

## Contribution to the knowledge of *Hypoxylon* and *Annulohypoxylon* in Guilan province (N Iran)

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### Abstract

During a study on wood inhabiting fungi in Guilan province, several specimens of the *Hypoxylon* and *Annulohypoxylon*, were examined. A total of eight taxa viz. *Hypoxylon fragiforme* on *Fagus orientalis*; *H. howeanum* on *Alnus* sp., *Carpinus betulus*, *Castanea sativa*, *Parrotia persica*, *Gleditschia caspica*, *Ilex aquifolium* and *Laurocerasus officinalis*; *H. perforatum* on *Zelkova carpinifolia* and *Gleditschia caspica*; *H. rubiginosum* on *Quercus castaneifolia* and *Populus* sp.; *Annulohypoxylon annulatum* on *Quercus castaneifolia*; *A. minutellum* on *Carpinus betulus*; *A. moriforme* on *Quercus* sp. and *A. moriforme* var. *microdiscum* on *Pterocarya fraxinifolia*, were identified. Among them *H. perforatum*, *A. annulatum*, *A. minutellum*, *A. moriforme* and *A. moriforme* var. *microdiscum* are new to the Iranian mycobiota.

**Keywords:** Biodiversity, mycobiota, wood degrading fungi, *Xylariaceae*

## کمک به شناسایی گونه‌های جنس *Hypoxylon* و *Annulohypoxylon* در استان گیلان\*

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### خلاصه

به منظور شناسایی قارچ‌های چوب‌زی در استان گیلان، نمونه‌های متعددی از جنس‌های *Hypoxylon* و *Annulohypoxylon* بررسی شدند. در مجموع، هشت گونه شناسایی شدند که عبارتند: *Hypoxylon fragiforme* روی *Fagus orientalis*؛ *H. howeanum* روی *Alnus* sp.؛ *Carpinus betulus*؛ *Castanea sativa*؛ *Parrotia persica*؛ *Gleditschia caspica*؛ *Ilex aquifolium* و *Laurocerasus officinalis* روی *H. perforatum*؛ *Zelkova carpinifolia* و *Gleditschia caspica*؛ *H. rubiginosum* روی *Quercus castaneifolia*؛ *Populus* sp.؛ *Annulohypoxylon annulatum* روی *Quercus castaneifolia*؛ *A. minutellum* روی *Carpinus betulus*؛ *A. moriforme* روی *Quercus* sp. و *A. moriforme* var. *microdiscum* روی *Pterocarya fraxinifolia* از میان آرایه‌های فوق *H. perforatum*؛ *A. annulatum*؛ *A. minutellum*؛ *A. moriforme* و *A. moriforme* var. *microdiscum* برای نخستین بار از ایران گزارش می‌شوند.

**واژه‌های کلیدی:** تنوع زیستی، قارچ‌های چوب‌زی، میکوبیوتا، *Xylariaceae*

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## Introduction

The genus *Hypoxylon* s.l., well known as a wood degrading fungus, is a member of the family *Xylariaceae* with widespread distribution (Lee 1999, Whalley 1996), being especially common in subtropical and tropical regions (Suwannasai *et al.* 2005, Rogers 2000). *Hypoxylon* species are saprophytes and occasionally parasites (Rogers 1979) or endophytes (Petrini & Petrini 1985, Petrini 1996). The taxonomy of *Hypoxylon* has been subject to several rearrangements by mycologists (Mazzaglia *et al.* 2000). *Hypoxylon* was broadly defined by Miller (1961) to consist of several taxa that are currently situated in *Biscogniauxia*, *Camillea*, *Kretzschmaria*, *Nemania*, etc. (Hsieh *et al.* 2005). Ju & Rogers (1996) divided the genus into two sections *Hypoxylon* and *Annulata*. Recently the genus *Annulohypoxylon* was erected by Hsieh *et al.* (2005) to accommodate species previously included in *Hypoxylon* section *Annulata*. The genus *Annulohypoxylon* differs from *Hypoxylon* in three major characters including ostioles that are encircled with a truncate area, carbonized stromatal tissue that discretely encloses each individual perithecium, and a thickening on the ascospore's perispore that is absent in most species of *Hypoxylon* (Hsieh *et al.* 2005).

A few studies on the xylariaceous taxa have been conducted in Iran; therefore, some sporadic reports of these fungi are available. Daneshpazhuh (1980) has reported seven species of *Hypoxylon* from Iran. Ershad (2009) has listed sixteen species of *Hypoxylon* from Iran that is largely based on Daneshpazhuh (*l.c.*). More recently, Miraabolfathy *et al.* (2011) reported *Biscogniauxia mediteranea* from Golestan province.

The objective of this study was to investigate the biodiversity of *Hypoxylon* s.l. and some allied genera in Guilan province of Iran. Hence in reporting a trial results, we only report parts of results that relate to our findings on *Hypoxylon* and *Annulohypoxylon* species.

