

The genus *Daldinia* in Guilan province (N Iran)

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Abstract

In order to identify *Daldinia* species in Guilan province, several specimens were collected during 2011–13. A total of six taxa, including *Daldinia caldariorum* (on *Morus alba*); *D. childiae* (on *Parrotia persica*, *Pterocarya fraxinifolia*, *Morus alba*, *Quercus* sp., *Prunus* sp., *Citrus sinensis*, *Gleditschia caspica*, *Buxus sempervirens*, *Carpinus betulus* and *Alnus subcordata*); *D. gelatinoides* (on *M. alba*); *D. cf. loculata* (on fallen wood); *D. pyrenaica* (on *B. sempervirens* and *Pt. fraxinifolia*), and *D. vernicosa* (on *Ulmus* sp., *Ficus* sp., and *P. persica*), were identified. All identified species are new to the Iranian mycobiota.

Keywords: Ascomycetes, biodiversity, taxonomy, *Xylariaceae*

جنس *Daldinia* در استان گیلان*

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خلاصه

به منظور شناسایی گونه‌های *Daldinia* در استان گیلان، نمونه‌برداری از نقاط مختلف جنگلی این استان طی سال‌های ۹۲–۱۳۹۰ انجام شد. در مجموع، شش گونه شناسایی شدند که عبارتند از: *Daldinia caldariorum* (روی *Morus alba*)، *D. childiae* (روی *Parrotia persica*، *Pterocarya fraxinifolia*، *M. alba*، *Quercus* sp.، *Prunus* sp.، *Citrus sinensis*، *Gleditschia caspica*، *Buxus sempervirens*، *Carpinus betulus* و *Alnus subcordata*)، *D. gelatinoides* (روی *Morus alba*)، *D. cf. loculata* (روی چوب)، *D. pyrenaica* (روی *B. sempervirens* و *Pt. fraxinifolia*) و *D. vernicosa* (روی *Ulmus* sp.، *Ficus* sp. و *P. persica*). تمامی گونه‌های شناسایی شده در این تحقیق برای فلور قارچ‌های ایران جدید هستند.

واژه‌های کلیدی: تاکسونومی، تنوع زیستی، قارچ‌های آسکومیستی، *Xylariaceae*

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Introduction

The genus *Daldinia* Ces. & De Not., member of the family *Xylariaceae* (*Xylariales*, *Ascomycota*), is characterized by conspicuous, spherical, subspherical, clavate, or cylindrical stromata which usually develop on dead or fallen woody plant materials. All *Daldinia* species have been regarded as saprobes that cause white rot on dead host plants, however, it has been shown that most species of the genus are endophytes, living in the host tissue without causing any symptoms or producing stromata (Whalley 1996, Stadler *et al.* 2014). The first monograph of *Daldinia* was published by Child (1932) and the second by Ju *et al.* (1997). The latest worldwide taxonomic revision of the genus has been made by Stadler *et al.* (2014) in which a polyphasic approach, based on morphological, chemical and molecular characteristics, has been applied to resolve the species recognition of the genus *Daldinia*.

Daldinia was primarily distinguished from the closely related genera such as *Hypoxylon*, *Thamnomycetes*, *Rhopalostroma*, *Phylacia* by having zonate stromatal internal structure with alternating, differently colored zones (Ju *et al.* 1997). However, Stadler *et al.* (2014) recently emended generic description. According to this new concept, some species with homogeneous internal stromatal structure are also included in *Daldinia*.

A few studies on the xylariaceous taxa have been conducted in Iran (Daneshpazhuh 1980, Zare & Asgari 2007, Mirabolfathy *et al.* 2011, Raei *et al.* 2012). Daneshpazhuh (1980) has reported few species of xylariaceous fungi, including *D. concentrica* from Iran. Another species, *D. cf. vernicosa*, has been reported by Zare & Asgari (2007) on which the hyperparasitic fungus, *Acrostalagmus luteoalbus* (Link : Fr.) Zare, W. Gams & Schroers was identified. The objective of this study was to investigate the species composition of *Daldinia* in Guilan province, Iran.

