Fungi associated with grapevine trunk diseases (GTDs) with emphasize on pestalotioid species in Kurdistan Province, Iran

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Research

Keywords: Seimatosporium, Sporocadus, Sporocadaceae, Truncatella, Xenoseimatosporium

DOI: https://doi.org/10.21203/rs.3.rs-192033/v1

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Abstract

Grapevine trunk diseases (GTDs) are destructive and important economically with worldwide distribution. In this survey 233 fungal isolates were obtained from grapevine cultivars showing trunk diseases symptoms in Kurdistan Province, Iran. Based on sequences data and morphology 24 species belong to 20 genera were characterized. *Botryosphaeriaceae*, *Alternaria*, *Sporocadaceae* and *Phaeoacremonium* members were the most prevalent identified fungal groups. At the species level *Botryosphaeria dothidea*, *Alternaria malorum*, *Phaeoacremonium aleophilum* and *Acremonium sclerotigenum* were the most frequent identified species. All species are new records in Kurdistan Province. *Clonostachys rosea* and *Neoscytalidium novae-hollandiae* are new records on grapevine in Iran. *Acremonium sclerotigenum*, *Alternaria chlamydosporigena*, *Ascochyta herbicola* and *Paecilomyces formosus* are new records on grapevine around the world. In phylogenetic analyses based on LSU, ITS, *TEF-1a* and *TUB2* sequence data four pestalotioid species belong to *Sporocadaceae* were identified. Of these, three species are new for science and introduced here as *Seimatosporium marivanicum*, *Sporocadus kurdistani* and *Xenoseimatosporium kurdistanicum*. Furthermore, three new combinations in *Sporocadus* are proposed.

Introduction

Grapevine trunk diseases (GTDs) including esca disease, eutypa and botryosphaeria dieback are the most destructive fungal diseases causing dieback and rapid or gradual decline in grapevine (Mugnai et al. 1999; Úrbez-Torres et al. 2012; Bertsch et al. 2013; Úrbez-Torres et al. 2014). These fungal diseases are major threat to the grapevine-related industries with a worldwide distribution, which have long been considered by researchers and dates back more than a century ago (Dubos and Larignon 1988; Mugnai et al. 1999; Graniti et al. 2000; Mostert et al. 2006; Surico et al. 2006; Surico 2009; Bertsch et al. 2013; Gramaje et al. 2015; Fischer and Peighami Ashnaei 2019). As can be concluded from literature usually different basidiomycetous taxa, more often *Fomitiporia mediterranea*, *Fomitiporia punctata* and *Phellinus igniarius*, and ascomycetous species belong to the most important and well-known genera *Phaeoacremonium* and *Pestalotioid fungi* are found as saprobes, endophytes and plant pathogens in association with mainly woody plants and human pathogens in different climates worldwide (De Hoog et al. 2000; Watanabe et al. 2010; Tanaka et al. 2011; Liu et al. 2019). These fungi comprising various anamorphic genera known by producing multi-septate conidia with appendages at both or either ends (Nag Rj 1993; Lee et al. 2006; Liu et al. 2019). Taxonomy of these fungi have been problematic and controversial in the past. In the past two decades, taxonomic studies based on DNA sequence data have contributed to clarify the ambiguities surrounding the systematic of pestalotioid fungi (Jeewon et al. 2002, 2003; Lee et al. 2006; Barber et al. 2011; Tanaka et al. 2011; Crous et al. 2015; Senanayake et al. 2015; Jaklitsch et al. 2016; Maharachchikumbura et al. 2016; Wijayawardene et al. 2016; Crous et al. 2018; Liu et al. 2019). In an extensive multigene phylogenetic study on coelomycetous fungi with appendage-bearing conidia Liu et al. (2019) discussed taxonomic history of these fungi in detail and placed them in the family *Sporocadaceae*, *Xylariales*. Liu et al. (2019) recognized 30 monophyletic genera in *Sporocadaceae* including *Seimatosporium*, *Sporocadus*, *Truncatella* and *Xenoseimatosporium*.

Kurdistan Province located in Iran is a part of Zagros Mountains occupied by early humans and ancient history in agriculture. Oak and grapevine are the two common trees that can be find growing across the Zagros Mountains. It is the first research on GTDs in this part of the world. In this survey during 2012–2014 some 230 fungal isolates were obtained. This study aimed to characterize these isolates based on morphology and DNA sequence data.

Materials And Methods

Sampling and fungal isolation

During a survey between 2012 and 2014 on grapevine trunk diseases in Kurdistan Province, twig and trunk samples of grapevines showing trunk diseases symptoms (cv. Askari, Bidaneh Sefid, Farkhi, Rasha and Sahabi) were collected from vineyards all over 10 years
old in 25 different villages. Grapevine cultivars showed different symptoms consisting of decline, reduced growth, interveinal yellow-brown or red-brown necrotic spots on leaves known as tiger-stripes pattern, spotting berries (black measles), sectorial and central brown necrosis of the trunks. Cross sections of samples were made and sliced to 0.5-1 cm pieces of infected wood. After surface sterilization, (3–4 min in 70% ethanol) four pieces were placed on 9 cm PDA plates supplemented with 100 mg chloramphenicol, streptomycin or tetracycline. Plates were incubated at 25 ± 2 °C in the dark. Colonies grown from wood pieces were transferred to PDA plates and incubated at 25 ± 2 °C in the dark. After 1–2 wk conidiomata were formed on PDA plates. To purify the isolates using single-spore method conidia were transferred to tap water agar (2% WA). After incubation at 25°C for 12 h single germinated conidia were transferred to PDA plates. Representative isolates were deposited in the culture collection (IRAN) of the Iranian Research Institute of Plant Protection (Tehran, Iran) and the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute (Utrecht, the Netherlands).

**Morphology**

Colonies were grown on PDA, MEA and OA at 25 ± 2°C for 1–2 wk. Structures were mounted in 100 % lactic acid or water and digital images were recorded with an Olympus DP72 camera on a Olympus BX51 microscope. Measurements were made with the Cell Sense Entry measurement module. For each isolate the mean, standard deviation, minimum and maximum values were calculated from measurements of at least 30 fungal structures. Conidial length was measured from the base of the basal cell to the base of the apical appendage, and conidial width was measured at the widest point of the conidium (Bonthond et al. 2018). Dimensions are presented as a range with extremes and mean ± standard deviation in parentheses. Depending fungal taxonomic groups the colony morphology and growth rate were determined on different culture media and temperature in the dark. For pestalotioloid fungi colony morphology and growth rate were determined on MEA and PDA at 21°C in the dark. After 2 wk mycelial growth was measured and cultural characteristics were recorded based on the colour charts of Rayner (1970).

