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

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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 *tef1*-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 *tef1*-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 *tef1*-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

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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.

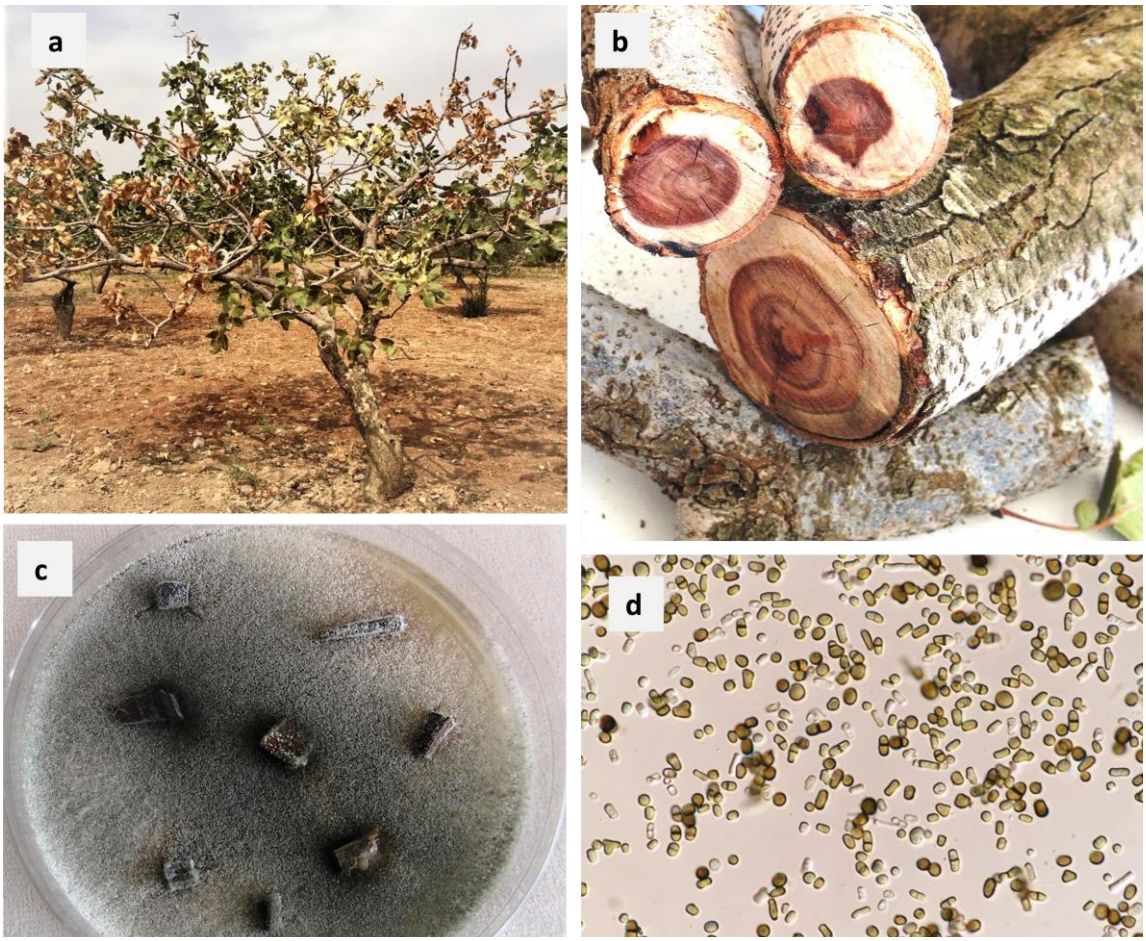

Fig. 1. *Neoscytalidium novaehollandiae*. **a.** Disease symptoms on pistachio tree, including shoot blight with scorching of leaves, branch wilt, decline and dieback, **b.** Brown vascular discoloration of the wood tissues of pistachio trees, **c.** Colony on PDA at 27 °C, **d.** Chains of arthroconidia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

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

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

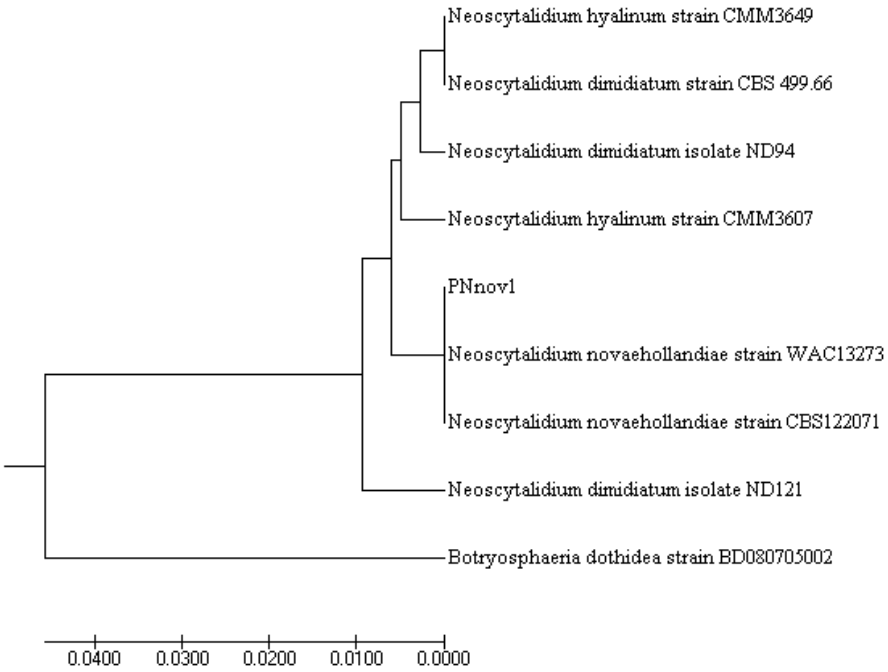

Fig. 2. Two-gene combined dataset: internal transcribed spacer (ITS) and partial translation elongation factor 1-alpha region (*tef1*). The phylogenetic tree was constructed by the Maximum likelihood method.

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