UDC 575.
https://doi.org/10.2298/GENSR2203101S
Original scientific article

NEW RECORD FOR MYCOBIOTA OF SERBIA: A RARE FUNGUS *Quambalaria cyanescens* FOUND IN *Pelophylax esculentus* (ANURA) SKIN MICROBIOME

Miloš STUPAR*, Željko SAVKOVIĆ, Katarina BREKA, Imre KRIZMANIĆ, Srđan STAMENKOVIĆ, Jelena VUKOJEVIĆ†, Milica LJALJEVIĆ GRBIĆ

University of Belgrade, Faculty of Biology, Belgrade, Serbia

Stupar M., Ž. Savković, K. Breka, I. Krizmanić, S. Stamenković, J. Vukojević, M. Ljaljević Grbić (2022). *New record for mycobiota of Serbia: a rare fungus Quambalaria cyanescens found in Pelophylax esculentus (Anura) skin microbiome*. - Genetika, Vol 54, No.3, 1101-1110.

A rare basidiomycete *Quambalaria cyanescens*, documented so far on various substrates worldwide, was isolated from the skin of edible frog (*Pelophylax esculentus*) captured in South Banat. The fungal identification was based on sequencing of *ITS* region and BLAST analyses. The presence of *Q. cyanescens* in the amphibian skin microbiome is not only the first finding of this fungus in Serbia but also the recording of new ecological habitat for this rare species of micromycetes. Phylogenetic analyses revealed the high similarity of isolate in this study with foliar pathogens of *Eucalyptus* in Australia.

Key words: basidiomycete, BLAST, edible frog, ITS, Quambalariaceae

INTRODUCTION

Taxonomy: The first *Quambalaria cyanescens* isolate was recovered from the human skin by HOOG and DE VRIES (1973) and described as *Sporothrix cyanescens* (strain CBS 357.73), division Ascomycota (FAN *et al*., 2014). However, as detailed morphological studies revealed the presence of dolipore septa, the newly discovered fungal species was reassigned to the division Basidiomycota. MOORE *et al*. (1987) proposed a new basidiomycete genus *Cerinosterus* with two species: *C. luteoalba* (DE HOOG) MOORE (anamorph: *Ditiola peziziformis* (LÉV.) REID) and C. *cyanescens* (DE HOOG and DE VRIES) MOORE) (former *Sporothrix cyanescens*). The genus *Cerinosterus* belongs to the order Dacrymycetales of the class Dacrymycetes (FAN *et al*., 2014). However, MIDDELHOVEN *et al*. (2000) based on 25S rRNA sequencing and nutritional profiling demonstrated that C. *cyanescens* is unrelated to *C. luteoalba*, and is a close relative of the

Corresponding author: Miloš Stupar, University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia, E-mail: smilos@bio.bg.ac.rs, tel: +381 11 3244 847, fax: +381 11 3243 603

† deceased

ustilaginomycetous fungus *Microstroma juglandis* (BÉRENGER) SACC (current name*: Pseudomicrostroma juglandis* (BÉRENGER) KIJPORN. and AIME from the order Microstomatales. To solve that problem, SIGLER and VERWEIJ (2003) erected the new genus *Fugomyces* within the order Microstomatales and named this fungus *F. cyanescens.* Further molecular-level studies of the ITS and LSU regions of the rDNA along with ultrastructural analysis led to the establishment of a new monotypic family Quambalariaceae (within the order Microstomatales in the class Exobasidiomycetes), in which *F. cyanescens* is placed under a novel binomial name *Quambalaria cyanescens* (DE BEER *et al*., 2006). According to Index Fungorum the current species name is *Quambalaria cyanescens* (DE HOOG & DE VRIES) Z.W. DE BEER, BEGEROW & BAUER (obtained from http://www.indexfungorum.org/Names/Names.asp). Apart from *Q. cyanescens*, another 6 species are recognized within the genus *Quambalaria* (*Q. coyrecup, Q. eucalypti, Q. pitereka, Q. pusilla, Q. rugosae, Q. simpsonii* and *Q. tasmaniae*) including the members of the former genus *Ramularia* (obtained from: http://www.mycobank.org).