**DNA extraction, PCR and sequencing**

The PCR reaction mixtures 25 µL contained 1×PCR buffer (PCR buffer with (NH4)2SO4), 3 mM MgCl2, 200 µM of each nucleotide, 5 pmol of each primer, 1 U of Taq polymerase and 1 µL of template DNA (50–100 ng/µL). Genomic DNA was extracted from 4–7 d old cultures grown in potato dextrose broth (PDB) using modified method of Raeder & Broda (1985) as described by Abdollahzadeh et al. (2009). The D1/D2 variable domains of the 28S nrDNA (LSU) and the ITS1, 5.8 and ITS2 region of ribosomal DNA and part of β-tubulin (TUB2) and the translation elongation factor 1-alpha (TEF-1α) were amplified and sequenced using the following primer pairs LR0R/LR5 for LSU (Vilgalys and Hester 1990), ITS5 or ITS1/ITS4 for ITS (White et al. 1990), T1/Bt2b for TUB2 (Glass and Donaldson 1995, O'Donnell and Cigelnik 1997), EF-1/EF-2 for TEF-1α (O'Donnell et al. 1998). The PCR reaction mixtures 12.5 µL contained 1×PCR buffer (PCR buffer with (NH4)2SO4), 3 mM MgCl2, 200 µM of each nucleotide, 5 pmol of each primer, 1 U of Taq polymerase and 1 µL of template DNA (50–100 ng/µL). The PCR amplification conditions were 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 52°C for 45 s (LSU and ITS) or 55°C for 45 s (TEF-1α and TUB2), and 72°C for 1 min, and a final extension of 72°C for 7 min. The PCR products were sequenced with both forward and reverse primers using an Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific). Forward and reverse reads were paired and consensus sequences were obtained using the software BioEdit v. 7.0.0 (Hall 2004). All new sequences were submitted to GenBank (Table 1).
Table 1
Isolates used in phylogenetic analyses

| Species                  | Isolate No.1 | Host                  | Location                | GenBank accession number |
|--------------------------|--------------|-----------------------|-------------------------|-------------------------|
|                          |              |                       |                         | LSU         | ITS               | TUB2    | EF1-α         |
| Allelochaeta fusispora   | CBS 810.73^T | *Eucalyptus polyanthemos* | Australia               | MH554279   | MH554067          | MH554743 | MH554503     |
| *All. falcata*           | CPC 13580    | *E. alligatrix*       | Australia               | MH554284   | MH554073          | MH704626 | MH704601     |
| Bartalina robillardoides | CBS 122615   | *Cupressus lusitanica* | South Africa            | MH554207   | MH553989          | MH554657 | MH554415     |
| *Broomella vitalbae*     | HPC 1154     | -                     | -                       | MH554367   | MH554173          | MH554846 | MH554608     |
| Ciliochorella phanericola| MFLUCC 12–0310| Dead leaves           | Thailand                | KF827445   | KF827444          | KF827478 | KF827477     |
| *Diploceras hypericinum* | CBS 109058   | *Hypericum* sp.       | New Zealand             | MH554178   | MH553955          | MH554614 | MH554373     |
| D. hypericinum           | CBS 492.97   | *H. perforatum*       | Netherlands             | MH554267   | MH554054          | MH554730 | MH554489     |
| *Disaeta arbuti*         | CBS 143903   | *Acacia pycnantha*    | Australia               | MH554346   | MH554148          | MH554821 | MH554583     |
| Discosia sp. 1           | CBS 241.66   | *A. karroo*           | South Africa            | MH554244   | MH554022          | MH554698 | MH554456     |
| Discosia sp. 2           | CBS 684.70   | *Aesculus hippocastanum* | Netherlands           | MH554277   | MH554064          | MH554740 | MH554500     |
| Distononappendiculata banksiae | CBS 143906 | *Banksia formosa*     | Australia               | MH554354   | MH554158          | MH554831 | MH554593     |
| Diversimediispora humicola | CBS 302.86^T | Soil                  | USA                     | MH554247   | MH554028          | MH554705 | MH554463     |
| Heterotruncatella restionacearum | CBS 118150 | *Restio filiformis*   | South Africa            | MH554203   | DQ278914          | MH554649 | MH554407     |
| Hyalotiella transvalensis | CBS 303.65^T | Leaf litter and top   | South Africa            | MH554248   | MH554029          | MH554706 | MH554464     |
|                          |              | soil of *A. karroo*   |                         | MH554248   | MH554029          | MH554706 | MH554464     |
|                          |              | community             |                         |            |                   |         |               |
| Hymenopleella hipphophaeicola | CBS 113687 | *Hippophaë rhamnoides* | Sweden                 | MH554188   | MH553969          | MH554628 | MH554387     |
| Immersidiscosia eucalypti | CBS 104197   | *Ardisia japonica*    | Japan                   | AB593724   | AB594792          | NA       | NA            |
| Lepteutypa fuckelii      | CBS 140409^NT| *Tilia cordata*       | Belgium                 | KT949902   | NR_154123         | MH554677 | MH554435     |

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2 LSU large subunit ribosomal DNA; ITS internal transcribed spacer; EF1-α translation elongation factor 1-alpha; TUB2 β-tubulin 2; N/A not available; Newly generated sequences are indicated in bold.
| Species                        | Isolate No.¹ | Host                  | Location          | GenBank accession number² |
|-------------------------------|--------------|-----------------------|-------------------|---------------------------|
|                               |              |                       |                   | LSU     | ITS     | TUB2     | EF1-α    |
| **Monochaetia ilexae**        | CBS 101009   | Air                   | Japan             | MH554176 | MH553953 | MH554612 | MH554371 |
| **Morinia acaciae**           | CBS 100230   | Prunus salicina cv. Omega | New Zealand        | MH554174 | MH553950 | MH554609 | MH554368 |
| **Neopestalotiopsis zimbabwana** | CBS 111495T | Leucospermum cunciforme | Zimbabwe          | JX556249 | JX556231 | KM199456 | KM199545 |
| **Nonappendiculata quercina** | CBS 270.82   | Quercus pubescens     | Italy             | MH554246 | MH554025 | MH554701 | MH554459 |
| **Parabartalinia lateralis**  | CBS 399.71T  | A. karroo             | South Africa      | MH554256 | MH554043 | MH554719 | MH554478 |
| **Pestalotiopsis humicola**   | CBS 115450   | *ilex cinerea*        | Hong Kong         | KM116208 | KM199319 | KM199418 | KM199487 |
| **Pseudopestalotiopsis cocos** | CBS 272.29T | Cocos nucifera        | Indonesia         | KM116276 | KM199378 | KM199467 | KM199553 |
| **Pseudosarcostroma osyndicola** | CBS 103.76T | Osyris alba           | France            | MH554177 | MH553954 | MH554613 | MH554372 |
| **Robillarda terrae**         | CBS 587.71T  | Soil                  | India             | KJ710459 | KJ710484 | MH554734 | MH554493 |
| **Sarcostroma leucospermi**   | CBS 111290T  | Leucospermum cv. 'High Gold' | South Africa | MH554292 | MH554081 | MH554755 | MH554516 |
| **Sarcostroma proteae**       | CBS 113610T  | Protea magnifica      | Australia         | MH554187 | MH553968 | MH554627 | MH554386 |
| **Seimatosporium botan**      | NBRC 104200T | Paeonia suffruticosa  | Japan             | AB593731 | AB594799 | LC047770 | NA       |
| **Seimatosporium ficeae**     | MFLUCC 15-0519T | Ficus sp.            | China             | KR920686 | KR920800 | NA       | NA       |
| **Seimatosporium germanicum** | CBS 437.87T  | -                     | Germany           | MH554259 | MH554047 | MH554723 | MH554482 |
| **Seimatosporium luteosporum** | CBS 142599T | Vitis vinifera        | USA               | KY706309 | KY706284 | KY706259 | KY706334 |

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² LSU: large subunit ribosomal DNA; ITS: internal transcribed spacer; EF1-α: translation elongation factor 1-alpha; TUB2: β-tubulin 2; N/A: not available. Newly generated sequences are indicated in bold.

