ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

DOI: 10.5248/113.273

Volume 113, pp. 273–281 *July–September 2010*

Some new pyrenomycetous and loculoascomycetous fungi on the endemic Hawaiian plant *Hibiscadelphus giffardianus*

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Abstract — The fungal associates of the rare tree *Hibiscadelphus giffardianus* were studied. Three of these (*Eutypella giffardiani*, *Thyridaria hawaiiensis*, and *Valsonectria macrospora*) are described and illustrated as new to science.

Key words — *Ascomycota*, Hawai'i, taxonomy

Introduction

Several unknown pyrenomycetous and loculomycetous fungi inhabiting dead branches of *Hibiscadelphus giffardianus* Rock (a Hawaiian endemic in the *Malvaceae*) were collected by J.D. Rogers in 2005; three of these species are described below. The Hawaiian Islands have the very high (90%) degree of endemism associated with an exceptionally diverse flora (Kim et al. 1998). For the mushrooms, 46 of 310 species are endemic Hawaiian taxa (Hemmes & Desjardin 2002). Other fungal groups (*Capnodiales*, *Chaetothyriales*, *Coronophorales*, *Corticiales*, *Diaporthales*, *Diversisporales*, *Dothideales*, *Erysiphales*, *Helotiales*, *Hymenochaetales*, *Meliolales*, *Microthyriales*, *Myriangiales*, *Phyllachorales*, *Pleosporales*, *Uredinales, Xylariales*) also have endemic Hawaiian representatives (Stevens 1925; Petrak 1952, 1953; Goos 1970; Sutton & Hodges 1983; Hodges & Gardner 1984; Hodges 1985; Barr & Hodges 1987; Gardner 1988, 1990, 1996; Goos & Uecker 1992; Koske & Gemma 1995; Gilbertson & Nakasone 2003; Rogers et al. 2003, 2006, 2007, 2008; Scholler & Aime 2006). We are not aware of any other reports of fungi on *Hibiscadelphus*. This paper contributes to a better understanding of the uniqueness of Hawaiian mycobiota.

Materials and methods

Microscopic analyses were carried out using standard techniques. Observations, measurements, and photographs of asci and ascospores were made using Zeiss Primo Star and Leica MZ75 microscopes, G10 and Canon Power Short S40 digital cameras, as well as AxioVision software. Photographs of stromata were taken using a Nikon D40x digital camera. Measurements of asci and ascospores were made in water mounts. Colors follow those of Rayner (1970).

Taxonomic descriptions

Eutypella giffardiani Lar.N. Vassiljeva & J.D. Rogers, **sp. nov.** Figs. 1A–B

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Stromata cortice erumpentia, valsoidea, gregaria, parte immersa nigro limitata, circular vel ellipsoidea, disco ostiolato nigro, 1–2 mm diam., praedita. Perithecia profunde immersa, monosticha, globosa, 300–350 µm diam.; collis leniter elongatis, sulcatis, 230–250 µm diam. Asci fasciculati, paraphysati, unitunicati, octospori, clavati vel fusoidei, membrana apicem versus incrassata, annulo apicali in liquore iodato Melzeri cyanescente, partibus sporiferis 25–35 × 4.5–5 µm, stipitibus 15–25 µm longitudine. Ascosporae unicellulares, allantoideae, irregulariter biseriatae vel conglobatae, subolivaceae, 6–8 × 1.7–2 µm.

Holotype: Hawai'i, Island of Hawaii, Hawaii Volcanoes National Park, Kipuka Puaulu (Bird Park), dead branches of *Hibiscadelphus giffardianus*, 3 November 2005, Jack D. Rogers (BISH). **Isotype:** VLA.

Stromata erumpent through the bark, valsoid, aggregated, bounded internally by a black zone line, circular to elliptical, with ostiolar disc 1–2 mm diam., surface black. Perithecia deeply embedded, globose, 300–350 µm diam.; perithecial necks somewhat elongate, sulcate, 230–250 µm diam. Asci in paraphysate fascicles, unitunicate, eight-spored, clavate or spindle-shaped, with tiny amyloid apical ring, p. sp. 25–35 × 4.5–5 µm, with stipes 15–25 µm long. Ascospores one-celled, overlapping and irregularly biseriate or crowded, allantoid, subolivaceous, 6–8 × 1.7–2 µm.

