More records of xylariaceous fungi from North of Iran

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Abstract

This study was carried out to contribute to the knowledge of biodiversity of xylariaceous fungi from North of Iran. Plant materials with fruiting bodies of xylariaceous fungi were collected and examined. Eight species *viz*. *Annulohypoxylon nitens*, *Biscogniauxia anceps*, *B. capnodes* var. *rumpens*, *B. mediterranea*, *B. plana*, *Hypoxylon flavoargillaceum*, *Jumillera cinerea*, and *Nemania illita* were identified. All these except *B. mediterranea* are new to the Iranian mycobiota. A dichotomous identification key for all the xylariaceous fungi examined by the authors from North of Iran is presented.

Keywords: Ascomycetes, biodiversity, wood inhabiting fungi, *Xylariales*

گزارش‌های جدید از قارچ‌های *Xylariaceae* در شمال ایران

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خلاصه

تحقیق حاضر با هدف افزایش دانش از تنوع زیستی قارچ‌های *Xylariaceae* در شمال ایران انجام شد. به منظور اجرای این تحقیق، اندام‌های گیاهی دارای نشانه‌هایی از اندام‌های بارده قارچ‌های *Xylariaceae* جمع‌آوری و پس از انتقال به آزمایشگاه بررسی شدند. هشت گونه شامل *Annulohypoxylon nitens*، *Biscogniauxia anceps*، *B. capnodes* var. *rumpens*، *B. mediterranea*، *B. plana*، *Hypoxylon flavoargillaceum*، *Jumillera cinerea* و *Nemania illita* شناسایی شدند که تمامی آن‌ها به غیر از *B. mediterranea* برای فلور قارچ‌های ایران جدید هستند. در این مقاله کلید شناسایی قارچ‌های متعلق به تیره *Xylariaceae* در شمال کشور که توسط نگارندگان جمع‌آوری و شناسایی شده‌اند، ارایه می‌شود.

واژه‌های کلیدی: آسکومیست‌ها، تنوع زیستی، قارچ‌های چوبزی، *Xylariales*

Introduction

Xylariaceae is the type and largest family of the *Xylariales* with more than 75 genera and a total of 800 or more species (Lumbsch & Huhndorf 2007). Members of the *Xylariales* generally produce well-developed stromata, with thick-walled perithecial ascomata either immersed in the stroma or superficial and papillate ostioles. Asci are cylindrical with an amyloid apical ring; ascospores are mainly with germ-slits or pores. The anamorph is characterized by producing conidia holoblastically from a sympodially, or occasionally percurrently, proliferating conidiogenous region (Whalley 1996, Rogers 2000). Xylariaceous species are typically saprobes, but are also commonly isolated as endophytes and some species are pathogens (Rogers 1979, Whalley 1996). Some members of the *Xylariales* are very important pathogens, causing large economic losses. For example, *Biscogniauxia mediterranea* canker of the cork oak (*Quercus suber* L.) and Turkish oak (*Quercus cerris* L.) is a serious problem in the Mediterranean area (Jurc & Ogris 2006). Another species, *Rosellinia necatrix* is the causative agent of root rot on a broad range of commercial crops such as apple, grape vine, pear, plum, poplar and walnut (Whalley 1996).

A few studies on the xylariaceous taxa have been conducted in Iran. Daneshpazhuh (1980) has reported seven species of *Hypoxylon* from Iran. Ershad (2009) has listed 16 species of *Hypoxylon* from Iran. Mirabolfathy *et al.* (2011) reported *Biscogniauxia mediterranea* from northern Iran. The present study follows our previous paper (Raei *et al*. 2012), in which eight xylariaceous fungi including *H. fragiforme* (Pers.) J. Kickx f., *H. howeanum* Peck, *H. perforatum* (Schwein.) Fr., *H. rubiginosum* (Pers.) Fr., *A. annulatum* (Schwein.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *A. minutellum* (Syd. & P. Syd.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *A. moriforme* (Henn.) Y.M. Ju, J.D. Rogers & H.M. Hsieh and *A. moriforme* var. *microdiscus* (Y.M. Ju & J.D. Rogers) Y.M. Ju, J.D. Rogers & H.M. Hsieh were reported from North of Iran.

Materials and Methods

Specimens were collected from the western parts of Guilan and Mazandaran provinces (N Iran) in July to September, 2011–12. Parts of the branches, barks and trunks of infested trees with *Hypoxylon* and its allied genera were collected. 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 razor blade 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 Oatmeal Agar (OA, Difco) and Malt extract Agar (MA) and incubated at 20° C under 12 h fluorescent light. 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 measurements. To study KOH extractable pigments, ascospores germ-slit and dehiscence and asci apical ring, Ju & Rogers (1996) procedures was followed. Identification of the taxa is mainly based on Ju & Rogers (1996), Rogers *et al.* (1997), Ju *et al*. (1998), Ju & Rogers (2002), Ju *et al*. (2004), Vasilyeva *et al*. (2007) and Pereira *et al.* (2010). 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

Eight species *viz. Annulohypoxylon nitens*, *Biscogniauxia anceps*, *B. capnodes* var. *rumpens*, *B. mediterranea*, *B. plana*, *Hypoxylon flavoargillaceum*, *Jumillera cinerea*, and *Nemania illita* were identified. All these except *B. mediterranea* are new to the Iranian mycobiota. Description and illustration of all species and a dichotomous identification key for all the xylariaceous fungi examined in this study and species already reported by the authors from North of Iran is presented.

Hypoxylon flavoargillaceum J.H. Mill., in Chardón & Toro, Monograph Univ. Puerto Rico, Series B 2: 200 (1934)

Stromata glomerate to pulvinate, with inconspicuous perithecial mounds, 1–4 mm long, 1–3 mm wide and 0.6–1 mm thick; surface fawn or hazel; yellowish brown granules immediately beneath surface and between perithecia, with yellow or amber KOH-extractable pigments. Perithecia nearly spherical, 0.25–0.33 mm diam. and 0.33–0.44 mm high. Ostioles lower than the stromatal surface (umbilicate). Asci cylindrical, 8-spored, 117–156 × 7–7.5 µm, with discoid apical ring bluing in Melzer's iodine reagent (amyloid). Ascospores dark brown, one-celled, inequilaterally ellipsoidal, 12.3–16 × 5.5–7 µm, with narrowly rounded ends and straight to slightly sigmoid germ-slit extending to the tips of the ascospores; perispore dehiscent in 10% KOH; epispore smooth (Fig. 1).