Ecology and pathogenicity: So far, *Q. cyanescens* was isolated from a variety of substrates, and also documented in a broad range of ecological conditions (air, soil, plant material, and insects). The geographical distribution of this species is wide (North Africa, North America, South Asia, Australia, Europe and the Middle East). (NARMANI and ARZANLOU, 2019). In Australia, *Q. cyanescens* is predominantly reported as a plant symbiont of *Eucalyptus* spp. and *Corymbia* spp., both genera of the family Myrtaceae (DE BEER *et al*., 2006; PAAP, 2006). ANTROPOVA *et al*. (2014) reported the colonization of *Betula pendula* ROTH. (Betulaceae) pollen grains with *Q. cyanescens* in Russia. KOLAŘIK *et al*. (2006) found *Q. cyanescens* in association with phloemophagous bark beetles (*Chaetoptelius vestitus* MULSANT and REY, *Ernoporicus caucasicus* LINDEMANN, *Hypoborus ficus* ERICHSON, *Phloeotribus scarabaeoides* (BERN.) FAUV., *Scolytus* spp., and *Thamnurgus characiae* ROSENHAUER) at eleven studied localities in Bulgaria, Croatia, Hungary, Spain, Syria, and Turkey. LORENZINI *et al*. (2016) reported the presence of *Q. cyanescens* on *Vitis vinifera* L. in Italy. Furthermore, *Q. cyanescens* is reported as a potential opportunistic human pathogen in immunocompromised patients, and so far has been isolated from blood, skin and lung samples from individuals affected with pseudoepidemic nosocomial pneumonia, peritoneal inflammation, invasive pulmonary infection, and lymphoma (JACKSON *et al*., 1990; TAMBINI *et al*. 1996; KUAN *et al*., 2015). Also, FAN *et al*. (2014) isolated *Q. cyanescens* from a female patient after augmentation mammoplasty. Although, *Q. cyanescens* is so far isolated from a variety of substrates, due to the paucity of literature data concerning the ecology and distribution of this micromycete, *Q. cyanescens* is still considered a rare fungal species. Furthermore, due to the limited number of case reports regarding the presence of *Q. cyanescens* in different medical conditions with an uncertain role in pathogenesis, this fungus is regarded as one of the rare clinical basidiomycetous pathogens (FAN *et al*., 2014; KUAN *et al*. 2015). Additionally, *Q. cyanescens* produces a numerous of biologically active metabolites, for example, the recently discovered napthoquinones quambalarine A and quambalarine B which showed strong antifungal activity against the human pathogen *Aspergillus fumigatus* FRESEN. and the entomopathogenic fungus *Beauveria bassiana* (BALS.-CRIV.) VUILL. (STODŮLKOVÁ *et al*., 2015). Novel findings by PROCHÁZKOVÁ *et al*. (2020) suggested that quambalarines are natural pigments with significant cytotoxic and antimicrobial properties.

The *Pelophylax esculentus* complex (green frogs) consists of 2 parental species: the marsh frog *P. ridibundus* (PALLAS, 1771) and the pool frog *P. lessonae* (CAMERANO, 1882) and a hybrid - the edible frog *P. esculentus* (LINNAEUS, 1758). Previous studies suggested, that mucous frog skin can harbor a variety of microorganisms, but since research regarding frog skin microbiota are predominantly targeted at detecting the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) LONGCORE, PESSIER and NICHOLS, non-*Bd* inhabitants are neglected.

In order to better understand the ecology and distribution of *Q. cyanescens*, more reports linking species with other substrates and potential hosts are required. According to BREKA *et al.*, (2019) rare fungal species could also be "hidden dwellers of amphibian integument". In that sense, the main goal of this research is, through targeting the skin of green frogs inhabiting localities in South Banat (Serbia) as a potential source of novel mycological findings in this region, to detect and register new species and to expand knowledge about their ecology.