Comments—Numerous species of *Eutypella* have been described. They are difficult to differentiate, and the identification key by Rappaz (1987) offers the most useful information. Two ranges of ascospore average length (5–8 µm and 7–11 µm), which are repeated many times in the key, characterize large groups in the genus. The specimen from Hawaii falls into the group with smaller size range. The ascospore width correlates well with length, and almost all species with ascospores 5–8 µm long are narrower than 2 µm, whereas species with an ascospore length of 7–11 µm have ascospores wider than 2 µm (most often 2–2.5 µm).

As there are 21 species with an ascospore length of 5–8 µm (Rappaz 1987), it is important to find other differences to distinguish between them. Two species [*E. comosa* (Speg.) Rappaz, *E. leucaenae* Rehm] are rare exceptions that can

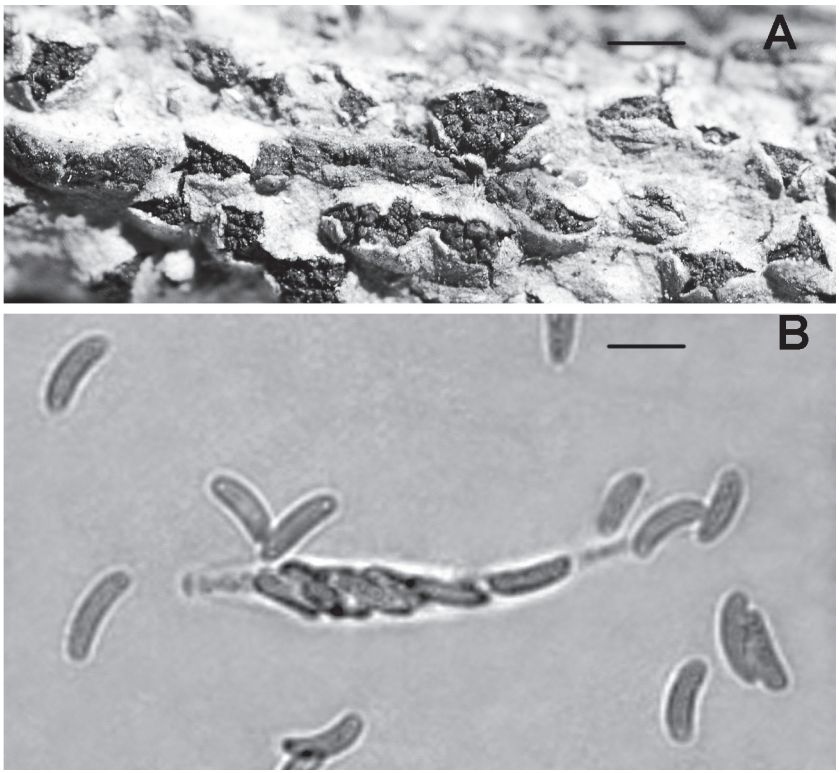

Fig. 1. *Eutypella giffardiani*. A. Stromata. B. Ascus and ascospores.
Scale bars: A = 1 mm; B = 6 µm.

be easily separated by ascospores that are more than 2 µm broad, and another species, *E. hunanensis* Rappaz, produces very narrow ascospores less than 1.2 µm wide. Six species [*E. arecae* (Syd. & P. Syd.) Rappaz, *E. bonariensis* Speg., *E. gliricidae* Rehm, *E. prunastri* (Pers.) Sacc., *E. sorbi* (Alb. & Schwein.) Sacc., *E. theobromicola* Wakef.] display a J-negative reaction of the ascal apical ring that contrasts with a J-positive one in the Hawaiian specimen.