Hypoxylon flavoargillaceum shares some characters with *H. notatum* Berk. & M.A. Curtis and *H. shearii* Y.M. Ju & J.D. Rogers; however, in the latter species the ascus apical ring is highly reduced and not bluing in Melzer's iodine reagent (Ju & Rogers 1996).

Specimen examined: Iran: Guilan province, Masuleh forest, on dead branches of *Mespilus germanica* L., 14 Jul. 2011; Rasht, Sarawan forest, on dead branches of *Quercus castaneifolia* C.A. Mey., 9 Apr. 2012. Both collected by S. Raei.

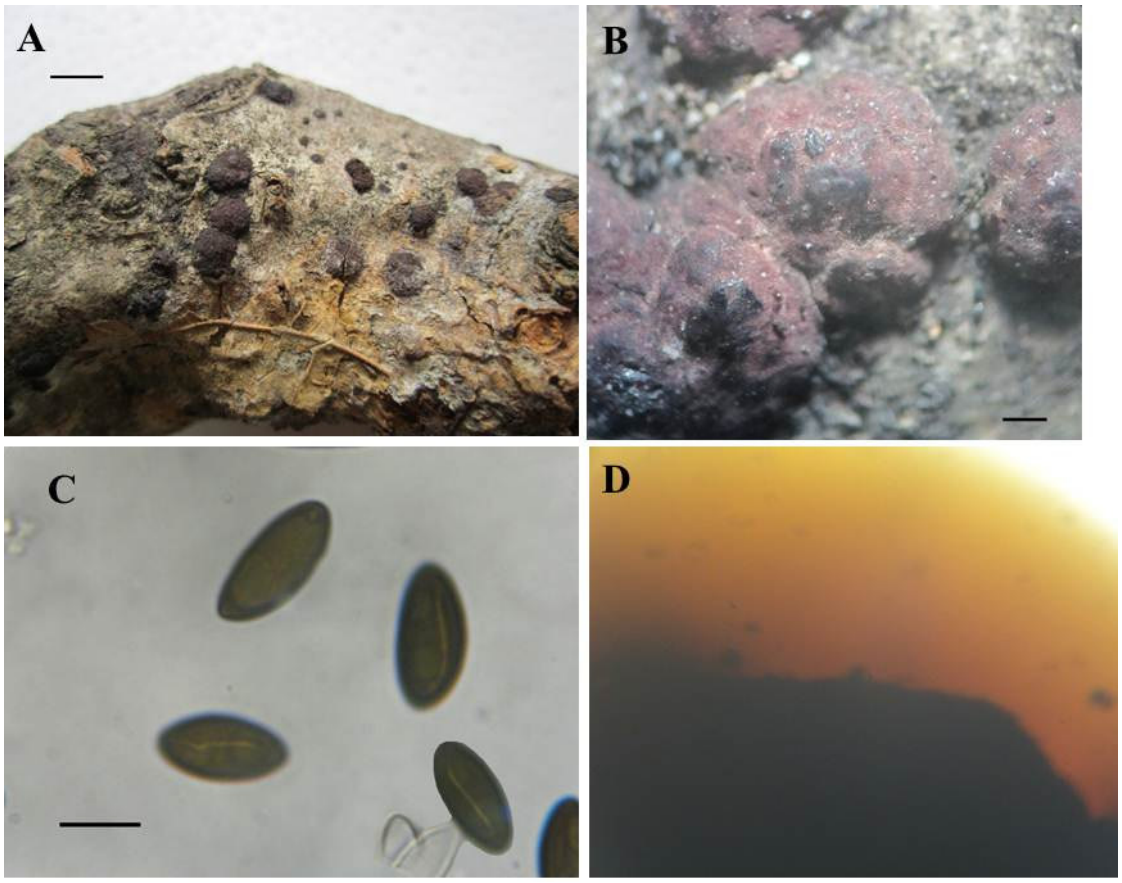

Fig. 1. *Hypoxylon flavoargillaceum*: A. Stromata on wood (Bar = 5 mm), B. Umbilicate ostioles (Bar = 500 µm), C. Ascospores with slightly sigmoid germ-slit spore-length and a dehiscent perispore in 10% KOH (Bar = 10 µm), D. Pigments in 10% KOH.

Annulohypoxylon nitens (Ces.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, Mycologia 97(4): 861 (2005)

Stromata effused-pulvinate, with conspicuous perithecial mounds, 1–9 cm long, 1–2 cm wide and 1–8 mm thick; surface dark brown vinaceous when young, then becoming blackish, with brown tone; blackish granules immediately beneath surface, with KOH-extractable pigments greenish olivaceous. Perithecia spherical to obovoid, 0.33–0.55 mm diam. and 0.5–0.7 mm high. Ostioles conical-papillate, encircled with a disc 0.25–0.33 mm diam. Asci not seen. Ascospores pale brown, one-celled, inequilaterally ellipsoidal, 7–9 × 3–3.5 µm, with narrowly to broadly rounded ends and straight germ-slit spore-length; perispore dehiscent in 10% KOH; epispore smooth (Fig. 2).

Specimen examined: Iran: Guilan province, Rasht, Sarawan forest, on dead branches of *Quercus castaneifolia* C.A. Mey., 9 Apr. 2012, coll. S. Raei.

The specimen examined here had the same characters of *A. nitens* except the perithecial mounds that were absent or inconspicuous (vs. the conspicuous and 1/4–1/2 exposed perithecial mounds of *A. nitens*).

