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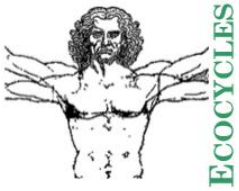

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CASE STUDY

Genetic analysis of the tree leaf disease microfungus *Rhytisma acerinum*

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Abstract **–** In the work presented *in silico* DNA sequence and dendrogram analyses were carried out to reveal molecular diversity and genetic distances among *Rhytisma* entries and related species based on gene bank data mining. The study revealed that *R. acerinum* shows the closest genetic distance to *R. polare*. DNA nucleotide variation detected raised a need for consensus between the *Linnean* morphological and the new molecular systematics.

Keywords **–** *Rhytisma acerinum*, microfungi, global carbon cycle

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INTRODUCTION

Unlike macro/higher/true fungi (*Fukasawa, 2021; Gyulai et al., 2018*) which comprise about 0.1 million species, microfungi (molds, mildews, rusts, smuts, *etc*.) comprise about a fifteenfold number of 1.5 million species. The only difference between macro- and microfungi is only in the body structures with the absence of a multicellular 'fruiting body' in microfungi (*Moore et al., 2011*). Here we focus on the genetics and environmental roles of the *Rhytisma acerinum* (*tar spot diseases of* '*Norway maple disease*') (Ascomycota) which seriously infects tree leaves of *Acers* (*Bartha, 2021; Simoncsics, 2017*). In this way, *Rhytisma* takes a role in the decomposition of last year's leaves by breaking down leaf components of *cellulose, hemicellulose, lignin, starch, pectin, inulin, chitin, tannin, humic acid,* and *fulvic acid* (*Tennakoon et al., 2021*). *Rhytisma* species are biotrophic leaf parasites. In the life cycle, ascopsores overwinter in apothecia on the leaf litter and are released by the wind in spring next year infect new leaves through the stoma (*Leith and Fowler, 1988*).

GENETIC ANALYSIS OF *RHYTISMA* SPECIES

The number and gene sequences of *Rhytisma* species (Pers., Fries) in *NCBI* gene data bank (*National Center for Biotechnology Information*; *https://www.ncbi.nlm.nih.gov*) are poor (by Dec. 2021) (Fig. 1). *Pers.* in the systematic name stands for the name of mycologist *Persoon, Christiaan Hendrik* (1761 – 1836) who described start spot fungus. *Fries* stands for the botanist *Fries, Elias Magnus* (1794 – 1879). The available *Rhytisma* species sequences and the number of [genes] are as follows: *R. acerinum* [23]*; R. americanum* [6]; *R. andromedae* [2]; *R. filamentosum* [4] (Fig. 2a,b); *R. huangshanense* [3] (Fig. 2a,b); *R. lonicerae* [1]; *R. panamense* [2]; *R. polare* [18] (Fig. 2a,b); *R. punctatum* [3]; *R. cf. punctatum* Lantz *et al*., 414 (UPS) [2]; *R. salicinum* [5]; *unclassified Rhytisma* (Fungi); *Rhytisma sp.* Hou 564 [1]; *Rhytisma sp*. [9]. However, it allowed comparative analysis in which *R. acerinum* accessions showed the closest genetic distance to *R. polare* (Fig. 2a).

