Hypoxylon subdisciforme sp. nov., *Nemania abortiva* sp. nov., and *Xylotumulus gibbisporus* gen. et sp. nov. from Hawaii, Hawaiian Islands

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Three undescribed taxa of family Xylariaceae are described from Hawaii, Hawaiian Islands. *Hypoxylon subdisciforme* is a discoid to subdiscoid species with plate-like ascus apical ring that does not stain or stains lightly in iodine. *Nemania abortiva* features asci with 4 or fewer ascospores, the first described *Nemania* species with consistently fewer than 8 ascospores. *Xylotumulus gibbisporus* shows mound-shaped erumpent stromata with 1-5 perithecia, ascus apical ring that does not stain in iodine, gibbous ascospores with spiraling germination slit, and anamorph produced on a sporodochium.

Key words: Hypoxylon, Nemania, Pyrenomycetes, Xylariaceae, Xylotumulus.

The new taxa to be described herein are the result of collections made in the Bird Park area of Hawaii Vocanoes National Park, Hawaii during August and November, 2005. Permission to do limited collecting was graciously given by park personnel who are cited in the Acknowledgements.

Materials and Methods

Cultures were initiated from ascospores extracted by a sterile needle from hydrated perithecia from which the upper surface had been aseptically removed. Ascospore masses were stabbed into plates of SME agar (Kenerley & Rogers, 1976). When colonies developed transfers were made to 2 % Oatmeal agar (Difco) (OMA), a standard medium for cultivating xylariaceous fungi. A minimum of

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15 ascospores and conidia were measured in water. Size ranges are given with extreme dimensions in parentheses. Asci were mounted in Melzer's iodine reagent (Stevens, 1974) in order to assess the reaction or non-reaction of the apical ring to iodine. Observations were made by differential interference microscopy and photomicrographs via a Nikon digital camera Coolpix 4300. Cultures were deposited in American Type Culture Collection. Color designations follow Rayner (1970).

Taxonomic Part

Xylotumulus J.D. Rogers, Y.-M. Ju & Hemmes, gen. nov.

Stromata erumpentibus ramis 1-5 perithecia inclusa. Asci brevistipitati annulo apicali prominente in liquore iodato Melzeri incolorato. Ascosporae brunneae gibbae vel fusoideae extremis plus minusve truncatis, rima germinativa praeditae.

Stromata erumpent from stems enclosing 1-5 perithecia. Asci short-stipitate with well-developed apical ring that does not stain blue in Melzer's iodine reagent. Ascospores brown, gibbous to fusoid with more or less flattened ends, with germination slit.

Typus generis. – *Xylotumulus gibbisporus* J.D. Rogers, Y.-M. Ju & Hemmes

Etymology. – Xylo- = xylariaceous + tumulus = mound

Xylotumulus gibbisporus J. D. Rogers, Y.-M. Ju & Hemmes, sp. nov. Figs. 1 – 7

Stromata tumida vel pulvinata e ramo erumpentibus, 2-5 mm diam cum 1-5 peritheciis inclusis; externa brunneola cum nitore argenteo, interna alba praedita. Perithecia globosa usque ad 1 mm diam. Ostiola obscura vel parum papillata. Asci raro octospori saepe oligospori, brevistipitata, omnino ca. 235 µm longi, usque ad 22 µm crassi, annulo apicali in liquore iodato Melzeri incolorato. Ascosporae brunneae vel fuscae, unicellulares, gibbae vel fusoideae extremis plus minusve truncatis, interdum appendicula cellulose unica, leves, $(30-)35 - 40(-60) \times 12 - 15(-16)$ µm, rima germinativa spiralis vel sigmoidea raro recta praeditae. Holotypus.– U.S.A., Hawaii, 2 Aug 2005, leg. J.D. Rogers (BISH).

