



Original Scientific Report

## New reports of *Melampsora rust* (Pucciniomycetes) on the *Salix retusa* complex in Balkan countries

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### ABSTRACT:

*Melampsora epitea*, known to cause rust on the complex of *Salix retusa* prostrate willows distributed in the subalpine and alpine belt of the mountains of Central Europe, is here documented for the first time in Montenegro and North Macedonia growing at six localities. It is not new for Serbia, but the records come from a newly reported host, namely *Salix serpyllifolia*. The pathogen's distribution presumably is wider than initially believed, and further surveys need to be conducted.

### Keywords:

plant pathogen, distribution, *Melampsora epitea*, basidiomycete, snow willows

UDC: 528.28:582.681.81(234.42)

Received: 09 January 2020

Revision accepted: 04 February 2020

During field investigation of the complex of *Salix retusa* (family Salicaceae) on the Balkan Peninsula, six populations with many individuals infected with rust fungus were documented (Fig. 1).

The complex of *Salix retusa* includes *S. retusa* L. (s.str), *S. kitaibeliana* Willd. and *S. serpyllifolia* Scop., prostrate willows distributed in the subalpine and alpine vegetation belts of the mountains of Europe: the Pyrenees, Alps, Apennines, Dinarides, Scardians, Carpathians and Rila. The systematic positions and geographical distribution of these taxa are not fully resolved and are still disputed (KOSIŃSKI 2017, 2019). Although he states that *S. retusa* is common on the Balkan Peninsula in the Dinarid, Scardian and Rila Mts., a number of prostrate willows with smaller leaves than those of typical *S. retusa* are found commonly and have been classified as *S. serpyllifolia*. Accordingly, for prostrate willows with small spatulate leaves, we here use the name "*S. serpyllifolia*". According to PEI (2005), willow and poplar (family Salicaceae) around the world are infected with rust fungus of the genus *Melampsora*.

The conducted field study took place in the summer periods from 2016 to 2018. Herbarium material of infected *S. retusa* collected in the field were deposited in the BEOU herbarium, while leaf samples with visible rust symptoms

were collected, placed in bags and transported to a laboratory in the Department of Algology, Mycology and Lichenology of Faculty of Biology, University of Belgrade for proper screening of symptoms, documented and used for further analyses by light and scanning microscopy. Symptoms were detected both in the field and on herbarium specimens.