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| Species                      | Isolate No.1 | Host                  | Location            | GenBank accession number2 |
|------------------------------|--------------|-----------------------|---------------------|---------------------------|
|                              |              |                       |                     | LSU | ITS | TUB2 | EF1-α |
| **Seimatosporium marivanicum** | IRAN 2310C = CBS 143781 | *V. vinifera* | Iran, Mariwan       | MW361960 | MW361952 | MW375352 | MW375358 |
|                              | IRAN 2310C = CBS 143780 | *V. vinifera* | Iran, Mariwan       | MW361959 | MW361951 | MW375351 | MW375357 |
|                              |              |                       |                     | CBS 789.68 |               |          |         |
| **Seimatosporium physocarpi** | CBS 139968T  | Physocarpus opulifolius | Russia             | KT198723 | KT198722 | MH554676 | MH554434 |
| **Seimatosporium pistaciae**  | CPC 24457    | Pistacia vera         | Iran                | MH554331 | MH554126 | MH554799 | MH554561 |
| **Seimatosporium rhombisporum** | MFLUCC 15-0543T | Vaccinium myrtillus | Italy              | KR092780 | KR092792 | NA        | NA       |
| **Seimatosporium rosae**     | CBS 139823ET | Rosa kalmiussica      | Russia             | KT198727 | LT853105 | LT853253 | LT853203 |
| **Seimatosporium soli**      | CBS 941.69T  | Forest soil under     | Denmark             | MH554282 | MH554071 | NA        | MH554507 |
|                              |              | *Fagus sylvatica*     |                     |               |          |          |          |
| **Seimatosporium vitifusiforme** | CBS 142600T | *V. vinifera*         | USA                 | KY706321 | KY706296 | KY706271 | KY706346 |
| **Seimatosporium vitis**     | MFLUCC 14-0051 | *V. vinifera*       | Italy               | KR920362 | KR920363 | NA        | NA       |
| **Seimatosporium vitis-viniferae** | CBS 123004T | *V. vinifera*       | Spain               | MH554211 | MH553992 | MH554660 | MH554418 |
| **Seiridium pseudocardinale** | CBS 122613   | Cupressus sp.        | Portugal            | MH554206 | LT853096 | LT853243 | LT853193 |
| **Sporocadus biseptatus**    | CBS 110324T  | -                     | -                   | MH554179 | MH553956 | MH554615 | MH554374 |
| **Sporocadus cornicola**     | CBS 143889   | *Cornus sanguinea*   | Germany             | MH554326 | MH554121 | MH554794 | MH554555 |
| **Sporocadus comi**          | MFLUCC 14-0467T | *Cornus sp.* | Italy              | KR559739 | KT162918 | NA        | NA       |
| **Sporocadus cotini**        | CBS 139966T  | *Cotinus coggyria*   | Russia              | MH554222 | MH554003 | MH554675 | MH554433 |

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2 LSU large subunit ribosomal DNA; ITS internal transcribed spacer; EF1-α translation elongation factor 1-alpha; TUB2 β-tubulin 2; N/A not available; Newly generated sequences are indicated in bold.
| Species               | Isolate No.\(^1\) | Host                  | Location                  | GenBank accession number\(^2\) |
|----------------------|--------------------|-----------------------|---------------------------|--------------------------------|
|                      |                    |                       |                           | LSU | ITS | TUB2 | EF1-α   |
| Sporocadus incanus   | CBS 123003\(^T\)   | Prunus dulcis         | Spain                     | MH554210 | MH553991 | MH554659 | MH554417 |
| Sporocadus italicus  | MFLUCC 14-1196\(^T\) | Crategus sp.         | Italy                     | MF614829 | MF614831 | NA | NA |
| Sporocadus kurdistanicus | IRAN 2356C\(^T\) = CBS 143778 | V. vinifera         | Iran, Sanandaj            | MW361958 | MW361950 | MW375350 | MW375356 |
|                      | IRAN 2355C         | V. vinifera          | Iran, Mariwan             | NA | MW361949 | NA | NA |
|                      | IRAN 2354C         | V. vinifera          | Iran, Mariwan             | MW361957 | MW361948 | MW375349 | MW375355 |
|                      | IRAN 2313C         | V. vinifera          | Iran, Dehgolan            | MW361956 | MW361947 | MW375348 | MW375354 |
| Sporocadus lichenicola | NBRC 32625\(^ET\) | Fagus sylvatica      | Germany                   | MH554252 | MH554035 | MH554711 | MH554470 |
| Sporocadus mali      | CBS 446.70\(^T\)   | Malus sylvestris     | Netherlands               | MH554261 | MH554049 | MH554725 | MH554484 |
| Sporocadus microcyclus | CBS 424.95\(^T\)  | Sorbus aria          | Germany                   | MH554258 | MH554045 | MH554721 | MH554480 |
| Sporocadus multisepatus | CBS 143899\(^T\)  | Viburnum sp.         | Serbia                    | MH554343 | MH554141 | MH554814 | MH554576 |
| Sporocadus rosigena  | MFLU 16-0239\(^T\) | Rosa canina          | Italy                     | MG829069 | MG828958 | NA | NA |
| Sporocadus pseudocorni | MFLUCC 13-0529\(^T\) | Cornus sp.           | Italy                     | KU359033 | NA | NA | NA |
| Sporocadus rosarum   | CBS 113832         | Rosa canina          | Sweden                    | MH554189 | MH553970 | MH554629 | MH554388 |
|                      | MFLUCC 14-0466\(^T\) | Rosa canina         | Italy                     | KT281912 | KT284775 | NA | NA |
|                      | MFLUCC 15-0563\(^T\) | Rosa canina         | Italy                     | MG829071 | MG828960 | NA | NA |
|                      | MFLUCC 14-0468\(^T\) | Rosa villosa        | Italy                     | KU359035 | NA | NA | NA |
| Sporocadus rotundatus | CBS 616.83\(^T\)   | Arceuthobium pusillum | Canada                   | MH554273 | MH554060 | MH554737 | MH554496 |
| Sporocadus sorbi     | CBS 160.25         | -                     | -                         | MH554229 | MH554008 | MH554684 | MH554442 |

