Mycologia, 87(5), 1995, pp. 678–683.

Scutellospora hawaiiensis: A new species of arbuscular mycorrhizal fungus from Hawaii

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Abstract: *Scutellospora hawaiiensis*, a species common in coastal sand dunes of Hawaii, is described. The pale orange-brown to red-brown spores have six walls; an outermost unit wall (1.2–2.0 μm thick) appressed to a laminated wall (0.8–2.2 μm), a coriaceous wall (2.8–4.8 μm), a new type of wall (the "notching wall") (0.5–1.6 μm), whose broken edges consist of a series of rectangular and V-shaped notches, resembling a torn linen cloth, a coriaceous wall (2.0–3.3 μm) and a thick amorphous wall (3.0–4.0 μm, expanding up to 88 μm when crushed in acidic mountants).

Key Words: Gigasporaceae, Hawaii, sand dunes, *Scutellospora*

Examination of soil samples from sand dunes on the islands of Hawaii and Kauai (Hawaiian Islands) revealed an undescribed species of *Scutellospora* (Gigasporaceae) with spores possessing an unusual type of wall that had not previously been recognized in spores of arbuscular mycorrhizal fungi. The new wall type fractures readily to produce a margin that resembles torn linen. Spores were recovered by wet-sieving and water and sucrose centrifugation (Walker et al., 1982). Wall descriptions and observations are from specimens mounted in a polyvinyl alcohol/lactic acid/glycerol (PVLG) mounting medium (Koske and Tessier, 1983) or in Melzer's reagent. Wall descriptions and terminology are from Walker (1983, 1986) and Morton (1986). All dimensions are based on analysis of ca 300 spores. A holotype has been deposited in the herbarium of Oregon State University (OSC), and isotypes have been deposited at the Farlow Herbarium, the Bishop Museum (Honolulu, Hawaii), and at the herbarium of the National Tropical Botanical Garden (PTBG).

Accepted for publication April 28, 1995.

Scutellospora hawaiiensis Koske et Gemma, sp. nov. FIGS. 1–12

Sporae in solo singillatim in base bulbosa terminalites vel subterminalites efformatae, subglobosae vel irregulares, (200–)240(–300) × (180–)230(–290) μm, pallidae vel obscure aurantio-brunneae. Tunica sporae stratis sex in turmis tribus. Turma externa stratis duobus: stratum externum laeve vel parum asperum, aurantio-brunneum vel atrofuscum, 1.2–2.0 μm crassum; stratum internum lamellatum, hyalinum vel luteolum, 0.8-2.2 μm crassum. Turma intermedia stratis duobus: stratum externum hyalinum, coriaceum, 2.4-4.8 μm crassum; stratum internum hyalinum, 0.5–1.6 μm crassum. Turma interna stratis duobus: stratum externum hyalinum, coriaceum, 2.0–3.3 μm crassum; stratum internum hyalinum, amorphum, 3.0–4.0 μm crassum, in solutione Melzeri violascens. Basis bulbosa 50–65 μm crassa. Cellulae auxilares cinnamomeae vel atrocastaneae, singulares vel fasciculatae, 40–55 μm crassum.

Spores formed singly in the soil, terminally or subterminally on a bulbous base; translucent, pale orange-brown to dark orange-brown; subglobose to irregular; (200–)240(–360) × (180–)230(–290) μm. Spore wall structure consisting of six walls (1–6) in three wall groups (A, B, C) (murograph, FIG. 1). Group A composed of walls 1 and 2. Wall 1 an orange- to red-brown unit wall, smooth to slightly roughened, 1.2-2.0 μm thick, closely appressed to wall 2; wall 2 minutely laminated, hyaline to pale yellow, 0.8–2.2 μm thick. Group B composed of walls 3 and 4. Wall 3 hyaline, coriaceous, 2.8–4.8 μm thick; wall 4 hyaline, 0.5–1.6 μm thick, and fracturing readily to produce margins that consist of a series of rectangular and V-shaped notches and have the appearance of torn linen fabric. Group C composed of walls 5 and 6. Wall 5 hyaline, coriaceous, 2.0–3.3 μm thick, fused to wall 6; wall 6 hyaline, amorphous, 3–4 μm thick, up to 25 (–88) μm thick when crushed, staining reddish-purple in Melzer's reagent. Germination shield ellipsoidal, lobed, 40–60 × 60–80 μm, with a simple margin, concolorous with spore. Sporogenous base yellow-brown to orange-brown, 50–65 μm broad, with walls measuring 1–4 μm thick, thickest near base of spore. Auxiliary cells dark orange-brown to red-brown, 40–55 μm broad, smooth or ornamented with knobby projections, formed singly or in loose clusters of four to six at the ends of coiled hyphae.