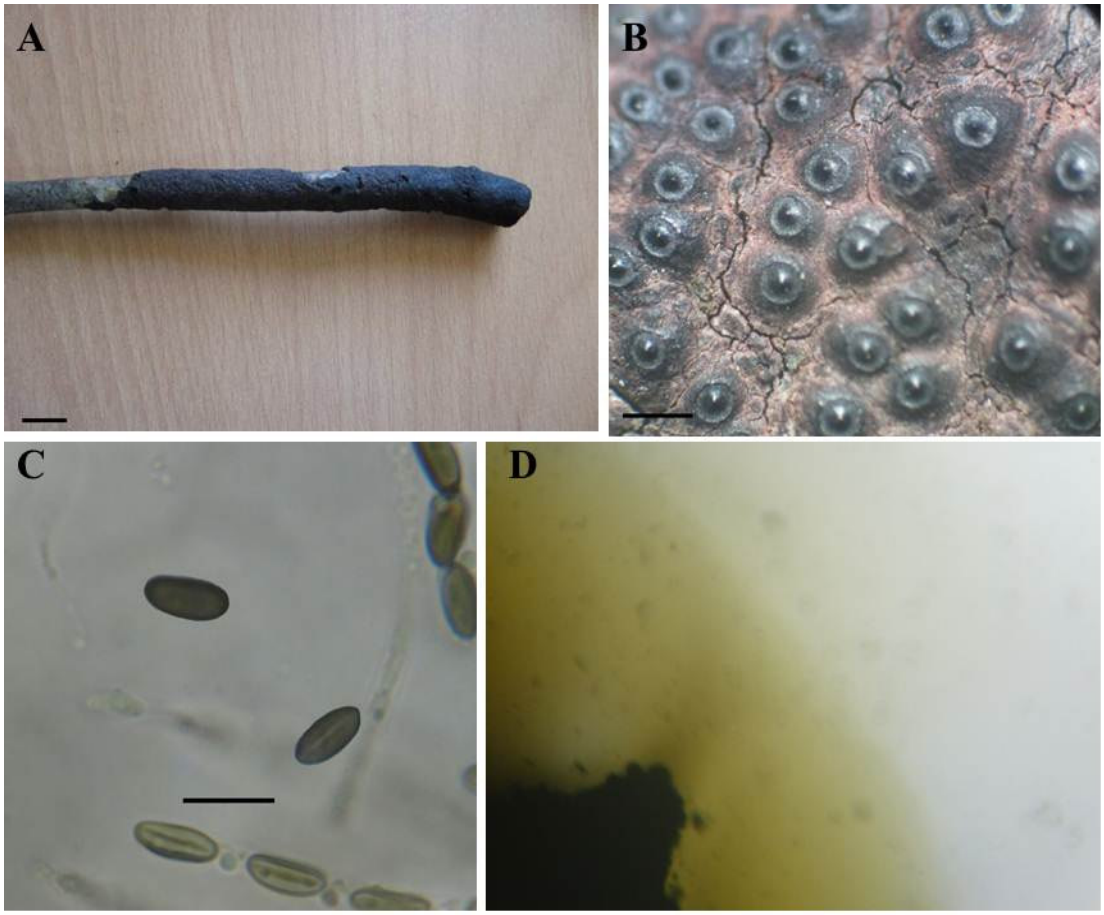

Fig. 2. *Annulohypoxylon nitens*: A. Stromata on wood (Bar = 1 cm), B. Perithecia with ostiolar discs (Bar = 500 µm), C. Ascospores (Bar = 10 µm), D. Pigments in 10% KOH.

Biscogniauxia anceps (Sacc.) J.D. Rogers, Y.M. Ju & Cand., Mycol. Res. 100(6): 669 (1996)

Stromata applanate, discoid to effuse, 0.5–10 cm long, 0.5–1.5 cm wide and 0.5–0.6 mm thick; without KOH-extractable pigments. Perithecia spherical, 0.2–0.4 mm diam. Ostioles umbilicate. Asci cylindrical, 8-spored, 104–143 × 8.5–10.4 µm, with an amyloid, discoid apical ring. Ascospores unequally two-celled, the larger cell sometimes dark brown to almost black and the smaller cell hyaline to sub-hyaline but most commonly both cells remaining hyaline, smooth, equi- or inequilaterally ellipsoidal to obovate. Ascospores two-celled, 13.4–17.5 µm total length, 6.2–8.2 µm wide at the broadest part, the larger cell 9.2–11.3 µm long and the

smaller cell 4.1–6.2 x 3.6–5.1 µm; larger cell when darkened with a straight full-length germ-slit; smaller cell are without germ-slit (Fig. 3).

Specimens examined: Iran: Guilan province, Masuleh forest, on dead branches of *Mespilus germanica* L., 14 Jul. 2011; Rasht, Sarawan forest, 13 Jul. 2011; Rasht, Sarawan forest, on dead branches of *Parrotia persica* C.A. Mey., 13 Jul. 2011; Astara forest, on dead branches of *Diospyros lotus* L., 10 Jul. 2012. All collected by S. Raei.

Biscogniauxia anceps, mainly characterized by two-celled ascospores, is finely differentiated from the closely related species, *B. uniapiculata* (Penz. & Sacc.) Whalley & Laessøe, by having larger ascospores mostly remaining hyaline at maturity.