## Materials and Methods

Specimens were collected from the western parts of Guilan province (N Iran) during the years 2011–12

from July to September. Parts of the branches, barks and trunks of infested trees with *Hypoxylon* and its allied genera were transferred to the laboratory. Specimens were initially examined by stereo-microscope for stromata appearance and development on the plant material. Several cross sections from stromata were made using a razorblade under a stereo-microscope to observe granules immediately beneath surface and between perithecia. The isolates were purified by single spore method on 2% water agar. Isolates were cultured on Difco Oatmeal Agar (OMA) and Scratch Malt Extract Agar (SMEA) and incubated at 20° C under 12 h fluorescent light. The morphological characteristics of fungi grown on OMA 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, ascospore germ slit and dehiscence and ascus apical ring Ju & Rogers (1996) procedures was followed. Identification of the taxa is based on Ju & Rogers (1996), Ju *et al.* (2004), Pereira *et al.* (2010) and Vasilyeva *et al.* (2007). All collected specimens are deposited in the Fungal Collection of the Department of Plant protection, College of Agriculture, University of Guilan. Accession numbers are presented in parentheses after collector name.

## Results and Discussion

In this study, eight taxa of *Hypoxylon* and *Annulohypoxylon* were identified. A brief description of each taxon is presented below.

*Hypoxylon fragiforme* (Pers.) J. Kickx f., Fl. Crypt. Louvain (Bruxelles): 116 (1835)

Stromata hemi-sphaerical to sphaerical, with conspicuous perithecial mounds, 2–4.5 mm diam. and 1.5–4.5 mm thick; with rust or dark brick surface;

orange-red granules immediately beneath surface and white granules between perithecia, with orange KOH-extractable pigments. Perithecia spherical to obovoid, 0.18–0.44 mm diam. and 0.3–0.6 mm high. Ostioles lower than or at the same level of the stromatal surface. Asci cylindrical, 124–200 × 5.5–8.5 μm, with discoid apical ring bluing in Melzer's iodine reagent. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 11.2–16.8 × 5–8 μm, with straight germ slit spore-length; perispore dehiscent in 10% KOH; episore smooth (Fig. 1).

Specimen examined: Guilan province, Rostamabad forest, on dead branches of *Fagus orientalis*, 23 Sept. 2011, coll. S. Raei (957).

***Hypoxylon howeanum*** Peck, Ann. Rep. N.Y. St. Mus. 24: 98 (1872)

Stromata superficial on bark, hemi-spherical to spherical, with inconspicuous to conspicuous perithecial mounds, 2–5 mm diam. and 1.5–3 mm thick; with fulvous to dark brick surface; orange-red granules immediately beneath surface and white granules between perithecia, with orange to rust KOH-extractable pigments. Perithecia spherical to obovoid, 0.18–0.35 mm diam. and 0.3–0.45 mm high. Ostioles lower than or at the same level of the stromatal surface. Asci cylindrical, 95–175 × 5–5.8 μm, with discoid apical ring bluing in Melzer's iodine reagent. Ascospores brown to dark brown, ellipsoid-inequilateral, with narrowly rounded ends, 6.5–10 × 3–5 μm, with straight germ slit spore-length; perispore dehiscent in 10% KOH; episore smooth (Fig. 2).

Colonies on OMA reaching the edge of Petri dish in two weeks, whitish, with diffuse margins, becoming fulvous to honey and producing synnemata. Conidiogenous structure *Nodulisporium*-like, conidia hyaline, smooth, ellipsoidal, 5.5–6.5 × 3–3.5 μm, with flattened base.

This species resembles *H. fragiforme*, but possess smaller ascospores and wider host range.

Specimens examined: Guilan province, Gisoum forest, on dead branches of *Alnus* sp., 2 Sept. 2011 (958); Shaft,

Emamzadeh Ebrahim forest, on dead branches of *Carpinus betulus*, 10 Sept. 2011 (959); Astara forest, 10 Jul. 2012 (960); Shaft, Visroud forest, on dead branches of *Castanea sativa* (961) and *Parrotia persica*, 19 Sept. 2011 (962); Shaft, Visroud forest, on dead branches of *Gleditschia caspica*, 19 Sept. 2011 (963); Shaft, Ghalerodkhan forest, 1 Aug. 2011 (964); Masal, on dead branches of *Ilex aquifolium*, 5 Sept. 2011 (965); Shaft, Emamzadeh Ebrahim forest, on dead branches of *Laurocerasus officinalis*, 10 Sept. 2011 (966). All collected by S. Raei.

