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ORIGINAL PAPER

Detection of *Paecilomyces formosus* in wood-boring beetles associated with oak dieback and decline in the Zagros forests of Iran

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Abstract

Oak dieback disease caused by the fungus *Paecilomyces formosus* threatens oak trees in the Zagros forests in western Iran. Various insects, such as wood-infesting beetles (Coleoptera), may play a role in dissemination of *P. formosus*. We collected larvae and adult insects from branch wood of oak trees with dieback symptoms in the Zagros forests. For larval identification, the mitochondrial gene cytochrome c oxidase I (COXI) was amplified by polymerase chain reaction (PCR). Fungal isolates from wood and insects collected from the sampled oaks were identified by morphology, acid production on creatine sucrose agar (CREA) medium, phylogeny of DNA sequence data for the β-tubulin gene and the internal transcribed spacer (ITS) rDNA. To detect *P. formosus* in larvae and adult insects, we used a nested PCR assay with the species-specific primer pairs PaMF and PaMR. The insects that most often tested positive for *P. formosus* were larvae of the buprestids *Acmaeodera* sp. and *Chrysobothris affinis*, and larvae of the cerambycid *Trichoferus campestris*. Adults of *C. affinis* and *Calchaenesthes diversicolis* (Cerambycidae), which were collected from within their galleries, also tested positive. Beetle larvae of *Anthaxia* sp. (Buprestidae), *Latipalpis plana* (Buprestidae), *Monochamus* sp. (Cerambycidae) and *Crypticus gibbulus* (Tenebrionidae) also tested positive. Larvae that tested negative for *P. formosus* were species of Cossidae (Lepidoptera), Elateridae (Coleoptera), Gasteruptiidae (Hymenoptera) and Syrphidae (Diptera). Future research is needed to determine whether any of these insects can serve as vectors of *P. formosus*. These results can be used to target-specific insects for monitoring.

Keywords Buprestidae · Cerambycidae · Cytochrome c oxidase I · Nested PCR · *Quercus brantii* · Oak dieback

Key message

- The fungus *Paecilomyces formosus* is a major causal agent of *Quercus brantii* dieback in the Zagros forests.
- *Quercus brantii* accounts for about 70% of the trees in the Zagros forests.
- Oak dieback disease poses a substantial threat to the health and stability of the Zagros forests.
- Various Buprestidae, Cerambycidae and Tenebrionidae tested positive for *P. formosus.*
- Pollarding practices need modification to reduce wounding that allow infection and attract insects.

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Introduction

The genus *Quercus* (oak) is one of the most important and dominant genera in the Fagaceae, a family with more than 1000 species worldwide (Coombes 2000). In Iran, oak trees occupy three major forested areas, including Zagros, Hyrcanian and Arsbaran (Sagheb Talebi et al. 2014). Iran's forests cover about 12 million ha, of which 5 million ha (40%) are in the mountainous region of Zagros in western Iran (Jazirei and Ebrahimi Rastaghi 2003). The

Zagros forests is one of the largest oak forests in the world, spreading from northwestern to southeastern Iran, almost parallel to the country's western border (Supplementary Fig. 1). The forests of this area are in the provinces of Chaharmahal and Bakhtiari, Fars, Ilam, Kermanshah, Khuzestan, Kohgiluyeh and Boyer-Ahmad, Lorestan and West Azarbaijan. In western Iran, the three dominant oak species are Persian oak (*Q. brantii* Lindl), gall oak (*Q. infectoria* Oliv.) and Lebanon oak (*Quercus libani* Oliv.) (Sagheb Talebi et al. 2014). The species *Q. brantii* is the most abundant oak species in the Zagros forests, occupying about 3.5 million ha (70%) of the total 5 million ha. The most important functions of the Zagros forests are watershed protection and providing climatic balance to Iran, serving as a defense barrier against micropollutants, providing raw materials used in construction and food products for humans and animals, and providing employment opportunities in the tourist and forest products industries (Sabernasab et al. 2019a, 2020).

Oak decline has been reported in nearly 40 countries since the beginning of the twentieth century (Kowsari and Karimi 2023). Among the most important abiotic factors involved in oak decline are climatic changes (mainly decreases in rainfall and groundwater and increases in drought frequency), increases in ambient temperature and various pollutants (Attarod et al. 2016, 2017; Rostamnia and Akhoondzadeh 2016; Goodarzi et al. 2019). Among the biotic factors, several pathogenic fungi and bark- and wood-infesting insects, primarily Buprestidae and Cerambycidae beetles (Coleoptera), are considered the most important factors involved in oak decline worldwide (Siwkcki and Ufnalski 1998; Denman et al. 2014; Haavik et al. 2015; Kowsari and Karimi 2023).

In Iran, there were reports as early as 2008 of oak dieback and decline in parts of the Zagros forests that covered more than one million ha (Sabernasab et al. 2020). Land use change, tree felling, livestock grazing, intentional and unintentional fires, and insect and disease outbreaks have all been listed as possible contributing factors to oak decline in the Zagros forests (Pourhashemi and Sadeghi 2020). On the other hand, the lack of tree regeneration in these forests has advanced the age distribution of the forest trees. In such situations, these older trees are likely more vulnerable to the environmental stressors mentioned above. The physiological changes in stressed oak trees can increase their susceptibility to certain groups of opportunistic fungal pathogens and insects, which can result in widespread dieback and decline (Panzavolta et al. 2018).

In recent years, efforts have been made to identify the fungal pathogens involved in the phenomenon of oak dieback and decline in the Zagros forests. Among the disease agents reported, the fungus *Paecilomyces formosus* Urquhart (Thermoascaceae), appears to be the most serious threat to oaks in western Iran. This pathogen causes wilting, yellowing, cankers and discoloration of the vascular tissue, which ultimately leads to decline and death of affected oaks and other forest trees (Rostami and Jamali 2023a). In Iran, *P. formosus* was first reported from pistachio trees (*Pistacia* spp.) in 2015 (Heidarian et al. 2015), although this pathogen may have been causing damage much earlier, but was reported under the name *P. variotii* Bainier (Aminaei and Ershad 1989). Based on molecular characters, *P. formosus* has been isolated and reported as the cause of dieback and decline of the following woody plants in Iran: *Amygdalus scoparia* Spach., *Acer monspessulanum* L., *Amygdalus lycioides* Spach., *Anagyris foetida* L., *Azadirachta indica* A. Juss., *Caesalpinia gilliesii* (Hook.) D.Dietr., *Cerasus avium* (L.) Moench., *Cerasus microcarpa* (C.A.Mey.) Boiss., *Crataegus pontica* K. Koch., *Ficus carica* L., *Haloxylon* sp. Bunge, *Malus domestica* (Suckow) Borkh., *Nerium oleander* L., *Paliurus spina-christi* Mill., *Pistacia atlantica* Desf., *Pistacia mutica* Fisch. & C.A.Mey., *Prunus amygdalus* (Mill.) D. A. Webb, *Punica granatum* L., *Quercus brantii*, *Quercus libani*, *Salix acmophylla* Boiss., *Tamarix aphylla* (L.) H.Karst. and *Tamarix hispida* Willd., *Tamarix ramosissima* Ledeb and *Ziziphus spina-christi* (L.) Desf. (Heidarian et al. 2018; Chakusary et al. 2019; Sabernasab et al. 2019a; Azizi et al. 2020; Ghasemi-Sardareh and Mohammadi 2020; Rostami and Jamali 2022, 2023a, 2023b; Jamali and Abbasi 2023). This fungus has been isolated from woody plants in many provinces of Iran, including Hormozgan, Isfahan, Kerman, Kermanshah, Khorasan Razavi, Markazi, Qazvin, Qom, Semnan, South Khorasan, Tehran, West Azarbaijan and Yazd (Heidarian et al. 2018; Sabernasab et al. 2019a).

Given the wide host range and distribution of this fungus in the Zagros forests, *P. formosus* spores are likely dispersed by wind and water. Various bark and wood-infesting insects that feed, oviposit or construct galleries in the crowns or trunks of trees may also serve as vectors of tree pathogens (Raffa et al. 2015; Akbulut et al. 2017; Panzavolta et al. 2018). For example, close associations between arthropods and fungal spores have been reported for fungi in the families Botryosphaeriaceae, Diatrypaceae and Togniniaceae, as well as the order Diaporthales (Kubátová et al. 2004; Moyo et al. 2014). In the case of *P. formosus* infections of forest trees in western Iran, the possibility of bark and wood-infesting insects being involved in spore dissemination exists, but the evidence to date is limited to isolation of the fungus from insect-infested trees. Therefore, the objectives of this study were to (1) collect branches from declining *Q. brantii* trees and test them for *P. formosus*, (2) collect and identify insects from within the sampled branches, and (3) attempt to detect *P. formosus* in the insects collected.

Materials and methods

Field sites and tree sampling

On multiple occasions during each season of the year from August 2021 through September 2023, sampling was carried out in four forest areas in Kermanshah Province in western Iran, including Kerend-e Gharb, Eslamabad-e Gharb, Chahar Zebar-e-Oliya and Gilan-e Gharb (Supplementary Fig. 1). The study area included a variety of tree species, but primarily *Q. brantii*. Some of the other common tree species were pistachio (*Pistacia atlantica*), Montpellier maple (*Acer monspessulanum*), wild pear (*Pyrus glabra*), hawthorn (*Crataegus pontica*), Judas tree (*Cercis siliquastrum*) and wild almond (*Amygdalus scoparia*). Thirty declining *Q. brantii* trees were randomly selected in each of the four areas. From each of the 120 sampled trees, a single branch (2–10 cm diameter) that had evidence of cankers and vascular tissue discoloration was cut. These 120 branches were transported to the Plant Pathology laboratory of the Department of Plant Protection, Razi University, Kermanshah, Iran, for the isolation of fungi and dissection to locate any associated wood-boring insects. We attempted fungal isolation from the wood of all 120 branches and from all insects found.