MATERIAL AND METHODS

Field sampling

A specimen of the water frog, belonging to *Pelophylax esculentus* complex was collected from the locality Jaruga, near the village Kusić on the rim of the protected natural landscape "Karaš-Nera" in South Banat, Serbia (44°52'30.8"N 21°28'16.0"E) in September of 2017. Jaruga is a canal originally made for regulating Nera river floodwaters and melioration of agricultural lands in the area, but since its primary purpose is lost; the canal flow is reduced and overgrown with wetland vegetation. The captured specimen was handled with nitrile gloves and rinsed with sterile distilled water, stored in a denim sack and immediately analyzed in a field laboratory. According to BREKA *et. al*. (2020) the collected individual, was identified as a male specimen of edible frog *Pelophylax esculentus* L.

Sampling of frog skin associated mycobiota

The frog specimen was swabbed, in laboratory conditions, with a sterile cotton swab, and then the swab was inoculated on Potato dextrose Agar (PDA) and incubated at 25 ±1°C (Memmert) within the 7 days. After swabbing captured specimens were released at the capturing site.

Isolation of micromycetes

After the incubation period, selected primary isolates were reinoculated on PDA. Among isolates prepared for molecular characterization, was an isolate forming snow-white velvety compact colonies with violet exudation and smooth-walled hyaline hyphae. The selected isolate was assigned as UIMPEJS017 (short from: **u**nidentified **i**solate from **m**ale specimen of ***P****elophylax* ***e****sculentus* from locality **J**aruga in **S**eptember 2**017**).

DNA extraction

Marginal mycelia (approximately 40 mg) were sampled and used for DNA extraction, according to the manufacturer's instructions (DNAeasy PLant Mini Kit, Qiagen GmbH, Hilden,

Germany). PCR amplification of the ITS region was conducted using the primers ITS1/ ITS4 (Table 1).

Table 1. Primers used for amplification with their corresponding PCR profiles.

Primer Name	Sequence (5' - 3')	Gene	Reference
ITS1-F	TCCGTAGGTGAACCTGCGG	ITS1, 5.8S rRNK	WHITE *et al*. (1990)
ITS4-R	TCCTCCGCTTATTGATATGC	and ITS2	

PCR amplification of ITS region was performed in a Mastercycler personal model (Eppendorf, Hamburg, Germany) in a 25 μl reaction mixture (5 ng DNA, 1 × PCR buffer, 1 μM of each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 1 unit of Taq polymerase) as follows: initial denaturation (95 ° C, 4 min), followed by 35 amplification cycles (95 ° C, 30 s; 52 ° C, 1 min; 72 ° C, 1 min) with a final extension (72 ° C, 7 min) (SAVKOVIĆ et al., 2021). The amplified DNA fragments were fractionated in 1% agarose gels in 0.5 × TBE buffer and visualized by Midori Green dye and UV illumination.

Sequence analyses

The resulting PCR products were shipped for purification and sequencing to Macrogene (Netherlands). Sequences were then compared with other related sequences deposited to the National Center for Biotechnology Information (NCBI) database using the BLAST analysis (BLAST+ 2.7.1 of the NCBI) for primary identification. Obtained fungal DNA sequence was deposited in the relevant GenBank database of NCBI.

Sequence alignment and phylogenetic analysis

Phylogenetic analysis was performed using the CLUSTALW integrated program in MEGA6 software (TAMURA *et al*., 2013). The phylogenetic tree was constructed based on the alignment and comparison of DNA sequences, using Maximum likelihood phylogeny of 1000 bootstrap replicas and *pairwise* deletions. By applying the Model test within MEGA6 program, a Kimura 2 parameter model was determined as the best model for nucleotide substitution.