***Hypoxylon perforatum*** (Schwein.) Fr., Summa veg. Scand., Section Post. (Stockholm): 384 (1849)

Stromata pulvinate to effused-pulvinate, with inconspicuous perithecial mounds, 3.5–7 mm long, 2.5–4 mm broad and 0.6–0.9 mm thick; surface brown-vinaceous; dark brown granules beneath surface and between perithecia, with KOH-extractable pigments amber to citrine. Perithecia nearly spherical, 0.19–0.3 mm diam. and 0.22–0.33 mm high. Ostioles lower than the stromatal surface, mainly covered with white substances. Asci cylindrical, 80–125 × 5–8.75 μm, with discoid apical ring bluing in Melzer's iodine reagent. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 10–12 × 3.5–5 μm, with straight to slightly sigmoid germ slit spore-length; perispore dehiscent in 10% KOH; episore smooth (Fig. 3).

Colonies on OMA reaching the edge of Petri dish in three weeks, whitish, with diffuse margins, becoming fulvous to honey and producing synnemata. Conidiogenous structures *Nodulisporium*-like, conidia hyaline, smooth, ellipsoidal, 5–6 × 3–3.5 mm, with flattened base.

Specimens examined: Guilan province, Shaft, Visroud forest, on dead branches of *Gleditschia caspica*, 19 Sept. 2011 (967); Talesh forest, on dead branches of *Zelkova carpinifolia*, 8 Aug. 2011 (968). Both collected by S. Raei.

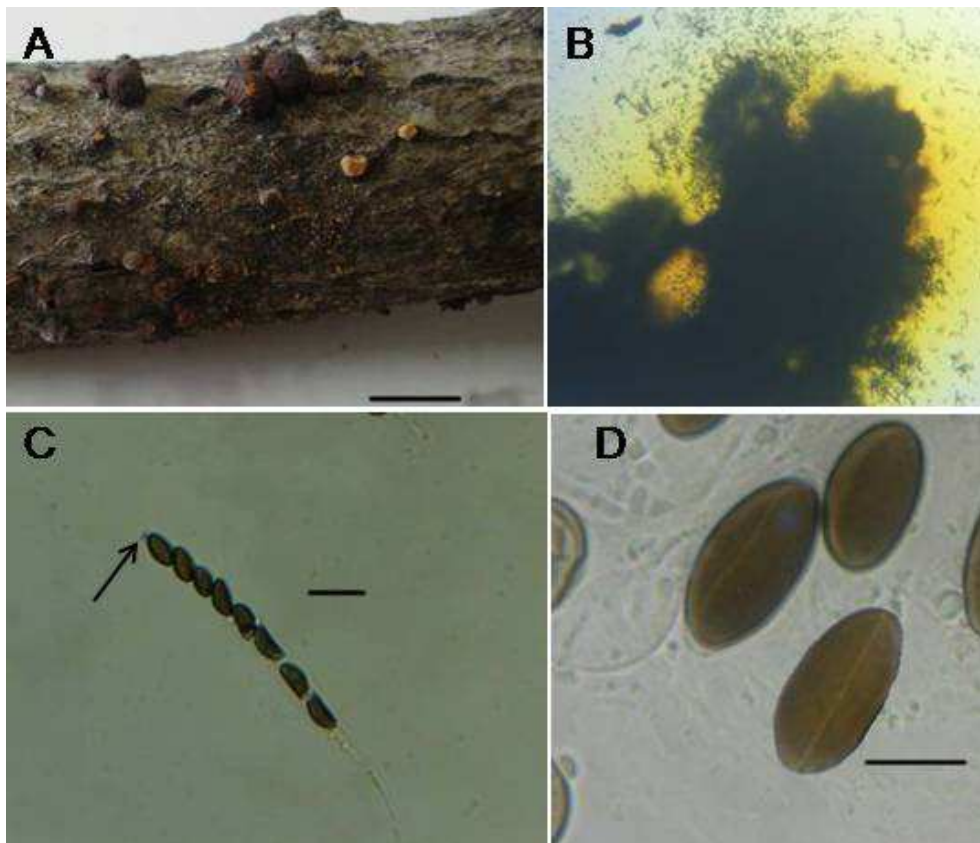


Fig. 1. *Hypoxylon fragiforme*: A. Stromata on wood (Bar = 10 mm), B. Pigments in 10% KOH, C. Asci with apical ring bluing in Melzer's iodine reagent (Bar = 10  $\mu$ m), D. Ascospores with germ slit (Bar = 10  $\mu$ m).