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\(^2\) LSU: large subunit ribosomal DNA; ITS: internal transcribed spacer; EF1-α: translation elongation factor 1-alpha; TUB2: β-tubulin 2; N/A: not available; Newly generated sequences are indicated in bold.
| Species                          | Isolate No.¹ | Host                          | Location                        | GenBank accession number² |
|---------------------------------|--------------|-------------------------------|---------------------------------|---------------------------|
|                                 |              |                               |                                 | LSU           | ITS          | TUB2          | EF1-α         |
| Sporocadus sp. 1                | CBS 506.71   | Euphorbia sp.                 | Italy                           | MH554268      | MH554055    | MH554731      | MH554490      |
| Sporocadus sp. 2                | CBS 466.96   | Inner tissue of zoocercidium, caused by Lasioptera rubi, on Rubus sp. | Netherlands                     | MH554265      | MH554052    | MH554728      | MH554487      |
| Sporocadus trimorphus           | CBS 114203   | *Rosa canina*                 | Sweden                          | MH554196      | MH553977    | MH554636      | MH554395      |
| Strickeria kochii               | CBS 140411   | *Robinia pseudoacacia*        | Austria                         | KT949918      | NR_154423   | MH554679      | MH554437      |
| Synnemapestaloides juniperi     | CBS 477.77   | *Juniperus phoenicea*         | France                          | MH554266      | MH554053    | MH554729      | MH554488      |
| Synnemapestaloides rhododendri  | MAFF 239201  | *Rhododendron brachycarpum*   | Japan                           | LC047744      | LC047753    | LC047761      | NA            |
| Truncatella angustata           | CBS 393.80   | *Gevuina avellana*            | Chile                           | MH554254      | MH554041    | MH554717      | MH554476      |
|                                 | CJA35        | *V. vinifera*                 | Iran, Sanandaj                  | NA            | MW361953    | NA            | NA            |
|                                 | CJA82        | *V. vinifera*                 | Iran, Sanandaj                  | NA            | MW361954    | NA            | NA            |
| Undetermined species            | CBS 387.77   | Skin of man                   | Finland                         | KM116277      | MH554040    | MH554716      | MH554475      |
|                                 | CBS 113991   | *Salix caprea*                | Sweden                          | MH554190      | MH553971    | MH554630      | MH554389      |
| Xenoseimatosporium kurdistanicum| IRAN 2353    | *V. vinifera*                 | Iran, Mariwan                   | MW361955      | MW361946    | MW375347      | MW375353      |
|                                 | IRAN 2305    | *V. vinifera*                 | Iran, Kamyaran                  | NA            | MW361945    | NA            | NA            |
| Xenoseimatosporium quercinum    | CBS 129117   | *Rhododendron sp.*            | Lativa                          | MH554216      | MH553997    | MH554666      | MH554424      |
|                                 | MFLUCC 14-1198 | *Quercus robur*             | Germany                         | NG_059681     | NR_155804   | NA            | NA            |

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² LSU large subunit ribosomal DNA; ITS internal transcribed spacer; EF1-α translation elongation factor 1-alpha; TUB2 β-tubulin 2; N/A not available; Newly generated sequences are indicated in bold.

**Phylogenetic analyses**

Consensus sequences together with retrieved sequences from GenBank (http://www.ncbi.nlm.nih.gov) were aligned using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html), and manually edited in MEGA v. 7.0.21. The aligned dataset was subjected to Bayesian analysis (BA) and Maximum Likelihood (ML) on the CIPRES Science Gateway portal (https://www.phylo.org/; Miller et al. 2012).
using MrBayes v. 3.2.6 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) and RAxML-HPC BlackBox v. 8.2.10 (Stamatakis 2014), respectively. The optimal nucleotide substitution models were determined for each locus using MrModelTest v. 2.3 (Nylander 2004). Bayesian analyses were implemented under the optimal nucleotide substitution models with four simultaneous Markov Chain Monte Carlo chains, 10 M generations and a sampling frequency of 1 000 generations, ending the run automatically when standard deviation of split frequencies dropped below 0.01. Burn-in was set to remove 25 % of the first sampled trees, after which the 50 % majority rule consensus trees and posterior probability (PP) values were calculated. The ML analyses were done using a GTR + GAMMA substitution model and four rate classes with 1 000 bootstrap iterations. The obtained phylogenetic trees were plotted using FigTree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree). Alignments and trees were deposited in TreeBASE (www.treebase.org; S27404) and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004).

Results

Fungal isolates and species identification

In this survey some 223 fungal isolates were obtained from grapevines showing trunk diseases symptoms (Fig. 1), which 30 isolates were morphologically pestalotioid belong to Sporocadaceae. Based on morphology and DNA sequence data 24 fungal species belong to 20 genera were identified (Fig. 2; Table 2). All fungal species characterized in this survey are new records for the fungal flora of Kurdistan Province. It is the first time Clonostachys rosea and Neoscytalidium novaehollandiae are reported on grapevine in Iran. Acremonium sclerotigenum, Alternaria chlamydosporigena, Ascochyta herbicola and Paecilomyces formosus are new records on grapevine around the world.
## Table 2
Fungal species associated with grapevine trunk diseases identified in this study

