



A new combination for *Microcyclospora rumicis* in *Sphaerulina* based on molecular and morphological data

M. Bakhshi ✉

Department of Botany, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

M. Arzanlou

Plant Protection Department, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

The cercosporoid species, *Microcyclospora rumicis* Arzanlou & Bakhshi was recently introduced on *Rumex crispus* L., from Iran (Guilan province, Talesh) (Arzanlou & Bakhshi 2011). Based on the morphological features, this fungus was described as a species of the genus *Microcyclospora* J. Frank et al. (Arzanlou & Bakhshi 2011). During a phylogenetic study of cercosporoid fungi from north of Iran, the genomic DNA of the type strain (CBS 131546) of the species, was extracted using the protocol developed by Möller et al. (1992). Amplification and sequencing of the internal transcribed spacer regions and intervening 5.8S nrRNA gene of the nrDNA operon (ITS) and partial nuclear ribosomal large subunit (LSU) were done with primers V9G + ITS4 and LR0R + LR5 respectively (Quaedvlieg et al. 2013). The resulting sequences were subjected to a BLAST search to find most similar sequences in GenBank. The obtained sequences from GenBank together with the novel generated sequences of this study, were aligned and subjected to multi-gene DNA sequence analyses using MrBayes v. 3.2.2. Based on the phylogenetic analyses, this fungus clusters together with *Sphaerulina* Sacc. species in a clade that is distinct (Fig. 1). Therefore, the fungus was transferred to the genus *Sphaerulina* and, a new combination, *Sphaerulina rumicis* is introduced here with following characteristics:

Sphaerulina rumicis (Arzanlou & Bakhshi) M. Bakhshi & Arzanlou, *comb. nov.* — MycoBank MB 823634.

Basionym: *Microcyclospora rumicis* Arzanlou & Bakhshi, Mycotaxon 118: 182. 2011.

Specimens examined. IRAN, Guilan, Talesh, on leaves of *Rumex crispus* L., Sept. 2010, M. Bakhshi, CCTU 1 = CBS 131546.

The LSU and ITS sequences of the type strain (CBS 131546) were deposited under GenBank accessions No. MG561649 and No. MG561648, respectively.

Notes: Based on its morphological features including microcyclic conidiation, conidiophores reduced to conidiogenous cells, scolecosporous, guttulate conidia and, unthickened nor darkened hila, Arzanlou & Bakhshi (2011) placed this fungus in *Microcyclospora*. To resolve the phylogenetic placement of this fungus, we generated phylogenetic tree based on LSU and ITS DNA sequence data (Fig 1). These data clearly revealed that the fungus was unrelated to *Microcyclospora* and is shown to belong to *Sphaerulina*. The phylogeny of the genus *Sphaerulina* (with type species *Sphaerulina myriadea* (DC.) Sacc.) has been clarified recently by Verkley et al. (2013) and Quaedvlieg et al. (2013). Other than the lack of pycnidial conidiomata, the morphological characters of the *Sphaerulina rumicis* are similar to other species of *Sphaerulina* (Verkley et al. 2013, Quaedvlieg et al. 2013). The placement of *Microcyclospora rumicis* in *Sphaerulina* according to the absence of pycnidial conidiomata, is somewhat notorious; however this phenomenon has also been observed in other species of fungi. For example, Crous et al. (2013) have shown that *Septoria pistacina* Allesch., a major pathogen of pistachio, despite of producing pycnidial conidiomata, belongs to *Pseudocercospora* (*P. pistacina* (Allesch.) Crous). Therefore, this is another example that questions the value of conidiomatal structure at generic level, and emphasizes the need for integration of morphological features with molecular data for accurate identification of fungal species.

REFERENCES

- Arzanlou M, Bakhshi M. 2011. *Microcyclospora rumicis*, a new species on *Rumex crispus* from Iran. Mycotaxon 118: 181–186.
Crous PW, Quaedvlieg W, Sarpkaya K, Can C, Erick

