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Original scientific paper

MOLECULAR TAXONOMY AND PHYLOGENETICS OF *Daedaleopsis confragosa* (Bolt.: Fr.) J. Schröt. FROM WILD CHERRY IN SERBIA

Vladislava GALOVIĆ[1]*, Miroslav MARKOVIĆ[1], Predrag PAP[1], Martin MULETT[4], Milana RAKIĆ[2], Aleksandar VASILJEVIĆ[3], Saša PEKEČ[1]

[1]University of Novi Sad, Institute of Lowland Forestry and Environment, Novi Sad, Serbia
[2]University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia
[3]Gljivarsko društvo Novi Sad, Novi Sad, Serbia
[4]Centre for Forestry and Climate Change (Centre for Forestry and Climate change Forest Research, Alice Holt Lodge Farnham Surrey GU104LH UK

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Daedaleopsis spp., a lignicolous fungus causes of white rot on wild cherry and other broadleaved species and makes economic losses in Serbian forestry. The paper presents results of two morphologically distinct fungi *Daedaleopsis confragosa* and *Daedaleopsis tricolor* isolated from native populations of wild cherry (*Prunus avium* L.) found in the sites of Protected Forests of Serbia. Morphological appearance of *D. tricolor* was found more abundant in comparison to *D. confragosa* species. Samples from Serbia were analysed using morphometric and molecular tools and compared with isolates from United Kingdom and published sequences from Sweden, Austria, Hungary, Germany, Canada, France, USA and Czech Republic to give the taxonomic insight and their genetic relatedness using fungal barcoding region ITS rDNA. Results from BLAST search confirmed morphology of the isolates to their taxonomic affiliation as *D. confragosa* while sequence analysis showed mutations at several polymorphic positions that indicates genetic divergence to *D. tricolor*. Phylogenetic analysis presents narrow genetic relations of Serbian isolates with the one from United Kingdom while distinctness from other countries investigated specimens. Future work needs variable regions for both species to be amplified in order to evaluate species boundaries or employing NGS technologies in more detailed sequence analyses.

Corresponding author: Vladislava Galović, University of Novi Sad, Institute of Lowland Forestry and Environment, Antona Čehova 13d, 21000 Novi Sad, Serbia, Phone: +381/21540383, E-mail: galovic@uns.ac.rs;

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INTRODUCTION

The wild cherry (*Prunus avium* L.) is of multi-facet importance in furniture and farmaceutical industry due to its highly respected wood quality and its decorative, melliferous and medicinal properties. Having high adaptive plasticity it can be found everywhere from fluvial deposits of lowland rivers to altitudes of 1400 m (STANKOVIĆ, 1981), 1500 m (BOJKOV and ZAHOV, 1952), 1700 m (ŠILIĆ, 2005), and 1900 m (RUSSELL, 2003) while being quite rare in the sub-Mediterranean zone. It has been classified into a group of fast-growing species, with a rotation of 40-60 years (JOVANOVIĆ, 2000). MIKIĆ (2008) showed that wild cherry trees rarely grow in groups and BALLIAN (2000, 2002) and BALLIAN *et al*., (2012) noted that the existence of high number of ecotypes and forms is a consequence of long-standing growth of natural populations in different ecological conditions. As this valuable species is nowadays represented in a small percentage in natural forests in Serbia, interest in wild cherry plantations as a valuable processing material is in raising demand. This situation has triggered the interest for studying potential problems in the cultivation and protection of this species with the special attention to disease. Given that previous research on wild cherry diseases in Serbia are scarce, as well as published papers devoted to this topic, not much is known about the different diseases invading *P. avium.* This study was carried out research *on Daedaleopsis confragosa* and its variety *D. confragosa* var. *tricolor* due to causing substantial damage on wild cherry trees. *Daedaleopsis confragosa* (Bolt.: Fr.) J. Schröt. is a lignicolous fungus causing white rot. It appears as a facultative parasite or saprophyte on dead trunks of deciduous trees and causes white rot. The *Daedaleopsis confragosa* or *Daedalea confragosa* fungus has under that name registered on more than 45 tree species worldwide. (according to http://nt.arsgrin.gov/fungaldatabases/new_allView.cfm?whichone=FungusHost&thisName=Daedalea%20confragosa&organismtype=Fungus&fromAllCount=yes).