Fungal isolation from oak tree branches

Several cross sections were made along the length of each branch with the cut surfaces visually inspected for internal wood discoloration and larval galleries of wood-boring insects. For each discolored area of wood, 10 small pieces (ca. 5 × 5 mm) of wood were cut along the outer margin, and after washing with running water, they were surface-sterilized by soaking in a 10% sodium hypochlorite solution (0.5% active chlorine) for 1 min and rinsed thrice in sterile water to remove any residual hypochlorite. After drying the wood pieces with sterile paper towels, they were placed in Petri dishes (5 pieces per dish) on potato dextrose agar medium (PDA: Merck, Darmstadt, Germany) supplemented with chloramphenicol (20 mgL^{-1}) and streptomycin sulfate (50 mgL^{-1}). The Petri dishes were kept in an incubator at 25 °C with a 12-h dark/12-h light cycle for three to five days until fungal colonies emerged, after which they were sub-cultured on PDA and purified using single-spore or hyphal-tipping methods.

Fungal isolation from insect specimens

When larval galleries were observed on the cut ends of a branch section, we split that particular section multiple times to search for any internal insect life stages, such as larvae, pupae or adults. For each insect life stage recovered, we first placed them individually into sterile tubes. For fungal isolation, 10–15 ml of sterile water was added to each tube with an insect and then shaken for 30 s (Hashemi et al. 2017). One milliliter of the suspension was then transferred onto PDA and spread evenly with a sterile L-shaped rod. Plates were then incubated at 25 °C, with any germinated spores later transferred to fresh medium to obtain pure cultures. In a subsequent method, each larva was surface-sterilized with 10% sodium hypochlorite solution for 1 min and then rinsed thrice in sterile water to remove residual hypochlorite. After drying with sterile paper towels, each specimen was cut with one-half placed in a Petri dish on PDA supplemented with chloramphenicol (20 mgL^{-1}) and streptomycin sulfate (50 mgL^{-1}), and the other half used for DNA extraction (see below).

Fungal identification and characterization

The initial identification of the fungal isolates from the wood and insect samples was made based on morphological characteristics. *Paecilomyces* isolates were identified based on cultural and microscopic structures (conidiophores, phialides, conidia and chlamydospores) as described by Samson et al. (2009). Ability of acid production by all isolates of *Paecilomyces* on creatine sucrose agar (CREA) medium was tested (Samson et al. 2009). The size of conidiophores, conidia, phialides and chlamydospores were measured using BioloMICS software (Robert et al. 2011). For molecular identification, DNA was extracted from representative isolates using a genomic DNA purification kit (Sinagen Co., Iran) following the manufacturer's instructions and amplified for two gene regions: the ITS region and a part of the β-tubulin gene. Standard polymerase chain reaction (PCR) protocols were followed using published primers (White et al. 1990; Glass and Donaldson 1995). The PCR products of representative isolates were purified and sequenced by Applied Biosystems (ABI prism 377) DNA sequencer according to the manufacturer's instruction (Microsunth, Swiss). The nucleotide sequences were edited with BioEdit software and deposited in GenBank (Supplementary Table 1). To confirm all 100 isolates morphologically identified as *P. formosus*, a nested PCR approach was employed using species-specific primers for this fungus (Rostami and Jamali 2023b). The first round of nested PCR was performed with universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The second round of amplification was carried out using species-specific primers PaMF = 5′-CCCATCCGTGTTGAACTACACC-3′ (forward) and PaMR = 5′-GGCCGACCCTACCAGAGCG-3′ (reverse). The amplification results were visualized through electrophoresis using 1% agarose gel in 1 × TAE buffer for 45 min at 90 V with 100-bp DNA ladder (CinnaGen, Iran). The controls, with no DNA, were included

in every set of amplification to check for DNA contamination in reagents and reaction buffers.

Effect of pH on *Paecilomyces* sp. mycelial growth

To evaluate the influence of pH on *Paecilomyces* isolate mycelial growth, a 5-mm-diameter mycelial block was cut from the margin of 3-day-old *Paecilomyces* colonies and placed on potato dextrose agar plates with pH adjusted to 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5 with 0.1 N HCl and NaOH before autoclaving, and incubated at 25 °C. The colony diameters of the *Paecilomyces* colonies were measured in 2 directions for each isolate replicated on 4 separate potato dextrose agar plates at each pH level 1 day after inoculation.

Insect identification

Adult insects were identified from photographs by various specialists. For larval identification, we extracted genomic DNA from each larva that tested positive for *P. formosus* (20 larval samples) and a few representative larvae of those larvae that did not test positive, using the DNA Extraction Kit (S-1034, Denazist Asia Co.) according to the manufacturer's instructions. Cytochrome c oxidase subunit I (COXI) was amplified using PCR protocols and sequenced after purification. The primers used for the PCR were as follows: COXI forward primer LCO1490 (5′-GGTCAACAAATCATAAAG ATATTGG-3′) and COXI reverse primer HCO2198 5′-TAA ACTTCAGGGTGACCAAAAAAT-3′ (Folmer et al. 1994). The PCRs were carried out in a T-Personal thermocycler (Biometra, Germany). Twenty-five microliter PCRs were performed using 12.5 μL Mastermix 2X (Sinaclon, Bioscience), 2.0 μL of each primer (10 pmol), 2.0 μL template DNA and 6.5 μL dH_2O. The amplification conditions were as follows: 5 min at 95 °C followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 60 s and elongation at 72 °C for 90 s, and a final elongation step at 72 °C for 10 min after cycling. The amplified DNA fragments were analyzed on a 1% agarose gel in TAE buffer (1 mM EDTA, 40 mM Tris, 20 mM acetic acid) stained with ethidium bromide and visualized on a UV transilluminator to confirm DNA amplification. The PCR products were purified and sequenced on an automated ABI prism 377 DNA sequencer according to the manufacture's instruction (Microsynth, Switzerland).

Phylogenetic analysis

All nucleotide sequences were edited manually using BioEdit Sequence Alignment Editor Software v. 7.0.9.1 (Hall 1999) and then were compared using the Basic Local Alignment Search Tool (BLAST) option in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Camacho et al. 2009). All sequences were deposited to GenBank under the accession numbers listed in Table 1. The sequences were aligned using ClustalW (http://www.clustal.org/) (Thompson et al. 1994) and manually edited with BioEdit Software. Following alignment using Gblocks v0.91b with the least stringent parameters (Castresana 2000), poorly aligned positions and divergent regions were eliminated to optimize the final alignment. Subsequently, phylogenetic trees were constructed using both maximum parsimony (MP) and maximum likelihood (ML) inference methods implemented in MEGA X software (Kumar et al. 2018) to determine the phylogenetic relationships and closest relatives of the specimens. The robustness of these phylogenetic trees was evaluated by bootstrap analysis with 1,000 replicates (Felsenstein 1985).

Detection of *P. formosus* in insect larvae and adults

To detect *P. formosus* in the recovered insect specimens (45 larvae and 5 adults), fungal genomic DNA was isolated from the insects using the above-mentioned methods and amplified using species-specific primers PaMF and PaMR both directly and as internal primers for nested PCR. The nested PCR was performed as described above. The genomic DNA from pure cultures of *P. formosus* was used as a positive control in all the experiments. For a negative control, genomic DNA was replaced with sterile distilled water.

Results

Tree dieback and decline symptoms and fungal isolation

Based on our field observations, the sampled *Q. brantii* trees showed a variety of external disease symptoms including leaf chlorosis, wilting, branch cankers, branch dieback, trunk cankers and whole-tree death (Fig. 1a-d). Examination of branch cross sections cut from the sampled *Q. brantii* trees all revealed wood discoloration (Fig. 1e-h). Of the 120 branches collected from the sampled oak trees, all 120 branches showed wood discoloration and 100 samples had galleries of wood-boring insects. In 50 of the 100 galleries, insect larvae and adults were found. *Paecilomyces* isolates were obtained from 75 of the 120 branch samples (62.5%). The attempts to isolate *P. formosus* from the wood and insects yielded 100 fungal isolates overall, with 75 from the 120 wood samples as mentioned above, 20 from insect larvae (44%, 20 of 45 larvae) and 5 from insect adults (100%, 5 of 5 adults). While *P. formosus* was successfully isolated from 100 samples (75 wood and 25 insects), the remaining 45 wood and 25 insect samples did not yield any fungal growth on PDA medium. Additionally, the *P.*

Table 1 Summary data for the wood-infesting insects that tested positive *P. formosus* including the collection site location, and GenBank sequence accession number for COXI sequence data sorted by insect family