Materials and Methods

Specimens were collected from Guilan province (N Iran) during 2011–13. Parts of the branches bark and trunks of infested trees with *Daldinia* type stromata were

transferred to the laboratory. Specimens were initially examined by stereo-microscope for stromata appearance. Several cross sections from stromata were made using a razorblade under a stereo-microscope to observe granules immediately beneath surface and concentric zones. The specimens were also cultured on Malt extract Agar (MA) and Oatmeal Agar (OA), and incubated at 20° C under 12 h fluorescent light. The isolates were isolated and purified on MA (Ju & Rogers 1996). The morphological characteristics of fungi grown on OA were studied using light microscope. For microscopic studies fungal structures were examined using a BH2 Olympus microscope equipped with a Sony digital camera (DSC-HX1). All measurements were taken in water and are based on at least 25–30 fungal structures such as perithecia, asci, ascospores, etc. To study KOH extractable pigments and dehiscence and ascus apical ring, Ju *et al.* (1997) procedures was followed. Colors of all structures were determined using the color charts of Rayner (1970). Identification of the taxa is based on Ju *et al.* (1997), Rogers *et al.* (1999), Vasilyeva & Stadler (2008), Suwannasai *et al.* (2012) and Stadler *et al.* (2014). All collected specimens are preserved at the Fungal Collection of the Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

Results and Discussion

In this study, six species of *Daldinia* were identified. Description and illustration of all species is presented here.

Daldinia caldariorum Henn., *Verhandlungen des Botanischen Vereins der Provinz Brandenburg* 40: 158 (1898)

Stroma depressed-spherical, short stipitate, 0.8 × 1.4 cm; surface smooth, sepia (63) in age; with KOH-extractable pigments livid purple (81) or vinaceous purple (101); tissue beneath perithecial layer composed of concentric zones, darker zones dark brown, 0.12–0.38 mm thick, lighter zones whitish or pale brown, 0.12–0.64 mm thick. Perithecia obovoid, cylindrical, 0.5–0.7 × 0.3–



Fig. 1. *Daldinia caldariorum*: A. Stromatal habit, B. Stroma in longitudinal section showing internal concentric zones and perithecial layer, C. Perithecial layer, D. Stromatal surface, with stromatal pigments in 10% KOH, E. Ascus with an amyloid apical ring, F. Ascospores in 10% KOH, G. Ascospores in water, with germ-slits (Bars: B = 0.6 mm; C = 1 mm; E = 20 μ m; F, G = 10 μ m).

0.45 mm, with inconspicuous ostioles. Asci with amyloid, discoid apical apparatus, $0.5\text{--}1 \times 1.5\text{--}2 \mu\text{m}$ long, stipe up to $135 \mu\text{m}$, and the spore-bearing portion measuring $49\text{--}65 \times 6.5\text{--}9 \mu\text{m}$. Ascospore smooth, pale to dark brown, ellipsoidal, with broadly rounded ends, $7\text{--}9 \times 3\text{--}4 \mu\text{m}$, with straight germ-slit spore-length; perispore indehiscent in 10% KOH (Fig. 1).

The specimen examined in this study, had the same morphological characters of *D. caldariorum* described by Stadler *et al.* (2014) except that its ascospores are narrower (vs. $8\text{--}11 \times 4\text{--}5.5 \mu\text{m}$). In this study one specimen (including one stroma) was collected and examined. This species is mainly distinguished from other species of *Daldinia* by having ascospores with ventral germ-slits (Ju *et al.* 1997). *Daldinia caldariorum* is a new record to the Iranian fungal flora.

Specimens examined: Iran: Guilan province, Siahkal, Ziaratgah forest, on branches of *Morus alba* L., 2 Oct. 2013, coll. M.J. Pourmoghaddam.

Daldinia childiae J.D. Rogers & Y.M. Ju, Mycotaxon 72: 512 (1999)