RESULTS AND DISCUSSION

The Internal transcribed spacer (ITS region) is considered a universal barcode for fungi and is commonly used for molecular identification of the division Basidiomycota members (BADOTTI *et al*., 2017). The molecular approach is a useful tool in situations when morphological characteristics are not informative enough, especially in cases when no reproductive structures are formed on nutrient media. Using ITS sequences initial BLAST searches showed the highest similarity (100%) of isolate UIMPEJS017 with the following isolates (MN162008.1, MN161999.1 (CROUS *et al*., 2019), DQ823419 (PAAP *et al*., 2008), EF444875.1 (PEGG *et al*., 2008) and AJ535500.1 (LANGRELL, unpublished data) deposited in GenBank database as *Quambalaria cyanescens* and *Sporothrix cyanescens*, respectively. Based on that homology, the tested isolate was identified as *Quambalaria cyanescens* (*Quambalaria*, *Quambalariaceae*, Microstromatales, Exobasidiomycetes, Ustilaginomycotina, Basidiomycota, Dikarya, Fungi, Eukaryota). The isolate was then deposited at the culture collection of the Institute of Botany,

Faculty of Biology, University of Belgrade as BEOFB5600m. The obtained sequence of internal transcribed spacer 1 partial sequence was deposited to the NCBI GenBank with accession number MW564022. The sequence was then compared with selected *Q. cyanescens* sequences (homology ranging from 97.23% to 100%) from the GenBank database (Table 2).

Table 2. Quambalaria cyanescens strains used in the ITS and rDNA phylogenetic study and their GenBank accession numbers.

Strain	Accession number	Homology (%)	Source	Reference
QC 11-3-2	KP641151.1	99.51	endophyte of *Pistacia vera* (Iran)	DOLATABAD *et al*., (2017)
2.2.217	KX674666.1	99.83	air of hospital newborn units (Turkey)	DEMIREL *et al*. (2017)
CPC 35399	MN162008.1	100	*Eucalyptus pilularis* (Australia)	CROUS *et al*. (2019)
AUMC 6294	JQ425382.1	98.85	soil of citrus and grapevine plantations (Egypt)	ABDEL-SATER *et al*. (2016)
UM 1095	KT186106.1	99.33	clinical isolate, peritoneal fluid in patient with nephrosclerosis (Malaysia)	KUAN *et al*. (2015)
11PU348	KU052084.1	99.32	clinical isolate (China)	HOU *et al*. (2016)
BRIP48398	EF444875.1	100	*Eucalyptus* spp. (Australia)	PEG *et al*. (2008)
WAC12952	DQ823419	100	*Corymbia calophylla* (Australia)	PAAP *et al*. (2008)
CBS876.73	DQ317623.1	99.82	*Eucalyptus pauciflora* (Australia)	DE BEER *et al*. (2006)
IPWS	HQ316147.1	97.23	endophyte of *Ipomea carnea* (India)	PADHI and TAYUK (2013)
MK 742	AM261920.1	99.82	association with phloemophagous bark beetles *Chaetoptelius vestitus*on *Pistacia vera* (Turkey)	KOLAŘIK *et al*. (2006)
CBS 127353	HG799003.1	99.12	*Betula pendula* pollen grains (Russia)	ANTROPOVA *et al*. (2014)
A12	KX611006.1	99.82	dust samples from *Quercus brantii* (Iran)	DOUST *et al*. (2017)

Corresponding phylogenetic tree is shown in Figure 1. Airborne isolate *Bjerkandera adusta* (*Bjerkandera*, Phanerochaetaceae, Polyporales, Agaricomycetes, Agaricomycotina, Basidiomycota, Dikarya, Fungi, Eukaryota) with a strain number BEOFB1603 and accession number MH605076.1 was used as an outgroup (SAVKOVIĆ *et al*., 2021).

