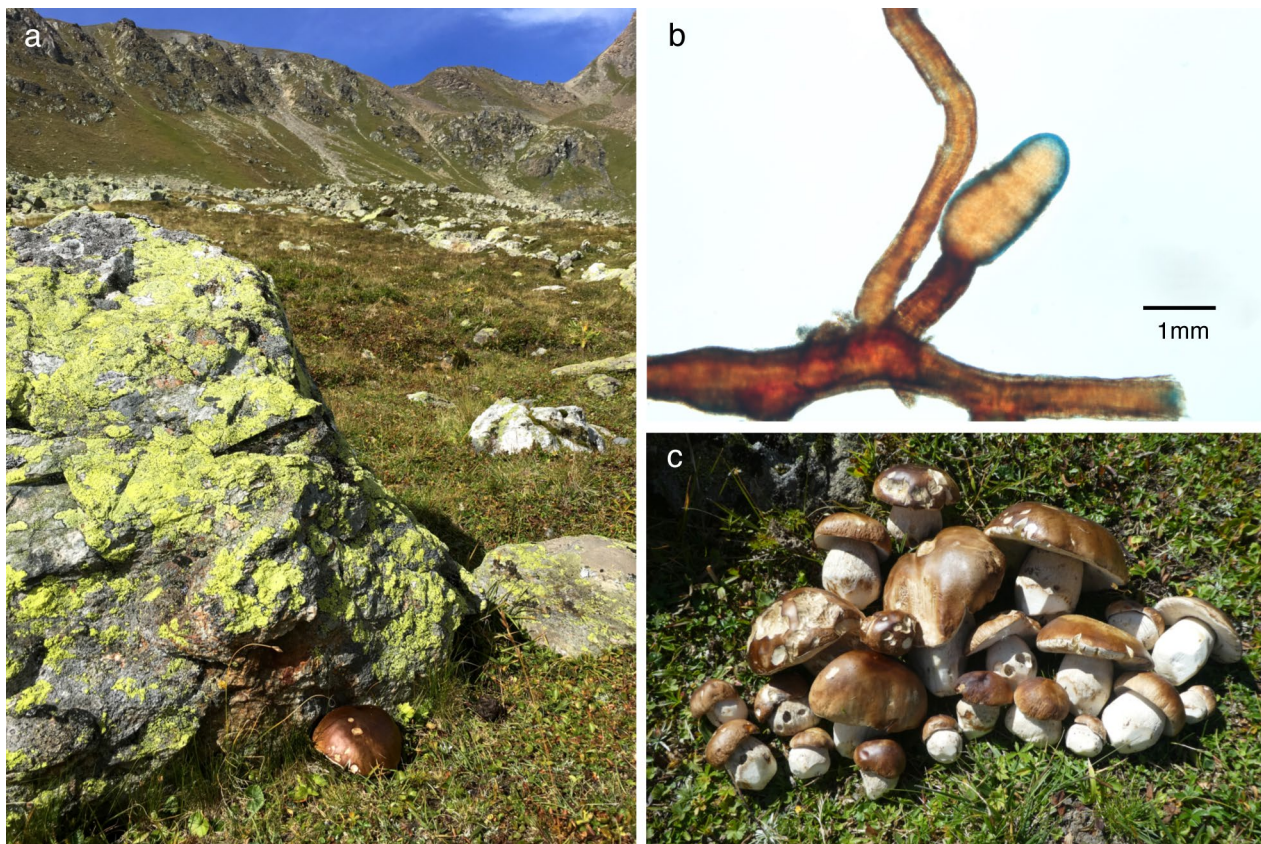
## Materials and methods

### Collection site and sampling

The collection site is an alpine meadow located about 2 km above Motta Naluns, Scuol, Ct. Grison, Switzerland (2440 m a.s.l.) (Fig. 1a). Vegetation at this location is made up of herbaceous plants and dwarf shrubs including *Poa alpina*, *Nardus stricta*, *Potentilla aurea*, *Homogyne alpina*, *Gentiana punctata*, *Vaccinium myrtillus*, *V. gaultherioides*, and the dominant shrub *S. herbacea*. Besides *B. edulis*, an ectomycorrhizal *Amanita* species (cf. *vaginata*) occurs at the same location. Fresh fruiting bodies of *B. edulis* were collected for DNA extraction on August 30, 2017 and immediately taken to the laboratory. Soil samples were taken around fruiting bodies by digging out a 10–20 cm chunk of soil containing embedded plant roots and above ground plant parts on August 29, 2018 and stored at 4 °C. Fine roots of *Salix herbacea* were washed free from soil and mycorrhizal root tips recovered using a stereo microscope (Fig. 1b). Roots of *Vaccinium* and *Potentilla* were also washed but no ectomycorrhizae were detected.

