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FULL PAPER

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Dwiroopa, a coelomycetous genus with two species

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Abstract The coelomycete *Dwiroopa* was determined to be the correct genus for *Harknessia lythri*, and a new combination is made for this species. The type species of *Dwiroopa*, *D. ramya*, is redescribed and illustrated based on the type specimen for which a lectotype is designated. A key to the two species of *Dwiroopa* is presented along with a discussion of their similarities and differences. The genus *Dwiroopa* is distinguished from *Harknessia* by conidial characteristics. In *Dwiroopa* the macroconidia have widely spaced, longitudinal slits around the conidia and lack a basal appendage, whereas in *Harknessia* the macroconidia are smooth or have closely spaced, longitudinal slits on only one side of the conidia and often have a true basal appendage. Both *Dwiroopa* and *Harknessia* are included in the Diaporthales.

Key words Diaporthales · *Harknessia* · Lectotype · Ornamentation · Slits

Introduction

Recently, a new species of *Harknessia* Cooke was described and illustrated as *H. lythri* D.F. Farr & Rossman (2001) that had been isolated from the noxious weed, purple loosestrife (*Lythrum salicaria* L., Lythraceae). Although similar to *Harknessia*, this coelomycetous species has peculiar, widely spaced, longitudinal slits around the macroconidia that are unlike the longitudinal, closely spaced striations or pallid bands present on macroconidia of species of *Harknessia* (Nag Raj 1993). Despite these differences *Harknessia* appeared to be the only genus that could accommodate *H. lythri*. After *H. lythri* was described, the monotypic genus *Dwiroopa* Subram. & Muthumary (1986) came to our attention in which the type species, *D. ramya* Subram. & Muthumary, has longitudinal slits on the macroconidia similar to those of *H. lythri*. One of the two portions of the type collection of *D. ramya* was examined and *H. lythri* was determined to be congeneric with that species. Molecular data published by Castlebury et al. (2002) also suggest that *H. lythri* is not congeneric with the type and a second species of *Harknessia*. Rather, *H. lythri* belongs in *Dwiroopa* and is transferred herein to that genus. In this article, the type species of *Dwiroopa*, *D. ramya,* is redescribed and illustrated. *Dwiroopa* is recognized as a genus distinct from *Harknessia* in the Diaporthales and the two species of *Dwiroopa* are compared.

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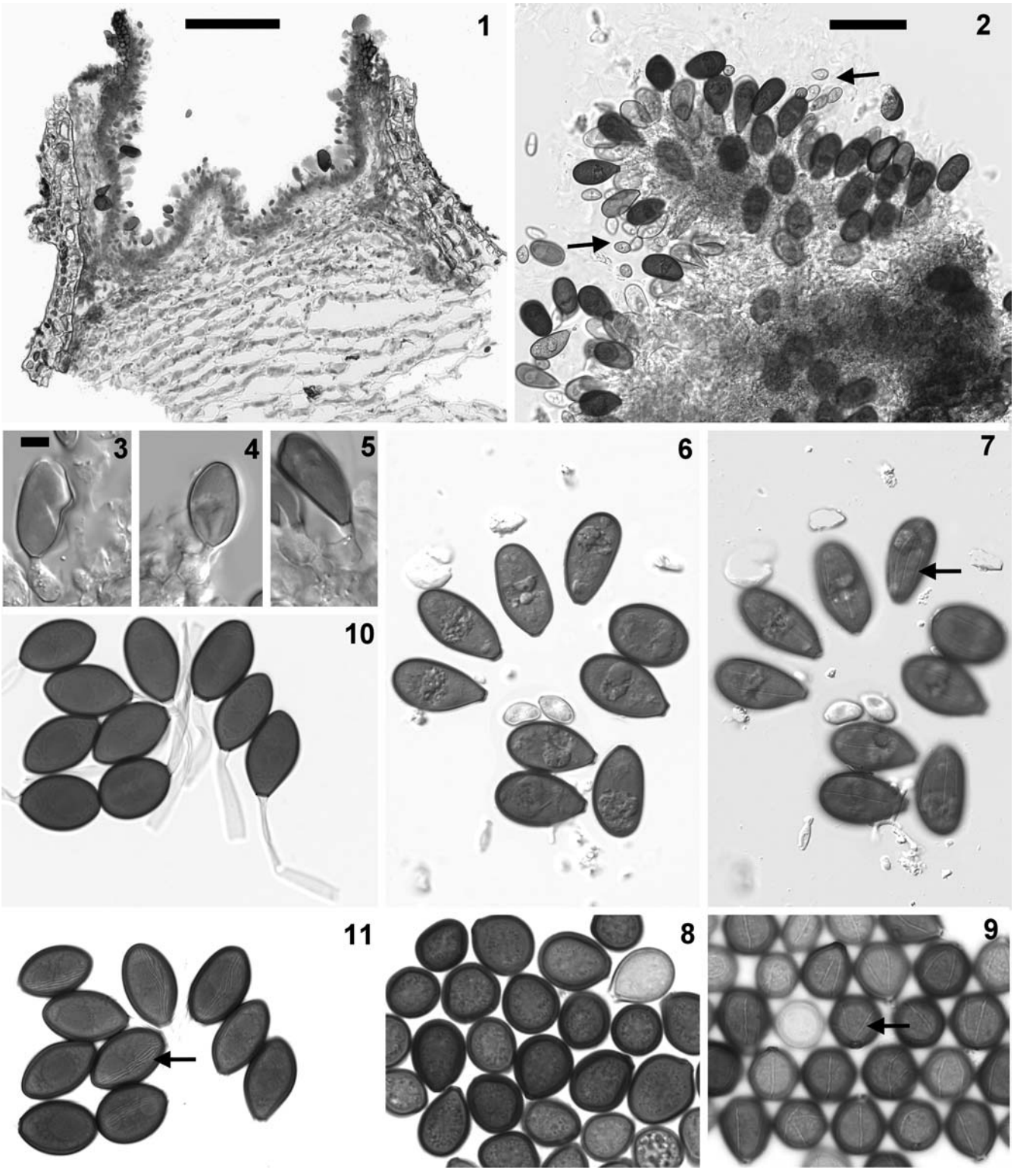
Materials and methods