Stromata spherical, depressed-spherical to turbinate, sessile or shortly stipitate, $0.5\text{--}2 \times 0.5\text{--}2.5 \text{cm}$; surface smooth, livid red (56), dark brick (60), sepia (63), brown vinaceous (84) or grayish sepia (106) in age; with orange brown or reddish brown granules immediately below surface and KOH-extractable pigments amber (47) or cinnamon (62); tissue beneath perithecial layer composed of concentric zones, darker zones dark brown to black, $0.12\text{--}0.51 \text{mm}$ thick, lighter zones brown to cream, $0.15\text{--}1 \text{mm}$ thick. Perithecia obovoid to lanceolate, $0.71\text{--}1.4 \times 0.23\text{--}0.64 \text{mm}$, with inconspicuous or slightly papillate ostioles. Asci with amyloid, discoid apical apparatus, $0.5\text{--}1.5 \times 2.5\text{--}3.5 \mu\text{m}$, long stipe up to $174 \mu\text{m}$ and, the spore-bearing portion measuring $60\text{--}104 \mu\text{m} \times 8\text{--}13 \mu\text{m}$. Ascospores brown to dark brown, ellipsoid-inequilateral, with narrowly rounded ends, $11\text{--}16 (-17) \times 5\text{--}7(-8) \mu\text{m}$, with straight germ-slit spore-length; perispore dehiscent in 10% KOH (Fig. 2).

Cultures and anamorph: Colonies on OA reaching 90mm diam. in 4 weeks, first white, felty and zonate, then becoming Honey (64) with concentric zones. Hyphae superficial, smooth to finely verruculose, up to $2.5 \mu\text{m}$ wide. Conidiophores laterally or terminally, hyaline, verruculose to verrucose, branched, branches short to medium, di- or trichotomously branched, $3 \mu\text{m}$ wide. Conidiogenous cells integrated, hyaline, with up to 4 loci, not crowded at the apex, $11\text{--}23 \times 2.5\text{--}4 \mu\text{m}$. Conidia hyaline, smooth, subglobose to ellipsoid or fusiform, with more or less flattened base, $6\text{--}9(-10) \times 3\text{--}5 \mu\text{m}$.

Morphology of the specimen examined with slightly differences agree with description provided by Rogers *et al.* (1999) and Stadler *et al.* (2014). This fungus has been previously reported from Iran as *D. concentrica* (Daneshpazhuh 1980); however, we examined all specimens of *D. concentrica* available at the fungus reference collection of the Ministry of Jihad-e-Agriculture ("IRAN") at the Iranian Research Institute of Plant Protection, Tehran (Iran). With regards to the new concepts of *D. concentrica* and *D. childiae* provided by Rogers *et al.* (1999) and Stadler *et al.* (2014), *D. childiae* differs from *D. concentrica* from released pigments in KOH 10%, cracks on the stromatal surface, size of conidia and host range. As a result all specimens examined here, are belong to *D. childiae*.

Specimens examined: Iran: Guilan province, Masal forest, on fallen wood of *Carpinus betulus* L., 5 Sept. 2011; Shaft, Emamzadeh Ebrahim forest, on fallen wood of *Alnus subcordata* C.A. Mey., 10 Sept. 2011; both collected by S. Raei; Siahkal, Deilaman forest, on dead branches of *Parrotia persica* C.A. Mey., 18 Aug. 2013; Roudsar, Rahimabad (Sefidab) forest, on dead branches of unknown tree, 30 Aug. 2013; Kelachai, Siahkalroud forest, on dead parts of branches of *Pterocarya fraxinifolia* C.A. Mey., 19 Sept. 2013; Chaboksar, Sarvelat forest, on fallen wood of unknown, 19 Sept. 2013; Amlash, Bolourdokan forest, on dead branches of *Prunus* sp., 3 Oct. 2013; Lahijan, Kohbijar

