



First record of *Neoscytalidium novaehollandiae* associated with pistachio dieback in the Southeastern Anatolia region of Turkey

S. Kurt ✉

A. Uysal

E. M. Soylu

M. Kara

S. Soylu

Department of Plant Protection, Faculty of Agriculture, Mustafa Kemal University, Hatay, Turkey

Turkey ranks the fourth in pistachio production in the world with 78,000 tonnes produced annually (Tuik, 2017). The production areas of pistachio (*Pistacia vera* L.) are predominantly in the Southeastern Anatolia region, where accounts for 97% of the total pistachio production in Turkey (Tuik, 2017). During the 2017 and 2018 years, a severe foliar blight, decline and dieback of pistachio trees were observed in the region, in 15-year-old commercial pistachio orchards established in Birecik county of Şanlıurfa province. Incidence of disease ranged from 16 to 64%. The observed symptoms included shoot blight with scorching of leaves, branch wilt, decline, trunk canker and death of trees (Fig. 1a). The twigs of infected pistachio trees showed necrosis and internal vascular discoloration (Fig. 1b). Gummy exudation was frequently associated with the affected tissues. In this study, we identified and characterized this fungal pathogen, based on its morphology, molecular characteristics, and pathogenicity.

Forty samples including infected root and stem tissues were surface-sterilized in 1% NaOCl solution for 1 – 2 min, rinsed in sterile distilled water, placed on Petri dishes containing potato dextrose agar (PDA), and incubated at 25 °C for 7 – 10 days. The fungus formed white to olivaceous aerial mycelium containing chains of arthroconidia and chlamydospores (Fig. 1c). Arthroconidia were aseptate to one septate, thick-walled, hyaline to brown, and circular, oval or cylindrical, 7.2 – 11.3 × 2.9–3.5 µm. Conidia were initially hyaline; ellipsoidal to globose with muriform septa, 4.5 – 10.4 × 2.6 – 3.6

µm (Fig. 1d). On the basis of morphological and cultural features, the fungus was identified tentatively to the *Neoscytalidium novaehollandiae* Pavlic, T. J. Burgess, & M. J. Wingf (Philips et al. 2013), and the subcultures were purified from single conidia of one isolate prepared on PDA. Representative culture of the *N. novaehollandiae* has been deposited in the Microbial Culture Collection of Plant Health Clinic, Hatay Mustafa Kemal University, Turkey, with isolate name BISAK_PNnov1.

Total genomic DNA of representative isolate (PNnov1) was extracted from the aerial mycelium using a DNeasy Plant Mini Kit-Quick-start protocol (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. The ITS-rDNA region (White et al. 1990) and *tefl-α* gene (Carbone and Kohn 1999) were amplified with ITS4/ITS5 and EF1-728F/EF1-986R primers, respectively, and sequenced. The obtained nucleotide sequences have been deposited in GenBank (MK530201 for ITS and MK535094 for *tefl-α*). The isolates and sequences from other studies used in the phylogenetic analyses for this study are described in Table 1. BLASTn queries of the sequences revealed 100% similarity with sequences of *N. novaehollandiae* strains in GenBank (Accession no. MH863173 for ITS and MF662596 for *tefl-α*). The phylogenetic tree was constructed by the maximum likelihood method (Fig. 2).

Pathogenicity was confirmed by placing the number of four 5-cm mycelial agar plugs of isolate PNnov1 in wounds made with a sterile blade under twig bark of 2-years-old potted pistachio. Inoculation sites were wrapped with parafilm to keep moisturized. The control plants inoculated with sterile PDA plugs and pathogen-inoculated plants were maintained in a growth chamber at 25±1 °C with 85 to 90% relative humidity. Fifteen days after inoculation, shoot lesions and gummosis developed on >70 of inoculated shoots. *Neoscytalidium novaehollandiae* caused 1.5–2.5 cm necrotic lesions on detached branches. The

Submitted 13 Sept. 2018, accepted for publication 15 Feb. 2019

✉ Corresponding Author E-mail: senerk31040@gmail.com

© 2019, Published by the Iranian Mycological Society

<http://mij.areeo.ac.ir>

control plants showed no symptoms of the disease. The fungus was re-isolated from symptomatic tissues of inoculated shoots, thus fulfilling Koch's postulates. Based on the morpho-cultural characteristics, molecular data and pathogenicity test, the pathogen was identified as *Neoscytalidium novaehollandiae*.

Neoscytalidium novaehollandiae has recently been reported on grapevine in Turkey (Akgul et al. 2019). To the best of our knowledge, this is the first record of pistachio dieback caused by *N.*

novaehollandiae from Turkey. In 2019, *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers was reported from pistachio in Turkey (Derviş et al. 2019). This fungus is an important pathogenic organism causing wound infections, therefore posing serious threat to newly established pistachio orchards. Further studies are required to reliably determine the potential threat posed by this pathogen for pistachio growers in Turkey.