For microscopic examination, material was rehydrated and mounted in 3% KOH. Conidiomata were sectioned at ~10 μm thick using a freezing microtome. Sections were mounted in lactic acid with cotton blue. Observations of microscopic features were made using a Zeiss Axioplan 2 microscope with bright-field illumination. Photographs and measurements of microscopic features were taken using a Spot 2 digital camera (Diagnostic Instruments, Sterling Heights, MI, USA) and ImagePro software (Media Cybernetics, Silver Spring, MD, USA). Specimens examined are listed following the description.

Taxonomy

Dwiroopa ramya Subram. & Muthumary, Proc. Indian Acad. Sci. (Plant Sci.) 96: 196, 1986. Figs. 1–7

Conidiomata immersed to semiimmersed, becoming erumpent, solitary, scattered, stromatic, brown to black, nonostiolate, with irregular opening, multiloculate,



Figs. 1–11. *Dwiroopa ramya, D. lythri*, and *Harknessia eucalypti*. **1–7** *D. ramya* IMI 255137 (lectotype). **1** Longitudinal section of conidiomata. **2** Squash mount of conidiogenous cells with developing macroconidia and mesoconidia. Mesoconidia indicated by *arrows*. **3–5** Immature macroconidia still attached to conidiogenous cells. **6** Median view of mature macroconidia. **7** Surface view of mature macroconidia showing widely spaced, longitudinal slits indicated by *arrow*. **8, 9** *Dwiroopa lythri* BPI 747560 (holotype). **8** Median view of mature macroconidia. **9** Surface view of mature macroconidia showing widely spaced, longitudinal slits indicated by *arrow*. **10, 11** *H. eucalypti* BPI 842225. **10** Median view of mature macroconidia with long basal appendage. **11** Surface view of mature macroconidia showing closely spaced, longitudinal slits indicated by *arrow*. *Bars* **1**, **2** 50 μm; **3–11** 10 μm

500–700 μm diameter. Conidiophores absent. Macroconidiogenous cells 8–11 × 5–7 μm, rectangular, ampulliform, not branched. Conidial ontogeny holoblastic. Macroconidia 21.2–26.9 μm ($\bar{x}$ = 24.0, SD 1.5, n = 48) × 12.0–17.2 μm ($\bar{x}$ = 14.6, SD 1.22), brown to dark brown, obovoid to ovoid-oblong, nonseptate; wall up to 1 μm thick, with six to ten longitudinal slits, most of which extend from the base to the apex; apex broadly rounded; base truncate with a short flange. Mesoconidia 7.6–12.7 μm ($\bar{x}$ = 10.5, SD 1.4, n = 20) × 3.4–5.9 μm ($\bar{x}$ = 5.3 μm, SD 0.74), pale brown, ellipsoidal, nonseptate; apex rounded; base truncate. Microconidia not observed.