While researching parasitic and saprophytic fungi on wild cherry trees MARKOVIĆ (2012) determined fungus *D. confragosa* and its variety *tricolor* as prevalent on this tree species. As genus, *Daedaleopsis* was first described by SCHRÖTER in 1888. This genus is widely distributed and consists of six species: *D. confragosa* (Bolt.: Fr.) J. Schröt., *D. nipponica* Imazeki, *D. papyraceoresupinata* (S. Ito & S. Imai) Imazeki, *D. pergamenea* (Berk. & Broome) (RYVARDEN and MELO, 2014), *D. septentrionalis* (P. Karst.) Niemelä and *D. sinensis* (Lloyd) Y.C. Dai. (http://en.wikipedia.org/wiki/Daedaleopsis).

Due to lack of data about the morphological and molecular features of the *D. confragosa* in Serbia, the aim of this research was to morphologically describe, prove taxonomical belonging and genetic relatedness of the fungus using molecular approach. Furthermore to evaluate the species boundary between two closely related taxa, *D. confragosa* and *D. tricolor* using nuclear ribosomal internal transcribed spacer (ITS rDNA) marker gene from Serbian specimens in comparison with isolates from United Kingdom and published sequences from the NCBI Gene bank from other countries.

MATERIALS AND METHODS

Material

All together 24 sequences were investigated in this study. Six specimens of *Daedaleopsis tricolor* and one of *Daedaleopsis confragosa* were sampled from protected forests

of Serbia (Fruška Gora, Crni Vrh, Kopaonik). Four isolates from United Kingdom of *D. confragosa*, originated from several regions (the Surrey region – 2 isolates, Berkshire and Hampshire – 1 isolate each) and inoculated in the laboratory of the Centre for Forestry and Climate Change, United Kingdom, were included in this research. The coordinates of sampling sites are provided in and mapped. Thirteen sequences from other countries from NCBI data base were included in research work as well.

Table 1. The isolates and sampling sites

Internal isolate code	Sampling sites	Altitude	Site affiliation
FG4	45°09′33,56″ N 19°46′58,43″ E	461 m	Fruska Gora - Zmajevac
FG5	45°09′19,40″ N 19°44′58,99″ E	468 m	Fruska Gora - Brankovac
FG D.c. prof.	45°09′36,75″ N 19°47′30,87″ E	442 m	Fruska Gora - Prema jezeru
2-1109 DC	44°08′22,36″ N 21°59′20,58″ I	719 m	Crni vrh (Bor)
FG 2/2 301109	45°09′22,86″ N 19°47′42,79″ E	491 m	Fruska Gora - Meteoroloska
DC FG g P. avium 30910	45°09′44,98″ S 19°54′23,06″ E	411 m	Fruska Gora - Sparkasa
UK23052011	51°12′13″N 0°48′12″W	-	Surrey1 - Farnham Flat UK
UK23052011	51°12′17″N 0°47′55″W	-	Surrey2 - Farnham Steep UK
UK23052011	51°24′33″N 0°38′29″W	-	Berkshire - Silwood park UK
UK23052011	51°11′45″N 1°00′26″W	-	Hampshire - Weston Common UK
Kop II DT 05022011	43°16′42,38″ N 20°52′11,47″ E	1.082 m	Kopaonik - Mramor

Methods for isolation of the fungus

The isolation of fungi has been done on a conventional PDA (potato-dextrose-agar) (BOOTH, 1971) growth substrate by using a standard method (a wooden fragment taken from the immediate vicinity of fruiting bodies or a small part of a fruiting body has had its surface flame-sterilised by a spirit lamp and then placed on the substrate). Upon obtaining pure fungal cultures, the isolates were transferred to the tubes containing MEA (Malt Extract Agar) (BOOTH, 1971) growth substrate, labelled and deposited into the mycological collection of the Institute of Lowland Forestry and Environment in Novi Sad. Fragments of pure fungal cultures have been cultivated "*in vitro*" in a liquid Malt Extract (ME) growth substrate (20 g/L of Oxoid Malt Extract) (Photo 3). Each sterile covered plastic box was filled with 30-40 mL of prepared growth substrate, and then inoculated by two pure fungal culture fragments. The fungi grew at room temperature (22-27°C), form a regular spherical shape with constant mixing (@100 rpm) applied to mycelia in order to get higher yield of pure cultures (KEČA, 2005).

Isolation of DNA, amplification and sequencing

For DNA isolation the "*in vitro*" liquid grown cultures have been used exclusively. The DNA isolation was done one month after the mycelia has grown in liquid ME growth substrate.

The mycelia was rinsed in sterile distilled water and ethanol, and then frozen in sterile 1.5 mL Eppendorf tubes until DNA isolation.