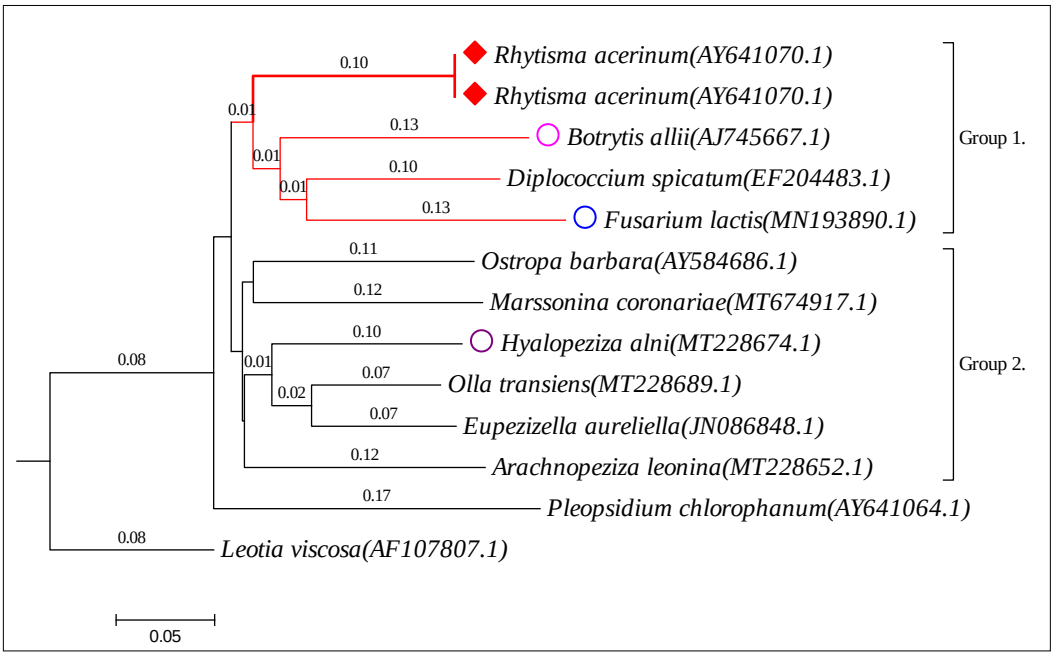

Figure 1. Genetic distances among *Rhytisma acerinum* (Ascomycota) and related microfungi species. Sequences of *DNA dependent RNA polymerase II gene* (largest subunit; 2168 nucleotides) were aligned to that of *R. acerinum* (*#AY641070.1*). The dendrogram was run by *Neighbor Joining* algorithm (*NCBI*) with indications of branch length and finally edited by *MEGA7* computer program (*Kumar et al., 2016*). Unit of genetic distances is indicated (0.05) which gives the numbers of nucleotide substitutions along a 100 nt DNA stretch. The two main groups and three other widespread microfungi are indicated (O).

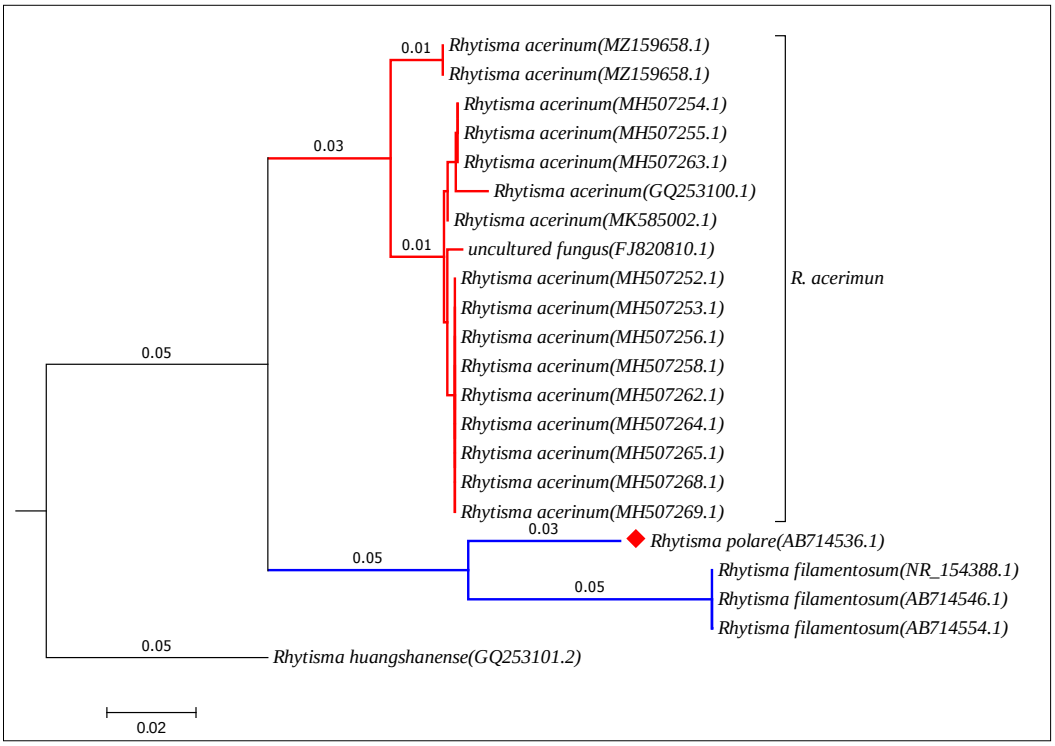

Figure 2a. Genetic distances among *Rhytisma acerinum* accessions and *Rhytisma* species based on genes of ITS1–5.8S–ITS2 ribosomal DNA (rDNA) sequences after aligning to *R. acerinum* (*NCBI# MZ159658.1*; 525 nt). The dendrogram was run by *Neighbor Joining* algorithm (*NCBI*) with indications of branch length and *NCBI* GenBank accession numbers and edited finally by *MEGA7* computer program (*Kumar et al., 2016*). Unit of genetic distance is indicated (0.02) which gives the numbers of nucleotide substitutions along a 100 nt DNA stretch.