Specimen examined: India, Karnataka State, Agumbe, on branch of unknown tree, Oct. 31, 1979, coll. D. Jayarama Bhat (IMI 255137). In the original publication, portions of the type collection were placed in two different herbaria, but neither one was designated as the holotype. Thus, IMI 255137 is herein designated as the lectotype of *D. ramya*. A second portion of the type collection listed as MUBL 2870 was not examined.

Based on an examination of the lectotype specimen of *D. ramya*, it was determined that *H. lythri* should be placed in the genus *Dwiroopa* as follows: *Dwiroopa lythri* (D.F. Farr & Rossman) D.F. Farr & Rossman, comb. nov. Basionym: *Harknessia lythri* D.F. Farr & Rossman, Mycologia 93: 997, 2001. Conidiomata and cultural characteristics are described in Farr and Rossman (2001).

Diagnostic characteristics of *D. lythri* illustrated in Figs. 8 and 9 are as follows: macroconidia 10.6–18.5 μm ($\bar{x}$ = 14.7, SD = 1.5, n = 156) × 8.9–15.4 μm ($\bar{x}$ = 11.6, SD = 1.25), brown to dark brown, subglobose to irregularly ellipsoidal, nonseptate; wall up to 1.5 μm thick, with two to seven widely spaced longitudinal slits extending from the apex to the base; base truncate, with a short flange, occasionally with a short basal appendage. Mesoconidia and microconidia not observed.

Specimen examined: St. Paul, MN, USA, on *Lythrum salicaria* in greenhouse, 1996, coll. E.J. Katovich (holotype BPI 747560).

Both *Dwiroopa ramya* and *D. lythri* were compared with a specimen of the type species of *Harknessia, H. eucalypti* Cooke, as illustrated in Figs. 10 and 11.

Specimen examined of *Harknessia eucalypti*: Scripps Ranch, San Diego Co., CA, USA on newly fallen leaves, Feb. 21, 2003, coll. Payam Fallah and Magzoub Ismail (BPI 842225).

Key to species of *Dwiroopa*

1. Macroconidia greater than 20 μm long, obovoid to oval-oblong; mesoconidia present, often intermixed with macroconidia ..... *D. ramya*
1. Macroconidia less than 20 μm long, subglobose to irregularly ellipsoidal; mesoconidia not observed. ... *D. lythri*

Discussion

The two species of *Dwiroopa* are similar in conidiomatal features, conidiogenesis, and conidial characteristics; however, *D. ramya* is easily distinguished from *D. lythri* by macroconidial size and shape. The macroconidia of *D. ramya* are primarily ovoid, 21–27 × 12–17 μm, whereas in *D. lythri* the macroconidia are subglobose to ellipsoid, 11–19 × 9–15 μm, smaller than those of *D. ramya*. Conidiogenesis is holoblastic in both species of *Dwiroopa*. The macroconidiogenous cells are similar in size and shape in the two species of *Dwiroopa.* Annellides are present on macroconidiogenous cells in *D. lythri* but were not observed in *D. ramya*. The large conidia produced by *D. ramya* were termed α-conidia by Subramanian and Muthumary (1986). These conidia are analogous to the macroconidia in *D. lythri* and species of *Harknessia*; thus, the terminology used here is consistent with Nag Raj (1993). *Dwiroopa ramya* also produces two kinds of smaller conidia, herein termed mesoconidia and microconidia. In *D. ramya* the mesoconidia are 7.6–12.7 × 3.4–5.9 μm, hyaline to pale brown, ellipsoidal conidia intermixed with the large dark brown macroconidia. The mesoconidial ontogeny appears to be holoblastic with conidiogenous cells similar to those producing the macroconidia (see Fig. 2; also illustrated in Subramanian and Muthumary 1986). Although termed β-conidia by Subramanian and Muthumary (1986), we refer to these as mesoconidia because they are unlike the filiform β-conidia that occur in *Phomopsis* (Sacc.) Bubák (see figs. 13, 14 in Castlebury et al. 2003). According to the original description (Subramanian and Muthumary 1986), *D. ramya* also produces a third kind of conidia called phialoconidia. Based on the illustrations in Subramanian and Muthumary (1986), these appear to be comparable to the microconidia occurring in some species of *Harknessia*, e.g., *H. antarctica* Speg., *H. arctostaphyli* Cooke & Harkn., and *H. gharsei* Golatkar (Nag Raj 1993). Microconidia were not observed by the authors in the type specimens of *D. ramya* or *D. lythri*. Paraphysis-like structures were described for *D. ramya*; these were not observed in the lectotype specimen. Such structures are lacking in *D. lythri* and are not known in any species of *Harknessia*. The conidiomata of *D. ramya* and *D. lythri* are variable in size and structure, ranging from uni- to multilocular. This variability was especially evident in conidiomata of *D. lythri* which extends from uniloculate, 75–90 μm diam on natural substratum to multiloculate, up to 1200 μm diameter on agar media (Farr and Rossman 2001). Neither *Dwiroopa ramya* nor *D. lythri* is known from other than their respective type collections.