Specimen code	Location	Species	Latitude	Longitude	Insect stage	Identification method	Accession numbers	Family
RU-MoGa1	Dalahu	*Monochamus* sp.	34° 20′ 59.3″N	46°23′ 22.8″E	Larva	Molecular	OR889154	Cerambycidae
RU-TrCa1	Khosro Abad	*Trichoferus campestris*	34° 10′ 39.1″N	46°22′ 03.5″E	Larva	Molecular	OR885308	Cerambycidae
RU-TrCa2	Dalahu	*Trichoferus campestris*	34° 21′ 00.3″N	46°23′ 28.9″E	Larva	Molecular	OR885629	Cerambycidae
RU-TrCa3	Kermanshah	*Trichoferus campestris*	34° 13′ 55.7″N	46°40′ 56.9″E	Larva	Molecular	OR885630	Cerambycidae
RU-TrCa4	Kermanshah	*Trichoferus campestris*	34° 14′ 49.0″N	46°40′ 44.5″E	Larva	Molecular	OR885631	Cerambycidae
RU-TrCa5	Chahar Zebar-e-Oliya	*Trichoferus campestris*	34° 15′ 01.0″N	46°40′ 41.3″E	Larva	Molecular	OR885632	Cerambycidae
RU-CaDi1	Dalahu	*Calchaenesthes diversicolis*	34° 20′ 59.0″N	46°23′ 23.5″E	Adult	Morphological	–	Cerambycidae
RU-CaDi2	Dalahu	*Calchaenesthes diversicolis*	34° 20′ 56.7″N	46° 23′ 23.2″E	Adult	Morphological	–	Cerambycidae
RU-ChAf-1	Chahar Zebar-e-Oliya	*Chrysobothris affinis*	34° 13′ 58.8″N	46° 40′ 58.3″E	Larva	Molecular	OR654604	Buprestidae
RU-ChAf-2	Babashahahmad,	*Chrysobothris affinis*	34° 20′ 59.1″N	46° 23′ 26.0″E	Larva	Molecular	OR889725	Buprestidae
RU-ChAf-3	Dalahu	*Chrysobothris affinis*	34° 20′ 58.9″N	46° 23′ 22.6″E	Larva	Molecular	OR889726	Buprestidae
RU-ChAf-4	Dalahu	*Chrysobothris affinis*	34° 20′ 59.3″N	46° 23′ 22.8″E	Adult	Morphological	–	Buprestidae
RU-ChAf-5	Dalahu	*Chrysobothris affinis*	34° 20′ 55.93″N	46° 23′ 22.5″E	Adult	Morphological	–	Buprestidae
RU-ChAf-6	Dalahu	*Chrysobothris affinis*	34° 20′ 58.9″N	46° 23′ 22.6″E	Adult	Morphological	–	Buprestidae
RU-LaPl-1	Khosro Abad	*Latipalpis plana*	34° 10′ 40.2″N	46° 22′ 03.1″E	Larva	Molecular	OR661268	Buprestidae
RU-AnHe-1	Sarmil	*Anthaxia* sp.	34° 19′ 27.8″N	46° 06′ 50.5″E	Larva	Molecular	OR661810	Buprestidae
RU-Ac-1	Dalahu -Babasha-hahmad,	*Acmaeodera* sp.	34° 20′ 55.7″N	46° 23′ 24.2″E	Larva	Molecular	OR670077	Buprestidae
RU-Ac-2	Gilan-e *Gharb*	*Acmaeodera* sp.	34° 01′ 42.0″N	46° 08′ 05.0″E	Larva	Molecular	OR670078	Buprestidae
RU-Ac-3	Gilan-e *Gharb*	*Acmaeodera* sp.	34° 05′ 20.0″N	46° 04′ 34.0″E	Larva	Molecular	OR670079	Buprestidae
RU-Ac-4	Gilan-e *Gharb*	*Acmaeodera* sp.	34° 07′ 29.0″N	45° 57′ 25.0″E	Larva	Molecular	OR670080	Buprestidae
RU-AC-5	Chahar Zebar-e-Oliya	*Acmaeodera* sp.	34° 13′ 11.8″N	46° 40′40.5″ E	Larva	Molecular	PP729950	Buprestidae
RU-Te1	Chahar Zebar-e-Oliya	Tenebrionidae sp.	34° 13′ 25.5″N	46° 40′ 45.0″E	Larva	Molecular	OR946320	Tenebrionidae
RU-Te2	Chahar Zebar-e-Oliya	Tenebrionidae sp.	34° 13′ 23.9″N	46° 40′ 44.7″E	Larva	Molecular	OR946321	Tenebrionidae
RU-Te3	Chahar Zebar-e-Oliya	Tenebrionidae sp.	34° 13′ 16.5″N	46° 40′ 40.4″E	Larva	Molecular	OR946322	Tenebrionidae
RU-CrGi1	Babashahahmad	*Crypticus gibbulus*	34° 20′ 55.6″N	46° 23′ 24.6″E	Larva	Molecular	ON738570	Tenebrionidea

Fig. 1 Examples of the symptomatic oak trees (*Quercus brantii*) that were sampled in the Zagros forests in western Iran **a**-**d**, examples of longitudinal and cross-sectional cuts from branches of declining *Quercus brantii* trees with evidence of insect galleries collected in the Zagros forests **e**–**h**, and a few of the insect specimens collected from branch samples of declining oaks in the Zagros forests that tested positive for *Paecilomyces formosus*. Shown are adults and larvae of Buprestidae **i**, **j**, Cerambycidae **k**, Tenebrionidae **l**

formosus-specific primer did not amplify in the remaining 25 insect samples.

Morphological identification and characterization of isolates

The 100 isolates of *Paecilomyces* spp. recovered from the wood and insect samples appeared as light yellow to yellow–brown fast-growing cultures on PDA. The conidiophores were verticillate or irregular with several phialides. The phialides had a cylindrical basal portion that tapered abruptly into a long cylindrical neck. The conidia were in long divergent chains, ellipsoidal to cylindrical with truncate ends, smooth and hyaline to yellow. Chlamydospores were present, thick-walled, globose, dark and smooth-walled. All isolates grew on creatine sucrose agar medium

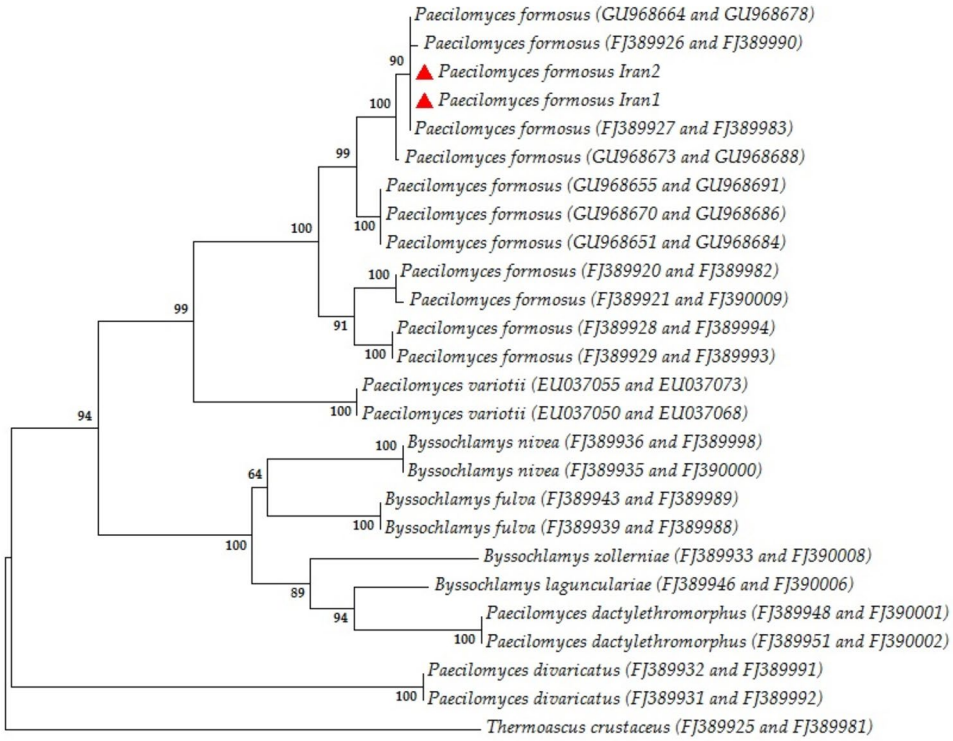

Fig. 2 Maximum parsimony phylogram generated in Mega X program from the alignment of 25 combined internal transcribed spacer (ITS) and β-tubulin gene sequences of *Paecilomyces* species with complete deletion gap handling and 1000-replication bootstrapping (consistency index, CI = 0.76; retention index, RI = 0.89; rescaled consistency index, RCI = 0.68)

and turned the medium a yellow color (Supplementary Fig. 2a-f). The optimum pH for growth was 6.5–7 and no growth occurred at pH 3.5 and 10. The above characters match with earlier reports for *P. formosus* (Samson et al. 2009).

Molecular identification of the fungal isolates

To confirm the isolates of *P. formosus* that were identified using morphological characteristics, we tested species-specific primers of PaMF/PaMR by amplification of 441-bp fragments, which confirmed the morphological identity as *P. formosus*. No PCR product was observed in the negative control (dH_2O as a negative control). Phylogenetic analysis of the combined ITS and β-tubulin sequences of two representative isolates with sequences in GenBank (Supplemental Table 1) affirmed that the fungal species in this study matches *P. formosus* with strong bootstrap (100%) (Fig. 2). The ITS and Bt sequences from our isolates were deposited at NCBI under accession numbers OR922490-OR922491 (ITS) and PP025482-PP025483 (Bt), respectively. Positive amplification using species-specific PCR supported the phylogenetic analysis.