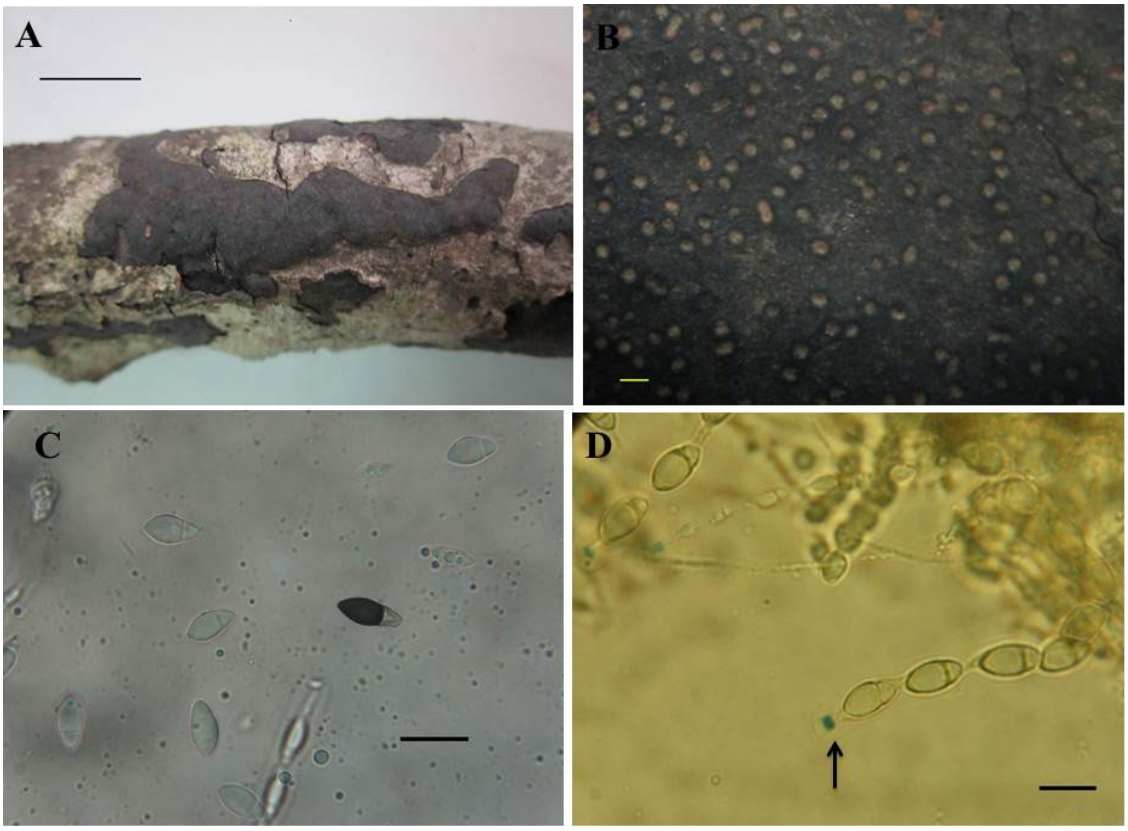

Fig. 3. *Biscogniauxia anceps*: A. Stromata on wood (Bar = 1 cm), B. Umbilicate ostioles (Bar = 500 µm), C. Ascospores (Bar = 20 µm), D. Asci with an amyloid apical ring (Bar = 20 µm).

Biscogniauxia capnodes var. ***rumpens*** (Cooke) Y.M. Ju & J.D. Rogers, in Ju, Rogers, San Martín & Granmo, Mycotaxon 66: 27 (1998)

Stromata applanate, 0.5–10 cm long, 0.5–5 cm wide and 0.5–0.9 mm thick; surface black, carbonaceous immediately beneath surface and between perithecia. Perithecia obovoid to tubular, 0.23–0.25 mm diam. and 0.5–0.65 mm high. Ostioles slightly higher than the stromatal surface with opening slightly papillate, or lower than the stromatal surface with openings punctate. Asci cylindrical, 8-spored, 130–170 × 9–10.5 µm, with an amyloid, discoid apical ring. Ascospores brown to dark brown, one-celled, ellipsoidal to lemon-shaped, nearly equilateral, 10.5–21 × 8–9 µm, with straight germ-slit spore-length (Fig. 4).

Specimen examined: Iran: Guilan province, Rasht, Sarawan forest, on dead branches of *Quercus castaneifolia* C.A. Mey., 13 Jul. 2011, coll. S. Raei.

Var. *rumpens* is differentiated from other varieties attributed to *B. capnodes* (Ju *et al.* 1998), *viz.* var. *capnodes* (Berk.) Y.M. Ju & J.D. Rogers, var. *limoniispora* Y.M. Ju & J.D. Rogers and var. *theissenii* (Syd. & P. Syd.) Y.M. Ju & J.D. Rogers, by having larger ascospores.

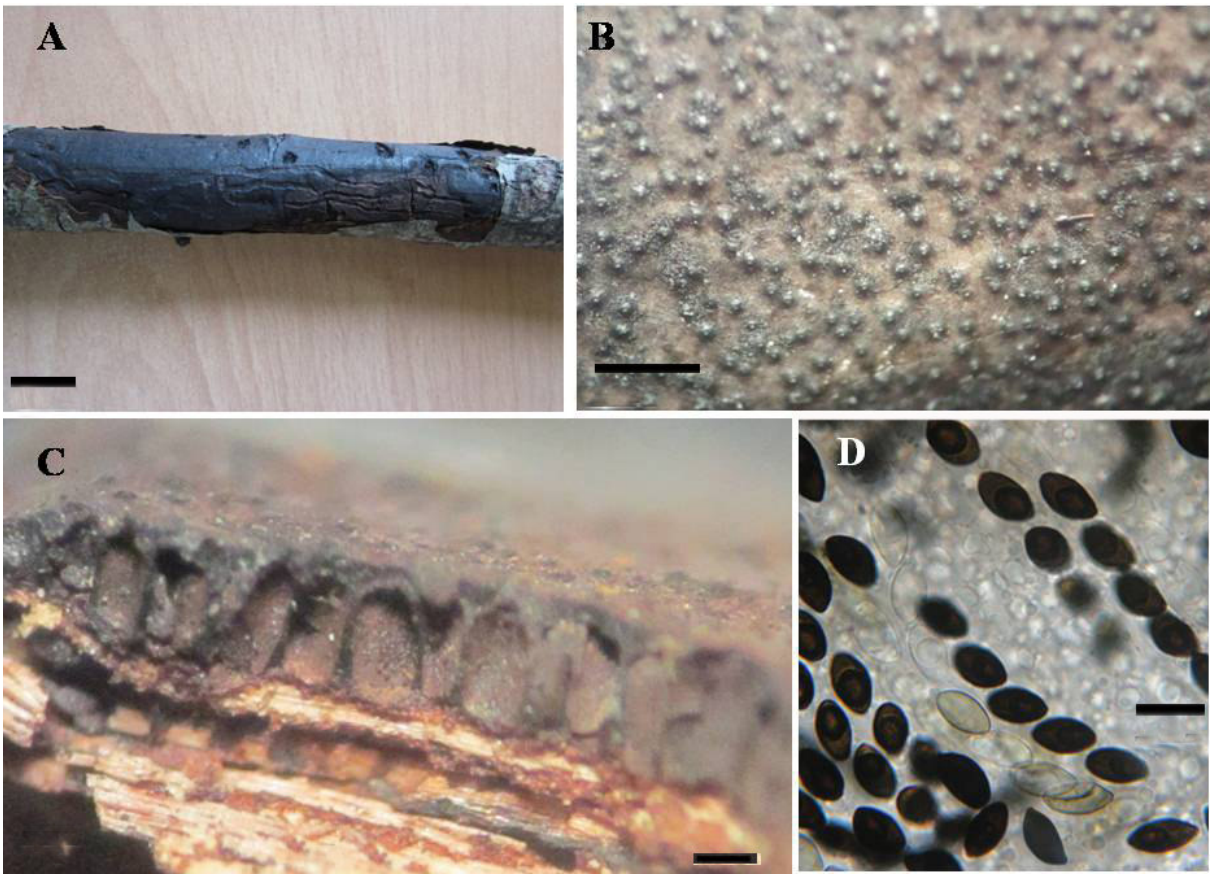

Fig. 4. *Biscogniauxia capnodes* var. *rumpens*: A. Stromata on wood (Bar = 1 cm), B. Stromatal surface with opening slightly papillate (Bar = 2 mm), C. Vertical section through the stroma showing perithecia (Bar = 200 µm, D. Ascospores (Bar = 20 µm).