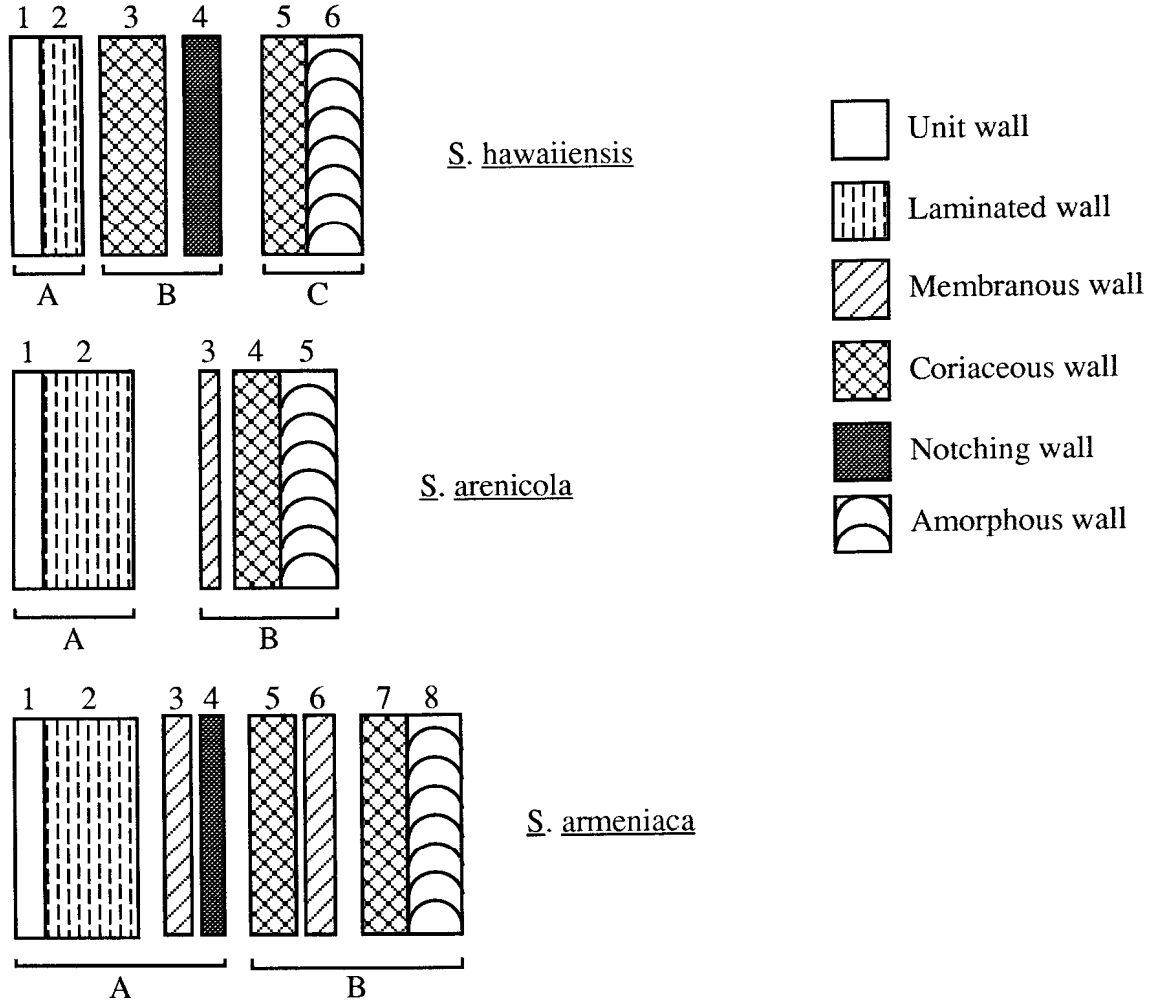


FIG. 1. Murographs of spores of *Scutellospora hawaiiensis, S. arenicola,* and *S. armeniaca.* The murograph of *S. armeniaca* differs from the original description in possessing an additional wall in position 4.