Stromata mound-shaped to pulvinate, erumpent from stems, 2-5 mm diam, containing 1-5 perithecia; externally Umber (9) to Chestnut (40) with a Pale Olivaceous Grey (120) sheen, internally white. – Perithecia globose, up to 1 mm diam. Ostioles obscure to slightly papillate. – Asci seldom 8-spored, often with irregular ascospore numbers (fewer than 8), short-stipitate, in total about 235 µm long, up to 22 µm wide, with apical ring not bluing in Melzer's iodine reagent. – Ascospores brown to dark brown, unicellular, gibbous to fusoid, with the apices more or less truncate, occasionally

with a cellular appendage at one end, smooth, $(30-)35-40(-60) \times 12-15(-16) \mu m$ (n = 21), with germination slit spiral or sigmoid or, rarely, straight.

Colony on OMA incubated in natural cycle of sunlight: darkness at ca. 20 C covering 9 cm diam Petri plate in ca. 2 weeks, white, appressed, becoming thickened; after ca. 4 weeks becoming Apricot (42) in various areas, especially at margins. After ca. 8 weeks Apricot (42) sporodochia 1-5 mm diam bearing conidia produced. – Conidiophores in palisades, hyaline, unbranched or sparingly branched, $25-45 \times 3-4 \mu m$, producing conidia holoblastically, proliferating sympodially and becoming slightly geniculate. – Conidia hyaline, long ellipsoid with flattened secession scar, (8-)9- $9.5(-10.5) \times 2-3 \mu m$ (n = 15).

Etymology. – For the gibbous shape of the ascospores.

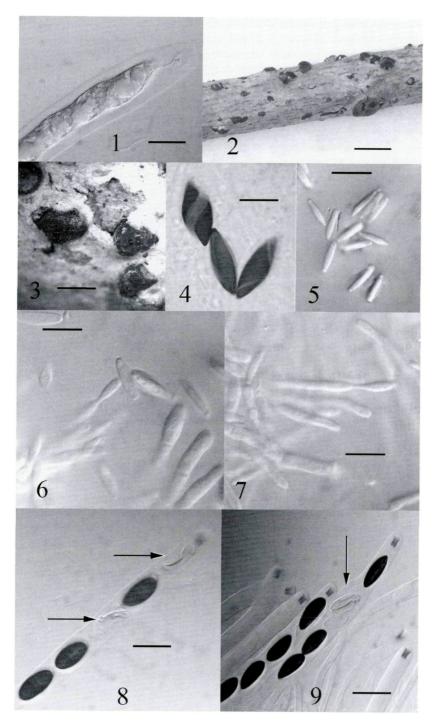
Habitat or Host plant. – On unidentified angiospermous stems.

Distribution. - Known only from type location in Hawaii.

Material examined. – U.S.A., Hawaii, Island of Hawaii, Hawaii Volcanoes National Park, Kipuka Puaulu (Bird Park), on dead angiosperm stems, 2 Aug 2005, J.D. Rogers, BISH holotype; WSP 71222, isotype.

Xylotumulus, as typified by *X. gibbisporus*, differs from other described xylariaceous genera in the following combination of characters: erumpent mound-shaped, mostly multiperitheciate stromata; gibbous to fusoid ascospores with flattened ends, one of which sometimes bears a cellular appendage; spiraling ascospore germ slit; short-stipitate ascus with an iodine-negative apical ring, usually bearing fewer than 8 ascospores; and sporodochial conidiomata. It is obviously closely related morphologically to *Amphirosellinia* Y.-M. Ju, J. D. Rogers, H.-M. Hsieh & Vasilyeva (Ju et al, 2004), differing primarily in the iodine-negative ascus apical ring, the variable number of ascospores per ascus, and the sporodochial conidioma.

Figs. 1 – 9. *Xylotumulus gibbisporus* and *Nemania abortiva*. Figs. 1 – 7. *Xylotumulus gibbisporus*. 1. Apical part of immature ascus showing ascus apex. 2 and 3. Stromata on twigs. 4. Ascospores, the uppermost one showing open spiral germ slit and the lowermost one showing ends of spiral slit. 5. Conidia. 6 and 7. Conidia and conidiogenous cells. Figs. 8 and 9. *Nemania abortiva*. 8. Part of ascus showing three maturing ascospores and two aborted ascospores (arrows). 9. Asci with apical rings. One ascus with four mature ascospores and another ascus with three mature ascospores (arrow). Figs. 1, 4 – 9 by DIC. Figs. 2 and 3 by macrophotography. Figs. 1, 8 and 9 from material in Melzer's reagent. Figs. 4 – 7 from material in water. Scale bars: 6 and 7: 8 μ m; 5: 10 μ m; 8: 11 μ m; 9: 14 μ m; 1 and 4: 18 μ m; 3: 3 mm; 2: 20 mm.