Biscogniauxia mediterranea (De Not.) Kuntze, Revis. gen. pl. (Leipzig) 2: 398 (1891)

Stromata applanate, black, variable in length, 0.7–1 mm thick; carbonaceous immediately beneath surface and between perithecia. Perithecia obovoid to tubular, 0.15–0.18 mm diam. and 0.51–0.56 mm high. Ostioles higher than the stromatal surface with openings coarsely papillate. Asci cylindrical, 8-spored, 120–174 × 8–12 µm, with an amyloid, discoid apical ring. Ascospores brown to dark brown, one-celled, ellipsoidal, nearly equilateral, 12.8–16 × 5.6–7.2 µm, with narrowly to broadly rounded ends and straight germ-slit spore-length (Fig. 5).

Colonies on 2% MA reaching 90 mm diam. in seven days, white and thin, becoming gray at the position of conidial formation. Advancing margins are more or less obvious as mycelial cords. On OA growth is less rapid, white, becoming fulvous to honey when producing synnemata. Conidiogenous structure *Nodulisporium*-like, conidia smooth, ellipsoid to lemon-shaped, 5–6 × 2.5–3.5 µm.

This species has been briefly described by Mirabolfathy *et al.* (2011). However, both teleomorphic and anamorphic characteristics are described herein. Charcoal disease, caused by *B. mediterranea*, is a serious disease of cork oak (*Quercus suber*) and Turkish oak (*Quercus cerris*) in the Mediterranean area (Jurc & Ogris 2006). During the last two years, it has caused extensive mortality of oak trees (*Quercus castaneifolia*) in forests of Guilan province. Regarding *Cryphonecteria parasitica* as the major pathogenic fungus on chestnut trees in Iran (Kazempour *et al*. 2006, Qezi *et al*. 2009), *B. mediterranea* is here introduced as the second agent threatening forest trees of Guilan province.

Specimen examined: Iran: Guilan province, Rasht, Sarawan forest, on trunk and branches of *Quercus castaneifolia* C.A. Mey., 13 Jul. 2011; Rasht-Fuman ring road, 31 Jul. 2011. Both collected by S. Raei.

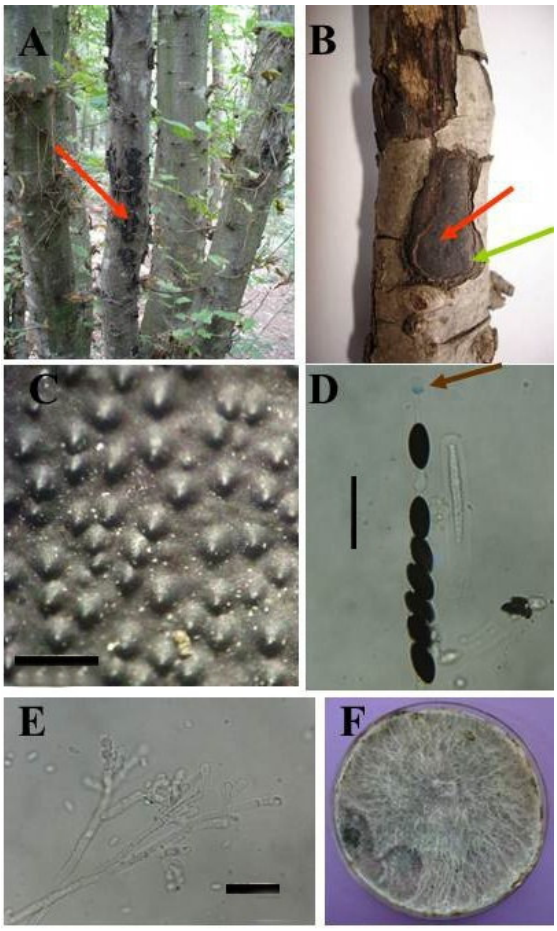

Fig. 5. *Biscogniauxia mediterranea*: A. Stroma on dead trunk, B. Close view of a bipartite stroma on a branch, green and red arrow show outer and inner layers respectively, C. Stromatal surface with openings coarsely papillate (Bar = 2 mm), D. Asci with an amyloid apical ring (Bar = 20 µm), E. *Nodulisporium*-like anamorph (Bar = 20 µm), (F) one-week old colony on 2% MA.

Biscogniauxia plana (Petch) Y.M. Ju & J.D. Rogers, in Ju, Rogers, San Martín & Granmo, Mycotaxon 66: 48 (1998)

Stromata applanate, 0.5–5.5 cm long, 0.5–3.5 cm wide and 0.5–0.6 mm thick; mature surface black, carbonaceous immediately beneath surface and between perithecia. Perithecia tubular, 0.07–0.1 mm diam. and 0.3–0.5 mm high, arranged in rosettes and discharging through a single ostiolar canal. Ostioles slightly lower to slightly higher than the stromatal surface with openings punctate or slightly papillate. Asci not seen. Ascospores brown to dark brown, one-celled, ellipsoidal, nearly equilateral, 10.5–13 × 5–6.5 µm, with narrowly to broadly rounded ends and straight germ-slit spore-length (Fig. 6).

Specimen examined: Iran: Guilan province, Astara forest, on dead branches of *Diospyros lotus* L., 11 Jul. 2012, coll. S. Raei.

Biscogniauxia plana resembles *B. communapertura* Y.M. Ju & J.D. Rogers by having perithecia arranged in rosettes and discharging through a single ostiolar canal; however, *B. communapertura* has papillate ostioles, larger perithecia and smaller ascospores.