### *Chorological data:*

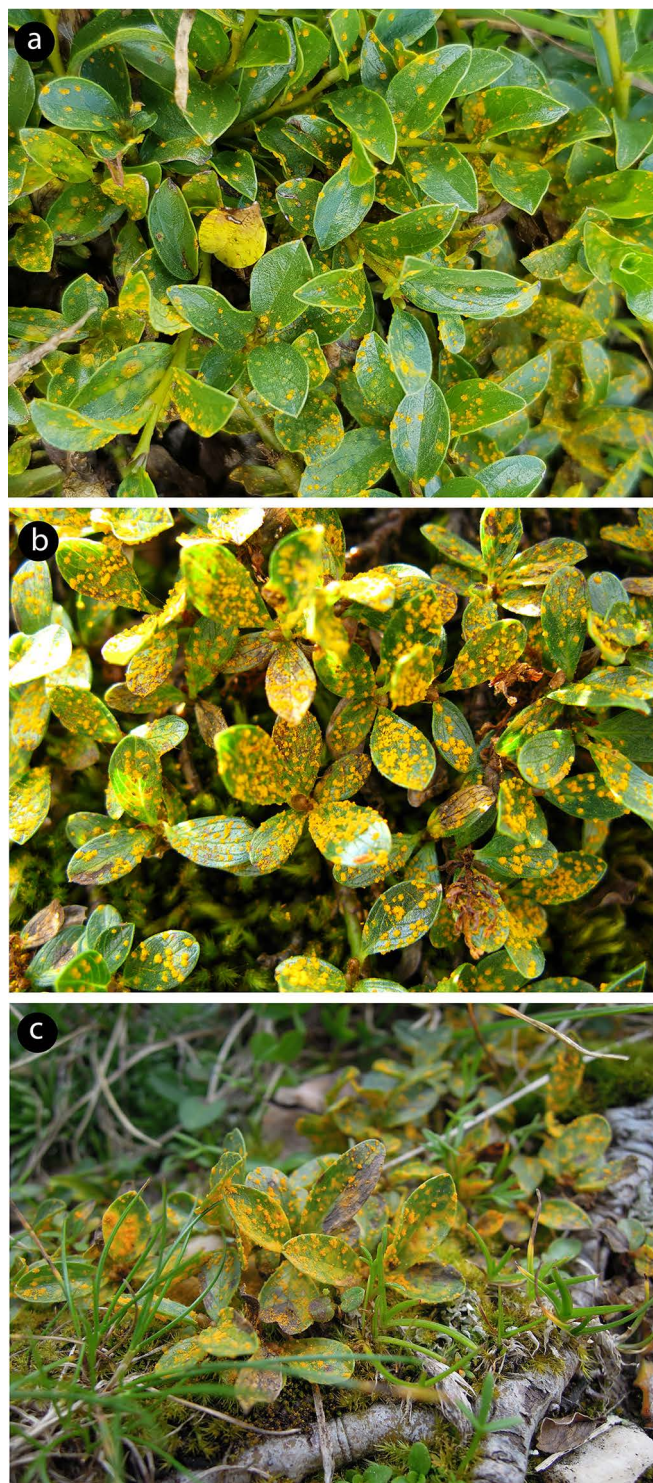
#### Specimens examined and pathogen identified:

#### ***Salix retusa* s. str.**

North Macedonia: Mt. Jakupica, Solunska glava N41.715351, E21.398691, 2221 m a.s.l., alpine-subnival snow-bed vegetation (Salicetea herbaceae), marble, leg. Lakušić D., Kuzmanović N., Janković I., 22.07.2016, BEOU-46336.

Montenegro: Prokletije Mts., Maja Karanfili, Skrožnje, N42.501556, E19.79057, 1985 m a.s.l., alpine-subnival snow-bed vegetation (Salicetea herbaceae), limestone, leg. Lakušić D., Kuzmanović N., Janković I., 29.07.2016, BEOU-46512.

Montenegro: Durmitor, Gornja Ališnica, N43.143605, E19.032165, 2050.2 m a.s.l., alpine calcareous grasslands,



**Fig. 1.** *Melampsora epitea* rust symptoms. a) infected *Salix retusa* from Montenegro, Durmitor, Ališnica (photo: D. Lakušić, 6.8.2018); b) infected “*Salix serpyllifolia*” from Montenegro, Komovi (photo: D. Lakušić, 11.07.2016); c) infected “*Salix serpyllifolia*” from Serbia (Kosovo: Šara Mts., Piribeg) (photo: M. Lazarević, 13.08.2018).

Scabioso silenifoliae-Caricetum laevis, limestone, leg. Lakušić, D., 06.08.2018, (field observation, photo documented - Fig. 1).



**Fig. 2.** Powdery pustules corresponding to uredinia documented on infected *Salix retusa* leaves collected in the Međukomlje Mts., Montenegro (magnification, 51.2×).

#### “*Salix serpyllifolia*”

Montenegro: Komovi Mts., Kom Vasojevički, N42.691946, E19.670435, 2163 m a.s.l., alpine-subnival snow-bed vegetation (*Salicetea herbaceae*), limestone, leg. Lakušić D., Kuzmanović N., Janković I., 11.07.2016, BEOU-46257.

#### Symptoms observed in the field:

#### *Salix retusa* s. str.

Montenegro: Durmitor Mts., Bobotov kuk, Velika Prevlja, [UTM: CN47] N43.126501, E19.037171, 2350 m a.s.l., alpine limestone grassland *Festuco-Seslerietea*, limestone, leg. Lakušić D., 14.08.2017, BEOU-46656.

