

NEW FINDINGS OF RUSTS (Pucciniales) ON TREES AND OTHER PLANTS IN SLOVENIA

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ABSTRACT

Rusts (Pucciniales) are obligate biotrophs, representing one of the largest groups of plant pathogenic fungi and are one of the most economically important pathogens of many native and cultivated plants. Introduction of alien rusts to new environments can lead to large-scale epiphytotic. Their life cycle is complex, requiring usually more than one host to be completed. The taxonomy and identification of rusts is notoriously difficult, and only the implementation of morphological and molecular data can reveal the correct taxonomic emplacement. From 2014 to 2016 trees and other plants with rust infections were sampled. Identifications of rusts were performed based on morphological characteristics and phylogenetical comparisons to reference ITS-rDNA sequences. First official records of alien rust species *Melampsorium hiratsukanum* and *Phragmidium mexicanum* are reported.

Keywords: alien species, *Melampsorium hiratsukanum*, *Phragmidium mexicanum*, phylogeny, rusts, trees

IZVLEČEK

NOVE NAJDBE RIJ (Pucciniales) NA DREVESNIH IN DRUGIH VRSTAH V SLOVENIJI

Rje (Pucciniales) so obligatni biotrofi in predstavljajo največjo skupino glivnih patogenov rastlin, ki povzročajo ekonomske izgube tako pri gojenih kot tudi samoniklih rastlinah. Vnos tujerodnih rij v nova okolja lahko vodi do epifitocij velikih razsežnosti. Življenjski krog rij je zapleten in številne vrste potrebujejo več kot le enega gostitelja. Taksonomija in identifikacija rij je težavna in je pogosto možna le z uporabo tako morfoloških kot molekularnih podatkov. V obdobju od 2014 do 2016 smo vzorčili drevesne in druge rastline z znaki okužb z rjami. Identifikacijo vzorcev rij smo izvedli na podlagi filogenetske primerjave z referenčnimi nukleotidnimi zaporedji regije ITS-rDNA ter z opazovanjem mikromorfoloških struktur urediniospor. V Sloveniji smo tako prvič zabeležili zastopanost tujerodnih vrst rij *Melampsorium hiratsukanum* in *Phragmidium mexicanum*.

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Ključne besede: drevesa, filogenija, *Melampsorium hiratsukanum*, *Phragmidium mexicanum*, rje, tujerodne vrste

1 INTRODUCTION

The most comprehensive work about rust fungi and their distribution and hosts in Slovenia is a publication from 19th century (Voss, 1889-1892). Afterwards, few data was published by Janežič (Janežič, 1953; 1955; 1957; 1970), Maček (Maček, 1965; 1968; 1969; 1970; 1974) and in some reports gathered in Public Forestry Service at Slovenian Forestry Institute or by individuals (Jurc, 1995; Jurc in Weber, 2001; Jurc, 2007a; b; Ogris in Jurc, 2008).

From 2014 to 2016 we have performed a survey of rusts connected to woody plants in Slovenia and samples of rusts infecting willows (*Salix* spp.), poplars (*Populus* spp.), alders (*Alnus* spp.), silver birch (*Betula pendula*) and some other plants (eg. *Potentilla indica*) were collected.

Here we present the phylogenetic based identification of two new rusts for Slovenia, which were found on grey alder (*Alnus incana*) and mock strawberry (*Potentilla indica*). Also, occurrence of species from the genus *Melampsorium* on different hosts in Slovenia is unraveled with this contribution.

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2 MATERIALS & METHODS

Infected leaves, showing yellow pustules, were collected from *Alnus incana*, *A. glutinosa*, *Betula pendula* (nine locations) and from *Potentilla indica* (one location). Leaves were dried and herbarized and representative specimens were deposited in Mycotheca and Herbarium of Slovenian Forestry Institute (LJF).

Pieces cca. 2 mm × 2 mm were excised from fresh or dried leaves infected with rusts and dipped into liquid propane cooled by liquid nitrogen and followed by lyophilization. Small pieces of leaves were then attached to specimen stubs with carbon conductive tape and affixed with colloidal silver paint. Samples were sputter coated with platinum in a pure argon atmosphere (sputter coater SCD 050, BAL-TEC, Germany). Images were taken randomly at various magnifications with scanning electron microscope JSM-7500F (JEOL, Japan).