| Species                        | Isolates no. | Frequency (%) | Grapevine cv.                      | Location                                                                 |
|-------------------------------|--------------|---------------|------------------------------------|---------------------------------------------------------------------------|
| Acremonium sclerotigenum      | 20           | 8.55          | Rasha, Sahabi, Farkhi              | Dehgolan, Kamyaran, Marivan, Sanandaj                                    |
| Alternaria chlamydosporigena  | 15           | 6.4           | Rasha                              | Bijar, Dehgolan, Kamyaran, Qorveh                                        |
| Alternaria malorum            | 27           | 11.55         | Rasha, Sahabi, Farkhi, Bidaneh Sefid | Bijar, Dehgolan, Divandareh, Kamyaran, Marivan, Sanandaj, Saqez           |
| Ascochyta herbicola           | 4            | 1.7           | Rasha                              | Marivan                                                                   |
| Botryosphaeria dothidea       | 27           | 11.55         | Rasha, Sahabi, Bidaneh Sefid       | Bijar, Marivan, Sanandaj, Saqez                                          |
| Cadophora malorum             | 7            | 3             | Rasha, Bidaneh Sefid               | Baneh, Kamyaran, Marivan, Saqez                                          |
| Clonostachys rosea            | 14           | 6             | Rasha, Bidaneh Sefid               | Kamyaran, Marivan, Sanandaj                                               |
| Didymella glomerata           | 10           | 4.3           | Rasha                              | Dehgolan, Kamyaran, Marivan                                               |
| D. pinodella                  | 5            | 2.1           | Rasha, Bidaneh Sefid               | Kamyaran                                                                  |
| Diplodia seriata              | 4            | 1.7           | Rasha                              | Marivan                                                                   |
| Juxtiphoma eupyrena           | 4            | 1.7           | Rasha                              | Marivan, Sanandaj                                                         |
| Kalmusia variispora           | 2            | 0.85          | Rasha                              | Kamyaran                                                                  |
| Microsphaeropsis olivacea     | 7            | 3             | Rasha, Bidaneh Sefid               | Kamyaran, Sanandaj                                                        |
| Neoscytalidium hyalinum       | 14           | 6             | Rasha, Sahabi                      | Marivan                                                                   |
| N. novaehollandiae            | 6            | 2.5           | Rasha                              | Baneh, Qorveh                                                             |
| Paecilomyces formosus         | 6            | 2.5           | Rasha                              | Marivan                                                                   |
| Phaeoacremonium aleophilum    | 23           | 9.9           | Rasha, Sahabi, Bidaneh Sefid       | Dehgolan, Kamyaran, Marivan, Sanandaj                                     |
| Ph. parasiticicum             | 2            | 0.85          | Rasha                              | Marivan                                                                   |
| Ph. rubrigenum                | 5            | 2.1           | Rasha                              | Marivan                                                                   |
| Phaeomoniella chlamydospora   | 2            | 0.85          | Rasha                              | Kamyaran, Marivan                                                         |
| Seimatosporium marivanicum    | 10           | 4.3           | Rasha, Sahabi                      | Marivan                                                                   |
| Sporocadus kurdistanicus      | 6            | 2.6           | Rasha                              | Dehgolan, Marivan, Sanandaj                                               |
| Truncatella angustata         | 4            | 1.7           | Rasha, Sahabi, Bidaneh Sefid       | Marivan, Sanandaj                                                         |
| Xenoseimatosporium kurdistanicum | 10     | 4.3           | Rasha, Sahabi                      | Kamyaran, Marivan                                                         |

Given that three new pestalotioid species were identified for science here we focused on phylogeny and description of the pestalotioid fungi isolated in this study. Based on morphology, cultural characteristics, grapevine cultivar and sampling geographical location 10 out of 30 isolates were selected for sequencing and phylogenetic studies (Table 1).

### Phylogeny

Two datasets were subjected to phylogenetic analyses. The first dataset consisted of concatenated LSU, ITS, TEF-1α and TUB2, containing 55 taxa representing 30 genera and one undetermined clade recognized by Liu et al. 2019 and Leptotyphla fuckelii CBS.
Seimatosporium marivanicum Abdollahz., Nahvi M. & Khaledi E., sp. nov.

MycoBank MB 838232

Etymology: Name refers to Marivan in Kurdistan Province, Iran where this species was first found.

Diagnosis: In the multigene phylogenetic tree Sei. marivanicum constituted a highly supported distinct calde grouped with a clade containing Seimatosporium luteosporum and Seimatosporium vitifusiforme (Fig. 4). Sei. marivanicum has 4, 5, 20 and 17 bp differences with Sei. luteosporum in LSU, ITS, TEF-1a and TUB2 sequences, respectively. LSU and ITS sequences of Sei. marivanicum are identical to the Sei. vitifusiforme, but there are 6 and 2 bp differences between these two species in TEF-1a and TUB2, respectively. Sei. marivanicum can be easily differentiated from Sei. luteosporum by conidial dimensions (24 × 3.5 μm vs. 19.9 × 5.3 μm) (Table 3). Conidial size of Sei. marivanicum is almost indistinguishable from Sei. vitifusiforme, but conidia in Sei. vitifusiforme are 3-eusepta, while in Sei. marivanicum we have conidia with up to 6 eusepta (Table 3). Moreover, both appendages (apical/basal) of Sei. marivanicum are more longer than Sei. vitifusiforme (15/16 μm vs. 10/9.5 μm ) (Table 3). Sei. luteosporum has been reported on Prunus persica and Vitis vinifera from California and Sei. vitifusiforme has only reported on Vitis vinifera from California (Farr and Rossman, 2020). To differentiate Seimatosporium species reported on grapevine we have presented conidial characteristics in Table 3.
Table 3 Conidial characteristics of *Seimatosporium* species reported on grapevine

| Species          | Conidial dimensions (µm) | Septum no. | Type of appendages                  | Apical appendage length (µm) | Basal appendage length (µm) | Conidia L/W ratio | Reference                        |
|------------------|--------------------------|------------|-------------------------------------|-----------------------------|-----------------------------|-------------------|----------------------------------|
| *S. botan*       | 16–20 × 5–7 (av. = 18 × 6) | 3          | basal                               | 4–8 (av. = 5.8)             | 4–8 (av. = 5.4)             | 2.6–3.8 (av. = 3) | Hatakeyama and Harada 2004       |
|                  | 16–20 × 4–5 (av. = 18 × 4) | 3          | apical and basal                    |                            |                            | 4–5 (av. = 4.6)    |                                  |
| *S. hysteroides* | 12–14 × 5–6              | 3          | often lacking, occasionally basal or with both types | 5–12                        | 5–12                        | –                 | Shoemaker 1964                   |
| *S. lonicerae*   | 9–16 × 3.5–5 (av. = 13 × 4.4) | 3, (2)*   | both types or basal only             | 3–7 (av. = 5.5)             | 2–12 (av. = 7)              | 3                 | Nag Raj 1993                     |
| *S. luteosporum* | 16.7–25.4 × 4.7–5.6 (av. = 19.9 × 5.3) | 3          | apical and basal                    | 10.1–24.2 (av. = 17.9)      | 9.8–23.6 (av. = 16.7)       | –                 | Lawrence et al. 2018             |
| *S. macrosporum* | 28–39 × 9–12.5            | 5          | lacking appendages                  | –                           | –                           | –                 | Sutton 1975                     |
| *S. marivanicum* | 16–31 × 3–7 (av. = 24 × 3.5) | 3 (–6)    | apical and basal                    | 7–20 (av. = 15)             | 5–20 (av. = 16)             | 5 (–6)            | This study                       |
| *S. parasiticum* | 22–35 × 5–6 (–7) (av. = 27.5 × 5.5) | 5, (3/4)* | apical and basal                    | 2–5 (av. = 3.5)             | 2–8 (av. = 4.5)             | 5                 | Nag Raj 1993                     |
| *S. vitifusiforme* | 18.6–30.3 × 3.7–5.1 (av. = 24.9 × 4.2) | 3          | apical and basal                    | 7–12.6 (av. = 10)           | 3.9–16.6 (av. = 9.5)        | –                 | Lawrence et al. 2018             |
| *S. vitis-viniferae* | 13.5–26 × 4.5–6 (av. = 16.5 × 5.2) | 3 (–6)    | basal or with both types            | 4–11 (av. = 7)              | 4–10 (av. = 7.9)            | 3.2               | Liu et al. 2019                  |
| *S. vitis*       | 34–40 × 14–17 (av. = 37 × 15) | 3          | basal                               | –                           | 4–8 (av. = 5)               |                   | Senanayake et al. 2015           |

Type. Iran: Kurdistan Province. Marivan, Nzhmar, *Vitis vinifera* (cv. Rasha), 11 Sep. 2012, J. Nahvi Moghadam (IRAN 17872F—holotype; IRAN 2310C = CBS 143781—ex-type culture).