For DNA isolation, a Qiagen DNeasy Plant Mini Kit has been used according to manufacturer's protocol. The yield and quality of isolated genomic DNA was measured by BioSpec-nano Micro-volume UV-VIS spectrophotometer (www.shimadzu.com) and purity using 1% ethidium bromide agarose gel by horizontal electrophoresis system. The gel was photo documented using DOC print system (SERVA Electrophoresis, www.serva.de). When working with the DNA, Qiagen reagents (www.Qiagen.com) (DNeasy Plant Mini Kit, PCR Purification Kit, and Gel Extraction Kit) were used from extraction to sequencing. All successfully amplified PCR products obtained by using selected primer sets were sequenced. For molecular characterisation ITS rDNA, PCR and SNPs methods were used. PCR amplification of nuclear ribosomal internal transcribed spacer (ITS) region was applied utilizing a combination of ITS1F and ITS4 primer pair. For the PCR mixture and multiplication of DNA fragments, a protocol published by GALOVIĆ *et al*. (2010) was followed. The region sequenced was 600-650bp long, varying based on the length of sequence amplified in certain taxa. The amplicons were purified and the corresponding fragments isolated from the gel using Qiagen kit reagents and sent for Sanger sequencing. The ITS amplicons were sequenced (http://dna.macrogen.com) and taxonomic affiliation has been determined for each isolate separately, using the Basic Local Alignment Search Tool (BLAST) program within the National Centre for Biotechnology Information (NCBI) gene bank (https://www.ncbi.nlm.nih.gov). All sequences generated in this study were deposited in the same, NCBI data base.

Sequence alignment and phylogenetic analyses

Involving 11 newly generated sequences in this study, marked with asterix, and 13 additional nucleotide sequences from the NCBI Gene Bank was shown in Table 2. Sequence alignment was done by MUSCLE. The preferred nucleotide substitution model, with the lowest Bayesian Information Criterion (BIC) was Kimura 2 with Gamma distribution (K2+G). After aligning the sequences, phylogeny reconstruction was conducted by Maximum Composite Likelihood Model. Gaps/Missing data treatment was handled as Partial Delition option. Evolutionary analyses were conducted in MEGA5 (TAMURA *et al*., 2011).

Phylogenetic tree was reconstructed to reveal the genetic relatedness of the isolates collected in Serbia and United Kingdom in comparison with the ones showed the highest homology in the BLAST process.

Tab 2. ITS sequences used in phylogenetic analyses

Morphological determination	Origin	Strain	GenBank Acc. No.
D. confragosa	United Kingdom/Farnham Flat	23052011_1	KY816877*
D. confragosa	United Kingdom/farnham Steep	23052011_2	KY816878*
D. confragosa	United Kingdom/Silwood Park	23052011_3	KY816879*
D. confragosa	United Kingdom/Weston Common	23052011_4	KY816880*
D. confragosa var. tricolor	Serbia/Fruška gora - Zmajevac	FG4	KY816881*
D. confragosa	Serbia/Fruška gora- Brankovac	FG5	KY816882*
D. confragosa var. *tricolor*	Serbia/Fruška gora - Prema jezeru	FG	KY816883*
D. confragosa var. *tricolor*	Serbia/ Crni vrh (Bor)	2-1109_DC_SRB	KY816884*
D. confragosa var. *tricolor*	Serbia/Fruška gora - Meteoroloska	FG_2-2_301109_SRB	KY816885*
D. confragosa var. *tricolor*	Serbia- Sparkasa	DC_FG_30910_SRB	KY816886*

D. confragosa var. tricolor	Serbia-Kopaonik	Kop_II_DT_05022011	KY816887*
Daedaleopsis confragosa	Czech Republic	Dt1	HG973502.1
D. confragosa var. tricolor	Czech Republic	Dt8	HG973496
Daedaleopsis confragosa	Canada	X-78	KC176348.1
Daedaleopsis confragosa	France	BRFM 1131	JX082373.1
Daedaleopsis confragosa	Deutschland	4	FR686551.1
Datronia mollis	USA	RLG6304sp	JN165002.1
Polyporus arcularius	Canada	CulTENN10299 SBI 2	AB070865.1
Earliella scabrosa	USA	PR1209	JN165009.1
Lenzites tricolor	France	CIRM-BRFM 954	GU731548.1
Lenzites tricolor	France	BRFM:954	JN645096.1
D. confragosa var. tricolor	Austria	WU:19193	KR108005.1
Daedaleopsis confragosa	Austria	WU:8934	KR108000.1
D. confragosa var. tricolor	Austria	WU:26903	KR108006.1

RESULTS

Morphological characterisation of the fungus

Biology of the fungus *Daedaleopsis confragosa* has been described in details by many authors, e.g. GORLENKO *et al.*, (1980), PHILLIPS (1981), ELLIS and ELLIS (1990), KUO, (2005), KARADŽIĆ (2010), MARKOVIĆ (2012).