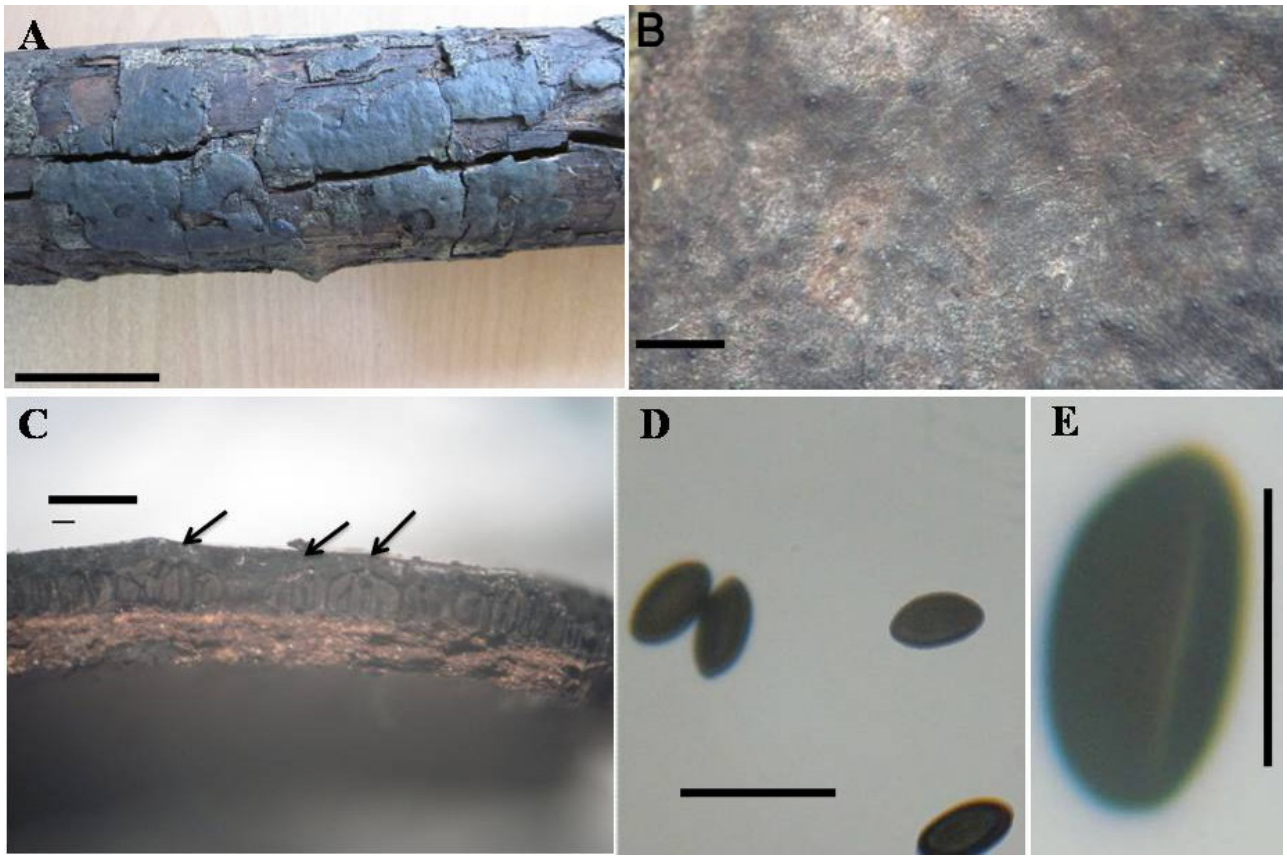

Fig. 6. *Biscogniauxia plana*: A. Stromata on wood (Bar = 3 cm), B. Stromatal surface with openings slightly papillate (Bar = 3 mm), C. Numerous perithecia arranged in rosettes with single ostiolar canal (Bar = 400 µm), D. Ascospores (Bar = 20 µm), E. Ascospore with straight germ-slit spore-length (Bar = 10 µm).

Nemania illita (Schwein.) Pouzar, Česká Mykol. 39(1): 24 (1985)

Stromata effused-pulvinate, discoid to hemispherical; confluent, rarely solitary, 0.2–2.5 cm long, 0.2–1.5 cm wide and 0.4–1.2 mm thick; surface black, with inconspicuous perithecial mounds; carbonaceous immediately beneath surface and white between perithecia; mature stromata lacking KOH-extractable pigments. Perithecia obovoid, 0.4–0.7 mm diam. and 0.5–0.9 mm high. Ostioles higher than stromatal surface and with openings papillate, with or without encircling disc. Asci 8-spored, cylindrical, 100–150 × 5–6 µm, with an amyloid apical ring which has a height/width ratio greater than 1/2. Ascospores pale brown, fusoid, crescent-shaped, inequilateral, 12–16 × 3–4.5 µm, with a straight, inconspicuous germ-slit; perispore indehiscent in 10% KOH; epispore smooth (Fig. 7).

Colonies on 2% MA reaching 90 mm diam. in three weeks, white, felt like, zonate, with lobate margins. Specimen examined: Iran: Guilan province, Rasht, Sarawan forest, on dead branches of *Quercus castaneifolia* C.A. Mey., 9 Apr. 2012, coll. S. Raei.

Nemania illita is well characterized by papillate ostioles, white tissue between the perithecia and pale brown, crescent-shaped ascospores with narrowly rounded ends.

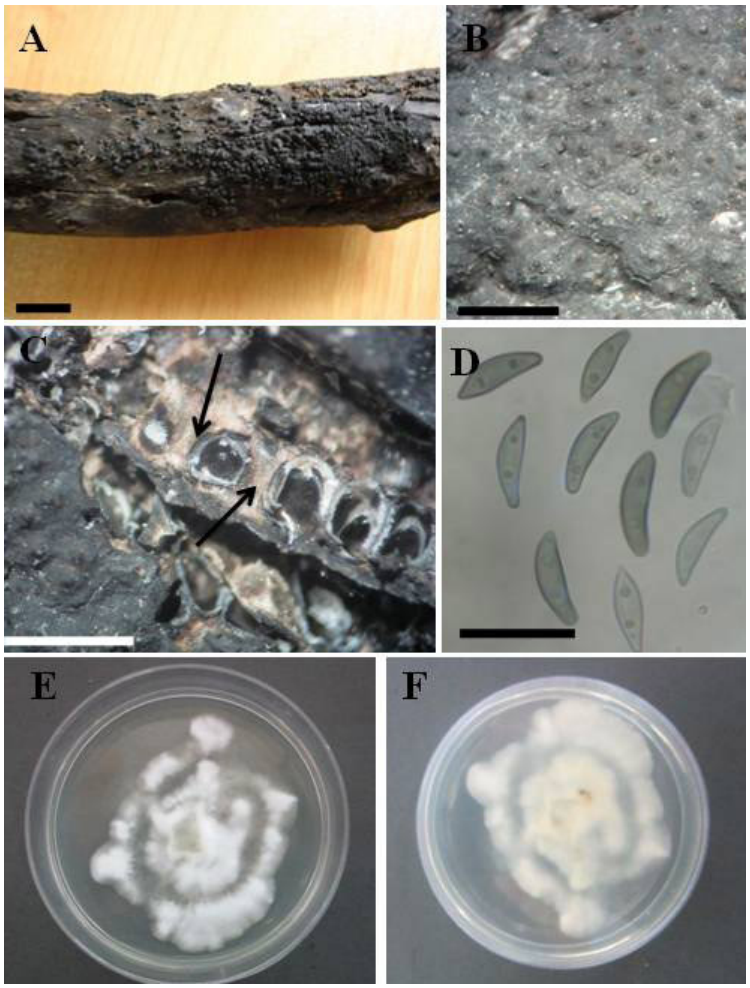

Fig. 7. *Nemania illita*: A. Stromata on wood (Bar = 1 cm), B. Stromatal surface with papillate openings (Bar = 3 mm), C. White tissue between perithecia (Bar = 1500 µm), D. Ascospores (Bar = 20 µm), E, F. Two week old colony on 2% MA