We used only two isolates in this portion of the study because all 100 isolates in the present study exhibited identical morphological characteristics, growth patterns and acid production activity on creatine sucrose agar (CREA) medium. However, for accurate and rapid identification of the 100 fungal isolates obtained from the wood and insects in the present study, we used species-specific primers for *P. formosus* as detailed in Rostami and Jamali (2023b). These species-specific primers successfully identified all 100 isolates as *P. formosus*, further confirming their identity. Employing species-specific primers is a common practice in many studies (Zang et al. 2012; Bao et al. 2022), as it serves to reduce costs, expedite the research process and facilitate rapid pathogen identification. In addition, in many studies employing species-specific primers, the need for additional markers for identification is eliminated (Zhao et al. 2016).

Molecular identification of insect larvae

After amplification of the larval cytochrome c oxidase I region, all sequences were 660 bp long with 388 conserved and 271 variable sites. Using the BLAST search tool in GenBank, most larvae with a high percentage of similarity were identified to the species level, but some only to the genus or family level (Table 1). In the phylogenetic analysis based on COXI sequences, our specimens were placed in monophyletic groups together with other authentic specimens from other countries with high bootstrap values (Figs. 3, 4, Supplemental Table 1). COXI sequences of the larvae that tested positive for *P. formosus* were deposited at NCBI. The result of the phylogenetic analysis was in accordance with the molecular identification based on DNA sequences in BLAST search.

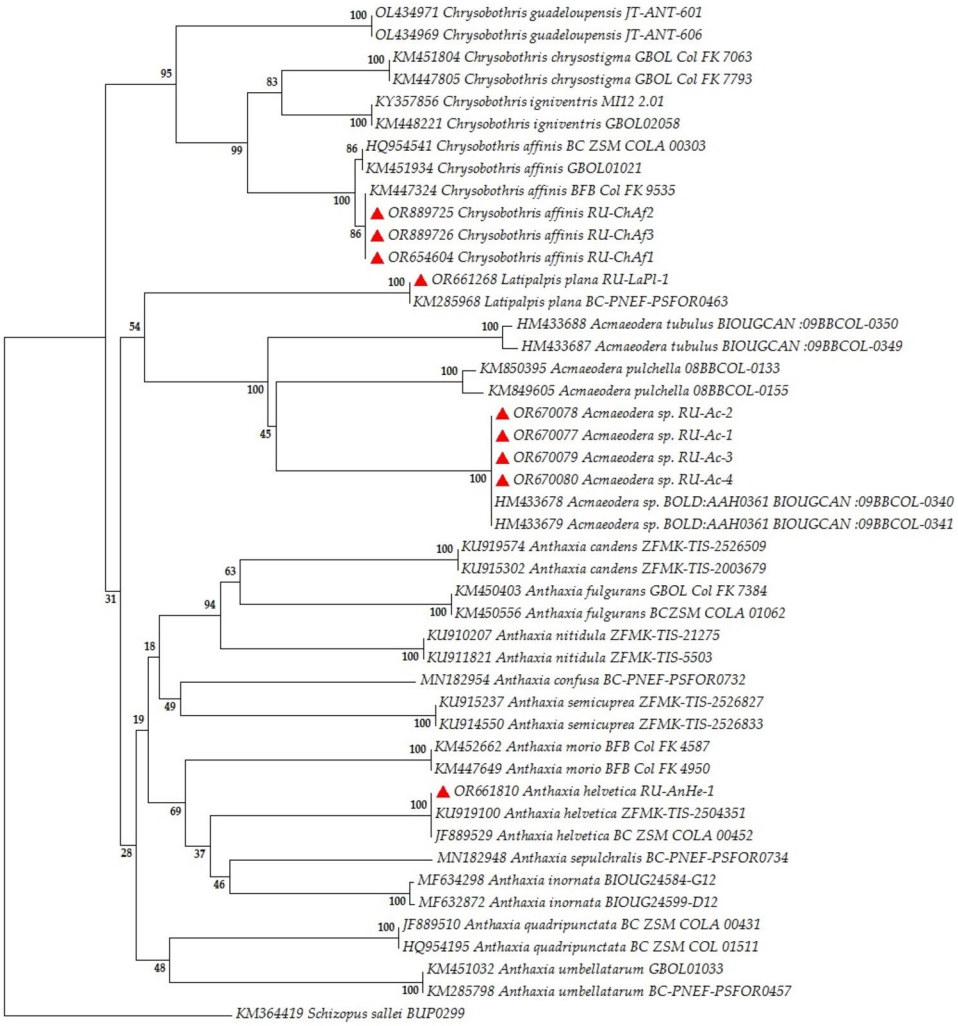

Fig. 3 Maximum parsimony phylogram generated in Mega X from the alignment of 45 cytochrome c oxidase I (COXI) sequences of the Buprestidae specimens using Kimura two-parameter model with complete deletion gap handling and 1,000-replication bootstrapping (tree length = 689; consistency index, CI = 0.37; retention index, RI = 0.77; rescaled consistency index, RCI = 0.28)

Molecular detection of *Paecilomyces formosus* in larvae and adult beetles

Overall, 50 insects (45 larvae and 5 adults) that represented four insect orders were assayed using nested PCR analysis to confirm the presence or absence of *P. formosus*. PCR analysis detected *P. formosus* in all five adult beetles (three *Chrysobothris affinis* Fabricius buprestids and two *Calchaenesthes diversicolis* Holzschuh cerambycids) and 20 beetle larvae (44% of 45 larvae) from three families (Buprestidae, Cerambycidae and Tenebrionidae) (Table 1). Among the 45 larvae collected in this study (Fig. 1i-l), *P. formosus* was detected in 45% (10 of 22) of the buprestids and 50% (6 of 12) of the cerambycids. The buprestid larvae that most often tested positive for *P. formosus* were *Acmaeodera* spp. and *Chrysobothris affinis*, while the cerambycid larvae that most often tested positive were *Trichoferus campestris*. All five *C. affinis* and *C. diversicolis* adults that were collected from their galleries tested positive for *P. formosus*. In addition, larval specimens of *Anthaxia* sp. (Buprestidae), *Latipalpis plana* (Olivier) (Buprestidae), *Monochamus* sp. (Cerambycidae) and *Crypticus gibbulus* (Quensel) (Tenebrionidae) tested positive as well as a few other unidentified Tenebrionidae for *P. formosus*. Besides the Buprestidae, Cerambycidae and Tenebrionidae mentioned above, the other insect larvae that tested negative for *P. formosus* were Cossidae (Lepidoptera), Elateridae (Coleoptera), Gasteruptiidae (Hymenoptera) and Syrphidae (Diptera).

Discussion

Oak dieback and decline are a disease complex that has resulted in damage and death to many trees in Zagros forests in recent years. Changes in several environmental parameters are likely factors in the decline of these trees (Attarod et al. 2016, 2017). Among the environmental factors, the reduction in rainfall during the growing season has been identified as one of the main reasons for oak decline (Azizi et al. 2015). Drought stress results in many physiological

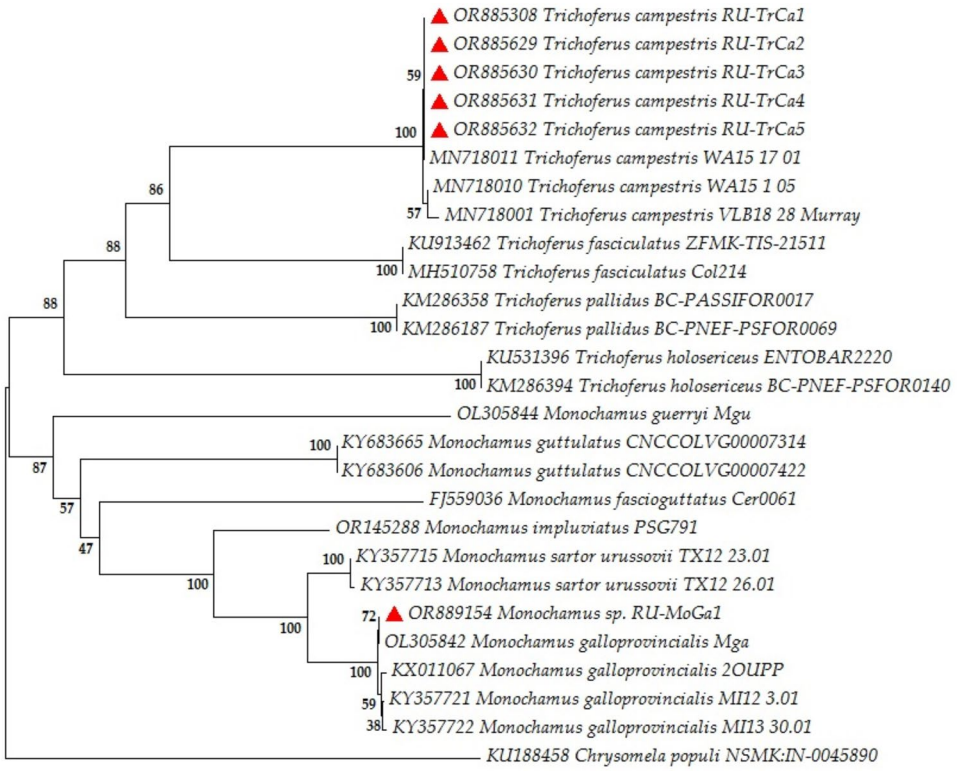

Fig. 4 Maximum parsimony phylogram generated in Mega X from the alignment of 26 cytochrome c oxidase I (COXI) sequences of the Cerambycidae specimens using Kimura two-parameter model with complete deletion gap handling and 1,000-replication bootstrapping (tree length = 613; Conserved 385/777; Variable 273/777; Parsimony informative 231/777; consistency index, CI = 0.59; retention index, RI = 0.83; rescaled consistency index, RCI = 0.49)

changes to trees that can make them more susceptible to insects and pathogens (Mattson and Haack 1987; Desprez-Loustau et al. 2006). Among the biotic factors, fungi and insects are recognized as the most important agents involved in oak decline.