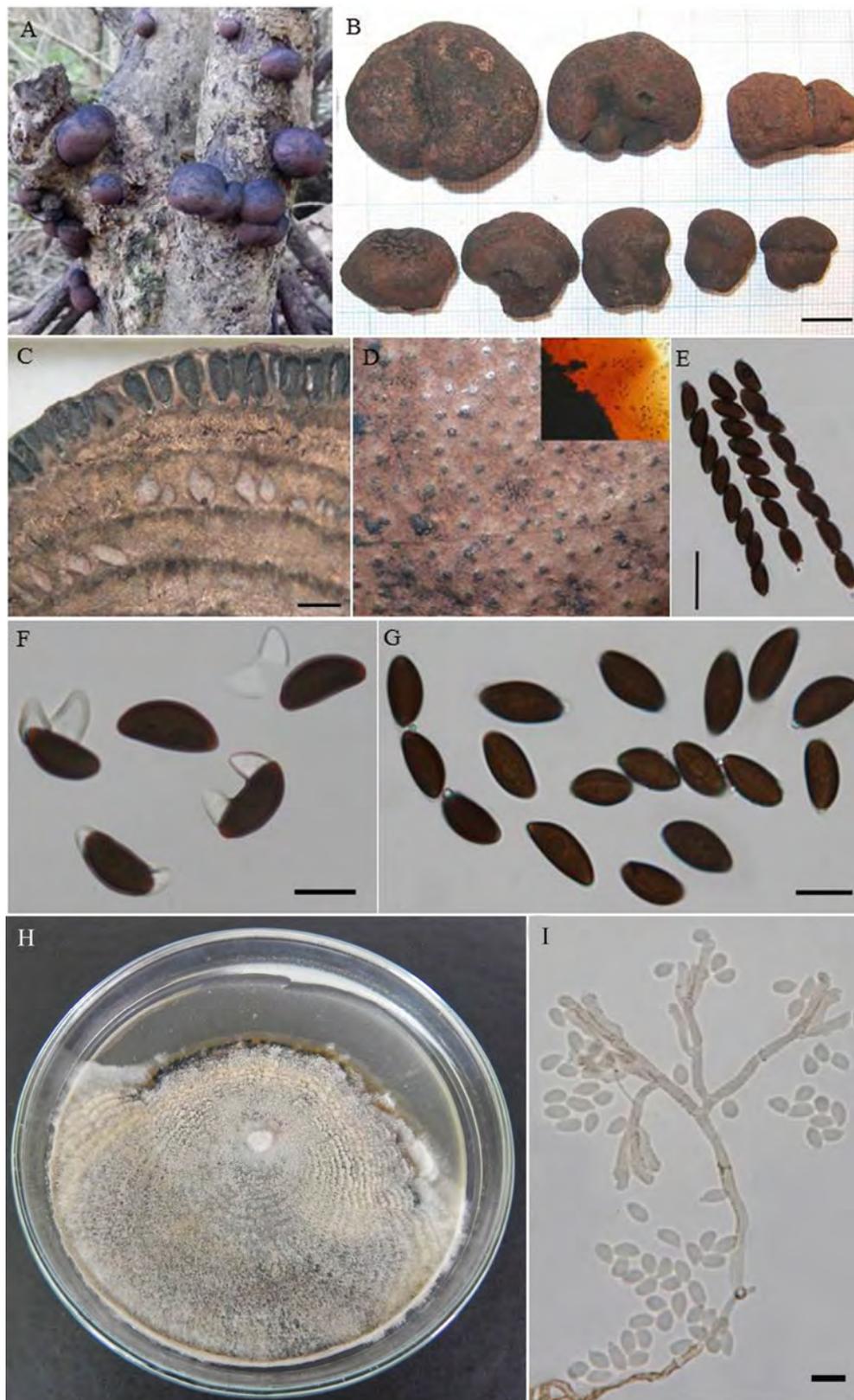


Fig. 2. *Daldinia childiae*: A, B. Stromatal habit, C. Stroma in longitudinal section showing internal concentric zones and perithecial layer, D. Stromatal surface, with stromatal pigments in 10% KOH, E. Ascus with an amyloid apical ring, F. Ascospores in 10% KOH, showing dehiscent perispore, G. Ascospores in water with germ-slit, H. colony on OA after 4 weeks, I. Conidiogenous structure and conidia (Bars: B = 10 mm; C = 1 mm; E = 20 μ m; F, G, I = 10 μ m).