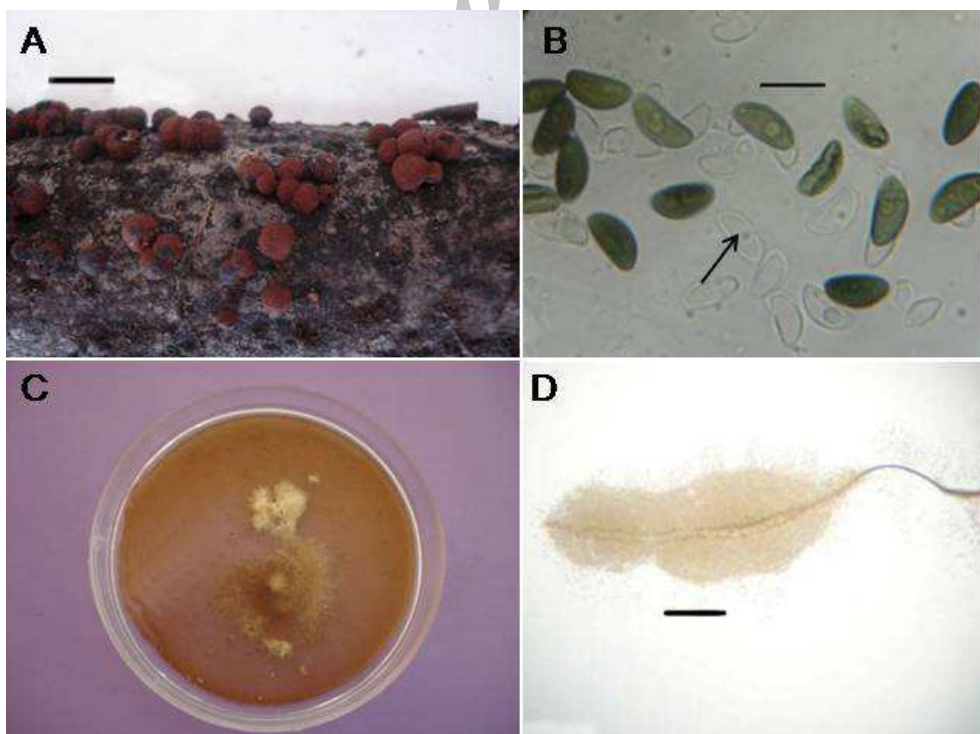


Fig. 2. *Hypoxylon howeanum*: A. Stromata on wood (Bar = 10 mm), B. Ascospores and perispore dehiscent in 10% KOH (Bar = 10  $\mu$ m), C. Colony of OMA after three weeks, D. Synnemata (Bar = 100  $\mu$ m).

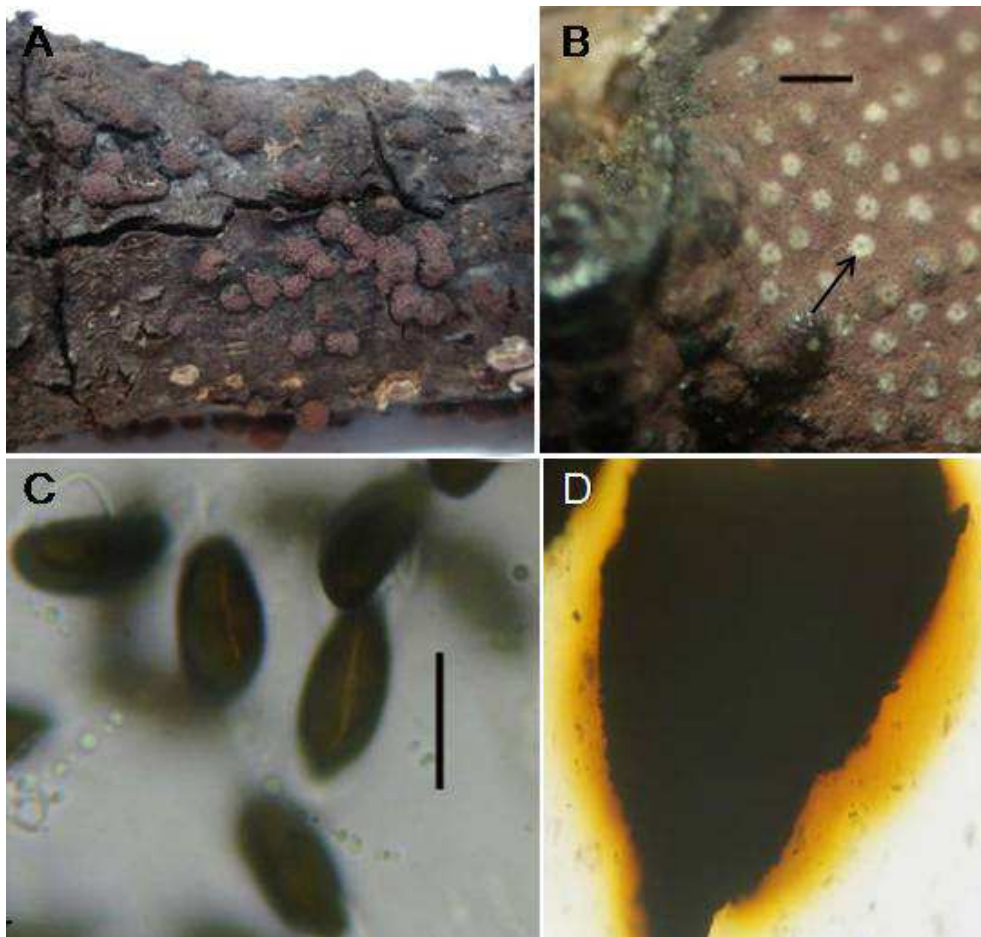


Fig. 3. *Hypoxylon perforatum*: A. Stromata on wood, B. Ostioles covered with white substances (Bar = 10 mm), C. Ascospores with slightly sigmoid germ slit spore-length and a dehiscent perispore in 10% KOH (Bar = 10  $\mu$ m), D. Pigments in 10% KOH.

