1 First Report of Collar and Root Rot of Lettuce Caused by *Plectosphaerella*
2 *cucumerina* in Serbia

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12 In March 2021, unusual plant stuning, collar and wet root rot of lettuce (*Lactuca sativa*
13 L.) during the rosette stage was observed in two commercial fields in Serbia (44°58'N,
14 20°32'E; 44°45'N, 20°43'E). Disease incidence in the fields (≈ 0.9 ha each) was approximately
15 15 and 20%, respectively. Initial above-ground symptoms were yellowing and wilting of
16 leaves, while below-ground symptoms were collar, wet root rot, and lesions became necrotic.
17 Eventually, whole plants wilted, collapsed and died. A total of 35 symptomatic plants were
18 collected from the fields and diseased tissues were cut into small pieces, surface sterilized and
19 plated on potato dextrose agar (PDA). Isolation resulted in 20 morphologically uniform
20 monoconidial isolates. The isolates formed white to creamy colonies, gradually becoming
21 salmon pink, slimy, or moist in appearance, with a sparse aerial mycelia. Numerous hyphal
22 coils with conidiophores and hyaline, smooth-surfaced, ellipsoid to ovoid, septate or aseptate
23 conidia were formed (4.5 to 10.1×1.2 to 3.7 μm (n=100)). To confirm the species identity, the
24 internal transcribed spacer (ITS) region and the D1/D2 region, of a selected representative
25 isolate 13-3-c were amplified and sequenced by using primer pairs ITS1/ITS4 (White et al.
26 1990) and N1/N2 (O'Donnell and Gray 1995), respectively. The sequences were deposited in
27 GenBank (ITS: OR880564 and D1/D2: OR880567). Sequence analysis revealed 100%
28 nucleotide identity with *P. cucumerina* isolates from different countries deposited in the
29 NCBI GenBank, including isolate MH860704 (Vu et al. 2019) (ITS region) and isolate
30 KY662256 (Su et al. 2017) (D1/D2 region). Neighbor-joining analysis was conducted based
31 on combined ITS and D1/D2 regions, and the tree was constructed with the substitution
32 models (1,000 bootstrap). Combined phylogeny confirmed that the sequences shared a
33 common clade with *P. cucumerina*. Hence, morphological, microscopic, and molecular
34 characterization confirmed the pathogen as *P. cucumerina* (Palm et al. 1995; Carlucci et al.
35 2012). In a pathogenicity assay 10 isolates were tested. Five 30-day-old lettuce plants (cv.
36 Majska Kraljica) per isolate were root-dipped in the conidial suspensions (1×10^5 conidia/ml).
37 The 10 inoculated plants were transplanted into 1 L pots containing sterile substrate
38 (Floragard, Germany). Plants treated with sterile distilled water were used as controls. Plants
39 were maintained in a greenhouse at 25 to 28°C under a 12-h photoperiod (Cai et al. 2021).
40 Four weeks after inoculation, stunting, chlorosis and wilting of plants were observed, while
41 collars and roots exhibited typical decaying symptoms. No symptoms were observed on the
42 control plants. The pathogen was reisolated from symptomatic tissue as previously described.
43 Koch's postulates were completed by confirming the identity of reisolates based on
44 morphological features. To our knowledge, this is the first report of *P. cucumerina* on lettuce
45 or any other crops in Serbia. *P. cucumerina* is already known as a pathogen of lettuce and
46 other hosts grown in many countries worldwide, as well as in some European countries
47 (Belgium, England, Italy, Netherland and Switzerland) (Zhang et al. 2019). This emerging
48 pathogen may cause significant economic losses in lettuce production in Serbia and in the

entire Balkan region. Our results may help to develop effective management strategies based on accurate and timely identification and regular pathogen monitoring.

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Fig. S1. Disease symptoms of *Plectospherella cucumerina* - collar, wet root rot, and became wilting and necrotic leaves
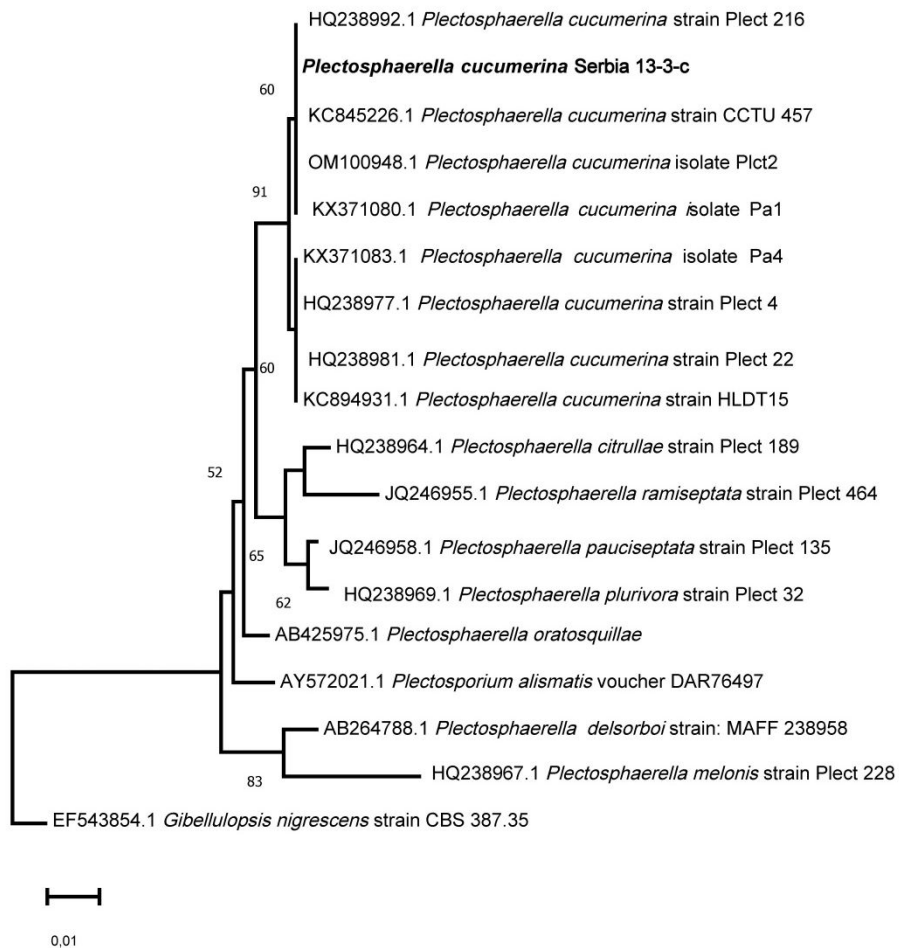

Fig S2. Neighbor-Joining method phylogenetic tree inferred from internal transcribed spacer (ITS) region of ribosomal RNA gene sequences of 18 selected *Plectosphaerella* sp. isolates. The phylogram was generated with MEGA 11 using the p-distance model. Bootstrap analysis was performed with 1,000 replicates, and bootstrap values (>50%) are shown next to the branches. There were a total of 377 positions in the final dataset. *Gibellulopsis*

nigrescens isolate CBS 387.35 is included as an outgroup. *Plectosphaerella cucumerina* isolates obtained in this study from Serbia are indicated in bold.