Another character differentiating species of *Eutypella* is the size of the ostioles. *Eutypella androssowii* Rehm and *E. tetraploa* (Berk. & M.A. Curtis) Sacc. have ostioles with a diam of 100–150 µm and 100–180 µm, respectively, whereas 200 µm diam ostioles occur in *E. andicola* Speg., *E. atropae* (Mont.) Sacc., and *E. capensis* Rappaz; 150–200 µm diam ostioles are observed in *E. padina* (Nitschke) Nannf. and *E. sarcobati* Ellis & Everh. Ostioles in *E. extensa* (Fr.) Sacc. are 180–220 µm diam, compared to a diameter ~220–250 µm in the Hawaiian specimen. As such, they correspond to *E. alsophila* (Durieu & Mont.) Berk. in the group with the range in ascospore length of 5–8 µm. However,

E. alsophila falls into the group with 400–600 µm diam perithecia, not the group with 200–400 µm diam perithecia.

The Hawaiian specimen belongs to the group with 200–400 µm diam and the combination of the other diagnostic features — ascospore, ostiole, and perithecial sizes and the J-positive apical ring — corresponds to the sole remaining species, *E. kochiana* Rehm. There is no information about ostiolar size in *E. kochiana*, but it has smaller ascospores than the Hawaiian specimen. Although Rappaz's key (step 6) identifies the length ascospore group (5–7.5 µm) where *E. kochiana* appears to belong, the length range of its 4.5–6 µm diam ascospores does not overlap with that in the Hawaiian specimen.

Recently, *E. alsophila, E. arecae, E. comosa, E. gliricidae,* and *E. kochiana* were transferred to the re-instated genus *Peroneutypa* Berl. based on ascus morphology (Carmarán et al. 2006). The asci in this genus were described as urn-shaped but with a truncated apex and wider in the middle where ascospores tend to cluster. The apical portion has a thick wall and very small apical ring and lacks any channel. The asci in *Eutypella giffardiani* from Hawaii have a thick-walled apical region that is penetrated by a narrow channel with cytoplasmic strands connecting the apex with the ascus cytoplasm. This kind of ascus is considered to be typical of true species of *Eutypella* (Carmarán et al. 2006).

After the publication of Rappaz's (1987) monograph, several new species of *Eutypella* were described (Agarwal & Gupta 1988; Rajak et al. 1988; Ananthapadmanaban 1989), some of which form ascospores averaging 5–8 µm long (e.g., *E. pongamiae* G.P. Agarwal & S. Gupta). However, as information about the iodine reaction of the ascal apical ring and ostiole size is lacking, it is difficult to make a proper comparison. *Eutypella ceibae* R.C. Rajak et al. has a comparable ascospore length (4–8 µm), but a width of 2.5–6 µm is indicated (a very unusual range, and data on the ascal apical ring in asci and ostiolar size are also wanting).

Three other species from India — "*E. annonae-squamosae*" A. Pande, "*E. colebrookiae-oppositifoliae*" A. Pande, and *E. rozabaghensis* (Srinivas. & P.G. Sathe) A. Pande, which were originally described in *Quaternaria* Tul. & C. Tul. (Srinivasulu & Sathe 1970, Kale & Kale 1972) and later transferred to *Eutypella* either invalidly (as nom. nov.) or as comb. nov. (Pande 2008) — produce ascospores averaging 7–11 µm long and wider than 3 µm. Therefore, the Hawaiian specimen differs from them.

Thyridaria hawaiiensis Lar.N. Vassiljeva & J.D. Rogers, **sp. nov.** Figs. 2A–E

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Stromata valsoidea, mollia, cortice erumpentia, disco ectostromatico pulvinato vel hemisphaerico, castaneo, up to 1.5 mm diam. praedita, intus rubiginosa, ligno circa peritheciis nigra. Perithecia globosa, 300–400 µm diam.; ostiolis leniter papillatis, nigris. Asci longe clavati vel cylindrici, sessiles vel short-stipitati, 100–110 × 12–14 µm, juvenilis