forest, on dead branches of unknown tree, 11 Oct. 2013; Langaroud, Parashkoh forest, on fallen wood of *Citrus sinensis* L., 13 Oct. 2013; Langaroud, Liseroud forest, on fallen wood of *Quercus* sp., 13 Oct. 2013; Siahkal, Lonak forest, on trunk and branches of *Buxus sempervirens* L., 17 Oct. 2013; Kelachai, Vajargah forest, on fallen wood of *Gleditschia caspica* Desf., 18 Oct. 2013; Siahkal, Deilaman forest, on dead branches of unknown tree, 23 Oct. 2013; Langaroud, Komle forest, on trunk of *Morus alba* L., 1 Nov. 2013; Langaroud, Khorma forest, on dead branches of unknown, 1 Nov. 2013, all collected by M.J. Pourmoghaddam; Gisom Kolab forests, on different kinds of forest trees, 1 Nov. 1982, coll. M. Izadyar (IRAN 947F); Asalem, on *Acer* sp., 15 Feb. 1973, coll. Hassanzadeh; Khalkhal, Asalam, on wood, 10 Jun. 1991, coll. M. Saber, A. Karavar, Z. Ghanbari & H. Tehrani (IRAN 11215F); E Azarbaijan province, Arasbaran Forest, Ainalou, on wood, 17 Oct. 2003, coll. M.R. Asef & H. Tehrani (IRAN 11933F); Mazandaran province, Nowshahr, Kheirudkenar, on Wood, 23 Oct. 2005, coll. M. Bahram (IRAN 5746F); Golestan province, Gorgan, Baran-Kuh forest, on wood, 30 Oct. 2007, coll. M. Sohrabi (IRAN 13383F); Esfahan province, Najafabad, on wood, 1 Mar. 1999, coll. S. Zangeneh (IRAN 10582F); Mazandaran province, Kheirudkenar, *Fagus orientalis* Lipsky., 10 Oct. 2011, coll. Abiavi (IRAN 15742F); Amol, Zardman, on Wood, 15 Jul. 2007, coll. M.R. Asef, M. Amini-Rad & A. Sadeghi (IRAN 14699F); Guilan province, Siahkal forest, *Diospyros kaki* L.f., 11 Oct. 1990 (IRAN 8115F); Lahijan, Siahkal, *Fagus* sp., 12 Nov. 1981 (IRAN 9286F), all collected by B. Daneshpazhuh & M. Abbasi; Mazandaran province, Sangdeh, Dehmian Forest, *Fagus* sp., 13 May 1978 (IRAN 949F); Guilan province, Asalem forest, *Fagus* sp., 18 May 1977 (IRAN 950F), all collected by B. Daneshpazhuh; Mazandaran province, Ramsar, Dalikhani forests, Fallen stumps of *Quercus* sp., 1 May 1982 (IRAN 946F); Ramsar, Cibon forests Khoshkedaran, Decorticated trunk, 27 April. 1982 (IRAN 948F); Golestan province, Gorgan, Shamooshak Forest, on rotten trunk, 1 Aug. 1982 (IRAN 954F);

Guilan province, Rasht, Emamzadeh-Hashem, Baragol Forest, on fallen wood, 9 Oct. 1985 (IRAN 9285F); Mazandaran province, Tonekabon, Porehsar, on rotten trunk of *Fagus* sp., 29 May 1982 (IRAN 6775F), all collected by B. Daneshpazhuh & A. Karavar; Golestan province, Gorgan, Khanbebin, Shirabad forests, Dried wood, 3 Jul. 1976 (IRAN 951F); Gorgan, Zengo forests, Dried wood, 4 Jul. 1976 (IRAN 952F); Mazandaran province, Amol, Mahmoudabad, *Diospyros kaki* L.f., 21 Sept. 1974 (IRAN 953F), all collected by D. Ershad; Mazandaran province, Firuzkola, on Wood, 8 Oct. 2009 (IRAN 14348F); Amol to Babol, Baliran, on Wood, 8 Oct. 2009 (IRAN 14349F); Noor, Chamestan, on wood, 13 Oct. 2010 (IRAN 14955F); Sangdeh, Flourd, on Wood, 10 Oct. 2010 (IRAN 14956F); Tonekabon, Liresar, on wood, 15 Oct. 2009 (IRAN 14700F), all collected by M.R. Asef & A. Torabi.