A key was presented to other genera that could be confused with Amphirosellinia (Ju et al, 2004) and it can also be applied to the separation of *Xylotumulus* from the genera included there. Although Xylaria species usually have highly developed stromata and longstipitate asci with iodine-positive aspical rings, there is no doubt that *Xylotumulus* has affinities with certain of them. For example, Xylaria boergesenii (Ferd. & Winge) Cannon seems similar in ascospore germ slit morphology; the stroma, however, is more highly developed; no anamorph was reported (Cannon, 1987). Xylaria coremiifera J.D. Rogers & Y.-M. Ju shows asci with 8 or fewer ascospores, whereas Xylaria umbonata J.D. Rogers & Y.-M. Ju has ascospores with a spiral germ slit (Rogers & Ju, 2004). Some species of Penzigia Sacc. - the type species of which is a Xylaria (Ju & Rogers, 2001) and most other species of which are probably *Xylaria* (Laessøe, 1994) – show features reminiscent of *Xylotumulus*. For example, *P. macrocarpa* Penz. & Sacc. has iodine-negative ascus rings (Tjibodas, 2/3/1897, PAD, ?holotype). Penzigia quercus Müller & Ahmad is reported to have broadly fusoid ascospore $24.5 - 39 \times 11 - 16 \,\mu\text{m}$; a long straight germ slit is illustrated (Müller & Ahmad, 1955). Stromata are described as superficial with a brief stipe. The ascus ring is said to stain in iodine and is illustrated (Müller & Ahmad, 1955). We have not seen material of this taxon.

Nemania abortiva J.D. Rogers, Y.-M. Ju & Hemmes, sp. nov. Figs. 8 and 9.

Stromata pulvinata, forma irregularia interdum serpentina, usque ad 1.5 cm longa, 1 cm diam , 1 – 2 mm crassa, externa furca, interna probiliter initio alba, carbonacea, leve. Perithecia 0.5 - 1 mm diam. Ostiola papillata. Asci fere quadrispori, interdum trispori, sexspori vel octospori, omnino $100 - 125 \,\mu$ m longi, $5.5 - 6.5 \,\mu$ m lati, partibus sporiferis ca. 60 μ m longis, stipitibus 41 – 51 μ m longis, annulo apicali in liquore iodato Melzeri cyanescente, inverse petasiformi, 3 μ m alto, 3 μ m lato. Ascospore brunneae vel fuscae unicellulares, late ellipsoideo-inequilaterales, levis, (9.5 –)10.5 – 12(– 13.5) × (4.5 –)6 – 6.5(– 7.5) μ m, rima germinativa recta longa inconspicua praeditae. Holotypus. – U.S.A., Hawaii, 2 Aug 2005, leg. J.D. Rogers (BISH).

Stromata pulvinate, irregular in shape, sometimes serpentine, up to 1.5 cm long, 1 cm diam, 1-2 mm thick, externally Fuscous Black (104), internally probably at first white, then carbonaceous, smooth. – Perithecia 0.5-1 mm diam. Ostioles papillate. Asci mostly 4-spored, sometimes 3-spored, 6-spored or 8-spored, in total $100-125 \mu$ m long, $5.5-6.5 \mu$ m wide, the spore-bearing part ca. 60μ m long, stipes $41-51 \mu$ m long, the apical ring bluing in Melzer's iodine reagent, inverse hat-shaped, 3μ m high, 3μ m wide. – Ascospores brown to dark brown, unicellular, broad ellipsoid-inequilateral, smooth, $(9.5 -)10.5 - 12(-13.5) \times (4.5 -)6 - 6.5(-7.5) \mu m$ (n = 21), with long inconspicuous germ slit. – Colony on SME under natural cycle of daylight: darkness at ca. 20 °C covering 9 cm diam Petri plate in ca. 2 weeks, white, thin. After 4 weeks developing Ochraceous (44) areas that bear a few geniculate conidiophores. After 20 weeks abundant sporulation on original isolation area. – Conidiophores subhyaline, geniculate, up to 117 µm long, 3 µm broad, producing conidia holoblastically with sympodial conidiogenous cell proliferation. – Conidia hyaline, lacrymoid to ellipsoid, with flattened secession scar, smooth, $6 - 6.5 (-7.5) \times 3 - 4.5 \mu m$ (n = 15).