Table1. PCR conditions used in this study (Vilgalys in Hester, 1990; White in sod., 1990; Gardes in Bruns, 1993; Moncalvo in sod., 1995; Aime, 2006). All listed primers were used for PCRs and sequencing, except those indicated with (*) were used only for sequencing.

Region	Primers (Sequencing)	Annealing T (°C)
ITS2 & 28S rRNA gene	Rust2inv, LR6, LR0R*, LR3*, LR5*	57
18S rRNA gene	Rust18S-R, NS1	57
18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 & 28S rRNA gene	ITS1F, ITS4B	60–50 (touch down PCR)

The extraction of DNA, PCR mixtures, sequencing of PCR products and phylogenetic analyses were performed as described by Piškur (2016). The primer combinations and PCR conditions used in this study are listed in Table 1. Sequences were deposited in

GenBank under Accession Numbers LN795901–LN795902 (for *Phragmidium mexicanum*) and KY607916–KY607932 (for *Melampsorium* sp.). Selected sequences were used for BLASTn search at the NCBI website. Additional reference sequences from publicly available databases were included in phylogenetic analyses.

3 RESULTS & DISCUSSION

Phragmidium mexicanum (Mains) H.Y. Yun, Minnis & Aime on *Potentilla indica* (Ljubljana, 30 May 2014, leg. D. Jurc, det. D. Jurc, B. Piškur, LJF5282, GenBank LN795901, LN795902)

The rust infection – small orange pustules of uredinia and brown telia – on the lower side of leaves of *Potentilla indica* have been observed since 2008 in Ljubljana in near vicinity of Slovenian Forestry Institute. Since then, different intensities of fungal infections have been observed every year during growth periods. Uredinia are always present, but telia can be seen only occasionally from spring to autumn on longer infected leaves. We suppose that environmental conditions (ti. long periods of wet weather) delaye withering and dieback of the infected leaves and fungus has thus enough time to form telia.

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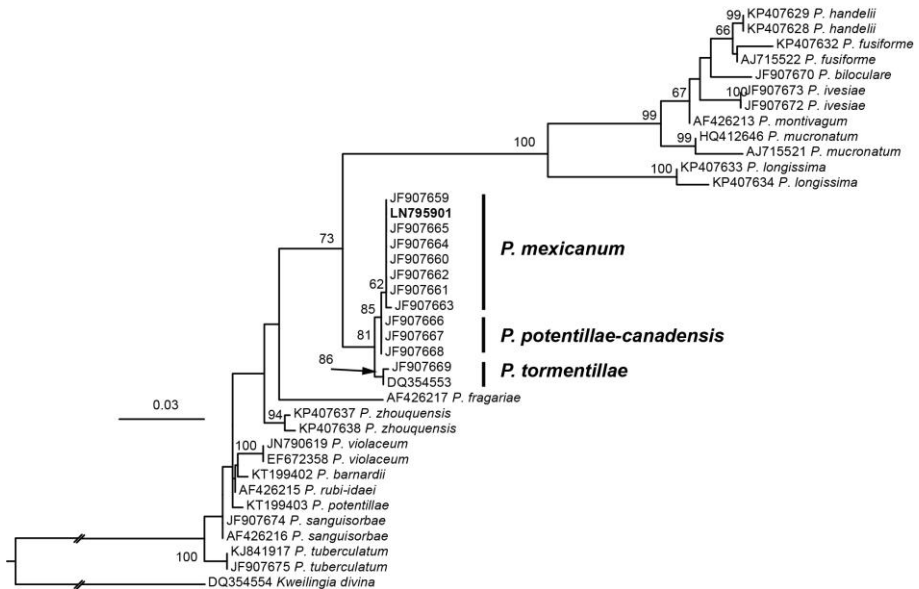


Figure 1: *Phragmidium* – the maximum likelihood tree based on the 28S rDNA dataset. Tree topology was obtained after 1,000 runs. *Kweilingia divina* was selected as an outgroup. The bootstrap values (> 50 %) are indicated at the nodes. Representative sequence from Slovenia is printed in bold.