Distribution and habitat. Known only from coastal sand dunes in the Hawaiian Islands.

Mycorrhizal associations. Associated in the field with roots of *Cocos nucifer* L., *Dodonaea viscosa* Jacq., *Ipomoea pes-caprae* (L.) R. Br. subsp. *brasiliensis* (L.) Ooststr., *Leucaena leucocephala* (Lam.) de Wit, *Scaevola sericea* Vahl, *Sida fallax* Walp., *Sporobolus virginicus* (L.) Kunth, *Verbesina encelioides* (Cav.) Benth. & Hook., *Vigna marina* (J. Burm.) Merr., and *Vitex rotundifolia* L. fil. Forming arbuscular mycorrhizae in pot culture with *Sorghum sudanense* Piper.

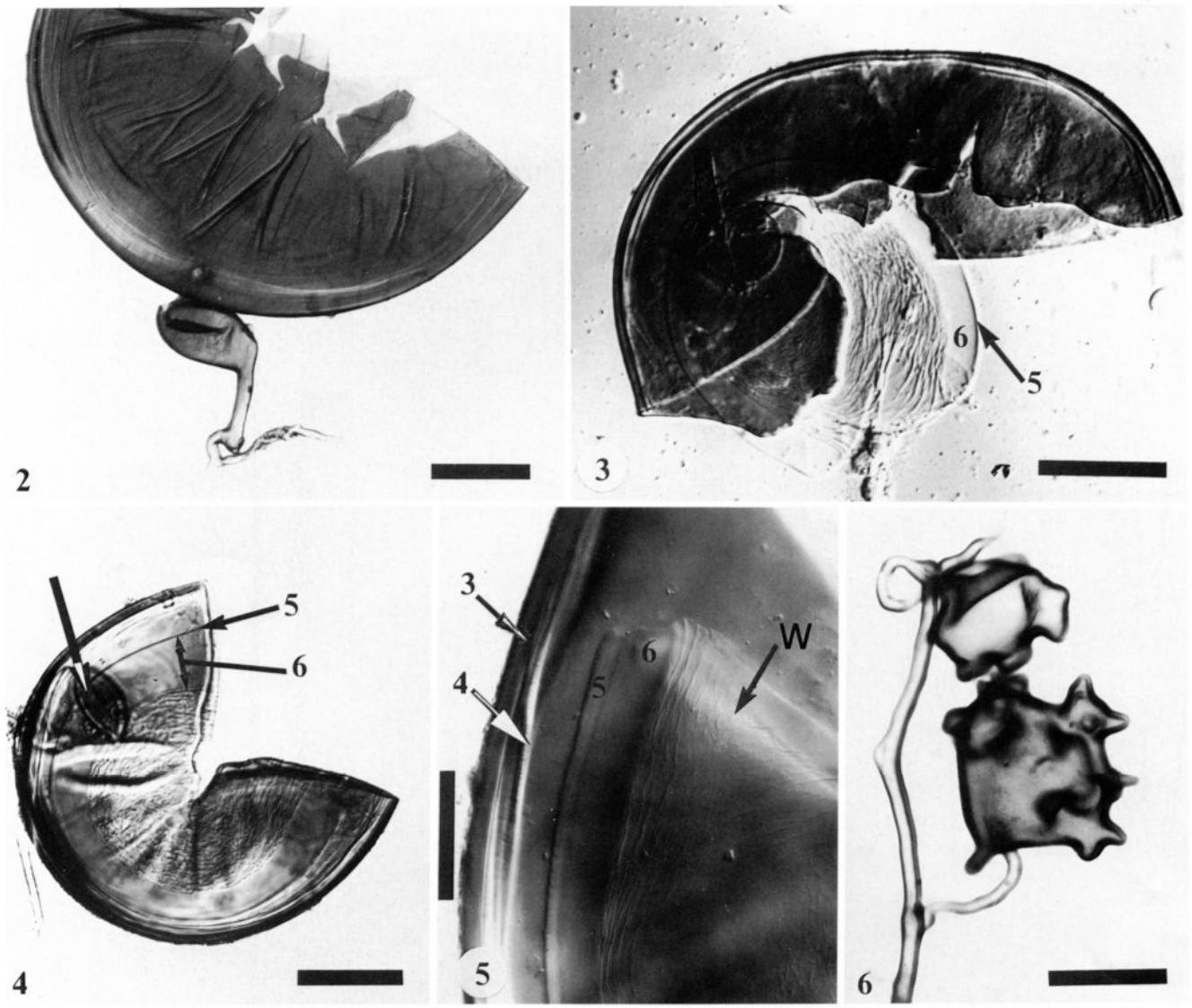
Collection sites. HAWAII: Hawaii Island: coastal sand dunes at Kuka'ilimoku Pt. (old Kona airport), Hapuna Beach State Park, Punalu'u Beach, Kalapana, and Kaimu; Kauai Island: coastal sand dunes at Polihale State Beach, Kapa'a Beach, Hanapepe Beach, Waioli Beach, and Makahu'ena Bay Beach; recovered from trap cultures of coastal sand dune soil collected from Polihale State Park.