The morphological description of the fungus *Daedaleopsis confragosa* and its variety *D. tricolor* invading wild cherry, originated from Serbia are given as following. Fruiting bodies of the *Daedaleopsis confragosa* fungus (Photo 1) are semicircular or bracket-shaped (sometimes their bodies form whole circles or even overlap a bit), console-shaped (although they can be slightly descending at times), measuring 4-15 x 3-10 x 2-4 cm. The upper side is more or less concentrically zoned, smooth, sometimes with short and bristly hairs in concentric zones, without shine, in brown hues mostly, although its colour may vary from ochre (towards the margin) to brownish red (towards the centre). The margin is always thin and sharp, predominantly lighter than the rest. The hymenophore consists of tubes measuring up to 10 mm in length and, depending on their position, can assume the shape of pipe organs. Whitish when young, grayish brown later, and pinkish brown in injured places. The pores are roundish very infrequently, mostly elongated, and often maze-like (Photo 2). The trama is tough, thin, and in grayish brown hues. The fruiting bodies grow individually (when having a regular consoled shape) or in groups (when overlapping like tiles or coalescing). The basidia are elongated or club-shaped, having 4 sterigmata at the top and a basal clamp connection. The basidiospores are hyaline, cylindrical to slightly curved, smooth, measuring (5.9) 7.1-9.4 (11.8) x (1.4) 2.3-2.8 (4.1) μm.

The *Daedaleopsis confragosa* var. *tricolor* species (Photo 2) differs from *D. confragosa* by being smaller, having concentric brownish red to chocolate brown zones on the top surface, and its margin is often in light ochre to almost white colour. The hymenophore consists of lamellae (which sometimes have transverse, concentric cross-bridges). The lamellae are pale to reddish brown, serrate, often irregularly crowded, and turn red when injured. The basidia are somewhat larger in size, while the spores are very similar to the spores of *D. confragosa*. The trama is darker and slightly corky.

Origin of the isolates were mapped in Figure 1.

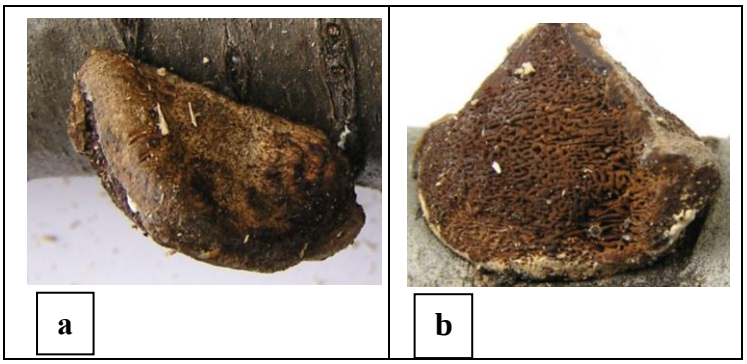

Photo 1. Fruiting body of *D. confragosa*: a) Upper side; b) Himenofor;

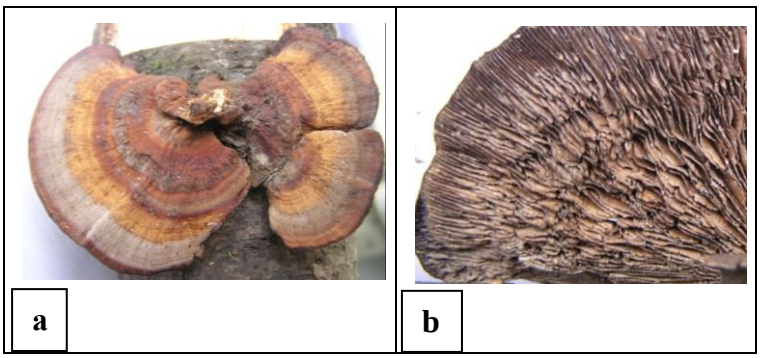

Photo 2. Fruiting body of *D. confragosa var. tricolor*: a) Upper side; b) Himenofor;

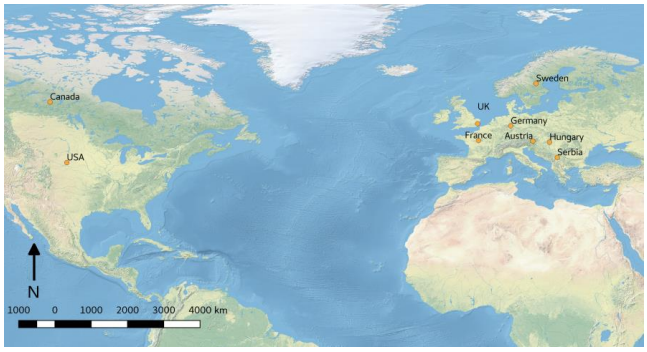

Figure 1 The origin of the isolates in this study.