Jumillera cinerea (Ellis & Everh.) J.D. Rogers, Y.M. Ju & F. San Martín, Mycotaxon 64: 43 (1997)

Stromata applanate or effused-pulvinate, solitary or confluent, 0.2–8 cm long, 0.2–2 cm wide and 0.3–0.5 mm thick, mature surface gray, carbonaceous immediately beneath surface, tissue between perithecia and beneath the perithecial layer mainly composed of host tissue, mature stromata lacking KOH-extractable pigments. Perithecia 0.2–0.3 mm diam. Ostioles lower than stromatal surface, with ostiolar openings punctate. Asci cylindrical, 8-spored, 90–120 × 5–5.5 µm, with an amyloid, discoid apical ring. Ascospores brown to dark brown, one-celled, ellipsoidal, nearly equilateral, with narrowly to broadly rounded ends, 9–11 × 4–4.5 µm, with straight germ-slit spore-length; perispore indehiscent in 10% KOH; epispore smooth (Fig. 8).

Specimen examined: Iran: Mazandaran province, Nour, Chamestan, on dead branches of *Citrus* sp., 3 Apr. 2012, coll. S. Raei.

Jumillera cinerea is differentiated from the closest species *J. albida* J.D. Rogers, Y.M. Ju & F. San Martín by having gray stromatal surface color (vs. white in the latter species).

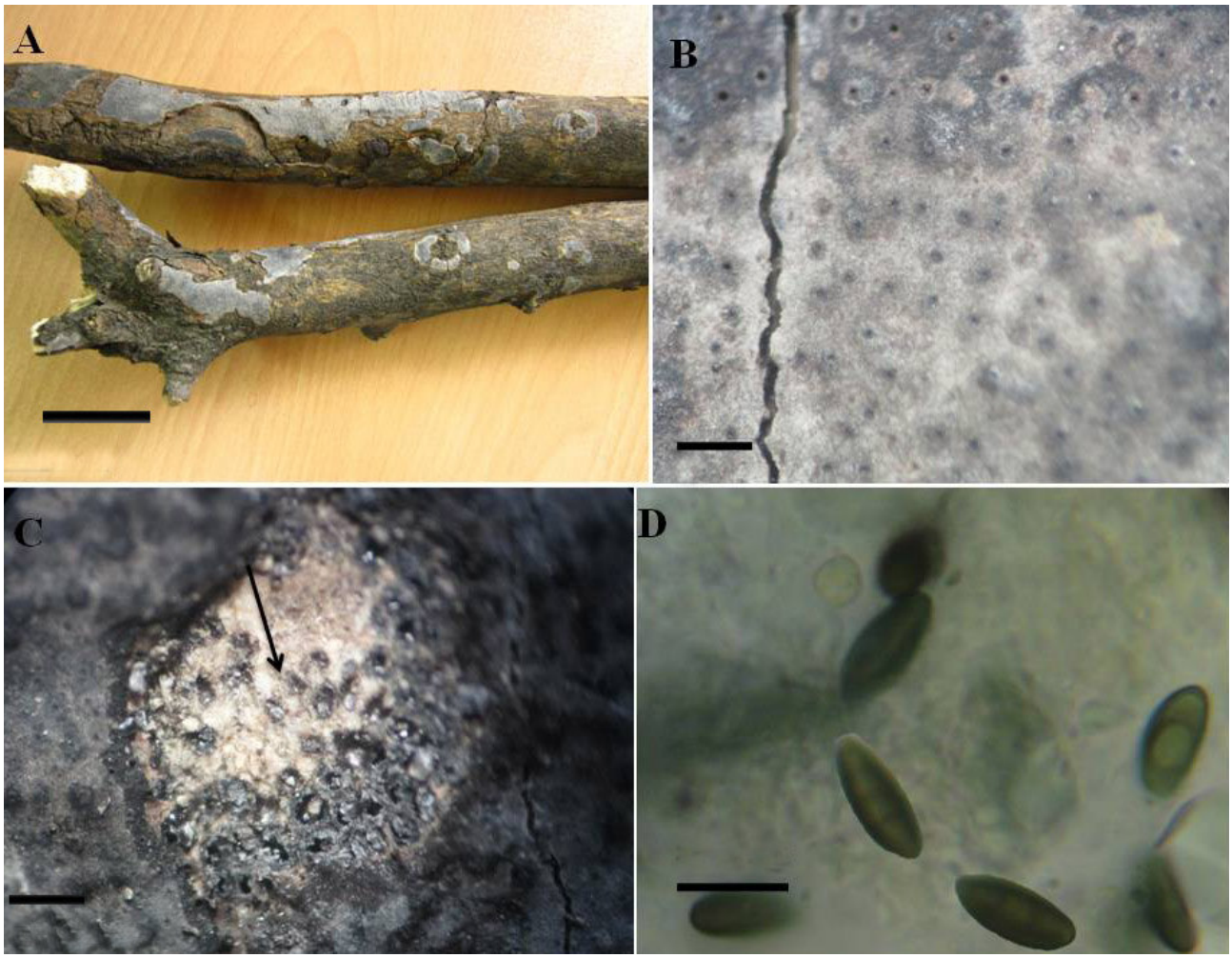

Fig. 8. *Jumillera cinerea*: A. Stromata on wood (Bar = 3 cm), B. Stromatal surface with punctuate ostiolar openings (Bar = 1500 µm, C. Perithecia embedded within host tissue (Bar = 500 µm), D. Ascospores (Bar = 10 µm).