Fig. 1. *Neoscytalidium novaehollandiae*. **a.** Disease symptoms on pistachio tree, including shoot blight with scorching of leaves, branch wilt, decline and dieback, **b.** Brown vascular discoloration of the wood tissues of pistachio trees, **c.** Colony on PDA at 27 °C, **d.** Chains of arthroconidia and chlamydospores on PDA.

Table 1. *Neoscytalidium* strains and ex-type strain used in the phylogenetic analyses.

| Species | Isolate/strain no. | Host | Origin | Genbank Accession Number | |
|--------------------------------|--------------------|-------------------------------|-----------|--------------------------|----------|
| | | | | ITS | TEF |
| <i>N. hyalinum</i> | CMM3649 | <i>Jatropha curcas</i> | Brazil | KF234550 | KF226707 |
| <i>N. dimidiatum</i> | CBS 499.66 | <i>Mangifera indica</i> | Portugal | KF531820 | KF531798 |
| <i>N. dimidiatum</i> | ND94 | <i>Solanum lycopersicum</i> | Turkey | MH114590 | MH114594 |
| <i>N. hyalinum</i> | CMM3607 | <i>Jatropha curcas</i> | Brazil | KF234542 | KF226688 |
| <i>N. novaehollandiae</i> | WAC13273 | <i>Mangifera indica</i> | Australia | GU172397 | GU172429 |
| <i>N. novaehollandiae</i> | CBS122071 | <i>Crotalaria medicaginea</i> | Australia | EF585540 | EF585580 |
| <i>N. dimidiatum</i> | ND121 | <i>Solanum lycopersicum</i> | Turkey | MH114591 | MH114595 |
| <i>Botryosphaeria dothidea</i> | BD080705002 | Poplar | China | FJ493245 | - |

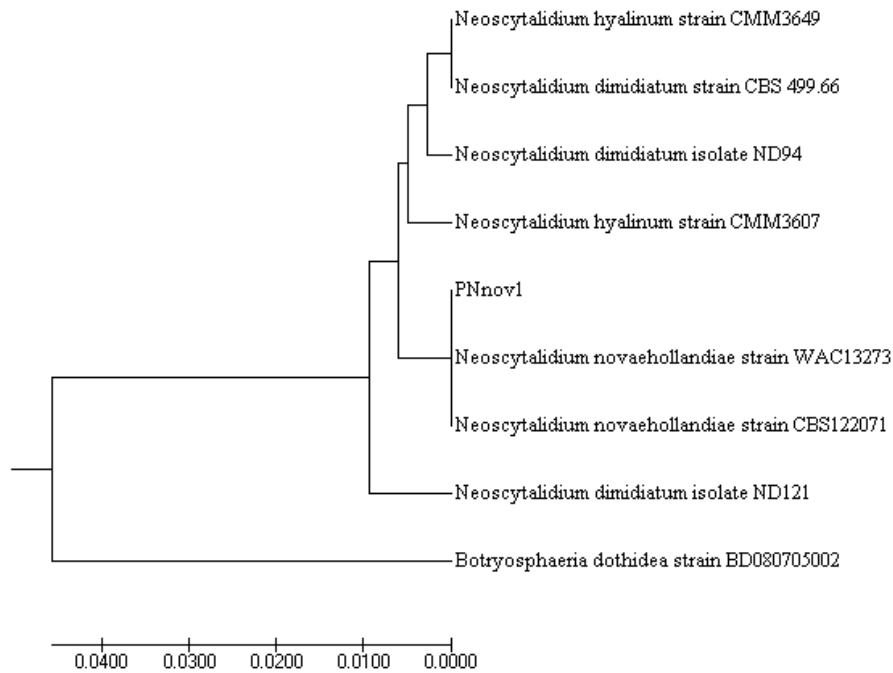


Fig. 2. Two-gene combined dataset: internal transcribed spacer (ITS) and partial translation elongation factor 1-alpha region (*tef1*). The phylogenetic tree was constructed by the Maximum likelihood method.

REFERENCES

Akgul DS, Savas NG, Ozarslandan M. 2019. First Report of Wood Canker Caused by *Lasiodiplodia exigua* and *Neoscytalidium novaehollandiae* on Grapevine in Turkey. *Plant Disease* 103:1036.

Carbone I, Kohn LM, 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91: 553–556.

Derviş S, Türkölmez Ş, Çiftçi O, Ulubaş Serçe, Ç, Dikilitaş M. 2019. First Report of *Neoscytalidium dimidiatum* Causing Canker, Shoot Blight, and Root Rot of Pistachio in Turkey. *Plant Disease* 103 (6): 1411.

Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW. 2013. The Botryosphaeriaceae: genera and species known from culture. *Studies in Mycology* 76: 51–167.

Tuik 2017. Turkish Statistical Institute Databases. Pistachio production statistics of Turkey. Ankara. (<http://www.turkstat.gov.tr/>).

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR Protocols: A Guide to Methods and Applications*. New York, USA: Academic Press Inc., 315-322.