Daldinia gelatinoides Lar. N. Vassiljeva, Nizshie Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii, Griby. Tom 4. Pirenomitsety i Lokuloaskomitsety (Sankt-Peterburg): 177 (1998)

Stroma turbinate, stipitate, stout stipe, 1–2.4 × 0.8–2.7 cm; surface smooth, sepia (63) in age; with KOH-extractable pigments vinaceous purple (101); tissue beneath perithecial layer is not composed of alternating zones but consists of a hollow cavity, with remnants of zonate tissue at base of stroma. Perithecia obovoid to lanceolate, 0.7–1.15 × 0.38–0.64 mm, with inconspicuous ostioles. Asci with amyloid, discoid apical apparatus, 0.5–1.5 × 2–3.5 μm long, stipe up to 117 μm, and the spore-bearing portion measuring 62.4–78 μm × 10.4–13 μm. Ascospores dark brown to black, ellipsoid-inequilateral, with broadly to narrowly rounded ends, (11–)12–13(–14) × (5.5–)6–7 μm, with straight germ-slit spore-length; perispore indehiscent in 10% KOH (Fig. 3).

This species is similar to *D. vernicosa* in having the same spore morphology and indehiscent perispore. However, it is distinguished from the latter species by an *Entonaema*-like habit of its stromata that are highly gelatinous, almost entirely hollow inside and filled with



Fig. 3. *Daldinia gelatinoids*: A. Stromatal habit, B. Stroma in longitudinal section showing interior hollow, perithecial layer and remnants of internal concentric zones at base, C. Perithecial layer, D. Stromatal surface, with stromatal pigments in 10% KOH, E. Ascus with an amyloid apical ring, F. and G. Ascospores in 10% KOH and water, respectively (Bars: B = 10 mm; C = 0.5 mm; E = 20 μ m; F, G = 10 μ m).

liquid when fresh (Stadler *et al.* 2014). This is the first report of this species from Iran.

Specimens examined: Iran: Guilan province, Siahkal, Ziaratgah forest, on branches of *Morus alba* L., 11 Oct. 2013, coll. M.J. Pourmoghaddam.

Daldinia cf. loculata (Lév.) Sacc., Syll. fung. (Abellini) 1: 394 (1882)

Stromata turbinate, usually stipitate, 0.6–1.3 × 0.7–1.1 cm; surface smooth, brown vinaceous (84) in age; with KOH-extractable pigments livid purple (81) or vinaceous purple (101); tissue beneath perithecial layer composed of concentric zones, darker zones dark brown to black, 0.12–0.51 mm thick, lighter zones, light brown to dark brown, 0.38–0.76 mm thick. Perithecia lanceolate, 0.71–0.84 × 0.35–0.51 mm, with inconspicuous or slightly papillate ostioles. Asci not observed. Ascospores dark brown, ellipsoid-inequilateral, with narrowly rounded ends, 12–16(–16.5) × 6–8 μm, with straight germ-slit spore-length; perispore indehiscent in 10% KOH (Fig. 4).

Based on Stadler *et al.* (2014) spores of this species are ellipsoid-equilateral to slightly inequilateral but in specimen examined spores are ellipsoid-inequilateral. This is the first report of this species from Iran.

Specimens examined: Iran: Guilan province, Astaneh Ashrafiyeh, Safrabaste forest, on fallen wood, 15 May 2012, coll. S.A. Hashemi.

Daldinia pyrenaica M. Stadler & Wollw., Mycotaxon 80: 180 (2001)

Stromata hemispherical to depressed-spherical, sessile, 0.7–1.1 × 1.2–1.5 cm, surface smooth, dark brick (60) in age; with KOH-extractable pigments amber (47), cinnamon (62) or honey (64); tissue beneath perithecial layer composed of alternating concentric zones, darker zones dark brown to black, 0.12–0.51 mm thick, lighter zones light brown, 0.25–0.76 mm thick. Perithecia lanceolate to obovoid 0.76–1.3 × 0.25–0.58 mm, with inconspicuous or slightly papillate ostioles. Asci with amyloid, discoid apical apparatus, 0.75–1.5 × 2.5–4 μm, long stipe up to 182 μm, and the spore-bearing portion

measuring 83–91 μm × with 9–13 μm wide. Ascospores brown, ellipsoid-inequilateral with narrowly rounded ends, 12–17 × 6–8(–9) μm, with straight germ-slit spore-length, perispore dehiscent in 10% KOH (Fig. 5).

This species resembles of *D. childiae* by having darker and lighter concentric zones, released pigments in KOH 10%, dehiscent perispore, the shape of ascospores. However, stroma in *D. childiae* are distinctly stipitate and ascospores are slightly smaller than those of *D. pyrenaica* (Stadler *et al.* 2014). *Daldinia pyrenaica* is a new record to the Iranian mycobiota.