Description. Sexual morph: unknown. Asexual morph. Conidiomata acervular, stromatic, immersed, semi-immersed to erumpent, dark brown to black. Conidiophores branched, hyaline, smooth. Conidiogenous cells discrete, mostly cylindrical or oblong, 4–20 × 1–2 µm (av.
= 10 ± 1.5 × 1.5 ± 0.2 μm), hyaline, smooth. Conidia allantoid, subcylindrical, curved to straight, 3(–6)-septate, wall smooth, some constricted at the septa, 16–31 x 3–7 μm (av. = 24 ± 1.5 x 3.5 ± 0.4 μm), bearing appendages; basal cell obconic with truncate base or trapezoid, thin-walled, hyaline to pale brown, 2–4 μm (av. = 3 ± 0.2 μm) long; median cells 2(–5), cylindrical or doliiform to ovoid, thick-walled, pale to mid-brown, ± equal, each 5–10 μm (av. = 8 ± 0.7 μm) long; apical cell conic with an acute or rounded apex, thin-walled, hyaline to pale brown, 3–7 μm (av. = 4 ± 0.5 μm) long; apical appendage single, attenuated, smooth, flexuous, unbranched, hyaline, (4–) 7–20 μm (av. = 15 ± 2.5 μm) long; basal appendage single, attenuated, smooth, excentric, 5–20 μm (av. = 16 ± 3 μm) long; mean conidium length/width ratio = 5(–6):1.

Culture characteristics: Colonies on MEA at with fluffy aerial mycelium and entire edge, white to buff (19''f), honey (19-21''b) to vinaceous buff (15-17”d) at the center, reaching 61–64 mm diam after 14 d at 21 °C; on PDA at with fluffy aerial mycelium and entire edge, white at the edge to olivaceous grey (21”i) at the center, reaching 58 mm diam after 14 d at 21 °C.

Specimens examined: Iran: Kurdistan Province: Marivan, Barda Rash, Vitis vinifera (cv. Rasha), 12 Sep. 2012, J. Nahvi Moghadam (IRAN 2300C = CBS 143780).

Sporocadus kurdistanicus Abdollahz., Nahvi M. & Khaledi E., sp. nov.

MycoBank MB838233

Etymology: Name refers to Kurdistan Province in Iran where this species was first found.

Diagnosis: Four isolates of Spo. kurdistanicus clustered in a highly supported clade separated from all Sporocadus species (Fig. 4). Three Sporocadus species including Sporocadus lichenicola, Sporocadus rhododendri and Sporocadus rosigena have previously been reported from grapevine. Spo. lichenicola shows 4 bp (substitution), 8 bp (substitution), 81 bp (28 deletion/insertion, 53 substitutions) and 68 bp (13 deletion/insertion, 55 substitutions) differences with Spo. kurdistanicus. TEF-1α and TUB2 sequences are not available for Spo. rosigena, but in LSU and ITS the type of Spo. rosigena (MFLU 16-0239) has 6 bp and 5 bp differences with Spo. kurdistanicus, respectively. In terms of morphology, conidial dimension of Spo. kurdistanicus is similar with Spo. lichenicola, but conidia of Spo. kurdistanicus are 3-euseptate while in Spo. lichenicola they are 3–4-euseptate and occasionally 5-euseptate. Spo. kurdistanicus is differentiated easily from Spo. rosigena by having larger conidia (Table 4). No sequences are available for Spo. rhododendri, but Spo. kurdistanicus can be distinguished from Spo. rhododendri by producing larger conidia (Table 4).

Table 4 Conidial characteristics of Sporocadus species reported on grapevine

| Species           | Conidial dimensions (μm) | Septum no. | Type of appendages | Conidia L/W ratio | Reference                  |
|-------------------|--------------------------|------------|--------------------|-------------------|----------------------------|
| S. kurdistanicus  | 18–24×6.5–9.5 (av. = 21.5×8) | 3          | lacking app        | 3                 | This study                 |
| S. lichenicola    | 18–25×5.5–8 (av. 21.6×7.2) | 3(–4), occasionally 5 | lacking app        | 3                 | Liu et al 2019             |
| S. rhododendri    | 15.5–20×6.5–8.5           | 3          | lacking app        | –                 | Pirozynski and Shoemaker 1970 |
| S. rosigena       | 12–14×5–7.5 (av. = 13×6.5) | 3, occasionally 2 | lacking app        | –                 | Wanasinghe et al 2018      |

Type: Iran: Kurdistan Province: Sanandaj, Bavarez, Vitis vinifera (cv. Rasha), 28 Sep. 2012, J. Nahvi Moghadam (IRAN 17870F–holotype; IRAN 2356C = CBS 143778–ex-type culture).

Description: Sexual morph: unknown. Asexual morph: Conidiomata acervular, stromatic, immersed, semi-immersed to erumpent, dark brown to black. Paraphyses 30–40 μm, filiform, cylindrical, aseptate, hyaline, smooth-walled. Conidiophores cylindrical or reduced to conidiogenous cells, hyaline, smooth. Conidiogenous cells discrete, mostly cylindrical, sometimes ampulliform, 5–20 x 1–4 μm (av. = 11.6 ± 3.72 x 2.8 ± 0.58 μm), hyaline, smooth. Conidia fusoid, ellipsoidal to obovoid, subcylindrical, rarely slightly curved, 3-septate, wall
smooth, 18–24 × 6.5–9.5 μm (av. = 21.5 ± 0.9 × 8 ± 0.5 μm), lacking appendages; basal cell obconic with a truncate base, pale brown, 2.5–6.5 μm (av. = 4.8 ± 0.92 μm) long; median cells 2, fairly thick-walled, pale brown to brown, doliiform, mostly ± equal, each 6–8 μm (av. = 7 ± 0.5 μm) long, occasionally variable in size, together 10–15 μm (av. = 13.5 ± 1.5 μm) long; apical cell not conic with rounded apex, or conic with obtuse apex, concolourous with median cells, 3–7 μm (av. = 4.5 ± 0.5 μm) long; mean conidium length/width ratio = 3:1.

**Culture characteristics:** Colonies on MEA flat, appressed to fluffy, folded, edge sinuate, white to buff (19''f) to sinnamon (13-15''i) at the edge, reaching 43–47 mm diam after 14 d at 21 °C; on PDA flat with fluffy aerial mycelium and a few radial circular line from the center, edge sinuate, buff (19''f) to vinaceous buff (15-17”d), wet and cinnamon (13-15''i) to sepia (13-15''k), at the center reaching 33–45 mm diam after 14 d at 21 °C.