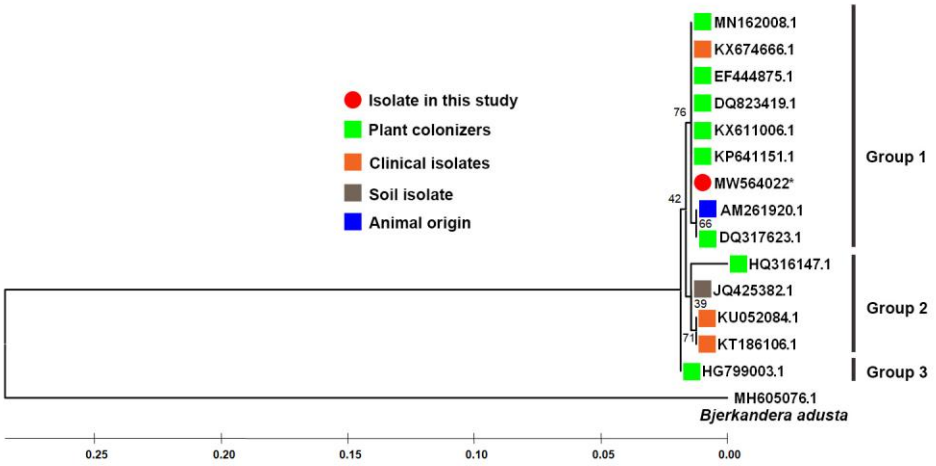

Figure 1. Maximum likelihood phylogenetic trees of internal transcribed spacer (ITS1) region of *Quambalaria cyanescens* isolates; isolate *Bjerkandera adusta* MH605076.1 served as the outgroup. The isolate obtained in this study is marked with a red circle and it's Gene Bank Accession No. with an asterisk.

The isolates of eight species (MN162008.1, KX674666.1, EF444875.1, DQ823419, KX611006.1, AM261920.1 and DQ317623.1) clustered together with the isolate presented in this study with similarity ranging from 99.51 – 100% and formed Group 1. Within this group, the highest similarity of the tested *Q. cyanescens* ITS sequence (100%) was documented with those of Australian isolates: foliar pathogens of *Eucalyptus* spp. (CROUS *et al*., 2019; PEG *et al*., 2008) and causative agents of canker and shoot blight diseases of *Corymbia* spp. (PAAP *et al*., 2008). An additional cluster – Group 2, was formed by four isolates (HQ316147.1, JQ425382.1, KU052084.1, KT186106.1) and among them were clinical isolates from China and Malaysia. On the other hand, the isolate HG799003.1 (*Q. cyanescens* isolated from *Betula pendula* pollen grains in Russia (ANTROPOVA *et al*., 2014) did not cluster together with any of the other sequences.

The association of amphibians with *Q. cyanescens* is for the first time documented in this research, although the nature of this association is not clear and requires further studies. Up to this date, *Quamabalaria* species have not been reported on any substrates investigated in Serbia, nor has it been detected on the amphibian skin, worldwide. Previous studies regarding the amphibian mycobiota associated with *Pelophylax esculentus* complex integument in this region were focused on the registration of pathogens, such as the notorious *Batrachochytrium dendrobatidis* (MALI *et al*., 2017), and potential non-*Bd* pathogens *Fonseceaea* sp. (STUPAR *et al*., 2017) and *Aphanomyces* sp. (STUPAR *et al*., 2020). However, no members of the Basidiomycota division are so far registered as potential amphibian pathogens, so it could be assumed that detected *Q. cyanescens* is most likely a transient. Bearing in mind the amphibian lifestyle and

close contact with various environmental mediums (water, soil, vegetation, mud etc) as well as interactions with other animals (insects, mollusks, crustaceans, fish, other amphibians, reptiles, birds, mammals…), amphibian skin is regarded as a suitable substrate for microbial colonization of diverse origins. Although ambiguous, literature data of the ecology of *Q. cyanescens* regards this fungus as predominantly a plant colonizer, so the potential source of basidiospores could be nearby vegetation. Having in mind, that members of Myrtaceae family, *Pistacia vera*, and *Ipomena* spp. are not native to Serbian flora, it could be assumed that the source of *Q. cyanescens* could be *Betula pendula*, *Vitis vinifera*, or novel "plant candidates" harboring *Q. cyanescens* in this region yet to be discovered. Further studies are required to determine whether this fungus is a constant or transient element of the amphibian skin microbiome, and what impact it has on *P. esculentus* performance as well as on other taxa from the complex. More specifically, the biological interaction of the *Q. cyanescens* – *P. esculentus* association needs to be established, i.e. whether the relationship is that of neutralism, commensalism, parasitism, or symbiosis. To date, this is the third report of potential human and/or amphibian skin-associated fungal pathogens from two species at two localities (STUPAR *et al*., 2017; 2020, this paper) arising from a three-year study of REL (*P. ridibundus* – *P. esculentus* – *P. lessonae*) population systems of green frogs in the South Banat region of Serbia. Although all reported fungal pathogens were sporadic in occurrence and generally designated as transient elements of the microbiome, more research is needed before definite conclusions can be drawn. Additionally, although all species belonging to members *Pelophylax esculentus* complex are present on the locality Jaruga (REL), it should be emphasized that *Q. cyanescens* was isolated only from the hybrid species *P. esculentus*.