Specimens examined: Iran: Guilan province, Siahkal, Lonak forest, on dead branches of *Pterocarya fraxinifolia* C.A. Mey., 17 Oct. 2013; Langaroud, Khorma forest, on dead branches of *Buxus sempervirens* L., 1 Nov. 2013. Both collected by M.J. Pourmoghaddam.

Daldinia vernicosa Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 198 (1863)

Stromata turbinate or peltate, usually stipitate, 1.3–1.8 × 1.3–3.5 cm; surface smooth or wrinkled, sepia (63) or fuscous black (104) in age; with KOH-extractable pigments dark livid (80); tissue beneath perithecial layer composed of concentric zones, darker zones dark brown to black, 0.17–0.38 mm thick, lighter zones white, gelatinous, disintegrating and becoming loculate when dry, 0.33–1.2 mm thick. Perithecia obovoid to lanceolate, 0.76–1 × 0.28–0.71 mm, with inconspicuous or slightly papillate ostioles. Asci with amyloid, discoid apical apparatus, 0.5–1.5 × 2.5–3.5 μm, long stipe up to 208 μm, and the spore-bearing portion measuring 65–93 μm × 9–13 μm wide. Ascospores dark brown to black, ellipsoid-inequilateral, with broadly to narrowly rounded ends, 11–14(–15) × 6–8 μm, with straight germ-slit spore-length; perispore indehiscent in 10% KOH (Fig. 6). This is the first report of this species from Iran.

Specimens examined: Iran: Guilan province, Siahkal, Ziaratgah forest, on trunk and branches of *Ficus* sp., 11 Oct. 2013; Siahkal, Ziaratgah forest, on trunk and branches of *Parrotia persica* C.A. Mey., 11 Oct. 2013; Langaroud, Komle forest, on trunk and branches of *Ulmus* sp., 1 Nov. 2013. All collected by M.J. Pourmoghaddam.

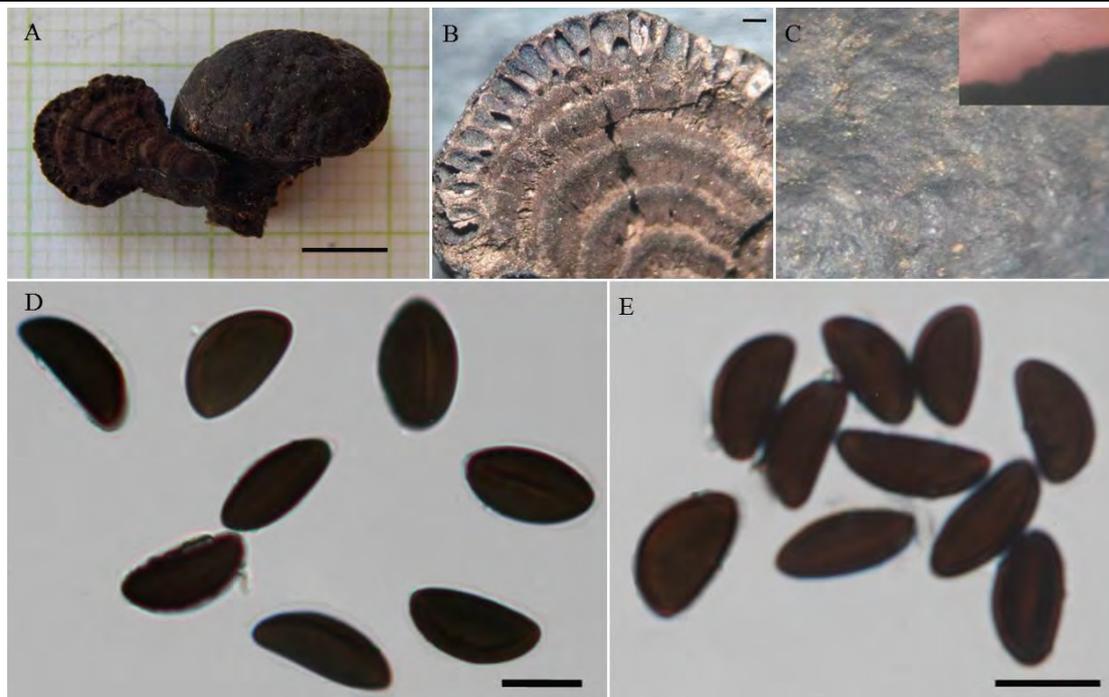


Fig. 4. *Daldinia cf. loculata*: A. Stromatal habit, B. Stroma in longitudinal section showing internal concentric zones and perithecial layer, C. Stromatal surface, with stromatal pigments in 10% KOH, D. Ascospores in 10% KOH with germ-slit, E. Ascospores in water with germ-slits (Bars: A = 5 mm; B = 0.5 mm; D, E = 10 μ m).