*Hypoxylon rubiginosum* (Pers.) Fr., Summa veg. Scand., Section Post. (Stockholm): 384 (1849)

Stromata pulvinate to effused-pulvinate, with inconspicuous to conspicuous perithecial mounds, 0.5–3.5 cm long, 0.4–1 cm broad and 1–1.5 mm thick; surface dark brick; yellowish brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange. Perithecia spherical to obovoid, 0.25–0.5 mm diam. Ostioles lower than or at the same level of the stromatal surface. Asci cylindrical, 100–137.5  $\times$  6.5–8.5  $\mu$ m, with discoid apical ring bluing in Melzer's iodine reagent. Ascospores brown, unicellular, ellipsoid-inequilateral, 9–13  $\times$  4–6  $\mu$ m, with straight germ slit spore-length; perispore dehiscent in 10% KOH; episporium smooth (Fig. 4).

Colonies on OMA reaching the edge of Petri dish in three weeks, whitish, with diffuse margins. Sporulating regions scattered over entire surface of colony. Conidiogenous structure *Nodulisporium*-like, conidia hyaline, smooth, ellipsoidal to lemon-shaped, 4.5–6  $\times$  2–3  $\mu$ m, with flattened base. Specimens examined: Guilan province, Rasht, Sarawan forest, on dead branches of *Quercus castaneifolia*, 9 Apr. 2012 (969); Rasht-Fuman ring road, on dead branches of *Quercus castaneifolia*, 31 Jul. 2011 (970); Rasht, on dead branches of *Populus* sp., 12 Oct. 2011 (971). All collected by S. Raei.

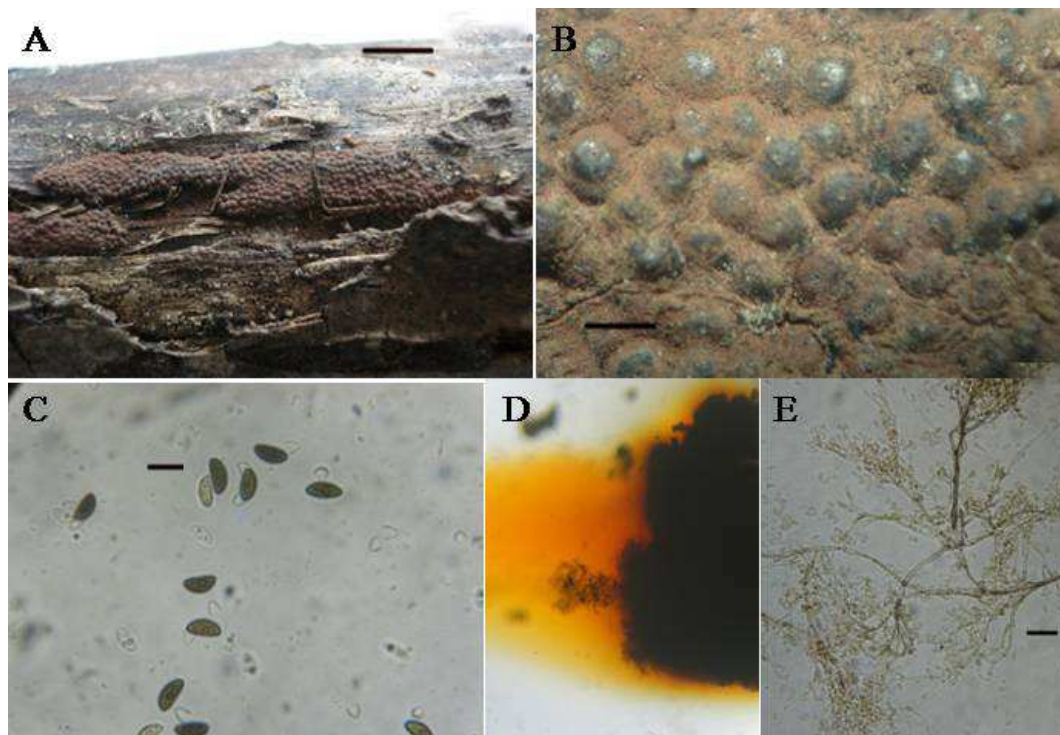


Fig. 4. *Hypoxylon rubiginosum*: A. Stromata on wood (Bar = 500  $\mu$ m), B. Umbilicate ostioles (Bar = 2 mm), C. Ascospores with dehiscent perispore in 10% KOH (Bar = 10  $\mu$ m), D. Pigments in 10% KOH, E. *Nodulisporium*-like anamorph (Bar = 20  $\mu$ m).