ACKNOWLEDGEMENTS

The study was supported by Ministry of Education, Science and Technological Development of Serbia (451-03-9/2021-14/ 200178).

Received, May13th, 2021
Accepted April 28th, 2022

REFERENCES

ABDEL-SATER, M.A., A.H., MOUBASHER, Z.S.M., SOLIMAN (2016): Diversity of filamentous and yeast fungi in soil of citrus and grapevine plantations in the Assiut region, Egypt. Ceska Mykol., *68*: 183–214.

ANTROPOVA, A.B., E.N., BILANENKO, V.L., MOKEEVA, L.N., CHEKUNOVA, A.V., KACHALKIN, O.V., SHTAER, O.V., KAMZOLKINA (2014): Report of *Quambalaria cyanescens* in association with the birch (*Betula pendula*). Microbiol., *83*(5): 690-698.

BADOTTI, F., F.S., DE OLIVEIRA, C.F., GARCIA, A.B.M., VAZ, P.L.C., FONSECA, L.A., NAHUM,... A., GÓES-NETO (2017): Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of Basidiomycota (Fungi). BMC Microbiol., *17*(1): 1-12.

BREKA, K., M., STUPAR, D., VIDAKOVIĆ, M., LJALJEVIĆ GRBIĆ, J., KRIZMANIĆ, I., KRIZMANIĆ, S., STAMENKOVIĆ (2019): Microbial community of green frog's skin. XX European Congress of Herpetology. Milan, Italy. 2.-6.Sept. 2019. Program & Abstracts. p239.

BREKA, K., I., KRIZMANIĆ, T., VUKOV, S., STAMENKOVIĆ (2020): A procedure for taxon assessment based on morphological variation in European water frogs (*Pelophylax esculentus* complex). T. J. Zool, *44*(*3*): 214-223.

CROUS, P.W., M.J., WINGFIELD, R., CHEEWANGKOON, A.J., CARNEGIE, T.I., BURGESS, B.A., SUMMERELL, J., EDWARDS, P.W.J., TAYLOR, J. Z., GROENEWALD (2019): Foliar pathogens of eucalypts. Stud Mycol., *94*: 125-298.

DE BEER, Z.W., D., BEGEROW, R., BAUER, G.S., PEGG, P.W., CROUS, M.J., WINGFIELD (2006): Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia, Stud Mycol., *55*: 289–298.

DE HOOG, G.S., G.A., DE VRIES (1973): Two new species of *Sporothrix* and their relation to *Blastobotrys nivea*. Antonie Van Leeuwenhoek., *39*: 515–520.

DEMIREL, R., B., SEN, D., KADAIFCILER, A., YOLTAS, S., OKTEN, E., OZKALE, D., R., BERIKTEN, A.H., SAMSON, N., UZTAN, O.A., YILMAZ, H., GUNYAR, AYDOGDU, A., ASAN, M., KIVANC, S., OZDIL, E., SAKARTEPE (2017): Indoor airborne fungal pollution in newborn units in Turkey. Enviro. Monit. Assess., *189*(7): 1-11.

DOLATABAD, H.K., M., JAVAN-NIKKHAH, W.T., SHIER (2017): Evaluation of antifungal, phosphate solubilisation, and siderophore and chitinase release activities of endophytic fungi from *Pistacia vera*. Mycological Progress., *16*(*8*): 777-790.

DOUST, N.H., M., AKBARINIA, N., SAFAIE, H., YOUSEFZADEH, M., BÁLINT (2017): Community analysis of Persian oak fungal microbiome under dust storm conditions. Fungal Ecol., *29*:1-9.