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(I.)                                    250        260        270        280        290        300
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|
>MZ159658.1 Rhytisma acerinum   TCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGT
>MH507269.1 R. acerinum         ..............................................................
>AB714536.1 R. polare           ..T...........................................................A..
>GQ253101.2 R. huangshannense   ..T..............................................................
>AB714546.1 R. filamentosum     ..T...........................................................A..
>EF191241.1 Coccomyces australis ..T..........................................................A..
>MH682238.1 Hypoderma cordylines ..T..........................................................A..
>GU138741.1 Hypoderma rubi      ..T..........................................................A..
>MK584985.1 Lophodermium herbarum ..T.....................................................A..

(II.)                                   430        440        450        460        470        480
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|
>MZ159658.1 Rhytisma acerinum   GCCTCAAAAACAGTGGCGGCC-CCGTCCGGTCTCAAGCGTAGTAATACTCGCCGCTTGTT
>MH507269.1 R. acerinum         .........G..........-.....................C......T.......C
>AB714536.1 R. polare           ...CT....T..........T-......A..............T..............
>GQ253101.2 R. huangshannense   ...C.....C.......A...G...C..T.....C....C....T.G.........G..C
>AB714546.1 R. filamentosum     ...C.....GA.........T-......A....T..................T........
>EF191241.1 Coccomyces australis .........T..........T-......A..........C...........T........
>MH682238.1 Hypoderma cordylines .........T..........T-..................C......G....T........
>GU138741.1 Hypoderma rubi      .........T..........T-..................C...........T........
>MK584985.1 Lophodermium herbarum ........GT...........-...C.............C......G....T.......C
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Figure 2b. How many DNA nt differences need to be identified for determining a systematically new species? Samples of two DNA stretches (60 nt each) with low (I.) and high (II.) levels of nucleotide diversity of ITS1-5.8S-ITS2 ribosomal DNA (rDNA) sequences. Two *Rhytisma acerinum* isolates and some related species were compared by MSA (multiple sequence alignment) run by BioEdit (*Hall, 1999*) program after aligned to *R. acerinum* sequence *NCBI# MZ159658.1* (525 nt).

Figure 3. Rhytisma acerinum-infected *Acer platanoides* tree leaves, at late summer [a,b] and late fall [c] – the fungus overwinters on fallen leaves (2021, Gödöllő, Hungary).

RHYTISMA AS A NEW SPECIES (2014)

A new species *of Rhytisma polare / polaris* (Fig. 2a) – living on *Salix polaris* – was discovered recently and approved by morphological differences with some genetic data of ITS and LSU sequences of ribosomal rDNA (*Masumoto et al., 2014*). The study also supported the difficulties of minimal DNA sequence differences (Fig. 2b) need to identify a taxonomically new species from its related species (*Gyulai et al., 2019*). *R. polare* lives in extreme cold and dry conditions in the high-Arctic polar semi-desert of Spitsbergen, Norway. A study showed that free water availability from rainfall and snowmelt is essential to facilitate ascostromal maturation and ascospore dispersal that was observed also with short spore-dispersal distance

RHYTISMA AS A BIOLOGICAL INDICATOR

Similar to the toxic ammonium (*Bittsánszky et al., 2015*), the effect of city air pollutants sulfur dioxide (SO_2) was studied, which did not show correlations with the distribution of *R. acerinum* diseases on *Acer pseudoplatanus* (sycamore) grown in Edinburgh, UK (*Leith and Fowler, 1988*). However, the tolerance limit was reported to be approximately 90 µg m−3 SO_2 (*Bevan and Greenhalgh, 1970*). One of the other current serious air pollutants, nitrogen dioxide (NO_2) had reduced tar spot symptoms of *R. acerinum* growing on sycamore above a threshold concentration of about 20 µg m−3 in England (*Gosling et al., 2016*).

RHYTISMA AND LEAF LITTER DECOMPOSITION

Similar to leaf infecting microfungi, *R. acerinum* was found to be potential in taking a role in GCC (global carbon cycle) (*Pan et al., 2011*) by decomposing (Fig. 3./1c) of last year leaves (*i.e.*, leaf litter). Leaf litter decomposition of fungi (and bacteria) is significant in comparison to, *e.g.*, the slowly decaying *Platanus* and oak (*Quercus)* leaves in case of dry falls and winters. However, before leaf litter decomposition, leaves must run photosynthesis and produce oxygen at a high rate as possible (*Liu and van Iersel, 2021*).

CONCLUSIONS

The roles of tree leaf fungal disease *R. acerinum* and related species were studied concerning genetics and systematics. It revealed a wide range of DNA nucleotide differences, which raised a need for consensus to determine its minimal rate applied for molecular systematics. The study also revealed that *R. acerinum* shows the closest genetic distance to *R. polare.* Indications were made to the decomposing roles of leaf litter decaying microfungi in the global carbon cycle.

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