Etymology. – For the tendency to produce fewer than 8 mature ascospores.

Habitat or Host plant. – Decayed unidentified angiospermous wood.

Distribution. - Known only from type location in Hawaii.

Material examined. – U.S.A., Hawaii, Island of Hawaii, Hawaii Volcanoes National Park, Kipuka Puaulu (Bird Park), on decayed angiosperm wood, 2 Aug 2005, J.D. Rogers, BISH; holotype, WSP 71221, isotype.

Nemania abortiva is the only described Nemania species with consistently fewer than 8 ascospores. Examination of immature asci reveals that 8 ascospores are delimited, but fewer than 8 – most frequently 4 – become mature, i.e. some of them appear to abort. Thus, the origin of the specific epithet for this fungus. In culture, N. abortiva did not produce abundant conidia for many weeks, an unusually long time compared with other cultured Nemania species.

Hypoxylon subdisciforme J.D. Rogers, Y.-M. Ju & Hemmes, sp. nov. – Figs. 10 – 15.

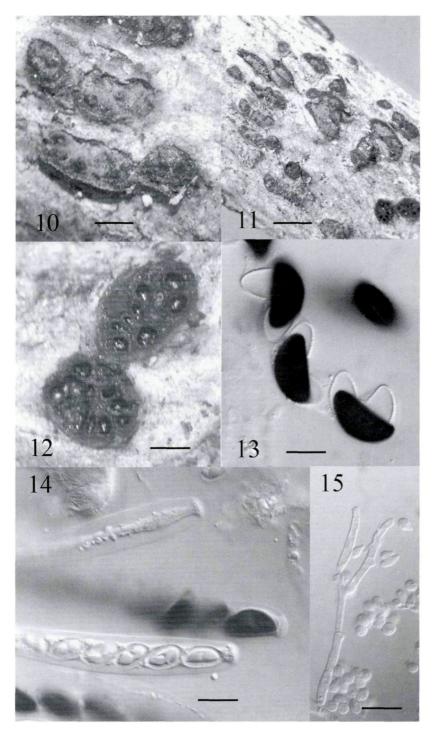
Stromata discoidea vel elongata vel irregularia, leviter pulvinata, 1-4 mm lata et longa, ca. 1 mm crassa, lignosa, erumpentia; externe umbrina vel castanea, sub superficie et inter perithecia granulis dilutis conspersa, granulis aurantiacis vel luteis in KOH dissolutis, textura sub peritheciis atra, ca 0.5 mm crassa. Perithecia globosa, 0.4-0.5 mm diam, aliquot vel multa per stroma. Ostiola umbilicata, interdum in foveis dispersa. Asci 8-spori, $120-140 \mu$ m longitudine tota, $7-8 \mu$ m crassi, partibus sporiferis $70-80 \mu$ m longitudine, annulo apicali in liquore iodato Melzeri leviter cyanescente vel non cyanescente, plano, minuto. Ascosporae brunneae vel fuscae, unicellulares, ellipsoideo-inequilaterales, levis, $11.5-13.5 \times 6-7(-7.5) \mu$ m, rima germinativa recta inconspicua praeditae; perisporium in KOH dehiscens, spiris inconspicuis vel conspicuis ornatum; episporium leve. Holotypus. –U.S.A., Hawaii, 1 Nov 2005, leg. J.D. Rogers (BISH).