The genus *Dwiroopa* is morphologically similar to *Harknessia* in producing dark brown, nonseptate, thick-walled macroconidia that are generally more than 10 μm long (see Figs. 10, 11). In both genera conidia are produced by means of holoblastic ontogeny with each conidiogenous cell having periclinal thickenings. Conidiophores are lacking. As already discussed, some species of *Harknessia* produce microconidia as described for *Dwiroopa ramya* as phialoconidia (Subramanian and Muthumary 1986). Both

Dwiroopa and *Harknessia* are characterized by solitary, uni- to multiloculate, thin-walled conidiomata that are initially immersed in the substratum, eventually becoming erumpent (see Fig. 1).

The genus *Dwiroopa* is distinguished from *Harknessia* by conidial characteristics. In *Dwiroopa* the macroconidia have widely spaced longitudinal slits around the conidia and lack a basal appendage (Figs. 6–9), whereas in *Harknessia* the macroconidia are smooth or have closely spaced longitudinal slits on only one side of the macroconidia and often have a true basal appendage (Figs. 10, 11). Some species of *Harknessia* including the type species, *H. eucalypti*, have longitudinal slits on the conidia, but, if present, these slits are almost always close together on only one side of the macroconidium. Exceptionally, one species of *Harknessia, H. gharsei,* has longitudinal bands that are evenly spaced around the conidium (Nag Raj 1993). Macroconidia of most species of *Harknessia* have a basal appendage that may vary considerably in length although it may also be absent (Nag Raj 1993). Although both species of *Dwiroopa* occasionally have a frill or flange at the base of each macroconidium, these tend to be short or lacking and are interpreted as a remnant of the macroconidiogenous cell rather than a true basal appendage.

The genera *Harknessia* and *Dwiroopa* are anamorphic fungi that are derived from within the Diaporthales, as shown by Castlebury et al. (2002), who provided an overview of that order based an analysis of nuclear large subunit ribosomal DNA sequences. In Castlebury et al. (2002), *Dwiroopa lythri* (as *H. lythri*) did not cluster with a group that included the type species of *Harknessia, H. eucalypti*, and a second species of *Harknessia, H. molokaiensis* Crous & J.D. Rogers (listed as its sexual state, *Wuestneia molokaiensis* Crous & J.D. Rogers). These molecular data support excluding *D. lythri* from *Harknessia*. Living material of *D. ramya* is not available for use in molecular studies.

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References

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002) A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences. Mycologia 94:1017–1031

Castlebury LA, Farr DF, Rossman AY, Jaklitsch W (2003) *Diaporthe angelicae* comb. nov., a modern description and placement of *Diaporthopsis* in *Diaporthe*. Mycoscience 44:203–208

Farr DF, Rossman AY (2001) *Harknessia lythri*, a new species on purple loosestrife. Mycologia 93:997–1001

Nag Raj TR (1993) Coelomycetous anamorphs with appendage-bearing conidia. Mycologue, Waterloo

Subramanian CV, Muthumary J (1986) *Dwiroopa*, a new genus of the Coelomycetes. Proc Indian Acad Sci (Plant Sci) 96:191–197