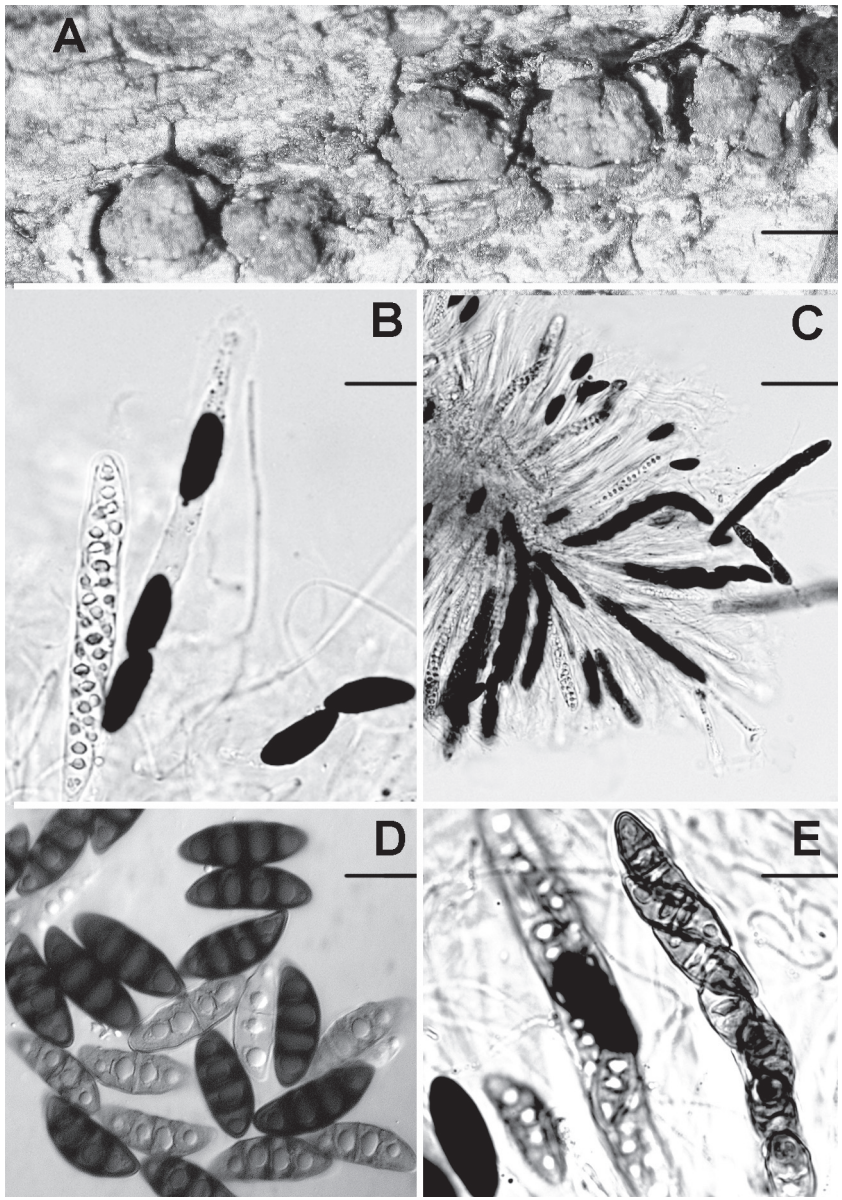

Fig. 2. *Thyridaria hawaiiensis*. A. Stromata. B–C, E. Asci and ascospores. D. Ascospores. Scale bars: A = 1 mm; B = 15 µm; C = 50 µm; D,E = 12 µm.

crasse tunicati; paraphysibus numerosis, hyalinis, longissimis, sinuosis, ca. 1 µm latis. Ascosporae uni- vel biseriatae, ellipsodeae vel fusoideae, rectae, inaequilateralia vel leniter cirvatae, 3-septatae, interdum leniter constrictae, pallide- vel atro-fuscae, etiam opacae, levigatae, (19–)20–25(–27) × 6–9 µm.