Montenegro: Mt. Maganik, Kurozeb (Petrov vrh) 1800-2100 m a.s.l., heaths above *Pinus heldreichii* H. Christ belt, on limestone, Niketić M., Tomović G. 19.08. 2010, BEOU-31409.

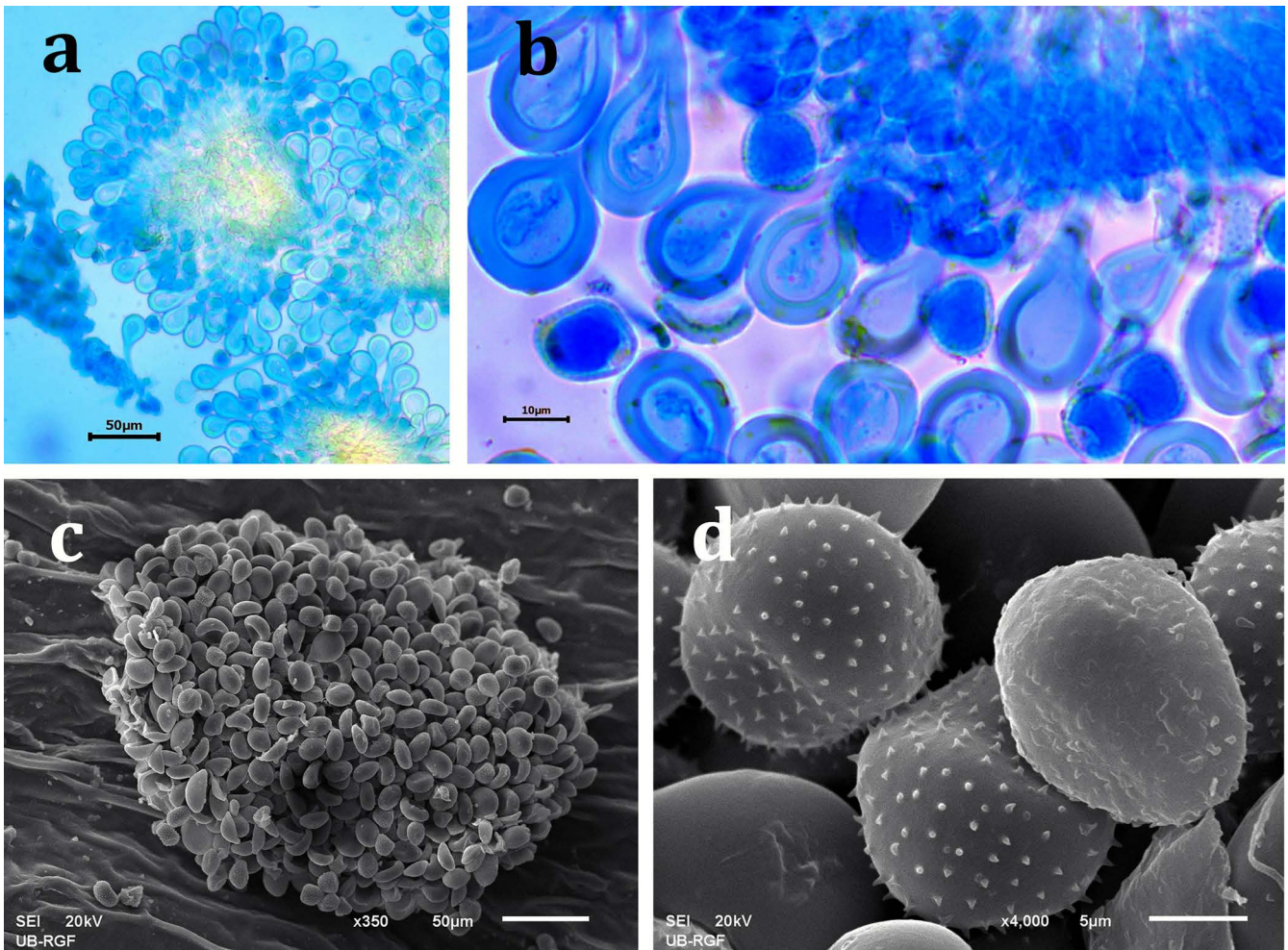
#### “*Salix serpyllifolia*”