Photo 3. Pure fungal cultures in liquid Malt Extract (ME) growth substrate

Molecular identification and phylogeny

The sequences of ITS amplicons, derived from our laboratory, were compared with sequences in the NCBI gene bank using BLAST searching tool, and the result are provided in Table 3. In order to obtain a more detailed insight into the similarities and differences between analysed isolates, i.e. the polymorphism of their sequences, the alignment of sequences has been displayed in Figure 3, showing the conserved regions, but also the polymorphic sites based on which the phenogram of the observed species has been drawn.

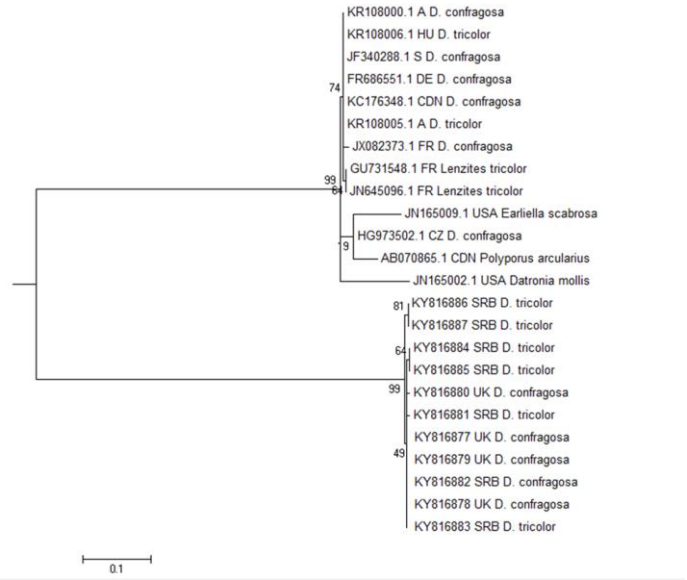

Fig. 2. Phenogram revealed using the Maximum Likelihood method from an analysis of the nuclear ITS rDNA matrix of *Daedaleopsis confragosa*, *Daedaleopsis tricolor, Earliella scabrosa*. *Polyporus arcularius and Datronia mollis* were used to root the trees. Numbers above branches indicate Maximum Likelihood (MLBS)

Table 3. The BLAST analysis of sequenced ITS regions of rDNA of Daedaleopsis confragosa isolates from Serbia and United Kingdom

Isolate No.	Internal isolate code	Host	Site	ITS1 and ITS2 Acc No. in NCBI /internal sequence code	Identities (%)	Gaps (%)	BLAST results
1	FG4	*Prunus avium*	Fruška Gora	KY816881/ 1291ZAB006	630/633 (99%)	1/633 (0%)	FJ810177.1 D. confragosa strain dd08088
2	FG5	*Prunus avium,*	Fruška Gora	KY816882/ 1291ZAB004	632/633 (99%)	1/633 (0%)	FJ810177.1 D. confragosa strain dd08088
3	FG D.c. prof.	*Prunus avium*	Fruška Gora	KY816883/ 1291ZAB011	632/632 (100%)	0/632 (0%)	FJ810177.1 D. confragosa strain dd08088
4	2-1109 DC	*Prunus avium*	Crni Vrh	KY816884/ 1291ZAB012	629/632 (99%)	0/632 (0%)	FJ810177.1 D. confragosa strain dd08088
5	FG 2/2 301109	*Prunus avium*	Fruška Gora	KY816885/ 1291ZAB016	630/632 (99%)	0/632 (0%)	FJ810177.1 D. confragosa strain dd08088
6	DC FG g P. avium 30910	*Prunus avium*	Fruška Gora	KY816886/ 1291ZAB022	631/633 (99%)	0/633 (0%)	FJ810177.1 D. confragosa strain dd08088
7	FF UK23052011_1	*Prunus spp.*	Farnham Flat UK.	KY816877/ 12B0ZAA009	582/582 (100%)	0/582 (0%)	FR686551.1 D. confragosa strain 4
8	FS UK23052011_2	*Prunus spp.*	Farnham Steep UK.	KY816878/ 12B0ZAA012	297/302 (98%)	4/302 (1%)	FR686551.1 D. confragosa strain 4
9	SP UK23052011_3	*Prunus spp.*	Silwood park UK	KY816879/ 12B0ZAA016	547/551 (99%)	1/551 (0%)	FR686551.1 D. confragosa strain 4
10	WC UK23052011_4	n n	Weston Common UK	KY816880/ 12B0ZAA017	347/356 (97%)	6/356 (2%)	FR686551.1 D. confragosa strain 4
11	KopIIDT 05022011	*Prunus avium*	Kopaonik-Mramor	KY816887/ 12B0ZAA018	630/631 (99%)	0/631 (0%)	FJ810177.1 D. confragosa strain dd08088