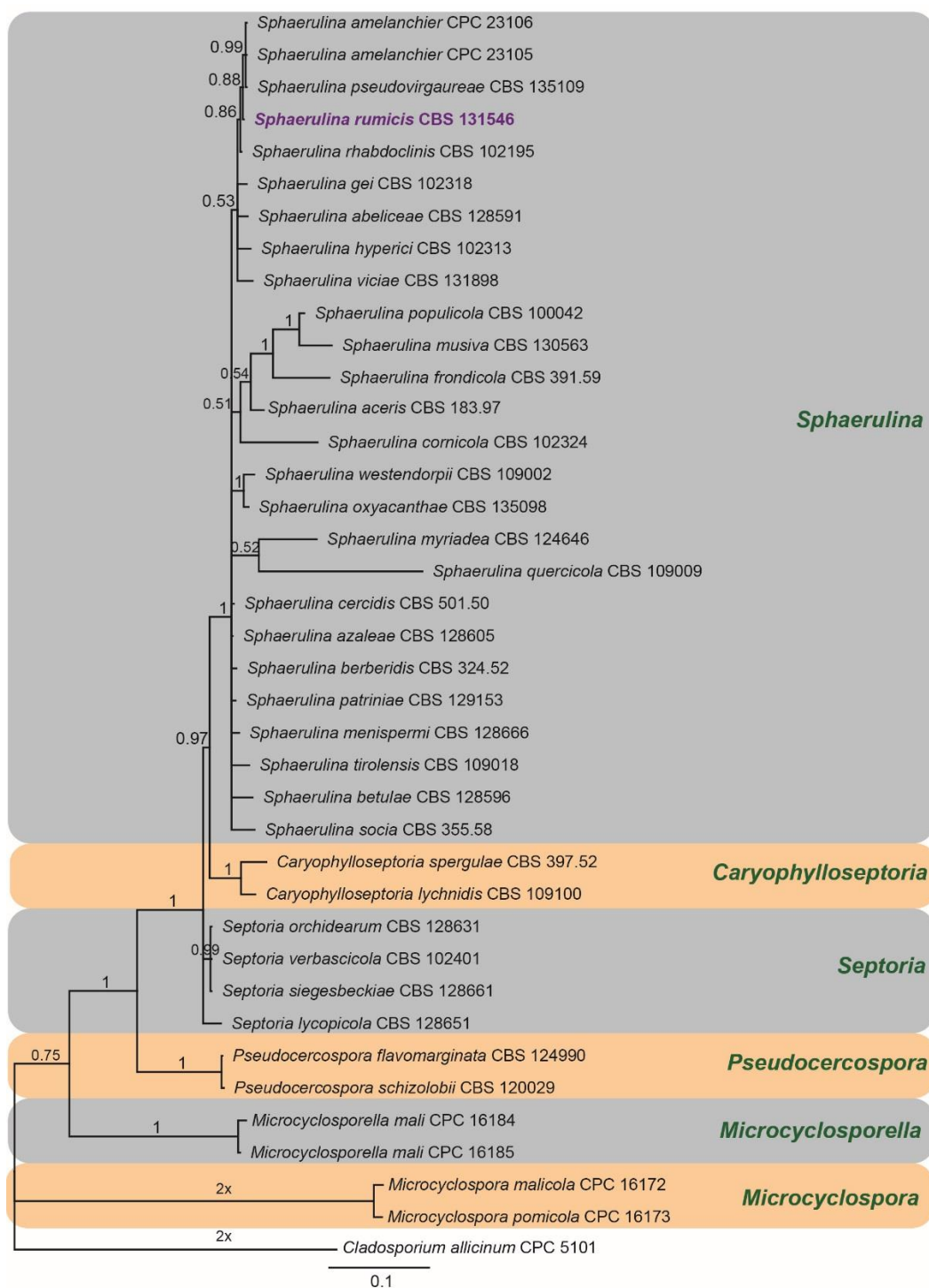


Fig. 1. Phylogenetic tree inferred by Bayesian analysis of the combined two-loci (LSU and ITS) sequence alignment using MrBayes v.3.2.2. The scale bar indicates 0.1 expected changes per site. *Cladosporium allacinum* (CPC 5101) was used as an outgroup.

A. 2013. Septoria-like pathogens causing leaf and fruit spot of pistachio. IMA fungus 4(2): 187–199.
 Möller EM, Bahnweg G, Sandermann H, Geiger H. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Research 20: 6115–6116.

Quaedvlieg W, Verkley GJM, Shin H-D, Barreto RW, Alfenas AC, Swart WJ, Groenewald JZ, Crous PW. 2013. Sizing up Septoria. Studies in Mycology 75: 307–390.

Verkley GJM, Quaedvlieg W, Shin H-D, Crous PW. 2013. A new approach to species delimitation in Septoria. Studies in Mycology 75: 213–305.