Dichotomous identification key to some xylariaceous taxa collected by the authors from Iran

1. Stromata bipartite, with an outer membrane dehiscing to expose ostiolar openings, without KOH-extractable pigments; ascospores with perispore not dehiscent in 10% KOH ……..……..…….. 2
1. Stromata essentially unipartite, with or without KOH-extractable pigments; ascospores with perispore dehiscent or not dehiscent in 10% KOH ……..……..…….. 3
2. Perithecia embedded in white fungal tissue or a mixture of fungal and host tissue; anamorph usually *Libertella*-like or *Geniculosporium*-like; colonies slow growing on MA ……..……..…… ***Jumillera cinerea***
2. Perithecia embedded in black carbonaceous or brown woody stromatal tissue; anamorph usually *Periconiella*-like or less frequently *Nodulisporium*-like; colonies fast growing on MA ……..……..…….. ***Biscogniauxia*** (A)
3. Stromata without KOH-extractable pigments; ascospores with perispore not dehiscent in 10% KOH; asci with apical rings that have a height/breadth ratio greater than 1/2; anamorph *Geniculosporium*-like ……..…….. ***Nemania illita***
3. Stromata usually with KOH-extractable pigments; ascospores usually with perispore dehiscent in 10% KOH; asci with discoid apical ring; anamorph *Nodulisporium*-like ……..……. 4
4. Stromata with surface colored when mature, usually not blacked, carbonization of stromatal layer directly surrounding perithecia absent; ostioles usually umbilicate, less frequently papillate or at the same level of stromatal surface, usually not encircled with an annulate disc; perispore when dehiscing, with conspicuous to very inconspicuous transverse coil-like ornamentation ……..……..…….. ***Hypoxylon*** (B)

4. Stromata with surface usually blackened when mature, infrequently colored, usually with carbonaceous stromatal layer directly surrounding each perithecium; ostioles always papillate, encircled by an annulate disc or annulate disc lacking; perispore when dehiscing, with a thickened area visible at the position of ca. 1/3 ascospore length from one end on the same side as the germ-slit ……..……..……..... ***Annulohypoxylon*** (C)

A_1. Ostioles lower than the level of stromatal surface; ascospores two-celled, mostly remaining hyaline when mature ……..……..…… ***B. anceps***

A_1. Ostioles at the same level or higher than the level of stromatal surface; ascospores one-celled, brown to dark when mature ……..……..……. A_2

A_2. Ostiolar openings coarsely papillate; stromatal tissue between perithecia composed entirely of carbonaceous tissue; ascospores 12.8–16 × 5.6–7.2 µm ……..……..…….. ***B. mediterranea***

A_2. Ostiolar openings slightly papillate or punctate; stromatal tissue between perithecia composed entirely of carbonaceous tissue; ascospores smaller ……..…...……...……..…….. A_3

A_3. Perithecia very narrow, ca. 0.1 mm diam., with numerous individuals arranged in rosettes and sharing a single ostiolar canal; ascospores 10.5–13 × 5–6.5 µm ……..……..……..…... ***B. plana***

A_3. Perithecia more than 0.2 mm diam., usually not arranged in rosettes and usually with individual ostioles; ascospores 10.5–21 × 8–9 µm ……..……..…. ***B. capnodes*** var. ***rumpens***

B_1. Stromata hemispherical to spherical, usually larger than 1.5 mm thick, with orange red granules immediately beneath surface, and white granules between perithecia ….……...……..….. B_2

B_1. Combination of characters differing from above ……..……...……..…… B_3

B_2. Ascospores 11.2–16.8 × 5–8 µm ……...……...……...…… ***H. fragiforme***

B_2. Ascospores 6.5–10 × 3–5 µm ……...……..…. ***H. howeianum***

B_3. Stromata containing orange red, orange, dull orange, orange brown, yellowish brown, rust, or less frequently dull brown granules, with KOH-extractable pigments orange, reddish, or rust, stromata pulvinate to effused-pulvinate, 0.5–3.5 cm long × 0.4–1 cm wide × 1–1.5 mm thick; ascospores 9–13 × 4–6 µm, with straight germ-slit spore-length ……...……...…..….. ***H. rubiginosum***

B_3. Stromata containing buff, light brown, dull brown, dull reddish brown, or black granules, with KOH-extractable pigments yellowish, greenish yellow, dull green, or olivaceous ……..……...….. B_4

B_4. Stromata pulvinate to effused-pulvinate, 3.5–7 mm long × 2.5–4 mm wide × 0.6–0.9 mm thick; surface brown vinaceous; ostioles usually overlain with conspicuous white substance, with dark brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments amber; ascospores 10–12 × 3.5–5 µm, with straight to slightly sigmoid germ-slit spore-length ……..……...……...…….. ***H. perforatum***

B_4. Stromata glomerate to pulvinate, 1–4 mm long × 1–3 mm wide × 0.6–1 mm thick; surface fawn or hazel; yellowish brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments yellow or amber; ascospores 12.3–16 × 5.5–7 µm, with straight to slightly sigmoid germ-slit spore-length …….. ……...……..……...……..… ***H. flavoargillaceum***

C_1. Ostioles not encircled by a disc; stromata usually larger than 2.5 mm thick; ascospores 6.5–8 × 3–4 µm. …… ……..……...…….…… ***A. minutellum***

C_1. Ostioles encircled by a disc ……..……...…… C_2

C_2. Ostiolar discs not exceeding 0.2 mm diam.; ascospores 10–12 × 4–4.5 µm ……..… ***A. moriforme*** var. ***microdiscus***

C_2. Ostiolar discs exceeding 0.2 mm diam. ……..……..…….. C_3

C_3. Mature stromata shiny black, with greenish olivaceous KOH-extractable pigments; ascospores 7–9 × 3–3.5 µm …… ……...……..……..…….. ***A. nitens***

C_3. Mature stromata dull brown, black or olive ……..……..…….…. C_4

C_4. Stromata glomerate, pulvinate or effused-pulvinate, 1–2.5 cm long × 0.3–1 cm wide × 1–1.5 mm thick; ascospores 7.7–10.3 × 2–3.6 µm; no apparent host specificity ……..……..….……..... ***A. moriforme***

C_4. Stromata hemispherical, 0.2–0.8 cm diam. × 0.2–0.8 cm thick; ascospores 9.3–11.3 × 4.1–4.6 µm, inhabiting *Quercus* ……..……..…..……..……..…..… ***A. annulatum***

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