### DNA extraction and sequencing

DNA was prepared from freeze-dried tissues of fruiting bodies and root samples using NucleoSpin Plant II Kit (Macherey–Nagel, Düren, Germany). The nuclear ribosomal RNA 5.8S gene and the two internal transcribed spacers (ITS1 and ITS2) were amplified using the *Boletus* specific primers Bedu1F and Bedu2R (Mello et al. 2006). Reactions were carried out in a GeneAmp PCR System 9700 thermocycler (PE Applied Biosystems, Foster City, CA, USA) at standard conditions as previously described (Leuchtman and Cléménçon 2012). Sequencing reactions of purified products were performed in 10 µl volumes using the BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems) and both strands were sequenced. Sequence products were separated on a 3130xl Genetic Analyzer (Applied Biosystems™, Foster City, California), and sequences assembled and edited using Geneious 9.1.7 (Biomatters Ltd., Auckland, New Zealand). The 505 bp consensus sequences were blasted against entries in the NCBI database for species identification. GenBank accession numbers were assigned to the new sequences (National Center for Biotechnology Information,



**Fig. 1** *Boletus edulis* (porcini mushroom). **a** Habitat at 2440 m a.s.l. in the Lower Engadin valley (Switzerland); **b** example of mycorrhizal root tip of *Salix herbacea* used for DNA extraction (stained with aniline blue); **c** fruiting bodies collected in the year 2018

Bethesda, Maryland, USA; <http://www.ncbi.nlm.nih.gov/>): MK675104 (fruiting body), MK675105 (mycorrhizal root tips). Specimens of *B. edulis* used for DNA extraction are deposited at the Herbarium of ETH Zurich (ZT Myc 60013). Phylogenetic analyses were performed with PAUP\* 4.0a as previously described (Leuchtman and Cléménçon 2012).

## Results

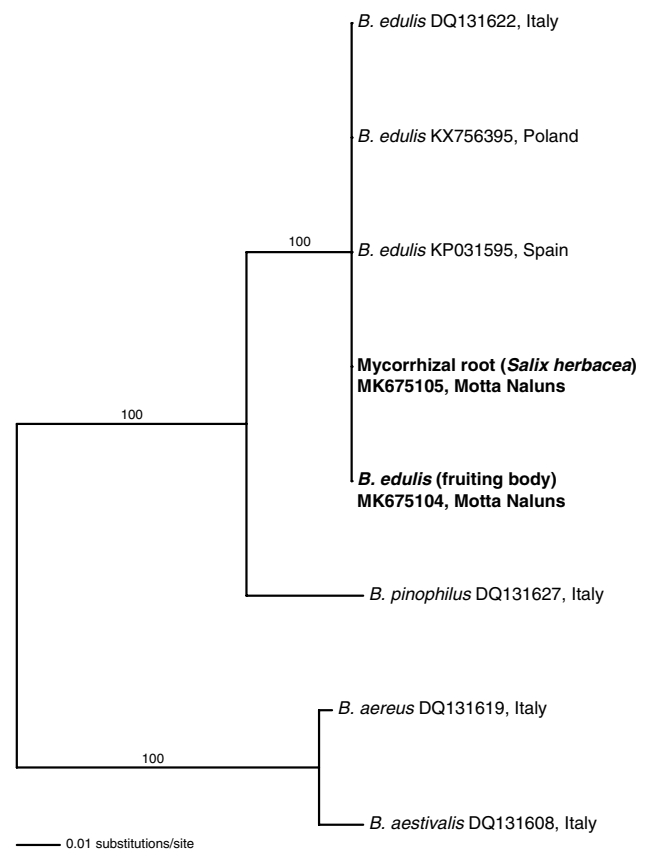
Mature, well-developed fruiting bodies of a bolete fungus were first discovered at a site near Motta Naluns at an elevation of 2440 m a.s.l. on September 1, 2016 and again for two consecutive years at the end of August. Fruiting bodies of different sizes were scattered in an area of approximately 2000 m<sup>2</sup> yielding a harvest of 1.32 kg fresh weight in 2018 (Fig. 1c). The fungus was identified as *B. edulis* based on its morphological characters (Breitenbach and Kränzlin 1991). In addition, ITS barcode sequences of DNA extracted from tissues of fruiting bodies showed 100% nucleotide identity to numerous sequences of *B. edulis* available from the NCBI database including reference sequences from Italy (Accession no. DQ131622), Poland (KX756395) and Spain (KP031595) (Fig. 2).

*Salix herbacea* was the dominant shrub in patches where fruiting bodies of *B. edulis* were collected, and the roots of this plant made up the majority of the root mass present in the soil around fruiting bodies. ITS sequences from DNA extracts of mycorrhizal root tips of *S. herbacea* from three different soil samples were identical with those of the *B. edulis* fruiting bodies (Fig. 2). This evidence indicates that the mycorrhizal partner of *B. edulis* at our site is *S. herbacea*.