Out of the 11 analysed isolates (Serbian and United Kingdom), 2 have been 100% identical to the blasted sequence, 7 showed 99%, one 98% and one 97% identity. Besides those ones it was included 13 sequences from other counrties in alignement process and phenogram reconstruction (Figure 2). All sequences have been compared by Maximum Likelihood method, based on the Tamura-Nei model (TAMURA and NEI, 1993).

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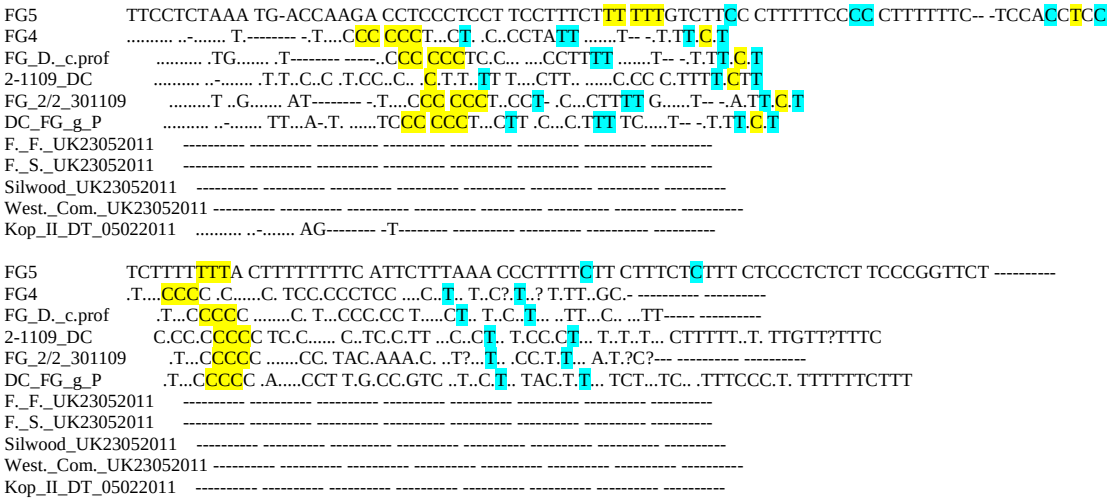

Fig. 3. The Clustal W multiple sequence alignment of nucleotide sequences and a view of the conserved and variable sites in the part of the sequenced fragment for UK and SRB isolates.

The phenogram divided the observed isolates into 2 clades and one subclade within first clade. The first and more abundant one represented 13 sequences from 8 countries and their genetic relatedness. It was shown that most of the sequences were monophyletic while the statistical program defined and separate two sequences of *Lenzites tricolor* from France (GU731548.1 and JN645096.1) as genetically close and distant from all others. Species *D. confragosa* (JX082373.1) from France was more distinct from all others as well. That indicate that ITS rDNA marker works on the species level. Bootstrapping was supportive and reliability was confirmed. The second clade grouped sequences from Serbia and United Kingdom where specimens from Fruška gora and Kopaonik were genetically very close and those specimens from Crni Vrh and Fruška gora as well. Morphologically those specimens were characterised as *D. tricolor*. One specimen from Fruška gora-Zmajevac (KY816881-FG4) as *D. tricolor* was most distinctive together with specimen from Weston Common UK (KY816880) indicating their genetic specificity. Sample from Serbia Fruška gora-Brankovac (KY816882) is morphologically and molecularly belons to *D. confragosa.*while isolate from Fruška gora-Prema jezeru (KY816883) morphologically as *D.* tricolor were grouped in the sam clade together with the rest of the UK samples that are generated as *D. confragosa* as well.