**Specimens examined:** Iran: Kurdistan Province: Marivan, Ahmadabad, *Vitis vinifera* (cv. Rasha), 23 Sep. 2012, J. Nahvi Moghadam (IRAN 2354C); Marivan, Nasl-Goshtkhani, *Vitis vinifera* (cv. Rasha), 14 Sep. 2012, J. Nahvi Moghadam (IRAN 2355C); Dehgolan, Javanmardabad, *Vitis vinifera*, 10 Sep. 2012, J. Abdollahzadeh & E. Khaledi (IRAN 2313C = CBS 143777).

*Sporocadus comri* (Wijayawardene, Camporesi & K.D. Hyde) Abdollahz., **comb. nov.** Mycobank, MB838310

**Basionym:** *Seimatosporium comri* Wijayaw., Camporesi & K.D. Hyde, *Fungal Diversity* **73:** 100 (2015).

**Type:** Italy: Pesaro-Urbino Province, Monte Nerone, on branches of *Cornus* sp., 11 June 2012, Erio Camporesi (MFLU 15–0742–holotype; MFLUCC 14–0467–ex-type culture).

**Description:** For a complete description, see Li et al. (2016).

*Sporocadus pseudocorni* (Wijayawardene, Camporesi & K.D. Hyde) Abdollahz., **comb. nov.** Mycobank, MB838308

**Basionym:** *Seimatosporium pseudocornii* Wijayaw., Camporesi & K.D. Hyde. *Fungal Diversity* **78:** 99 (2016).

**Type:** Italy: Forlì-Cesena Province, near Monte Riccio-Bagno di Romagna, on dead branch of *Cornus* sp. (*Cornaceae*), 5 Jan. 2013, Erio Camporesi (MFLU 15–3558–holotype; MFLUCC 13–0529–ex-type culture).

**Description:** For a complete description, see Senanayake et al. (2015).

**Notes:** *Sporocaduscomricola* and *Sporocaduspseudocorni* are identical in LSU sequence data. ITS, *TEF-1a* and *TUB2* sequences data are not available for *Spo. pseudocorni* but morphologically these are two distinct species (31–42 × 5–7 μm in *Spo. pseudocorni* vs. 34–51 × 13–18 μm in *Spo. cornicola)*.

*Sporocadus italicus* (Q.J. Shang & K.D. Hyde) Abdollahz., **comb. nov.**

Mycobank, MB838309

**Basionym:** *Seimatosporium italicum* Q.J. Shang & K.D. Hyde, *Fungal Diversity* **67:** 165 (2017).

**Type:** Italy: Papiano–Stia, Arezzo Province, on dead aerial branch of *Crategus* sp., 14 May 2014, E. Camporesi (MFLU 17-0499–holotype; MFLUCC 14-1196–ex-type culture).

**Description:** For a complete description, see Hyde et al. (2017).

*Xenoseimatosporium kurdistanicum* Abdollahz., Khaledi E. & Nahvi M., **sp. nov.**

MycoBank MB838234

**Etymology:** Name refers to Kurdistan Province in Iran where this species was first found.

**Diagnosis:** *Xen. kurdistanicum* is the second introduced species in *Xenoseimatosporium* after *Xenoseimatosporium quercinum*. These two species are clearly separated in phylogenetic analyses (Fig. 4). There are 1 bp (substitution), 15 bp (12 substitutions, 3 deletions/insertions), 20 bp (16 substitutions, 4 deletions/insertions) and 15 bp (14 substitutions, 1 deletion/insertion) differences in LSU, ITS, *TEF-1a* and *TUB2* sequences, respectively. These two species are easily distinguishable morphologically by conidial

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dimensions (29 × 6 μm in *Xen. kurdistanicum* vs. 18.2 × 4.5 μm in *Xen. quercinum*), number of septa in conidia (3 in *Xen. kurdistanicum* vs. 2-4 in *Xen. quercinum*) and apical/basal appendages length (20/25 μm in *Xen. kurdistanicum* vs. 13.4/12.1 μm in *Xen. quercinum*).

**Type:** Iran: Kurdistan Province. Marivan, Bara Rash, *Vitis vinifera* (cv. Rasha), 12 Sep. 2012, J. Nahvi Moghadam (IRAN 17871F—holotype; IRAN 2353C—ex-type culture).

**Description:** Sexual morph: unknown. Asexual morph: *Conidiomata* acervular, immersed, semi-immersed to erumpent. *Conidiophores* branched, hyaline, smooth. *Conidiogenous cells* discrete, cylindrical, oblong to lageniform, 8–15 × 1.5–2.5 μm (av. = 10 ± 1.5 × 2 ± 0.5 μm), hyaline, smooth. *Conidia* mostly allantoid, occasionally subcylindrical, curved to straight, 3-septate, smooth, some constricted at septa, 22–32 × 4–8 μm (av. = 29 ± 2 × 6 ± 0.9 μm), bearing appendages; basal cell obconic with truncate base or trapezoid, thin-walled, hyaline to pale brown, 2–4 μm (av. = 3 ± 0.3 μm) long; median cells 2, mostly cylindrical, occasionally doliiform, pale to mid-brown, thin-walled, ± equal, each 8–12 μm (av. = 10 ± 0.8 μm) long; apical cell conic with an acute or rounded apex, hyaline to pale brown, 2–4 μm (av. = 3 ± 0.5 μm); apical appendage single, attenuated, smooth, flexuous, unbranched, 15–30 μm (av. = 20 ± 2 μm); basal appendage single, attenuated, smooth, flexuous, unbranched, excrcent, 18–33 μm (av. = 25 ± 1.8 μm) long; mean conidium length/width ratio = 5(−6):1.

**Culture characteristics:** Colonies on MEA flat, appressed to fluffy, folded, edge sinuate, buff (19”f) to sinnamon (13-15”i), reaching 45–50 mm diam after 14 d at 21 °C; on PDA flat with entire edge, fluffy, buff (19”f) to vinaceous buff (15-17”d), reaching 55–61 mm diam after 14 d at 21 °C.

**Specimens examined:** Iran: Kurdistan Province. Kamyaran, Bovana, *Vitis vinifera*, 18 Sep. 2012, E. Khaleedi (IRAN 2305C).

**Discussion**

In an extensive study on grapevine trunk diseases (GTDs) of vineyards showing esca, petri, dieback and decline symptoms in Kurdistan Province we collected 233 fungal isolates including 30 *Pestalotia*-like isolates. Based on morphology and sequences data (LSU, ITS, TEF-1α and TUB2) 24 fungal species belong to 20 genera including well-known genera associated with grapevine trunk diseases such as *Botryosphaeria*, *Diplodia*, *Neoscytalidium*, *Phaeoacremonium* and *Phaeomoniella* were identified. *Botryosphaeraceae* (21.75%), *Alternaria* (17.95%), *Sporocadaceae* (12.9%) and *Phaeoacremonium* (12.85%) species were the most prevalent fungi isolated in this study. *Botryosphaeria dothidea* (11.55%). *Alternaria malorum* (11.55%), *Phaeoacremonium aleophilum* (9.9%) and *Acremonium sclerotigenum* (8.55%) were the most frequent identified species.