To date, several fungi have been reported as possible factors involved in oak decline in the Zagros forests, including *Biscogniauxia mediterranea* (De Not.) Kuntze, *Biscogniauxia rosacearum* Raimondo & Carlucci, *Discula quercina* (West.) Arx., *Inonotus krawtzewii* (Pilát) Pilát, *Kalmusia variispora* (Verkley, Göker & Stielow), *Obolarina persica* Mirabolfathy, Ju, Hsieh and Rogers, *Paecilomyces formosus*, *Paramicrosphaeropsis* spp. *Petriella sordida* (Zukal), *Phaeoacremonium tuscanicum* Essakhi, Mugnai, Surico and Crous and *Neoscytalidium novaehollandiae* Pavlic et al. (Mirabolfathy 2013; Safaee et al. 2017; Alidadi et al. 2019; Hanifeh et al. 2019; Sabernasab et al. 2019a, 2019b; Bashiri et al. 2022; Bahmani et al. 2021; Artand et al. 2023). Of these, *B. mediterranea* and *P. formosus* are considered the most important oak pathogens in Iran (Mirabolfathy 2013; Sabernasab et al. 2020). *Paecilomyces formosus* has been reported from many countries throughout the world as well as many host plants (Samson et al. 2009).

Few studies have explored the possibility that insects could be involved in transmission of some of the fungi involved in oak decline. For example, Panzavolta et al. (2018), working in a mixed oak woodland in central Italy, found several field-collected adult Buprestidae and Cerambycidae to be externally contaminated with spores of pathogenic fungi. In other countries, the ambrosia beetle *Platypus cylindrus* Fab. (Curculionidae) and cerambycids in the genus *Cerambyx* have been reported as vectors of *B. mediterranea* (Martín et al. 2005; Henriques et al. 2015). Wood-boring beetles in the families Buprestidae (Sadravi and Moradi 2017; Gheibi et al. 2018) and Cerambycidae (Soleymani et al. 2015) are commonly reported as oak pests in Iran, but their possible role in vectoring plant pathogenic fungi has not been explored. For example, in Zagros forests, buprestid beetles such as *Agrilus hastulifer* Fåhraeus, *Chalcophorella bagdadensis* (Laporte & Gory) and *Chrysobothris parvipuncta* (Obenberger) and the cerambycid *Prinobius myardi* Mulsant (= *Macrotoma scutellaris* Vayssière) are commonly found in oaks infected with *B. mediterranea* (Pourhashemi et al. 2017).

In the present study, we demonstrated an association between *P. formosus* and various beetles in the families Buprestidae, Cerambycidae and Tenebrionidae. Considering the buprestids first, we found specimens of *Anthaxia, Acmaeodera*, *Chrysobothris* and *Latipalpis* that all tested positive for *P. formosus*. In Iran, Abaei (2009) reported that *Anthaxia hungarica* (Scopoli) infested oaks in Kermanshah province. Two other *Anthaxia* species associated with oaks in Iran include *A. cichorii* (Olivier) and *A. kiesenwetteri* Marseul (Ghahari et al. 2015). In the study by Jozeyan et al. (2015) in western Iran, 66% of

the beetles associated with oak decline were from the Buprestidae family, including *A. hastulifer*, *C. bagdadensis, C. parvipuncta* and *Lampetis mimosae* (Klug). In a study by Valipour et al. (2022) in western Iran, the buprestid beetles, including *Acmaeodera degener* (Scopoli), *A. ottomana* (Frivaldszky), *Acmaeodera undulata* (Abeille de Perrin), *Acmaeodera wethloi* (Obenberger), *Acmaeoderella* (*impunctata* (Abeille de Perrin), *Capnodis anthracina* (Fischer von Waldheim), *Capnodis cariosa hauseri* Obenberger, *C. bagdadensis*, *Chalcophorella escalerae* (Abeille de Perrin), *Perotis lugubris longicollis* Kraatz and *Svatactesis johanidesi* (Bílý), were associated with declining oak trees. Among the above species, *A. wethloi* and *A. undulata* were the most collected.

Similarly, in the present study, most of the buprestid larvae collected were species of *Acmaeodera*. In Bulgaria, Bencheva and Doychev (2022) suggested that two species of *Acmaeodera*, including *Acmaeodera crinita* Gory and *Acmaeodera ottomana* (Frivaldszky), could be vectors of *B. mediterranea* on cork oak (*Quercus suber* L.). The buprestid *C. affinis* has been reported from various hosts in Iran, including *Albizia lebbeck* (L.) Benth. (Fabaceae), apple (*Malus* sp.), apricot (*Prunus* sp.), *Callistemon citrinus* (Curtis) Skeels (Myrtaceae), pear (*Pyrus*, Rosaceae), *Prosopis cineraria* (L.) Druce (Fabaceae) and white mulberry (*Morus* sp., Moraceae), as well as from several provinces: Chaharmahal and Bakhtiary, East Azarbaijan Guilan, Hamadan, Isfahan, Kerman, Khuzestan, Lorestan, Mazandaran, Qazvin, Sistan and Baluchestan, Tehran, and Yazd and Zanjan (Modarres Awal 1997; Borumand 2002; Barimani et al. 2008, 2009; Rajabpour et al. 2012). To date, there have been no reports of *C. affinis* on oak in Iran. After the *Acmaeodera* specimens tested in the present study, larvae and adults of *C. affinis* were the next most frequent buprestid taxon that tested positive for *P. formosus*. As for the buprestid genus *Latipalpis*, Ghahari et al. (2015) reported that *Latipalpis johanidesi* Niehuis, *Latipalpis persica* Bílý and *L. plana* occur in Iran. Although Ghahari et al. (2015) gave no host data for these buprestid species, Jalil and Ali (2020) reported that members of this genus infest the branches of oaks. This is the first report of *L. plana* infesting oak in Iran.

So far, more than 36,000 species of Cerambycidae are known worldwide, with many recognized as important pests of forest and urban trees as well as vectors of some tree disease pathogens (Akbulut et al. 2017; Haack 2017; Özdikmen 2023). For example, cerambycids in the genus *Cerambyx* infest trunks of living oak trees and appear associated with charcoal disease of oaks, caused by *B. mediterranum* (Martín et al. 2005).

In the present study, cerambycid larvae and adults that were PCR-positive for *P. formosus* included two Cerambycinae (*Trichoferus campestris* and *Calchaenesthes diversicolis*) and one Lamiinae (*Monochamus* sp.). The first report of *T. campestris* in Iran was in 2005 from northern Iran where it infested *Alnus* and *Populus* trees (Sama et al. 2005). *T. campestris* lays eggs on the bark surface of healthy, stressed and dying trees as well as on logs with bark (EPPO 2021). The host list for *T. campestris* includes over 50 genera of both hardwoods and conifers including *Quercus* (EPPO 2021). Our recovery of *T. campestris* from oaks in Zagros forests is the first report of oaks serving as a larval host plant for *T. campestris* in Iran. *T. campestris* was the most collected cerambycid larva in our study that tested positive for *P. formosus*. Among the cerambycid adults collected in our study that tested positive for *P. formosus*, *C. diversicollis* is known to infest deciduous trees in the family Fagaceae, including *Q. brantii*, and previously has been reported from the Iranian provinces of Ilam, Kermanshah, Kohgiluyeh and Boyer-Ahmad, Kordestan and Lorestan (Özdikmen and Cihan Tüzün 2018). As for the PCR-positive *Monochamus* larva recovered, this specimen had 99% sequence similarity of the cytochrome oxidase I to *M. galloprovincialis* (Olivier), a cerambycid that is only known to infest conifers (Akbulut et al. 2017, EFSA 2018). In Iran, both *M. galloprovincialis* and *Monochamus sutor* (L.) have been recorded (Farashian et al. 2005; Chelav et al. 2019). These two *Monochamus* species are reported to use conifers in Iran, but Farashian et al. (2005) also reported *M. sutor* infesting the hardwood tree *Parrotia persica* (DC.) C.A.Mey (Hamamelidaceae) in Iran. A number of *Monochamus* species, including *M. galloprovincialis*, transmit the pinewood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle (Akbulut et al. 2017. EFSA 2018). In Asia, at least three *Monochamus* species occasionally use *Quercus* spp. as hosts, although in each case pines (*Pinus*) are their main larval host trees (EFSA 2018). Our finding is the first report of a *Monochamus* larva in a declining oak tree in Iran, which also tested positive for *P. formosus*.