Molecular identification proved morphological description of Serbian and United Kingdom isolates. In this study it is found that prevalent species in Serbia belong to *Daedaleopsis confragosa* var. tricolor while all isolates from United Kingdom were revealed as *Daedaleopsis confragosa* species.

Phenogram generated one clade with genetic similar Serbian and United Kingdom isolates and showed genetic divergence to all other homologues isolates found in the data base. Eventhough their taxonomical belonging indicates same species, different rearrangement of the basis in their sequences structure makes them genetically divergent.

DISCUSSION

Daedaleopsis confragosa fungus is distributed worldwide in Europe, North America and Asia. MARKOVIĆ (2006), MILIJAŠEVIĆ and KARADŽIĆ (2007), KARADŽIĆ and ČOLIĆ (2009) and KARADŽIĆ, (2010, 2011) found this fungus also in territories of Serbia and Montenegro and Republika Srpska on various tree species like *Alnus*, *Betula*, *Salix*, *Tilia*, *Corylus*, *Carpinus*, *Fagus*, *Malus*, *Picea Quercus*, and *Prunus avium*. . On wild cherry trees, it is most frequently found in moist forests, on tree trunks in the vicinity of waterways. It is usually found on injured trees, as well as on fallen trunks (MARKOVIĆ, 2012). During this research, *D. confragosa* was found very frequently and in large numbers on tree trunks in lower-altitude forests (Fruška Gora, 468 m), while in habitats above 1,100 m it was relatively rare. Fruiting bodies were found even on healthy wild cherry trees. Morphological distinctness of the fruiting bodies of the species and its variety was obvious which is in accordance with findings of KARADŽIĆ (2010) and MARKOVIĆ (2012) but there was no significant difference in micromorphology.

According to published literature data there are several genomic regions that are applied in fungal taxonomy and phylogenetic investigations: ribosomal IGS (Intergenic spacer (ANDERSON and STASOVSKI, 1992; HENRION *et al*., 1992; MOUKHAMEDOV *et al*., 1994; EDEL *et al*., 1995); mitochondrial rDNA (WHITE *et al*., 1990); ITS and large subunits of rRNA - nLSU rDNA (ZMITROVICH and MALYSHEVA, 2013); ITS rDNA, RPB2, TEF (KOUKOL *et al*., 2014) and ITS rDNA (MENTRIDA *et al*., 2015).

By suggesting throughout the literature that *D. tricolor* is ecotype of *D. confragosa* (KO and JUNG, 1999) or a variety of *D. confragosa* (KOUKOL *et al*., 2014) most of the research were based on ITS rDNA region that signifies it as the most widely sequenced DNA region in molecular ecology of fungi and has been recommended as the universal fungal barcode sequence that is used for molecular systematics on the species level or even within species (geographic races) (SCHOCH *et al*., 2012).

The fact that *D. confragosa* is a highly variable species, and that its *tricolor* variety also often appears on wild cherry trees and that morphological analysis is not enough to separate between species and varieties, in this study we performed amplification and sequencing of ITS rDNA region in analysis of the isolates.

Sequence analysis and multiple sequence alignment showed containment of both variable and conservative regions which allowed their comparison and discrimination at different taxonomic levels. Our results of multiple sequence alignment support findings of MENTRIDA *et al*., (2015) where mutations at polymorphic positions indicates different species boundary between closely related taxa showed in Figure 3. The subclade evidently grouped sequences that cover different taxa and belongs to geographically distinct area as USA and Canada. They were separated in the subclade cause of their higher taxonomic level as well. Taking into account the appearance and structure of fruiting bodies, type of rot, and physiological properties, each of these isolates show macroscopic differences, indicating pronounced morphological variability of the two observed species. Based on the multiple sequence alignment a view of conserved regions in a part of the sequenced fragment was provided for each isolate, transition point, and transversion of nucleotides. The complete fragment contains ii=369 identical pairs, si=65 transitional pairs, and sv=58 transversional pairs where ratio between transition and transversion pairs is R=1.73 Analyzing nucleotide alignment, several point mutations were occurred which implicate a different genomic structure of the isolates.

Based on molecular analyses and alignment of all nucleotide sequences, the FG5 isolate was found to be the most genetically distinct from the rest in its clade in such a way that nucleotide substitution where pyrimidine nucleotide C was substituted by T and *vice versa* showing the transitional mutation at polymorphic positions, which can confirm morphological characterisation by genomic approach and its belonging to *D. confragosa* in comparison to the other isolates that morphologically belongs to the *D. confragosa* var. *tricolor* . With a final dataset, the phylogenetic tree indicates relatedness and similar genetic structure of the isolates derived from UK and Serbia.