*Annulohypoxylon annulatum* (Schwein.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *Mycologia* 97(4): 857 (2005)

Stromata hemispherical, containing several perithecia with inconspicuous perithecial mounds, 0.2–0.8 cm diam. and 0.2–0.8 cm thick; surface blackish brown with olivaceous tinges; blackish granules immediately beneath surface, with KOH extractable pigments green. Perithecia spherical to obovoid, 0.33–0.5 mm diam. and 0.38–0.66 mm high. Ostioles coarsely papillate, encircled with a convex disc 0.27–0.47 mm diam. Asci not seen. Ascospores brown, unicellular, ellipsoid-inequilateral,  $9.3\text{--}11.3 \times 4.1\text{--}4.6 \mu\text{m}$ , with straight germ slit spore-length; perispore dehiscent in 10% KOH; episore smooth (Fig. 5).

This fungus is usually associated with *Quercus* species (Ju & Rogers 1996).

Specimen examined: Guilan province, Rasht, Sarawan forest, on trunk and branches of *Quercus castaneifolia*, 13 Jul. 2011, collected by S. Raei (972).

*Annulohypoxylon minutellum* (Syd. & P. Syd.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *Mycologia* 97(4): 859 (2005)

Stromata peltate, constricted below, usually confluent, with inconspicuous perithecial mounds, 0.2–1 cm diam. and 2–3 mm thick, surface purplish black; blackish granules immediately beneath surface, with KOH-extractable pigments livid red. Perithecia spherical to obovoid, 0.28–0.44 mm diam. and 0.44–0.66 mm high. Ostioles papillate, not encircled by a disc. Asci cylindrical,  $130\text{--}169 \times 4\text{--}5 \mu\text{m}$ , with apical ring not bluing in Melzer's iodine reagent. Ascospores pale brown to brown, unicellular, ellipsoid-inequilateral,  $6.5\text{--}8 \times 3\text{--}4 \mu\text{m}$ , with straight germ slit less than spore-length; perispore dehiscent in 10% KOH; episore smooth (Fig. 6)

Specimen examined: Guilan province, Astara forest, on dead branches of *Carpinus betulus*, 15 Sept. 2012, coll. S. Raei (973).

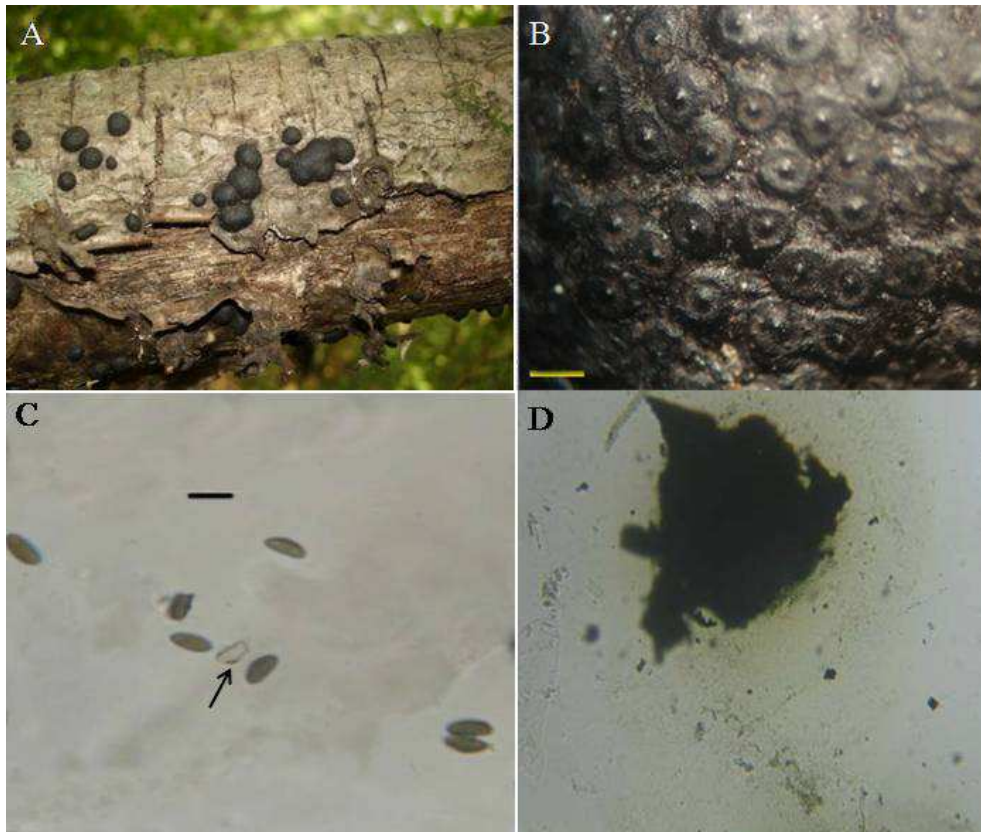


Fig. 5. *Annulohypoxyton annulatum*: A. Stromata on wood, B. Perithecia with ostiolar discs (Bar = 500  $\mu$ m), C. Ascospore in 10% KOH with dehiscent perispore bearing a thickening (arrow) (Bar = 10  $\mu$ m), D. Pigments in 10% KOH.