FAN, X., M., XIAO, F., KONG, T., KUDINHA, H., WANG, Y.-C., XU (2014): A rare fungal species, *Quambalaria cyanescens*, isolated from a patient after augmentation mammoplasty – environmental contaminant or pathogen? PLoS ONE, *9*: e106949.

HOU, X., M., XIAO, S.C.A., CHEN, H., WANG, L., ZHANG, X., FAN, Z-P., XU, J-W., CHENG, F., KONG, Y-P., ZHAO, Y-C., XU (2016): Sequencer-based capillary gel electrophoresis (SCGE) targeting the rDNA internal transcribed spacer (ITS) regions for accurate identification of clinically important yeast species. PLoS ONE, *11*(4): e0154385.

JACKSON, L., S.A., KLOTZ, R.E., NORMAND (1990): A pseudoepidemic of *Sporothrix cyanescens* pneumonia occurring during renovation of a bronchoscopy suite. J. Med. Vet. Mycol., *28*: 455–459.

KOLAŘIK, M., E.L.E.N.A., SLAVIKOVA, S., PAZOUTOVÁ (2006): The taxonomic and ecological characterisation of the clinically important heterobasiodiomycete *Fugomyces* cyanescens and its association with bark beetles. Ceska Mykol., *58*(1/2): 81.

KUAN, C.S., S.M., YEW, Y.F., TOH, C.L., CHAN, S.K., LIM, K.W., LEE, S.L., NA, C.-C., HOH, W.-Y., YEE, K.P., NG (2015): Identification and characterization of a rare fungus, *Quambalaria cyanescens*, isolated from the peritoneal fluid of a patient after nocturnal intermittent peritoneal dialysis. PLoS ONE, *10*: e0145932.

LORENZINI, M., M.S., CAPPELLO, A., LOGRIECO, G., ZAPPAROLI (2016): Polymorphism and phylogenetic species delimitation in filamentous fungi from predominant mycobiota in withered grapes. Int. J. Food Microbiol., *238*: 56-62.

MALI, I., A., VILLAMIZAR-GOMEZ, I., KRIZMANIĆ, R., AJTIĆ, M.R., FORSTNER (2017): Evidence of *Batrachochytrium dendrobatidis* Infection in Amphibians from Serbian Lowlands. J. Wildl Dis., *53*(*3*): 686-689.

MIDDELHOVEN, W.J., E., GUÉHO, G.S., DE HOOG (2000): Phylogenetic position and physiology of *Cerinosterus cyanescens.* Antonie van Leeuwenhoek, *77*: 313–320.

MOORE, R.T. (1987): Micromorphology of yeasts and yeast-like fungi and its taxonomic implications. Stud. Mycol., *30*: 203–226.

NARMANI, A., M., ARZANLOU (2019): *Quambalaria cyanescens*, a new fungal trunk pathogen associated with grapevine decline in Iran. Crop Prot., *124*: 104875.

PAAP, T. (2006): The incidence, severity and possible causes of canker disease of *Corymbia calophylla* (marri) in the southwest of Western Australia, *Ph. D. Thesis*, Perth, Murdoch Univ.

PAAP, T., T.I., BURGESS, J.A., MCCOMB, B.L., SHEARER, G.E.S.J., HARDY (2008): *Quambalaria* species, including *Q. coyrecup* sp. nov., implicated in canker and shoot blight diseases causing decline of *Corymbia* species in the southwest of Western Australia. Mycol. Res., *112*(*1*): 57-69.

PADHI, S., K., TAYUNG (2013): Antimicrobial activity and molecular characterization of an endophytic fungus, *Quambalaria* sp. isolated from *Ipomoea carnea*. Ann Microbiol., *63*(*2*): 793-800.

PEGG, G.S., C., O'DWYER, A.J., CARNEGIE, T.I., BURGESS, M.J., WINGFIELD, A., DRENTH (2008): *Quambalaria* species associated with plantation and native eucalypts in Australia. Plant Pathol., *57*(*4*): 702-714.

PROCHÁZKOVÁ, E., O., KUCHERAK, E., STODŮLKOVÁ, Z., TOŠNER, I., CÍSAŘOVÁ, M., FLIEGER, M., KOLAŘÍK, O., BASZCZYŇSKI (2020): NMR Structure Elucidation of Naphthoquinones from *Quambalaria cyanescens*. J. Nat. Prod., *84(1)*: 46-55.