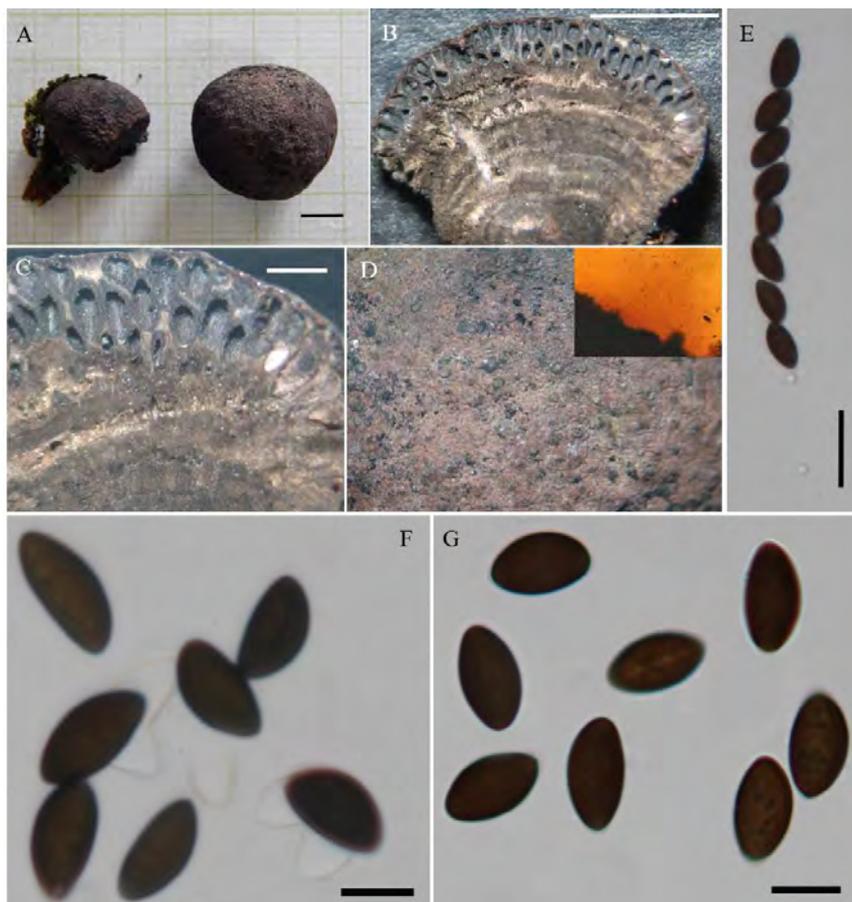


Fig. 5. *Daldinia pyrenaica*: A. stromatal habit, B. Stroma in longitudinal section showing internal concentric zones and perithecial layer, C. Perithecial layer, D. Stromatal surface, with stromatal pigments in 10% KOH, E. Ascus with an amyloid apical ring, F. Ascospores in 10% KOH, showing dehiscing perispore, G. Ascospores in water with germ-slits (Bars: A, B = 5 mm; C = 1 mm; E = 20 μ m; F, G = 10 μ m).



Fig. 6. *Daldinia vernicosa*: A, B. Stromatal habit, C. Perithecial layer, D. Stroma in longitudinal section showing interior locules, internal concentric zones and perithecial layer, E. Stromatal surface, with stromatal pigments in 10% KOH, F. Ascus with an amyloid apical ring, G. Ascospores in 10% KOH and germ-slits, H. Ascospores in water with germ-slits (Bars: A = 15 mm; B = 8 mm; C = 0.5 mm; D = 1 mm; F = 20 µm; G, H = 10 µm).

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