Etymology. Referring to its occurrence in the Hawaiian islands.

HOLOTYPE. USA. HAWAII: Kauai County, Makahu'ena Bay Beach near Mahalepu, among roots of *Sporobolus virginicus,* 27 May 1989, *K & G 908,* (OSC). ISOTYPES: FH, BIS, PTBG.

PARATYPES. HAWAII: Hawaii Island: 17–18 July 1984, *Koske 803, 807, 809, 810, 815, 816*; 19–22 July 1985, *Koske 820-828*; 16 Nov. 1987, *K & G 619*; Kauai Island: 12–14 July 1987, *K & G 139, 152, 163*; 23–27 May 1989, *K & G 836, 837, 839, 840, 841, 869, 873, 897, 898, 899, 900, 905, 906, 910*; 20 Feb. 1990, *K & G 1174*; 12 March 1990, *K & G 1260, 1261, 1263.*

Scutellospora hawaiiensis is distinguished from other species by its color and the distinctive spore wall structure, especially the presence of the "notching wall" (wall 4) with its unusual pattern of fracturing. The notching wall is characterized by the regular, angular appearance of the margin of the wall in crushed spores (FIGS. 7–10) The margin consists of a series of rectangular and V-shaped notches with many 90° angles. When this wall is viewed on edge where it has folded in crushed spores, the notching nature is manifested