SAVKOVIĆ, Ž., M., STUPAR, N., UNKOVIĆ, Ž., IVANOVIĆ, J., BLAGOJEVIĆ, S., POPOVIĆ, J., VUKOJEVIĆ, M., LJALJEVIĆ GRBIĆ (2021): Diversity and seasonal dynamics of culturable airborne fungi in a cultural heritage conservation facility. Int. Biodeter. Biodegr., *157*: 105163.

SIGLER, L., P.E., VERWEIJ (2003): *Aspergillus*, *Fusarium*, and other opportunistic moniliaceous fungi. – In: MURRAY P.R. *et al*. (eds), Manual of Clinical Microbiology vol. 2, pp. 1726–1760, ASM Press, Washington DC.

STODŮLKOVÁ, E., I., CÍSAŘOVÁ, M., KOLAŘÍK, M., CHUDÍČKOVÁ, P., NOVÁK, P., MAN, M., FLIEGER (2015): Biologically active metabolites produced by the basidiomycete Quambalaria cyanescens. PloS ONE, *10(2):* e0118913.

STUPAR, M., K., BREKA, I., KRIZMANIĆ, S., STAMENKOVIĆ, M., LJALJEVIĆ GRBIĆ (2020): First report of water mold (*Aphanomyces* sp.) documented on skin of pool frog (*Pelophylax lessonae*) in Serbia. North-West J. Zool., *16*(*2*): 216-219.

STUPAR, M., K., BREKA, I., KRIZMANIĆ, S., STAMENKOVIĆ, N., UNKOVIĆ, Ž., SAVKOVIĆ, J., VUKOJEVIĆ, M., LJALJEVIĆ GRBIĆ (2017): First case report on pathogenic fungus *Fonsecaea* sp. Negroni from skin of *Pelophylax* kl. *esculentus* L. in Serbia. Matica Srpska, J. Natural Sci., *133*: 307-313.

TAMURA, K., G., STECHER, D., PETERSON, A., FILIPSKI, S., KUMAR (2013): MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol., *30*: 2725-2729.

TAMBINI, R., C., FARINA, R., FIOCCHI, B., DUPONT, E., GUEHO, G., DELVECCHIO, F., MAMPRIN, G., GAVAZZENI (1996): Possible pathogenic role for *Sporothrix cyanescens* isolated from a lung lesion in a heart transplant patient. Med. Mycol., *34*: 195–198

WHITE, T.J., T., BRUNS, S., LEE, J.W., TAYLOR (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (eds), PCR protocols: a guide to methods and applications. New York: Academic Press, 315–322.

NOVI PODATAK ZA MIKOBIOTU SRBIJE: RETKA GLJIVA *Quambalaria cyanescens*, ZABELEŽENA U MIKROBIOMU KOŽE *Pelophylax esculentus* (ANURA)

Miloš STUPAR*, Željko SAVKOVIĆ, Katarina BREKA, Imre KRIZMANIĆ, Srđan STAMENKOVIĆ, Jelena VUKOJEVIĆ, Milica LJALJEVIĆ GRBIĆ

Univerzitet u Beogradu, Biološki fakultet, Beograd, Srbija

Izvod

Retka bazidiomiceta *Quambalaria cyanescens*, do sada zabaležena na različitim supstratima širom sveta, izolovana je sa kože jestive žabe (*Pelophylax esculentus*) ulovljene u Južnom Banatu. Izolat je identifikovan na osnovu sekvenciranja ITS regiona i BLAST analizom. Prisustvo *Q. cyanescens* u mikrobiomu kože žabe ne samo da predstavlja prvi nalaz ove gljive u Srbiji, već i evidentiranje nove ekološke niše za ovu retku vrstu mikromicete. Filogenetske analize pokazale su visok stepen sličnosti izolata u ovoj studiji sa folijarnim patogenima eukaliptusa u Asustraliji.

Primljeno 13.V.2021.
Odobreno 28. IV. 2022.