Although several buprestid and cerambycid species tested positive for *P. formosus* in the present study, it is not known whether they can transfer the fungus to uninfected trees in the field. Considering the typical buprestid and cerambycid life cycle, fungal transmission would most likely occur during adult maturation feeding on twigs and foliage or during oviposition. Consider first that maturation feeding on plant tissues by newly emerged adults appears required for nearly all buprestids and most cerambycids (Hanks 1999; Bílý 2002; Akbulut et al. 2017). In the case of newly emerged *Acmaeodera* and *Anthaxia* adults, these buprestids feed on flower pollen and petals, whereas new *Chrysobothris* adults usually feed on twig bark of their larval host plants (Maxwell and Fenton 1936; Westcott et al. 1979; Bílý 2002). Many adults in the cerambycid subfamily Cerambycinae first feed on pollen and nectar of plants that are not their larval hosts, whereas most members of the cerambycid subfamily Lamiinae feed on the bark and foliage of their larval host

plants (Hanks 1999; Haack 2017). As for the cerambycines collected in the present study, *C. diversicolis* and *T. campestris*, we found no reports of adult maturation feeding for either species. However, for the related species *Calchaenesthes pistacivora*, Holzschuh, Rad (2006) reported that adults feed on foliage to become sexually mature. As for *Monochamus* adults, which are in the subfamily Lamiinae, maturation feeding appears required and includes feeding on needles and twig bark of their larval host trees (Akbulut et al. 2017; Haack 2017).

Transmission of a pathogen could also occur during oviposition. As for tree-infesting buprestids, females usually lay eggs singly or in small clusters on the bark surface or in bark cracks and often cover the eggs with a secretion that aids in cementing the eggs to the bark and reducing desiccation (Bílý 2002; Chamorro et al. 2015). With respect to the cerambycids, most Cerambycinae lay eggs on the bark surface or in bark cracks and sometimes also cement eggs to the bark with a female-produced secretion (Lee and Lee 2020). In situations where the buprestid and cerambycid eggs are laid on the bark surface, larvae exit the eggs on the side touching the bark and then tunnel directly into the host plant. By contrast, most Lamiinae adult females use their mouthparts to chew a slit or a pit through the outer bark, and then turn and insert their ovipositor and lay one or more eggs under the bark (Akbulut et al. 2017; Hanks and Wang 2017). For adult *Monochamus* females that are vectoring pinewood nematodes, it is during construction of the oviposition pit and during oviposition itself when the nematodes exit the adult female's body and enter the host plant (Akbulut et al. 2017). Given the above, the wood borers that would be most successful at vectoring a fungus would be those that complete their maturation feeding on the larval host plant, as well as those that could contaminate the eggs or the oviposition site with fungal spores during oviposition.

In our study, some tenebrionid larvae, including *Cryptic us gibbulus*, tested positive for *P. formosus*. Tenebrionids develop in many habitats, with most species found in forested areas feeding on fungi or rotten wood (Steiner 2008, 2014). In the case of *C. gibbulus*, it has been collected most often under stones (Benyahia et al. 2015; Lillig 2019). To date, there have been no reports of tenebrionids on oak trees in Iran. In the present study, insects were identified using cytochrome *c* oxidase subunit I and cytochrome *b* markers, but of these two markers, Cytb was not amplified in all larvae. Possibly for this reason, as well as the fact that complete sequences were not available for all tenebrionids species in GenBank, we were not able to identify all specimens in this study beyond the genus or even family level.

The digestion of cellulose in wood substrates by insects requires hydrolytic enzymes, including endoglucanases (C_x-cellulases), cellobiohydrolases (C_1-cellulases) and β-glucosidases (cellobiases) (Reese and Mandels 1971; Ghose et al. 1981; Martin 1983). Many insects can produce C_x-cellulases and cellobiases, but few produce C_1-cellulases (Martin 1983). Many wood-inhabiting insects acquire fungal cellulase by either eating fungal tissue or fungal compounds that are present in the wood they inhabit (Martin 1983). *P. formosus* can digest cellulose and hence is a potential source of cellulolytic enzymes for the wood-infesting insects in the present study. The presence of *P. formosus* within the bodies of the larvae in our study could simply suggest that the insects have ingested the fungus, which then aids the insect in wood digestion. Nevertheless, our research was the first to demonstrate the occurrence of *P. formosus* within adult *C. affinis* and *C. diversicolis* beetles, as well as larvae of *Acmaeodera* sp., *Anthaxia* sp., *Crypticus gibbulus, Latipalpis plana, Monochamus* sp. and *Trichoferus campestris.* Future research should explore the possibility of *P. formosus* transmission by the above beetles.

In addition, practices that improve tree health should be encouraged in Iran. For example, cutting tree branches for firewood and livestock food is common throughout the Zagros forests (Valipour et al. 2014). The wounds that result from cutting could serve as entry points for airborne fungal spores and also attract insects, including various bark- and wood-infesting insects. Prohibiting such practices is impractical in these rural areas, but educating the public on proper pruning techniques could limit the number and size of such wounds and thereby promote faster healing. Similarly, adjusting grazing practices by farm animals in the forests could reduce soil compaction and promote forest regeneration (Valipour et al. 2014). Moreover, given the many recent advances in development of traps and lures for wood-boring insects (Rassati et al. 2019; Roques et al 2023), monitoring populations of such insects is often possible and may be warranted, especially if some of the beetles that tested positive for *P. formosus* in the present paper prove to be vectors of the fungus. It is also crucial to consider that other insects besides those studied here may play a role in *P. formosus* transmission.

Author contributions

GG conducted field experiments, sample preparation, laboratory works and sequencing of fungal isolates, SJ conceived the idea of the research, designed the scientific experiments and methodology, and took part in field surveys, software, phylogenetic investigation, GenBank sequences submission, writing—original draft preparation, and writing—review and editing, RAH helped design the field experiments and methodology, and contributed to writing and editing, and JV conducted field experiments.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval This article does not contain any studies with human participants or vertebrates performed by any of the authors.

References

Abaei M (2009) Pests of forest trees and shrubs. Iranian Research Institute of Plant Protection Press, Tehran, p 206

Akbulut S, Togashi K, Linit MJ (2017) (ed.) Cerambycids as plant disease vectors with special reference to pine wilt. In: Wang Q (ed) Cerambycidae of the world: biology and management. CRC Press, Boca Raton, FL, pp 223–266

Alidadi A, Kowsari M, Javan-Nikkhah M, Jouzani GS, Rastaghi ME (2019) New pathogenic and endophytic fungal species associated with Persian oak in Iran. Eur J Plant Pathol 155:1017–1032. https://doi.org/10.1007/s10658-019-01830-y

Aminaei MM, Ershad D (1989) Pistachio dieback disease in Kerman province. In: P. 82 Proceedings of 9th Iranian Plant Protection Congress, Mashhad, Iran

Artand S, Mehrabi-Koushki M, Tabein S (2023) Identification of some fungal species in Chaetomiaceae and Didymellaceae families associated with foliar and trunk diseases symptoms on forest trees in the Zagrosian region. J Appl Res Plant Protect 12(3):239–256https://doi.org/10.22034/arpp.2023.16635

Attarod P, Rostami F, Dolatshahi A, Sadeghi SMM, Zahedi Amiri G, Bayramzadeh V (2016) Do changes in meteorological parameters and evapotranspiration affect declining oak forests of Iran. J for Sci 62(12):553–561https://doi.org/10.17221/83/2016-JFS

Attarod P, Sadeghi SMM, Pypker TG, Bayramzadeh V (2017) Oak tree decline; a sign of climate variability impacts in the west of Iran. Caspian J Environ Sci 15(4):375–386https://doi.org/10.22124/cjes.2017.2662

Azizi G, Miri M, Mohamadi H, Pourhashemi M (2015) Analysis of relationship between forest decline and precipitation changes in Ilam Province. Iran J for Pop Res 23(3):502–515 https://doi.org/10.22092/ijfpr.2015.105655

Azizi R, Ghosta Y, Ahmadpour A (2020) New fungal canker pathogens of apple trees in Iran. J Crop Protect 9(4):669–681. http://dorl.net/dor/20.1001.1.22519041.2020.9.4.9.2

Bahmani Z, Abdollahzadeh J, Amini J, Evidente A (2021) *Biscogniauxia rosacearum* the charcoal canker agent as a pathogen associated with grapevine trunk diseases in Zagros region of Iran. Sci Rep 11(1):14098. https://doi.org/10.1038/s41598-021-93630-w

Bao J, Chen Y, Xing Y, Feng C, Hu Q, Li X, Jiang H (2022) Development of a nested PCR assay for specific detection of *Metschnikowia bicuspidata* infecting *Eriocheir sinensis*. Front Cell Infect Microbiol 12:930585. https://doi.org/10.3389/fcimb.2022.930585

Barimani H, Kalashian MY, Barari H (2009) Contribution to the knowledge of the jewel beetles (Coleoptera: Buprestidae) fauna of Mazandaran province of Iran. Caucasian Entomol Bull 5(1):63–68. http://dx.doi.org/10.23885/1814-3326-2009-5-1-63-68

Barimani H, Kalashian MY, Barari H (2008) The study of occurrence and distribution of *Chrysobothris affinis* (Fab, 1794) (Col.: Buprestidae) in Mazandaran province using red sticky trap. In: Proceedings of 18th Iranian Plant Protection Congress, p 201

Bashiri S, Abdollahzadeh J, Evidente A (2022) Diagnosing and pathogenicity of *Biscogniauxia* species the causal agents of oak charcoal canker and decline in Zagros forests of Iran. J Plant Pathol 104(3):1011–1025. https://doi.org/10.1007/s42161-022-01124-z

Bencheva S, Doychev D (2022) Distribution of *Biscogniauxia mediterranea* and its potential insect vectors on *Quercus suber* in Southwestern Bulgaria. Silva Balcan 23(1):57–65. https://doi.org/10.3897/silvabalcanica.23.e89314