Serbia (Kosovo): Šara Mts., Piribeg, N42.177782, E21.048534, alpine-subnival snow-bed vegetation (*Salicetea herbaceae*), limestone, leg. Lazarević M., Lazarević P. 13.08.2018 (field observation, photo documented Fig. 1).

On both sides of the leaves, formation of yellow to orange indistinguishable spots corresponding to orange powdery pustules (uredinia), scattered or rarely grouped and covering approximately 10 to 50% of the leaf surface, was documented (Fig. 2).

Pustules originating from infected leaf surfaces sampled at all investigated localities were gently removed with the aid of adhesive tape and by scraping, mounted with Lactophenol Cotton Blue and observed under a light





**Fig. 3.** *Melampsora epitea* s. str. - morphological characteristics of pathogen uredial stage documented on *Salix retusa*. a, c) uredinium; b) urediniospore with capitulate paraphyses dyed with Lactophenol Cotton Blue; d) urediniospore with dense spines and thick cell walls documented. a, b - light microscopy; c, d - SEM.

microscope (Nikon Eclipse E200 equipped with a Bresser MikroCam PROHDMI camera). To observe and analyse fungal structures present on *S. retusa* leaves using SEM, sample fragments were taken from leaf surface with visible symptoms by means of adhesive carbon tape on aluminum cylinders. SEM images were obtained at the Faculty of Mining and Geology, University of Belgrade, using a JEOL JSM-6610 LVSEM instrument (Tokyo, Japan). Samples ( $d=15\text{nm}$ ,  $\rho=19.2\text{g/cm}^3$ ) were gold-coated with a Leica EMS CD005 sputter coater (Wetzlar, Germany). Both types of microscopy, light and SEM, revealed in all tested samples the presence of numerous urediniospores and paraphyses emerging from amphigenous uredinia. Further morphological analyses of documented structures of the uredial stage were conducted using ImageJ software and included: characters of urediniospores (shape, dimensions, cell wall thickness, circularity-CIRC, spine distance-SD) and paraphyses (shape and dimensions). The CIRC value was normalised and ranged from 0.0 to 1.0, where the value 1.0 represents an ideal case of maxi-

imum symmetry, such as a perfect circle. Urediniospores with documented CIRC values in the case presented here ranged from 0.7 to 0.8, indicating more ovoid to ellipsoidal shape of the pathogen spore ( $14\text{--}24\times 11\text{--}20\ \mu\text{m}\ \text{Ø}$ ). The cell wall of urediniospores was approximately  $1\ \mu\text{m}$  thick, uniformly echinulate with dense spines. Mean SD ranged from 1.5 to  $2\ \mu\text{m}$ . Paraphyses were capitulate, with a thickened wall, placed intermixed or peripherally in the uredinia ( $30\text{--}61\times 9\text{--}26\ \mu\text{m}\ \text{Ø}$ ) (Fig. 3). Documented structures of rust fungus, urediniospores and paraphyses, and their morphological characters (shape, dimensions, CIRC, SD) from all leaf samples analysed microscopically showed no significant variation in morphology and size, thereby suggesting that *S. retusa* specimens from all investigated populations suffer from the same pathogen.