Research work of the group from Czech Republic (KOUKOL *et al*., 2014) using, besides RPB2 and TEF marker genes, ITS region as one of the choice marker for studies on molecular taxonomy concluded that no studied DNA region supports separation of *D. confragosa* and *D. tricolor* as distinct species but the later one as a variety of *D. confragosa*. Also Austrian group, MENTRIDA *et al*. (2015) state the similar conclusion that *D. confragosa* and *D. tricolor* cannot be separated on species level when using ITS data. The same authors found that *D. confragosa* and *D. tricolor* are genetically identical thus should be treated only as varieties of a single species. In this study we found that native specimens are not genetically identical having a pattern in which nucleotide transitions are favoured which is common in molecular evolution after STOLTZFUS and NORRIS (2015) so more likely to support eco-type theory. Even though we found some discrepancy in our study in comparison to other authors we cannot conclude our taxonomic and phylogenetic analyses with certainty due to the size of the sample and only one marker genes used. Also, it is necessary to generate more specific primers that could be able to distinguish variable regions in the species level or working on developing high-quality sequence databases and employing NGS technologies.

CONCLUSION

It can be pointed out that ITS rDNA marker used in this study have provided a clearer insight into molecular taxonomy of *Daedaleopsis* spp. sampled in Serbia, variability of taxa and have highlighted the need of consideration of careful sampling strategy and morphological and molecular characterisation before making taxonomic decisions. These insights also showed that micromorphology is not constant within the same fungal species, and those levels of homogeneity or heterogeneity depends on the culture development. Using molecular approach, particularly SNP of the ITS rDNA region, is available to distinguish between species from the same fruiting body and do and will have a very significant impact on further research work with a large number of morphologically recognized species whose isolates cannot be distinguishable by the culture solely.

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MOLECULARNA TAKSONOMIJA I FILOGENIJA GLJIVE *Daedaleopsis confragosa* (Bolt.: Fr.) J. Schröt. NA DIVLJOJ TREŠNJI U SRBIJI

Vladislava GALOVIĆ[1]*, Miroslav MARKOVIĆ[1], Predrag PAP[1], Martin MULETT[4], Milana RAKIĆ[2], Aleksandar VASILJEVIĆ[3], Saša PEKEČ[1]

[1]Univerzitet u Novom Sadu, Institut za nizijsko šumarstvo i životnu sredinu, Novi Sad, Serbia
[2]Univerzitet u Novom Sadu, PMF, Departman za biologiju i ekologiju, Novi Sad, Serbia
[3]Gljivarsko društvo Novi Sad, Novi Sad, Serbia
[4]Centar za šumarstvo i klimatske promene, Alice Holt Lodge Farnham Surrey GU104LH UK

Izvod

U radu su prikazani rezultati dve morfološki različite lignikolne gljive *Daedaleopsis confragosa* i *Daedaleopsis tricolor* izolovane iz matičnih populacija divlje trešnje (*Prunus avium* L.) pronađene na lokalitetima zaštićenih šuma Srbije čineći značajne ekonomske štete u dekompoziciji vrednog drveta ove biljne vrste. Morfološki izgled *D. tricolor* bio je prevalentan u poređenju sa vrstom *D. confragosa*. Uzorci iz Srbije analizirani su pomoću morfometrijskih i molekularnih metoda i upoređeni sa izolatima iz Velike Britanije i objavljenih sekvenci iz Švedske, Austrije, Mađarske, Nemačke, Kanade, Francuske, SAD i Češke da bi odredili taksonomsku pripadnosti i njihovu genetsku povezanost koristeći ITS rDNA barkod region u genomu istraživanih gljiva. Rezultati BLAST pretraživača sekvenci izolata potvrdili su morfološku karakterizaciju njihovoj taksonomskoj pripadnosti za vrstu *D. confragosa* dok je analiza sekvenci pokazala mutacije na polimorfnim pozicijama koje ukazuju na genetičku divergentnost za varijetet *D. tricolor*. Filogenetička analiza je pokazala usku genetsku srodnost srpskih izolata sa izolatima iz Velike Britanije, dok je razlika značajna u odnosu na sekvence izolata drugih zemalja uključenih u istraživanja. Za budući rad potrebno je umnožiti varijabilne regione obe vrste gljiva u cilju procenjivanja njihovih vrsnih granica ili primeniti nove NGS tehnologije za dublju i detaljniju analizu sekvenci.

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