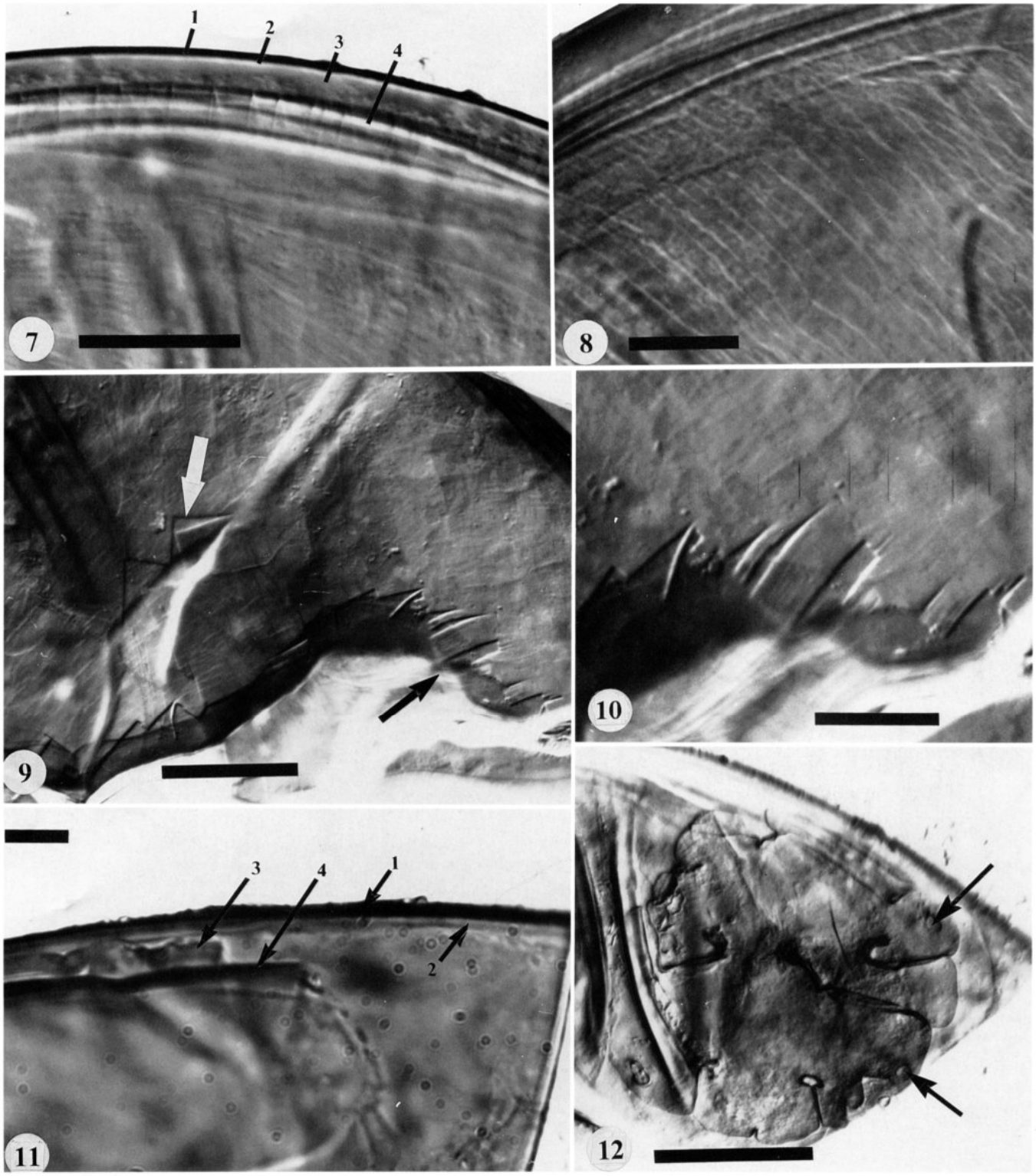


FIGS. 2–6. *Scutellospora hawaiiensis*. 2. Crushed spore with a subterminally attached sporogenous cell. Approximately half of the spores possess this type of cell, and the others have a terminal attachment. Coarse wrinkles result from the folding of wall 5, a thick coriaceous wall. Bar = 100 μm. 3. Crushed spore with wall group C extruded. Note wall 5 and the thick amorphous wall (wall 6). The inside of wall 6 is highly wrinkled. Bar = 100 μm. 4. Crushed spore with walls 5 and 6 remaining inside the outer walls. Note extensive spreading of wall 6 in this specimen. Dark object (arrow) is the sporogenous cell. Bar = 100 μm. 5. Walls in a crushed spore. Walls 1 and 2 are not clear in this photograph. Wall 4 is visible as a bright line with radial cracks. Wall 6 is thick and shows the wrinkling inner surface ("W") well. Bar = 50 μm. 6. Auxiliary cells on coiled hyphae. Bar = 20 μm.

as radial fissures (FIG. 7). When viewed in face view in crushed spores, the surface of the notching wall shows wrinkle-like tears and splits that have the appearance of marble (FIG. 8).

Another characteristic of the notching wall is evident when spores are crushed less forcefully (so that the notching of wall 4 illustrated in FIG. 8 does not occur), and the components of the wall separate partially into regions that are thick and thin. The surface of the wall then has an irregular cross-hatched appearance in face view (FIGS. 9, 10). A fibrillar component to the notching wall is suggested by the appearance of the fraying of the edge of the wall and the striations that are visible in face view of the wall (FIG. 10).

Wall 1 possesses most of the pigment that colors the spore. The notching wall (wall 4) typically is separated from wall 1 by a distance of 2-8 μm in crushed spores, a zone that is occupied by two walls (walls 2 and 3) that are sometimes very difficult to observe (FIG. 8). In many spores, walls 2 and 3 appear more as a gap between the more darkly pigmented outermost wall