Documented micromorphological features, as well as documented symptoms present on host plants identified as *S. retusa* correspond to the genus *Melampsora* (family Melampsoraceae, order Pucciniales, class Pucciniomycetes, division Basidiomycota). Species of the genus *Melampsora*

are biotrophic fungal pathogens of willows (*Salix* spp.) in the northern temperate zone (BENNET *et al.* 2011). Apart from *M. amygdalinae* Kleb, all *Melampsora* species that infect willows are heteroecious. Corresponding to the ploidy level of the fungus, *Salix* spp. are hosts for the dikaryotic stages of the fungus [uredinia (III) and telia (IV)], while the stages of spermogonia (0) and aecia (I) form on hosts in different lineages of seed plants, e.g., *Abies*, *Euonymus*, *Larix*, *Ribes*, *Saxifraga*, *Tsuga*, *Viola* and several genera of Orchidaceae (PEI 2005). For proper identification of documented *Melampsora* rust to the species level, awareness of teliospore morphology is required, as well as knowledge of the hosts' 0I stages of the pathogen. The conducted field investigation and sampling occurred in the summer period, and orange powdery pustulestelia bearing theliospores were not present and rust symptoms were not documented on other plants apart from *S. retusa*. However, HYLANDER *et al.* (1953) recognised *Melampsora* pathogens of *Salix* spp. similar in morphology as a species complex (*sensu stricto*) and named it the *M. epitea* complex. This name was adopted by WILSON & HENDERSON (1966) as the name for a collective species composed of various races of *Salix* spp. pathogens which are morphologically indistinguishable, but which alternate on different aecial hosts. These authors recognised two varieties within the *M. epitea* complex:

1. *M. epitea* var. *reticulatae* if the aecial host is *Saxifraga* spp.; and
2. *M. epitea* var. *epitea* if the aecial host is not *Saxifraga* spp.

However, a problem remains: *M. epitea* consists of different entities (DAMADI *et al.* 2011), and further delimitation of the taxonomic status within this group is urgently needed.

According to the Fungal Database (FARR & ROSSMAN 2016), members of the *M. epitea* complex have previously been documented on *S. retusa* in Bulgaria (DENCHEV *et al.* 1995), Poland (MULENKO *et al.* 2004, 2008) and Germany (BRAUN 1982; KRUSE 2013), and our data represent the first record of *Melampsora* rust on *S. retusa* in Serbia, Montenegro and North Macedonia. The previous records in Serbia (MARKOVIĆ & KARADŽIĆ 2006; MARKOVIĆ *et al.* 2007) come from the northern province of Vojvodina from *Salix* sp. They are surely from a different *Salix* host, inasmuch as there are no representatives of the *S. retusa* complex in Vojvodina. It can be assumed that distribution of the pathogen is wider, and new field investigations are expected to better document *Melampsora* rusts in the Balkans.

**Acknowledgement** – The Ministry of Education and Science of the Republic of Serbia supported this research. The authors would like to thank Dr. Maja Lazarević, who kindly gave us information about the appearance of rust symptoms on “*Salix serpyllifolia*” from Serbia (Kosovo: Šara Mts., Piribeg); and Dr. Snežana Vukojičić, who provided additional information about rust symptoms on *Salix retusa* from herbarium specimens deposited in BEOU.

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REZIME

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## Novi značajni nalazi izazivača rđe roda *Melampsora* (Pucciniomycetes) na vrstama kompleksa *Salix retusa* na Balkanu

Miloš STUPAR, Milica LJALJEVIĆ GRBIĆ, Jelena VUKOJEVIĆ i Dmitar LAKUŠIĆ

*Melampsora epitea* je opisana kao izazivač rđe na poleglim vrbama iz kompleksa *Salix retusa* u subalpskim i alpskim pojasevima planina centralne i južne Evrope. U ovom radu navode se prvi nalazi *M. epitea* na poleglim vrbama u Crnoj Gori i Severnoj Makedoniji. Nalaz iz Srbije postoji, ali se po prvi put dokumentuje ova vrsta patogena na vrsti *S. serpyllifolia*. Verovatno je da je rasprostranjenje patogena mnogo šire od do sada zabeleženog, što zahteva dalja istraživanja.

**Ključne reči:** biljni patogeni, rasprostranjenje, *Melampsora epitea*, bazidiomicete, polegle vrbe