All 24 characterized species are new fungal records in Kurdistan Province, Iran. *Clonostachys rosea* and *Neoscytalidium novaehollandiae* are reported as new records in association with grapevine in Iran.

Most of the identified species have previously been reported on grapevine, but *Acremonium sclerotigenum*, *Alternaria chlamydosporigena*, *Ascochyta herbicola* and *Paecilomyces formosus* are reported as new records on grapevine in the world.

In a multigene phylogeny based on LSU, ITS, TEF-1α and TUB2 sequences of representative species of all *Sporocadaceae* genera our representative pestalotioid isolates resided in four different genera *Seimatosporium*, *Sporocadus*, *Truncatella* and *Xenoseimatosporium*. To recognize our isolates at the species level we performed another multigene phylogeny based on LSU, ITS, TEF-1α and TUB2 sequences data of ex-type or authentic strains of all species belong to these four genera. Phylogenetic analyses showed that two isolates CJA35 and CJA82 are belong to *Sei. vitifusiform* and *Sei. macrospermum* species, respectively. The remaining eight isolates placed in three genera *Seimatosporium*, *Sporocadus* and *Xenoseimatosporium* and identified as new species namely, *Seimatosporium marivanicum*, *Sporocadus kurdistanicus* and *Xenoseimatosporium kurdistanicum*. Two isolates IRAN 2300C and IRAN 2310C constituted a distinct and well supported clade (BI-PP/ML-BS = 1/100) in *Seimatosporium* named as *Sei. marivanicum*. So far, 10 *Seimatosporium* species have reported on grapevine namely, *Seimatosporium botan*, *Seimatosporium hysterioides*, *Seimatosporium licaniae*, *Seimatosporium lichenicolus* (= *Sporocadus lichenicolus*), *Seimatosporium luteosporum*, *Seimatosporium macrosporum*, *Seimatosporium parasiticum*, *Seimatosporium vitifusiforme*, *Seimatosporium vitis* and *Seimatosporium vitis-viniferae*. Phylogenetically *Sei. marivanicum* is clearly distinct from all *Seimatosporium* species, but *Sei. luteosporum* and *Sei. vitifusiform* are the two closest species. Morphologically if we use conidial characteristics *Sei. marivanicum* can be distinguish from all other *Seimatosporium* species reported on grapevine. *Sei. marivanicum* is separated from *Sei. luteosporum* by having larger conidia. Although conidial morphology of *Sei. marivanicum* is more similar with *Sei. vitifusiform*, but number of eusepta and longer appendages can be used to differentiate these two species.
Four isolates IRAN 2313C, IRAN 2354C, IRAN 2355C and IRAN 2356C were grouped together in a separate and highly supported clade in both Bayesian (PP = 1) and RAxML (BS = 99) analyses within *Sporocadus* as a new species *Sporocadus kurdistanicus*. This species is the fourth *Sporocadus* species reported from grapevine along with *Spo. lichenicola*, *Spo. rhododendri* and *Spo. rosigena*. Phylogenetically *Spo. kurdistanicus* is well separated from *Spo. lichenicola* and *Spo. rosigena*. No sequences were available for *Spo. rhododendri* but it is possible to distinguish these two species by having larger conidia (18–24 × 6.5–9.5 µm vs. 15.5–20 × 6.5–8.5 µm) in *Spo. kurdistanicus*.

Phylogenetic analyses in this study revealed that *Sei. pseudocornii*, *Sei. italicum* and *Sei. cornii* are belong to *Sporocadus*, we therefore transferred them to *Sporocadu* as new combinations. Although asexual morph of *Sei. italicum* has not seen, as in most of *Sporocadus* species both apical and basal appendages absent in conidia of *S. cornii* and *S. pseudocornii* indicates their taxonomic position in *Sporocadus*.

Liu et al. (2019) used isolate CBS 466.96 as a representative isolate for *Spo. rosigena* despite three and two differences with the holotype (MFLU 16–0239) in LSU and ITS sequence data, respectively. In our analyses the holotype (MFLU 16–0239) and CBS 466.96 placed in two separate clades and thus isolate CBS 466.96 represents a distinct clade and can be introduced as a new *Sporocadus* species.

The type specimens of *Sei. pseudoraosae* (MFLUCC 14–0468), *Sei. pseudorusarum* (MFLUCC 14–0466) and *Sei. rosigenum* (MFLUCC 15–0563) were placed along with strain CBS 113832 in a clade named as *Sporocadus rosarum* by Liu et al. (2019). Since TEF-1α and TUB2 sequences are not available for *Sei. pseudorusarum* and *Sei. rosigenum* and only LSU is available for *Sei. pseudorosae* the identity of these species is not clear and we thus considered them as intraspecific variation in *Spo. rosarum* until these sequence data is available in the future studies.

*Xenoseimatosporium kurdistanicum* another new species we introduced here is the second species of *Xenoseimatosporium* a new pestalotioid genus recently introduced by Liu et al. (2019). These two species are easily distinguishable morphologically by conidial dimensions and appendages length as mentioned in the notes under *Xen. kurdistanicum*.

In a preliminary field experiment on pathogenicity of some identified species on two grapevine cultivars (Bidaneh Sed and Rasha), *N. novaehollandiae*, *B. dothidea* and *Ph. aleophilum* were the most virulent pathogenic species. Four pestalotioid species characterized in this study using an isolate from each species were nonpathogenic, but it is necessary to examine their pathogenicity with more isolates in greenhouse and field conditions individually and in combination with other species isolated from grapevine in this study.

**Declarations**

**Acknowledgements**

We thank Mr. Alireza Javadi for his assistance in preparation of holotypes and recording growth rate of new species introduced here. LSU, EF1-α and TUB2 sequences of new species described in this study were amplified and sequenced in Westerdijk Fungal Biodiversity Institute (Utrecht, the Netherlands) during Jafar Abdollahzadeh sabbatical leave in 2018.

**Adherence to national and international regulations**

All material for this study was collected in Iran in 2012, thus before the implementation of the Nagoya Protocol to the Convention on Biological Diversity.

**Author contributions**

JAb designed the project. JAb, EK and JNM collected the samples, photography and phylogenetic analyses. EK and JNM performed fungal isolation and all experiments. All authors contributed to the preparation of the manuscript.

**Funding**

This research was supported by the University of Kurdistan and Kurdistan Provincial Office under project 65/6/64197/2011.

**Availability of data and material**

All data generated or analyzed during this study are included in this published article. Requests for materials should be addressed to JAb.
Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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