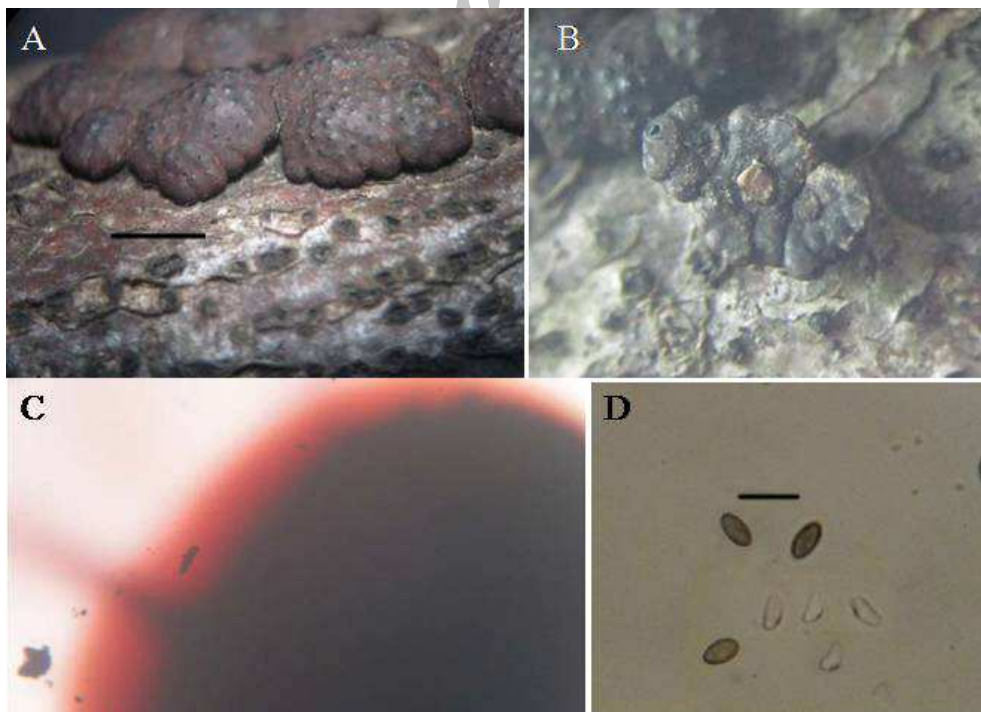


Fig. 6. *Annulohypoxyton minutellum*: A. Stromata on wood (Bar = 2 mm), B. Peltate stromata with obvious stalk (arrow), C. Pigments in 10% KOH, D. Ascospore in 10% KOH with dehiscent perispore bearing a thickening (Bar = 20  $\mu$ m).

***Annulohypoxylon moriforme*** (Henn.) Y.M. Ju, J.D.Rogers & H.M. Hsieh, *Mycologia* 97(4): 859 (2005)

Stromata effused-pulvinate, usually confluent, with inconspicuous perithecial mounds, 1–2.5 cm long, 0.3–1 cm broad and 1–1.5 mm thick; surface blackish with vinaceous tinges; blackish granules immediately beneath surface, with KOH-extractable pigments greenish olivaceous. Perithecia spherical, 0.4–0.6 mm

diam. Ostioles papillate, encircled with a disc 0.2–0.35 mm diam. Asci 98–143 × 3.5–5 μm, with apical ring bluing in Melzer's iodine reagent. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly to broadly rounded ends, 7.7–10.3 × 2–3.6 μm, with straight germ slit spore-length; perispore of some spores dehiscent in 10% KOH; episore smooth (Fig. 7).