Stromata discoid to elongate to irregular, slightly pulvinate, 1-4 mm long and wide, ca. 1 mm thick, woody, erumpent from host, exterior Umber (9) to Chestnut (40), interior blackish with scattered Ochraceus (44) granules, with pigment in KOH Orange (7) to Luteus (12). – Perithecia 0.4–0.5 mm diam, globose, several to many per stroma. Ostioles umbilicate, sometimes in depressions. – Asci 8-spored, 120–140 µm total length, 7–8 µm broad, the spore-bearing part 70–80 µm long, with ascus apical ring plate-like, slightly bluing in Melzer's iodine reagent or not bluing. – Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, smooth, 11.5–13.5 × 6-7(-7.5) µm (n = 20) with germination slit full-length, inconspicuous. Perispore dehiscent in KOH, with inconspicuous to conspicuous coil-like ornamentation; epispore smooth.

Colony on OMA under natural cycle of daylight: darkness at ca. 20 °C covering 9 cm diam Petri plate in ca. 4 weeks, at first white, thick, appressed, later developing Pale Luteus (11) and Umber (9) zones, with droplets of clear to Luteus (12) on paler zones and Chestnut (40) to Fuscous Black (104) on darker zones; reverse Fawn (87). Anamorph sparse to lacking, mostly near plate center. -Conidiogenous structures Virgariella-like (see Ju & Rogers, 1996). Conidiophores lacking a dominant main axis, unbranched or dichotomously branched one or two times to form a level of conidiogenous structures; additional branches repeatedly arising from previous level to form several levels. Conidiogenous regions hyaline, yellowish toward base, smooth, ca. 2.5 µm diam at base, terminating in 1 or 2 conidiogenous cells. - Conidiogenous cells cylindrical, hyaline, smooth, $15 - 30 \times 1.5 - 2.5 \,\mu\text{m}$, bearing poroid conidial secession scars. Conidia produced holoblastically in sympodial sequence, hyaline, smooth, subglobose to ellipsoid, with flattened secession scars, $3.5 - 5 \times 2.5 - 3.5 \mu m$ (n = 15).

Colony initiated from ascospore masses on SME under natural cycle of daylight: darkness thin, grey to tan, growing restrictedly 2-3 cm diam around inoculation site, staining agar blackish brown in 4 weeks. Anamorph-formation limited near periphery of colonies. – Conidiophores and conidogenous cells as described above. Conidia produced as described above, ellipsoid to lacrymoid to rotund with secession end more or less attenuated, hyaline to subhyaline, smooth, $(3.5 -)4.5 - 5(-6) \times 3 - 3.5 \ \mu m (n = 15)$.

Figs. 10 – 15. *Hypoxylon subdisciforme.* 10 and 11. Habit of stromata on bark. 12. Horizontal section of stroma exposing perithecia. 13. Ascospores showing dehisced perispores with minute ornamentation. 14. Asci showing non-staining apices. 15. Conidiophore with conidia. Figs. 13 – 15 by DIC. Figs. 10 – 12 by macrophotography. Figs 13 and 15 from water mounts. Fig. 14 from mount in Melzer's reagent. Scale bars: 14 and 15: 10 μm; 13: 6 μm; 12: 1 mm; 10: 1.5 mm; 11: 5 mm.



Etymology. – For the irregularly discoid shape of the stromata. Habitat or Host plant. – On unidentified dead angiospermous stems.

Distribution. - Known only from type location in Hawaii.

Material examined. – U.S.A, Hawaii, Island of Hawaii, Hawaii Volcanoes National Park, Kipuka Puaulu (Bird Park), on dead angiospermous stems, 1 Nov 2005, J.D. Rogers, BISH holotype; WSP 71224, isotype; 2 Aug 2005, WSP 71223.

Among extant *Hypoxylon* species *H. subdisciforme* most resembles *H. cercidicola* (Berk. & M. A. Curtis ex Peck) Y.-M. Ju & J. D. Rogers (as *H. cercidicolum* in Ju & Rogers, 1996) in the shape and color of the stroma, the manner of its emergence from the host periderm, and the highly reduced apical ring. *Hypoxylon cercidicola* differs in the strongly curved ascospores, cultural characteristics, *Fraxinus* host preference, and temperate distribution. The conidial state can be accommodated in the *Virgariella*-like form of *Nodulisporium* (Ju & Rogers, 1996).

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