Holotype: Hawai'i, Island of Hawaii, Hawaii Volcanoes National Park, Kipuka Puaulu (Bird Park), dead branches of *Hibiscadelphus giffardianus*, 3 November 2005, Jack D. Rogers (BISH). **Isotypes:** VLA; WSP.

Stromata valsoid, soft, erumpent from the bark with pulvinate or hemispherical, chestnut (40) ectostromatic 'disc' up to 1.5 mm diam., consisting of confluent and conspicuous tops of perithecial necks with slightly papillate, black ostioles, bay (6) or rust (39) under the surface, black around perithecia deep in the wood. Perithecia globose, 300–400 µm diam. Asci long clavate to cylindrical, sessile, 100–110 × 12–14 µm, thick-walled when young, surrounded by numerous, hyaline, long, sinuous and anastomosing paraphyses about 1 µm wide. Ascospores overlapping uniseriate or irregularly biseriate, ellipsoid-fusoid, straight to inequilateral or slightly curved, 3-septate, not at all or slightly constricted at the septa, light to dark brown, even opaque, smooth, (19–)20–25(–27) × 6–9 µm.

Comments— *Thyridaria hawaiiensis* resembles the type of the genus, (*T. incrustans* Sacc.) in ascospore shape, septation, and size, but the latter has a black, carbonaceous disc that is only covered "with a yellow-green pulverulence or pubescence when young" (Wehmeyer 1941). Also, the brown-black entostroma of *T. incrustans* was said to turn reddish in KOH. The stromata of *T. hawaiiensis* are rusty inside, chestnut-colored on the outside, and do not react to KOH.

An earlier name [i.e., *Thyridaria broussonetiae* (Sacc.) Traverso] exists for *T. incrustans* (Barr 1990), which is characterized by ascospores with verruculose walls and apical pores of the perithecia that are bright yellowish to orange pigmented, but the color of the whole stromata is not reported. The apical pore pigmentation is more similar to that found in some *Byssosphaeria* species that were placed together with *Thyridaria* in the *Melanommatales* (Barr 1990). The smooth ascospores in *T. hawaiiensis* distinguishes it from the verruculose spored *T. broussonetiae*.

Another endemic Hawaiian plant, *Acacia koa* A. Gray, was reported to support a separate *Thyridaria* species, namely *T. koae* Petr. characterized by smaller ascospores—14–21(mostly 18) × 6–9 µm (Petrak 1952).

Valsonectria macrospora J.D. Rogers & Lar.N. Vassiljeva, **sp. nov.** Figs. 3A–D

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Stromata cortice erumpentia, pulvinata, 1–2 mm diam, gregaria, lignose ubi siccata vel mollia ubi madefacta, extus initio aurantiaca demum castanea, intus aurantaca sine pigmento in KOH. Perithecia 0.2–0.3 mm diam, ambitus distinca vel indistincta,