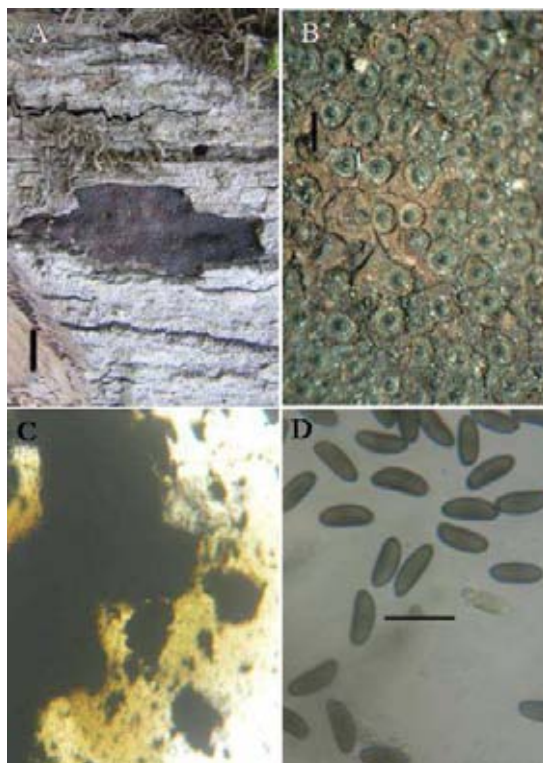


Fig. 7. *Annulohypoxylon moriforme*: A. Stromata on wood (Bar = 4 mm), B. Perithecia with ostiolar discs (Bar = 400 μm), C. Pigments in 10% KOH, D. Ascospores (Bar = 10 μm).

Colonies on OMA reaching the edge of Petri dish in two weeks, whitish, with diffuse margins, becoming fulvous to honey and producing synnemata. Conidiogenous structures *Nodulisporium*-like, conidia hyaline, smooth, ellipsoidal, 5.5–6 × 3–3.5 μm, with flattened base.

Specimen examined: Guilan province, Rezvanshahr, Douran forest, on trunk and branches of *Quercus* sp., 21 Jul. 2011, coll. S. Raei (974).

***Annulohypoxylon moriforme*** var. *microdiscum* (Y.M. Ju & J.D. Rogers) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *Mycologia* 97(4): 859 (2005)

Stromata effused-pulvinate, usually confluent, with conspicuous perithecial mounds, 0.5–4 cm long, 0.3–2 cm broad and 0.9–1.5 mm thick; surface blackish with

olivaceous tinges; blackish granules immediately beneath surface, with KOH-extractable pigments greenish olivaceous. Perithecia nearly spherical, 0.44–0.77 mm diam. Ostioles papillate, encircled with a disc 0.16–0.2 mm diam. Asci 130–187 × 5–6.5 μm, with apical ring bluing in Melzer's iodine reagent. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly to broadly rounded ends, 10–12 × 4–4.5 μm, with straight germ slit spore-length; perispore of some spores dehiscent in 10% KOH; episore smooth (Fig. 8).

This variety differs from its type variety in having smaller ostiolar discs and larger ascospores.

Specimen examined: Guilan province, Astara, on dead branches of *Pterocarya fraxinifolia*, 10 Jul. 2012, coll. S. Raei (975).



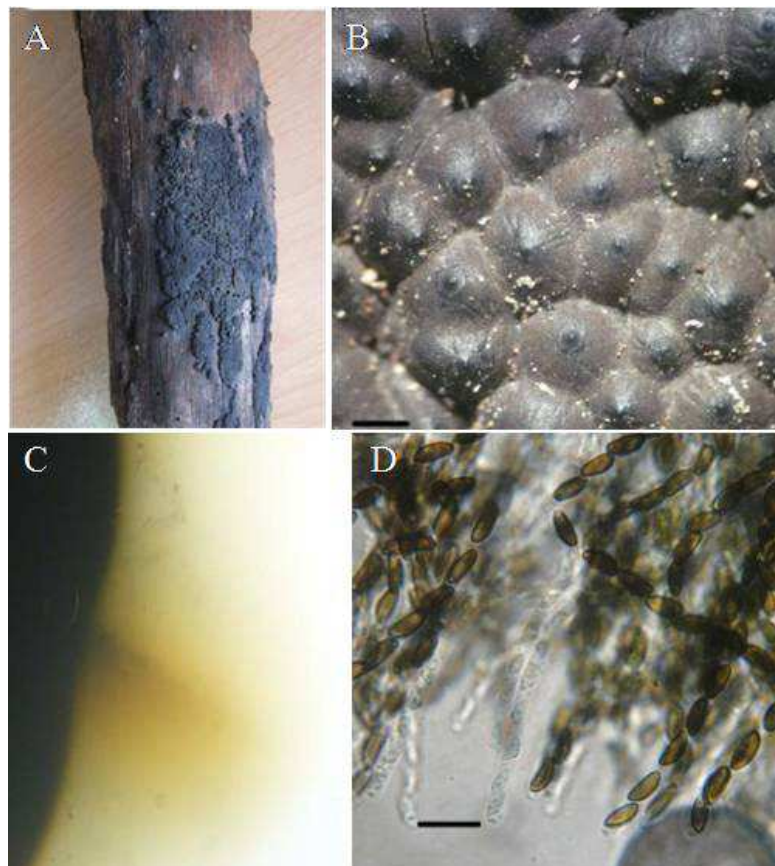


Fig. 8. *Annulohypoxyton moriforme* var. *microdiscum*: A. Stromata on wood, B. Perithecia with ostiolar discs (Bar = 400  $\mu$ m), C. Pigments in 10% KOH, D. Asci and ascospores (Bar = 20  $\mu$ m).

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