## Discussion

The discovery of *B. edulis* fruiting bodies at 2440 m a.s.l. is, to our knowledge, the highest documented record of this species in the Alps. *Boletus edulis* is typically associated with spruce (*Picea abies*) or pine (*Pinus* spp.) at the subalpine level and is rarely found above tree line. In the SwissFungi database (Gross et al. 2019) there are only four other entries of *B. edulis* referring to sites above 2000 m (Alt St. Johann 2010 m, Göschenen 2013 m, Castasegna 2140 m, Quinto 2210 m), and the Austrian database of fungi includes an additional five reports between 2000 and 2200 m a.s.l (Austrian Mycological Society 2015), one of which was reported to be associated with *A. uva-ursi* (Krpata et al. 2007). However, outside the Alps in the Rocky Mountains, *B. edulis* was found at altitudes of up to 3500 m a.s.l. (Hall 2003).

Using DNA markers, we have confirmed that *B. edulis* forms mycorrhizal associations with *S. herbacea*. This is



**Fig. 2** Maximum likelihood (ML) tree obtained from ITS sequence alignments of *Boletus edulis* fruiting body and mycorrhizal root tips collected in Motta Naluns, Switzerland, including sequences from other members of the *B. edulis* species complex. Sequences are designated by the taxon followed by the GenBank accession number and the geographic origin. Numbers at branches are ML bootstrap support percentages from 1000 replications

an unusual host for boletes, although it may support many other mycorrhizal fungi (Mühlmann and Peintner 2008). For example, Graf (1994) reported 55 species that occurred in associations with *S. herbacea* from snow-beds in the Swiss Alps, but none of them belonged to Boletales. However, *B. edulis* has been reported from Shetland and Lake District, in Scotland and Great Britain, respectively, where it appeared to be associated with *S. herbacea* based on circumstantial evidence (Watling 1992). Another bolete, *Boletus spadicus*, was reported from mainland Scotland on *S. herbacea* (Watling 1981).

An interesting question remains how *B. edulis* reached the exceptionally high altitude above the tree line. One hypothesis is that the *B. edulis* recorded here is descendant from a relict population that has been associated with conifers and then moved to *S. herbacea* when these trees disappeared. Individual trees of *Pinus* spp. and *Larix decidua* can reach 2400 m (Landolt 2003) but were not present at our site probably because of grazing by cattle that are herded in this



area during summer months. In fact, not far from our site (area Clünas) *Pinus mugo* is found at 2430 m (Reinalter 2007). Consistent with this hypothesis, *Amanita cf. vaginata*, another typical ectomycorrhizal fungus of coniferous forests was also found at this site. Another possibility is that fruiting bodies or spores of *B. edulis* were transferred by marmots or birds to this site where mycorrhizal association with *S. herbacea* became established in lack of other adequate mycorrhizal partners. For example, nutcrackers (*Nucifraga caryocatactes*) may collect seeds that are infested by spores and deposit them in the ground, or alpine choughs (*Pyrrhoxorax graculus*) may eat fruiting bodies and disperse spores with their feces (Halbwachs 2018). As mushroom-forming fungi rely upon air turbulence for spore dispersal, it is also possible that wind currents could have carried spores up the mountain. Large numbers of spores are released into the air and may be dispersed over long distances of up to 1000 m (Castaño et al. 2017), however, most spores will be deposited in relative proximity to the source and successful establishment beyond the source plant community may be rather unlikely (Peay et al. 2012). Although the origin of this *B. edulis* mycorrhizal association cannot be resolved with the available data, the fact that fruiting bodies have been observed at this site over at least three years and in relatively high abundance suggests that the mycelium is well-established and may continue to persist for the years to come.

It is obvious that our understanding of alpine mycorrhizal communities specifically, and the abundance and host associations of fungal species across the globe in general, remains limited. In the face of a globally changing climate, it is critical to shed light on the factors that determine the occurrence and diversity of fungi (Boddy et al. 2014). Recent studies provide evidence that climate change can drive range-expansion as well as host-shifts in fungal pathogens (Chakraborty and Newton 2011; Fisher et al. 2012) and wood-decaying fungi (Gange et al. 2011), but investigations of climate-induced changes in mycorrhizal associations are still missing. The unexpected finding of a basidiomycete typically found at lower altitudes extending its range into alpine habitats could indicate adaptation to a changing climate and other examples show that these observations may be more common than previously thought. For example *Limacella illinita* var. *rubescens* (Amanitaceae) that typically occurs in deciduous or coniferous woods was recently found at alpine level above 2100 m (Sassi 2018). Range expansion often involves switch to a new mycorrhizal partner as found in the case reported here, and understanding these processes should be a focus of future research. Long-term monitoring programs and accumulating data from public databases will provide invaluable resources to understand shifts in fungal distributional ranges associated with climate change and may help to establish strategies for the conservation of threatened species.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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