Benyahia Y, Soldati F, Rohi L, Valladarès L, Maatouf N, Courtin O, El Antry S, Brustel H (2015) First survey of darkling beetles (Coleoptera Tenebrionidae) of Talassemtane National Park Western Rif, Morocco. Check List 11:1778–1778

Bílý S (2002) Summary of the bionomy of the buprestid beetles of Central Europe (Coleoptera: Buprestidae). Acta Entomol Mus Natl Pragae 10:104

Borumand H (2002) Coleoptera (XXIV): Buprestoidea: Fam, (62): Buprestidae, In: Insects of Iran. The list of Coleoptera in the insect collection of Plant Pests & Diseases Research Institute, Ministry of Agricultural Jihad Agricultural Research Education & Extension Organization Plant Pests & Diseases Research Institute Insect Taxonomy Research Department Publ, 8: 1–63.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: architecture and applications. BMC Bioinform 10:1–9

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17(4):540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chakusary MK, Mohammadi H, Khodaparast SA (2019) Diversity and pathogenicity of Botryosphaeriaceae species on forest trees in the north of Iran. Eur J for Res 138:685–704. https://doi.org/10.1007/s10342-019-01200-7

Chamorro ML, Jendek E, Haack RA, Petrice TR, Woodley NE, Konstantinov AS, Volkovitsh MG, Yang XK, Grebennikov VV, Lingafelter SW (2015) Illustrated guide to the emerald ash borer *Agrilus planipennis* Fairmaire and related species (Coleoptera Buprestidae), Pensoft, Sofia

Chelav HS, Samin N, Warchalowski A, Hawkeswood TJ, Jêdryczkowski WB (2019) A contribution to the study of Carabidae Cerambycidae Chrysomelidae and Staphylinidae (Coleoptera) of Iran. Calodema 729:1–6

Coombes AJ (2000) Trees. Dorling Kindersley Handbooks, London

Denman S, Brown N, Kirk S, Jeger M, Webber J (2014) A description of the symptoms of acute oak decline in Britain and a comparative review on causes of similar disorders on oak in Europe. Forestry 87:535–551. https://doi.org/10.1093/forestry/cpu010

Desprez-Loustau ML, Marçais B, Nageleisen LM, Piou D, Vannini A (2006) Interactive effects of drought and pathogens in forest trees. Ann for Sci 63:597–612. https://doi.org/10.1051/forest:2006040

EFSA (European Food Safety Authority) (2018) Pest categorisation of non-EU *Monochamus* spp. EFSA J 16(11):e05435

EPPO (European and Mediterranean Plant Protection Organization) (2021) *Trichoferus campestris*. EPPO datasheets on pests recommended for regulation.

Farashian E, Yarmand H, Tavakkoli M, Sadghyan B, Ale Mansor H, Ahmadi SM (2005) An introducing to important xylophagous

pests (long horn beetles) of Iran. Iran J Wood Paper Sci Res 20:207–236 https://doi.org/10.22092/ijwpr.2005.117583
Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.http://dx.doi.org/10.2307/2408678
Folmer O, Black MB, Hoch W, Lutz RA, Vrijenhock RC (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from metazoan invertebrates. Mol Mar Biol Biotech 3:294–299
Ghahari H, Volkovitsh MG, Bellamy CL (2015) An annotated catalogue of the Buprestidae of Iran (Coleoptera: Buprestoidea). Zootaxa 3984:1–141.http://dx.doi.org/10.11646/zootaxa.3984.1.1
Ghasemi-Sardareh R, Mohammadi H (2020) Characterization and pathogenicity of fungal trunk pathogens associated with declining of neem (*Azadirachta indica* A. Juss) trees in Iran. J Plant Pathol 102:1159–1171. https://doi.org/10.1007/s42161-020-00598-z
Gheibi F, Pirzadian E, Movaseghi M (2018) Introducing joint action plan of the Forests Rangelands and Watersheds Organization for the Zagros. J for Range 116:8–19. http://dx.doi.org/10.52547/ifej.8.16.148
Ghose T, Montenecourt BS, Evelegh DE (1981) Measure of cellulase activity. In: International Union of Pure and Applied Chemistry Commission of Biotechnology. pp. 67–71
Glass NL, Donaldson GC (1995) Developmen of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330. https://doi.org/10.1128%2Faem.61.4.1323-1330.1995
Goodarzi M, Pourhashemi M, Azizi Z (2019) Investigation on Zagros forests cover changes under the recent droughts using satellite imagery. J for Sic 65(1):9–17. http://dx.doi.org/10.17221/61/2018-JFS
Haack RA (2017) Feeding biology of cerambycids. In: Wang Q (ed) Cerambycidae of the world: biology and management. CRC Press, Boca Raton FL, pp 105–132
Haavik LJ, Billings SA, Guldin JM, Stephen FM (2015) Emergent insects, pathogens and drought shape changing patterns in oak decline in North America and Europe. Forest Ecol Manag 354:190–205. http://dx.doi.org/10.1016/j.foreco.2015.06.019
Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
Hanifeh S, Zafari D, Soleimani MJ, Ravanlou A (2019) *Discula quercina* as a possible causal agent of dieback on oak trees in Iran. Forest Pathol 49(1):e12468. http://dx.doi.org/10.1111/efp.12468
Hanks LM (1999) Influence of the larval host plant on reproductive strategies of cerambycid beetles. Annu Rev Entomol 44:483–505. https://doi.org/10.1146/annurev.ento.44.1.483
Hanks LM, Wang Q (2017) Reproductive biology of cerambycid beetles. In: Wang Q (ed) Cerambycidae of the world: biology and management. CRC Press, Boca Raton, FL, pp 133–159
Hashemi H, Mohammadi H, Abdollahzadeh J (2017) Symptoms and fungi associated with elm trees decline in Iran. Eur J for Res 136:857–879. https://doi.org/10.1007/s10342-017-1075-y
Heidarian R, Fotouhifar K, Debets AJM, Aanen DK (2018) Phylogeny of *Paecilomyces* the causal agent of pistachio and some other trees dieback disease in Iran. PLoS ONE 13(7):e0200794. https://doi.org/10.1371/journal.pone.0200794
Heidarian R, Fotouhifar K, Mohammadi A, Javan-Nikkhah M, Debets F, Aanen D (2015) Phylogeny of genus *Paecilomyces* sp. the causal agent of pistachio die-back disease in Iran. P, 30, In: Proceedings of 2nd Iranian Mycological Congress August 23–25 University of Tehran, Karaj, Iran
Henriques J, Nóbrega F, Sousa E, Lima A (2015) Morphological and genetic diversity of *Biscogniauxia mediterranea* associated to *Quercus suber* in the Mediterranean Basin. Rev Cienc Agrar.http://hdl.handle.net/10400.5/10652
Hosseini A, Hosseini SM, Linares JC (2017) Site factors and stand conditions associated with Persian oak decline in Zagros mountain forests. For Syst 26(3):1–14. https://doi.org/10.5424/fs/2017263-11298
Jalil PA, Ali WK (2020) New description of the larval stage of *Latipalpis* (*Palpilatis*) *johanidesi* Niehuis 2002 (Coleoptera Buprestidae) from Erbil Province Kurdistan Region Iraq. Bull Iraq Nat History Museum 16:15–25https://doi.org/10.26842/binhm.7.2020.16.1.0015
Jamali S, Abbasi S (2023) First report of stinking bean trefoil (*Anagyris foetida* L.) dieback disease caused by *Paecilomyces formosus* in Iran. J Plant Pathol 105(1):371–371. https://doi.org/10.1007/s42161-022-01285-x
Jozeyan A, Shoushtar RV, Askary H (2015) Oaks wood borer beetle and relationship with dryness oak trees in Ilam province. J Trends Life Sci 4:273–280
Kowsari M, Karimi E (2023) A review on oak decline: The global situation causative factors and new research approaches. For Syst 32(3):1–11. https://doi.org/10.5424/fs/2023323-20265
Kubátová A, Kolařík M, Pažoutová S (2004) *Phaeoacremonium rubrigenum*-Hyphomycete associated with bark beetles found in Czechia. Folia Microbiol 49:99–104. https://doi.org/10.1007/BF02931381
Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096
Lee S, Lee S (2020) Multigene phylogeny uncovers oviposition-related evolutionary history of Cerambycinae (Coleoptera: Cerambycidae). Mol Phylo Evol 145:106707. https://doi.org/10.1016/j.ympev.2019.106707
Lillig M (2019) Tenebrionidae (Insecta: Coleoptera) from the Tunisian island group La Galite with comments on the zoogeography of the archipelago. Bull Entomol Soc Malta 10:35–50. http://dx.doi.org/10.17387/BULLENTSOCMALTA.2019.05.
Martin MM (1983) Cellulose digestion in insects. Comp Biochem Physiol, Part a: Physiol Mol Integr 75(3):313–324. https://doi.org/10.1016/0300-9629(83)90088-9
Martín J, Cabezas J, Buyolo T, Patón D (2005) The relationship between *Cerambyx* spp. damage and subsequent *Biscogniauxia mediterranum* infection on *Quercus suber* forests. For Ecol Manag 216(1–3):166–174. https://doi.org/10.1016/j.foreco.2005.05.027
Mattson WJ, Haack RA (1987) The role of drought in outbreaks of plant-eating insects. Bioscience 37:110–118. https://doi.org/10.2307/1310365
Maxwell M, Fenton FA (1936) Biology of *Chrysobothris femorata*. Proc Oklahoma Acad Sci 17:28–29
Mirabolfathy M (2013) Outbreak of charcoal disease on *Quercus* spp. and *Zelkova carpinifolia* trees in forest of Zagros and Alborz mountains in Iran. Iran J Plant Pathol 49:77–79
Modarres Awal M (1997) Buprestidae (Coleoptera). In: Modarres Awal M (ed) List of agricultural pests and their natural enemies in Iran. Ferdowsi University Press, Mashhad, pp 131–140
Moyo P, Allsopp E, Roets F, Mostert L, Halleen F (2014) Arthropods vector grapevine trunk disease pathogens. Phytopathology 104:1063–1069. https://doi.org/10.1094/PHYTO-11-13-0303-R
Özdikmen H (2023) Longhorned beetle (Cerambycoidea: Cerambycidae and Vesperidae) diversity of sixteen countries in the Middle-East on the base of number of species-group taxa and endemism. Munis Entomol Zool 18:1529–1616
Özdikmen H, Cihan Tüzün N (2018) Distribution of *Calchaenesthes* species (Coleoptera: Cerambycidae: Cerambycinae) in the South-Western Asia. Munis Entomol Zool 13:157–163
Panzavolta T, Panichi A, Bracalini M, Croci F, Benigno A, Ragazzi A, Tiberi R, Moricca S (2018) Tree pathogens and their