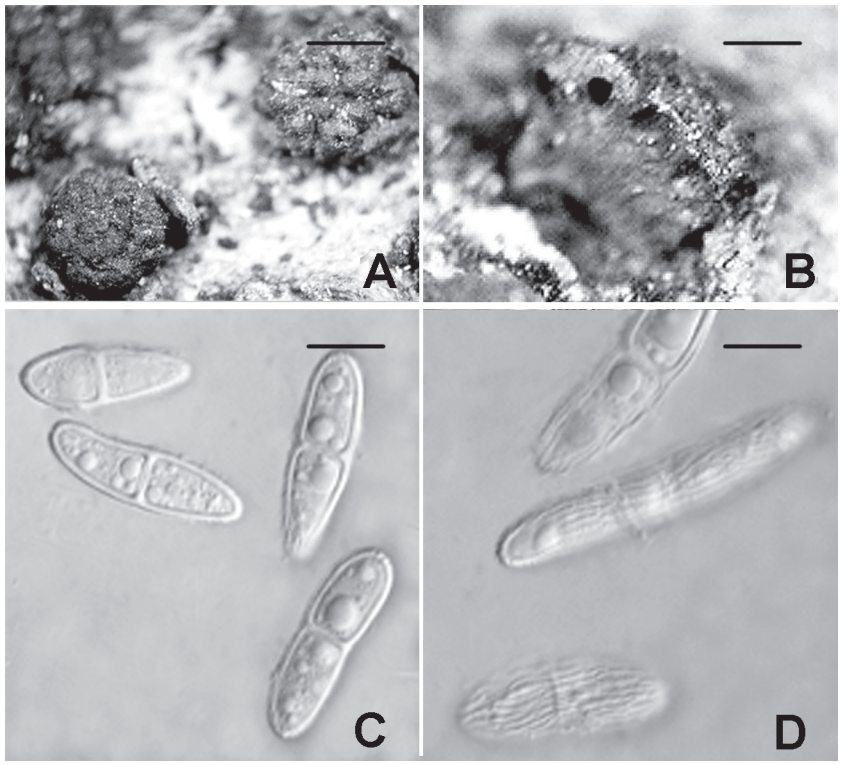

Fig. 3. *Valsonectria macrospora*. A–B. Stromata. C–D. Ascospores.
Scale bars: A = 1mm; B = 0.5 mm; C, D = 10 μm.

monosticha. Ostiola papillata, minuta. Asci octospori, ascosporis irreguleriter dispositis, ca. 100 μm longitudine tota, ca. 18 μm crassi, partibus sporiferis ca. 91 μm longitudine, annulo apicali nullo. Asci infrequenter observatis, probliter deliquesentibus. Ascosporae hyalinae,pariter bicellulares, ellipsoideae vel aliquantum inequilaterales, striis longitudinalibus ornatae, 28–31(–46) × 9–12(–15) μm, sine poro vel rima germinationis. Paraphyses vel pseudoparaphyses apparenter nullae.

Holotype: Hawai'i, Island of Hawaii, Hawaii Volcanoes National Park, Kipuka Puaulu (Bird Park), dead branches of *Hibiscadelphus giffardianus,* 3 November 2005, Jack D. Rogers (BISH).

Stromata erumpent from bark, pulvinate, 1–2 mm diam, gregarious, woody when dry, soft when wet, surface at first orange (7) becoming chestnut (40), interior orange (7), not releasing a pigment in KOH. Perithecia 0.2–0.3 mm diam with contours distinct or obscure, monostichous, ostioles papillate, minute. Asci eight-spored, the ascospores jumbled, ca. 100 μm total length, 18 μm broad, spore-bearing part ca. 91 μm long, with apex not bluing in Melzer's iodine reagent. Asci infrequently observed, probably deliquescent. Ascospores

hyaline, equally 2-celled, ellipsoid to somewhat inequilateral, ornamented with longitudinal striations, 28–31(–46) × 9–12(–15) µm, without germination pore or slit. Hamathecial elements not seen.

Comments—The material described herein, although abundant, seems overmature. It is included here because the ascospore average is much longer and broader than any other described *Valsonectria* species (Rossman et al. 1999). The striate ornamentation as observed by light microscopy (Figs. 3D) are, in reality, ribs when seen by SEM. Asci, which are not frequently encountered, appear to be unitunicate and are probably deliquescent. Hamathecial elements appear to be absent, but this might be due to the apparent overmaturity of the material. Our late colleague, Margaret Barr, examined our material and believed it to be a *Valsonectria*. It is noteworthy, however, that several species first described in *Valsonectria* have been transferred to *Valsaria* (Ju et al. 1996; Rossman et al. 1999). It is likewise noteworthy that none of the species of *Valsonectria* recognized by Seifert & Samuels (1997) has a valsoid arrangement of perithecia. Consequently, the name *Valsonectria* actually implies a feature that is not extant.

Acknowledgments

We thank Don Hemmes, Hilo, for obtaining permission for JDR to collect in Hawaii Volcanoes National Park, for his companionship, for identifying hosts, and for other aid that furthered these studies. We are also grateful to Dean A. Glawe, Washington State University, and Steven L. Stephenson, University of Arkansas, for their helpful comments to this paper.

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