The closest BLASTn hit for 28S rDNA sequence was sequence *Phragmidium mexicanum* JF907659. Phylogenetic analysis was based only on 591 bp of 28S rDNA

(from position 461 to 1051 in sequence LN795901) – due to short alignment coverage with other *Phragmidium* sequences. Phylogenetically closest species are *P. potentillae-canadiensis* Dietel with one and *P. tormentillae* with four parsimony informative sites regarding *P. mexicanum*. The phylogeny (Fig.1) agrees with previously published phylogenetic reconstructions (Yun in sod., 2011; Yang in sod., 2015). Since the 28S rDNA region is a conservative region, additional sequences representing more variable regions would give more detailed insight into phylogenetic relationships among *Phragmidium* species. Scanning electron microscopy of observed *P. mexicanum* specimens (for details see Fig. 3) revealed morphology, matching to the description by Yun et al. (2011), who recognized only one species with no varieties, due to the variable presence/absence of paraphyses and no sequence supported differences. Species *P. mexicanum*, in the taxonomic history placed also in the genus *Frommeëlla*, is infecting mock (false) strawberry (*P. indica*, formerly *Duchesnea indica*), an alien plant, originating in Asia. *P. mexicanum* was in Europe for the first time reported in 1952 in France and afterwards in Austria, Belgium, Czech Republic, Germany, Portugal, Poland (summarized after Wolczanska in Piątek, 2010) and Ukraine (Tykhonenko in Korytnyanska, 2012) and now in Slovenia.

Melampsoridium hiratsukanum Ito on *Alnus incana* (Zelenci, 12 Aug 2014, leg./det. D. Jurc, B. Piškur, LJF5283, GenBank KY607917; Javorniški Rovt, 14 Aug 2016, leg./det. B. Piškur, LJF5280, GenBank KY607921; Dovje, 29 Sep 2015, leg./det. D. Jurc, B. Piškur, GenBank KY607918–19; Trenta, 29 Sep 2015, leg./det. D. Jurc, B. Piškur, GenBank KY607920)

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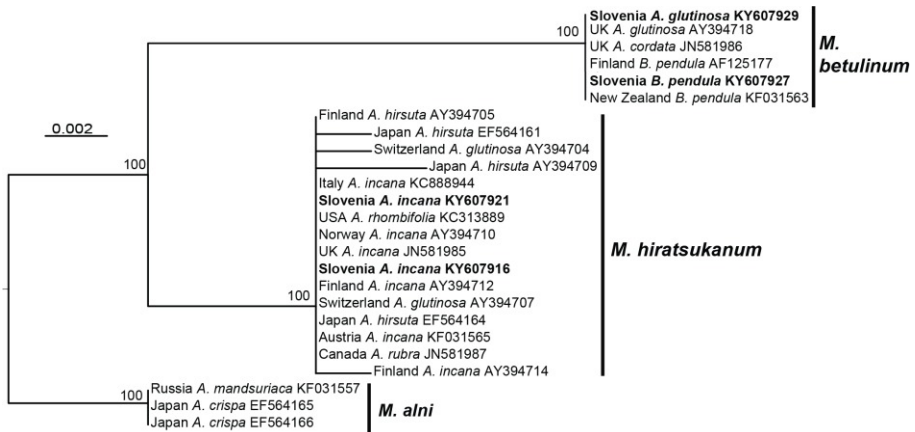


Figure 2: *Melampsoridium* – the maximum likelihood tree based on the ITS rDNA dataset. Tree topology was obtained after 1,000 runs and was midpoint rooted. The bootstrap values (> 50 %) are indicated at the nodes. Representative sequences from Slovenia are printed in bold.

On alders three morphologically similar species from the *Melampsorium* genus can appear: *M. betulinum* (Fr.) Kleb., *M. alni* (Thümen) Dietel, and *M. hiratsukanum*. The first species is believed to be native to Europe and the latter two in Asia (Hantula in sod., 2009). *M. hiratsukanum* has been spreading throughout Europe from around 1996. Species *M. alni* is believed to be restricted to eastern Asia and there are no reports about its presence in Europe (summarized after Hantula in sod., 2009). Alders, infected by *M. hiratsukanum*, can be strongly affected, as was reported from Austria, Italy, and Hungary, resulting in early defoliation already in August (Hantula in Scholler, 2006; Moricca in Maresi, 2010). With phylogenetically (Fig. 2) and morphologically (Fig. 3) based identification we have resolved the *Melampsorium* occurrence in Slovenia.