insect-mediated transport: Implications for oak tree die-off in a natural park area. Glob Ecol Conserv 15:e00437. https://doi.org/10.1016/j.gecco.2018.e00437
Pourhashemi M, Sadeghi SMM (2020) A review on ecological causes of oak decline phenomenon in forests of Iran. Ecol Iran for 8(16):148–164. http://dx.doi.org/10.52547/ifej.8.16.148
Pourhashemi M, Jahanbazi Goujani H, Hoseinzadeh J, Bordbar SK, Iranmanesh Y, Khodakarami Y (2017) The history of oak decline in Zagros forests. Iran Nature 21(2):30–37
Rad HH (2006) Study on the biology and distribution of long-horned beetles Calchaenesthes pistacivora n, sp, (Col: Cerambycidae): a new pistachio and wild pistachio pest in Kerman province. ISHS Acta Horticulturae 726:425–430. https://doi.org/10.17660/ActaHortic.2006.726.68
Raffa KF, Gregoire JC, Lindgren BS (2015) Natural history and ecology of bark beetles. In: Vega FE, Hofstetter RW (eds) Bark beetles. Academic Press, London, pp 1–40
Rajabpour A, Yarahmadi F, Seraj AA (2012) Faunestic investigation on important pests of Ahwaz's urban green landscape. Plant Pests Res 2(3):63–66
Rassati D, Marini L, Marchioro M, Rapuzzi P, Magnani G, Poloni R, Di Giovanni F, Mayo P, Sweeney J (2019) Developing trapping protocols for wood-boring beetles associated with broadleaf trees. J Pest Sci 92(1):267–279. https://doi.org/10.1007/s10340-018-0984-y
Reese ET, Mandels M (1971) Degradation of cellulose and its derivatives enzymic degradation. In: Bikales NM and Segal L (eds) High Polvmers, Wiley-Interscience, New York, London
Robert V, Szoke S, Jabas B, Vu D, Chouchen O, Blom E, Cardinali G (2011) BioloMICS Software: Biological data management identification classification and statistics. Open Appl Inform J 5:87–98. https://doi.org/10.2174/1874136301005010087
Roques A, Ren L, Rassati D, Shi J, Akulov E et al (2023) Worldwide tests of generic attractants, a promising tool for early detection of non-native cerambycid species. NeoBiota 84:169–209. https://doi.org/10.3897/neobiota.84.91096
Rostami T, Jamali S (2022) First report of dieback of *Salix acmophylla* caused by *Paecilomyces formosus* in Iran. Plant Dis 106(9):2518. https://doi.org/10.1094/PDIS-12-21-2812-PDN
Rostami T, Jamali S (2023a) Molecular diagnostics of *Paecilomyces formosus* in Zagros forests using species-specific primers. Iran J Plant Protect Sci 54(2):115–127https://doi.org/10.22059/IJPPS.2023.358063.1007028
Rostami T, Jamali S (2023b) Characterization pathogenicity and host range studies of *Paecilomyces formosus* associated with dieback of Christ's thorn trees (*Paliurus spina-christi* Mill,) in Iran. Forest Pathol 53(1):e12790. https://doi.org/10.1111/efp.12790
Rostamnia M, Akhoondzadeh HM (2016) Assessment of hazardous drought of Ilam province forests using landsat satellite images. J Geomatic Sci Tech 6(2):131–144
Sabernasab M, Jamali S, Marefat A, Abbasi S (2019a) Molecular and pathogenic characteristics of *Paecilomyces formosus* a new causal agent of oak tree dieback in Iran. Forest Sci 65(6):743–750. https://doi.org/10.1093/forsci/fxz045
Sabernasab M, Jamali S, Marefat A, Abbasi S (2019b) Morphological and molecular characterization of *Neoscytalidium novaehollandiae* the cause of *Quercus brantii* dieback in Iran. Phytopathol Mediterr 58(2):347–358https://doi.org/10.14601/Phytopathol_Mediter-10621
Sabernasab M, Jamali S, Marefat A, Abbasi S (2020) Pathogenicity evaluation of pathogenic fungi causing of oak tree dieback in Kermanshah province. Iran J Plant Protect Sci 51:79–92https://doi.org/10.22059/ijpps.2020.293088.1006920
Sadravi M, Moradi N (2017) Four important oak diseases in Iran. Plant Pathol Sci 6(2):14–23. http://dx.doi.org/10.29252/pps.6.2.14
Safaee D, Khodaparast SA, Mirabolfathy M, Mousanejad S (2017) Relationship between dieback of Persian oak (*Quercus brantii*) and apparent and latent infection of *Biscogniauxia mediterranea* in Zagros forests. Iran J Plant Pathol 52:535–549
Sagheb Talebi K, Sajedi T, Pourhashemi M (2014) Forests of Iran: a treasure from the past a hope for the future. Springer, Dordrecht
Sama G, Fallahzadeh M, Rapuzzi P (2005) Notes on some Cerambycidae (Coleoptera) from Iran with description of two new species. Quad Studi Nat Romagna 20:123–132
Samson RA, Houbraken J, Varga J, Frisvad JC (2009) Polyphasic taxonomy of the heat resistant ascomycete genus *Byssochlamys* and its *Paecilomyces* anamorphs. Pers Mol Phylogeny Evol Fungi 22(1):14–27. https://doi.org/10.3767%2F003158509X418925
Siwkcki R, Ufnalski K (1998) Review of oak stand decline with special reference to the role of drought in Poland. Eur J Forest Pathol 28:99–112. https://doi.org/10.1111/j.1439-0329.1998.tb01171.x
Soleymani H, Fallah Shamsi SR, Ahmadi S, Boustani M (2015) Evaluation of bark beetle damage in the decline of *Quercus persica* Forests (case study: Barm Plain Fars province). J Forest Rangeland 105:50–54
Steiner WE Jr (2008) A checklist of the darkling beetles (Insecta: Coleoptera: Tenebrionidae) of Maryland with notes on the species recorded from Plummers Island through the 20th century. Biol Soc Wash Bull 15:133–140. https://doi.org/10.2988/0097-0298(2008)15[133:ACOTDB]2.0.CO;2
Steiner WE Jr (2014) Larvae and pupae of two North American darkling beetles (Coleoptera Tenebrionidae Stenochiinae) *Glyptotus cribratus* LeConte and *Cibdelis blaschkei* Mannerheim with notes on ecological and behavioural similarities. ZooKeys 415:311–327. https://doi.org/10.3897/zookeys.415.6891
Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. Nucl Acids Res 22:4673–4680. https://doi.org/10.1093/nar/22.22.4673
Valipour A, Plieninger T, Shakeri Z, Ghazanfari H, Namiranian M, Lexer MJ (2014) Traditional silvopastoral management and its effects on forest stand structure in northern Zagros. Iran for Ecol Manag 327:221–230. https://doi.org/10.1016/j.foreco.2014.05.004
Valipour J, Zamani AA, Javanmiripour M, Lopez V (2022) Oak borer beetles of Buprestidae family in forest regions of Gilane Gharb city Kermanshah province. Iran J for Range Protect Res 19(2):339–353https://doi.org/10.22092/ijfrpr.2021.354622.1484
Westcott RL, Barr WF, Nelson GH, Verity DS (1979) Distributional and biological notes on North and Central American species of *Acmaeodera* (Coleoptera: Buprestidae). The Coleopterists' Bull 33:169–181. https://www.jstor.org/stable/4000017
White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc: A Guide Methods Appl 18:315–322
Zang R, Yin Z, Ke X, Wang X, Li Z, Kang Z, Huang L (2012) A nested PCR assay for detecting Valsa mali var. mali in different tissues of apple trees. Plant Dis 96(11):1645–1652. https://doi.org/10.1094/PDIS-05-11-0387-RE
Zhao ZH, Cui BY, Li ZH, Jiang F, Yang QQ, Kučerová Z, Stejskal V, Opit G, Cao Y, Li FJ (2016) The establishment of species-specific primers for the molecular identification of ten stored-product psocids based on ITS2 rDNA. Sci Rep 6(1):21022. https://doi.org/10.1038/srep21022