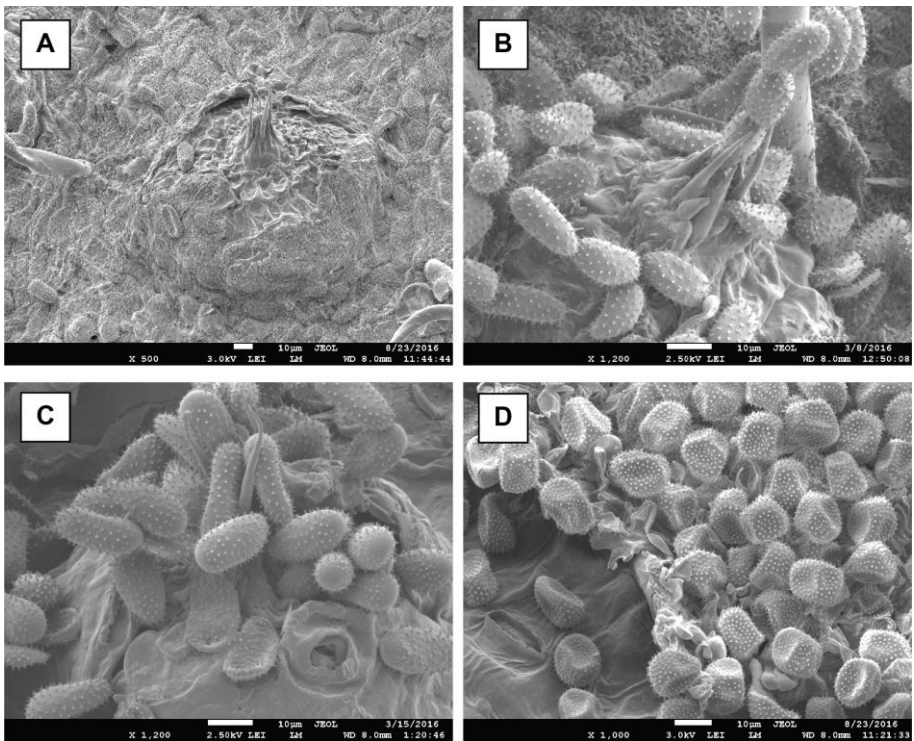


Figure 3: Scanning electron microscopy of: *Melampsorium hiratsukanum* – uredinium, peridial pore with ostiolar spines and urediniospores pressed out in chains. Urediniospores are regulary echinulate, ovoid to ellipsoid, can be arranged in chains (A, B). *M. betulinum* – urediniospores around peridial pore with ostiolar spines. Urediniospores are echinulate, smooth at one end, ovoid to ellipsoid (C). *Phragmidium mexicanum* – part of subepidermal uredinium with peripheral paraphyses and central mass of urediniospores (uniformly echinulate, non-catenulate and globoid) (D).

All rust infected samples of *A. incana* were identified as *M. hiratsukanum*. On *A. glutinosa* and *Betula pendula* only *M. betulinum* was found. Rust *M. alni* was not detected during our survey. This is the first written report of *M. hiratsukanum* in Slovenia.

Human activities, like global trade, are spreading fungi into new environments. Successful rust infection leads to extensive changes in host metabolism and can alter gene expressions in far remote organs of the infected host (Voegelé in sod., 2009). Asexual spores (urediniospores) are produced in massive amounts and their physiology enables a quick and successful adhesion to the host surface (Voegelé in sod., 2009) or to the occasional carriers. 18S ITS rDNA nucleotide sequence (LN795902) from our study showed 100% sequence identity to sequences from fungal clones obtained from swab from human skin on plantar heel (Findley in sod., 2013). This indicates that spores of *P. mexicanum* and other rusts can attach to human skin and can be as such long-distance dispersed. *M. hiratsukanum* is hypothesized to arrive in Europe with contaminated seedlings of ornamental *Alnus* spp. or forest trees and is believed to be spreading in Europe via urediniospores, dispersed by eg. wind (Hantula in Scholler, 2006). The similarity of *Melampsoridium* sequences to some sequences from publicly accessed databases showed that *Melampsoridium* spores can be found in environmental samples, like air samples and indoor house dust.

With this inventory, we got an insight into recent diversity of rusts in Slovenia, and gained diagnostic experience for further work with this challenging fungal group (Piškur, 2016).

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4 CONCLUSIONS

- Rusts *Melampsoridium hiratsukanum* and *Phragmidium mexicanum* are reported for the first time from Slovenia.
- Rust specimens from *Melampsoridium* and *Phragmidium* can be identified to the species level based on selected gene regions and valuable information can be obtained from SEM microphotographs.
- This study was part of a first comprehensive inventory of rusts on trees in Slovenia.

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