FIGS. 7–12. Spores of *Scutellospora hawaiiensis*. 7. Walls 1–4 are indicated. Notice how wall 3 can be interpreted as a space between walls 2 and 4 rather than as a discrete wall. Note radial cracks in the notching wall (wall 4) where it is folded over in the crushed spore. The folding of this wall makes it appear as three light-colored lines in the photograph. Only the outermost fold is indicated. Bar = 20 μm. 8. Face view of wall 4. Light-colored wavy lines are tears that develop in this wall when spores are crushed. Bar = 10 μm. 9. Margin of wall 4 in crushed spore showing characteristic fracturing. Note the shredding pattern (dark arrow) that suggests a fibrillar nature to the wall, and the right angle notching (light arrow) that indicates its brittle nature. Bar = 40 μm. 10. A higher magnification of the shredding pattern seen in Fig. 9. Note the checkerboard appearance of this wall. Bar = 20 μm. 11. Spores walls 1–4. Note the unusual nature of wall 3 in this specimen. Bar = 10 μm. 12. Germination shield. Germ tubes arising from this shield have broken off, leaving circular scars (two are indicated with arrows). Bar = 60 μm.

(wall 1) and the notching wall (wall 4) (FIGS. 7, 8). Wall 2 is firmly adherent to wall 1 and is finely laminated and lightly colored. Wall 3 is translucent and typically stays near the laminated wall. In some specimens, the elastic nature of wall 3 is apparent where it has retracted from the broken margin of the spore (FIG. 11). Associated with (but not adherent to) wall 3 is the notching wall.

The inner wall group of spores of *S. hawaiiensis* includes a coriaceous wall (wall 5) bound to an amorphous wall (wall 6) (FIGS. 2–4). The coriaceous wall occasionally is wrinkled on its outer surface, suggesting a very thin membranous wall. However, a separable membranous wall was not seen in more than 600 spores examined from the field and from pot culture. The amorphous wall often contains a surprising amount of material that expands when crushed in acidic mountants (FIG. 2). The thickness of this wall when crushed typically is 10–30 μm, but in some specimens it may be nearly 90 μm thick. The innermost side of the amorphous wall wrinkles markedly in crushed spores (FIGS. 2–5), and the wall stains reddish-purple rapidly in Melzer's reagent.

Spores of *S. hawaiiensis* are most likely to be confused with those of four other yellow-brown to red-brown spored species, *S. armeniaca* Błaszkowski, *S. arenicola* Koske & Halvorson, *S. castanea* Walker, and *S. erythropa* (Koske & Walker) Walker & Sanders. Spores of *S. erythropa* are larger and have a much different spore wall structure, lacking an amorphous wall and a notching wall. *Scutellospora castanea* produces spores similar in color and size to *S. hawaiiensis,* but spores possess only four walls (i.e., unit, laminated, and two membranous walls) (Walker et al., 1993). Spores of *S. hawaiiensis* and *S. arenicola* are similar in color, size and surface texture (smooth to slightly roughened), and in possessing an innermost wall group consisting of a coriaceous and an amorphous wall.. However, spores of *S. arenicola* possess an obviously laminated wall (wall 2) that is much thicker (3.5–)8(–12) μm than is the laminated wall of *S. hawaiiensis,* and the auxiliary cells of *S. arenicola* lack knobby projections. Other differences in spore wall structure (especially the presence of the notching wall in *S. hawaiiensis*) also separate the species. Spores of *S. armeniaca* resemble those of *S. hawaiiensis* in size, color, and in possessing an innermost amorphous wall attached to a coriaceous wall but differ from the latter in the much greater thickness of the laminated wall (5.4–13 μm) (Błaszkowski, 1992) and in having an additional pair of walls (pers. observ.). A very thin (<0.5 μm) notching wall (wall number 4) is present in spores of *S. armeniaca* (pers. observ., FIG. 1). This wall appears identical in structure to that in *S. hawaiiensis* except in its thinness, and it was overlooked in the original description. The notching nature of this wall in *S. armeniaca* is visible only with DIC microscopy at 1000× with critical illumination, whereas it is readily visible in *S. hawaiiensis* at 200×. The auxiliary cells of *S. armeniaca* resemble those of *S. hawaiiensis* in shape, but differ in their much lighter color (hyaline to pale yellow vs. dark orange-brown to red-brown) and smaller size (22.5–32.5 μm vs. 40–55 μm broad).

Scutellospora hawaiiensis is a common species in coastal sand dunes of Kauai (Koske and Gemma, 1996), and has previously been reported from Hawaii Island as *Scutellospora* 816 (Koske, 1988). In samples from dunes in which no spores of this species were found, its presence was indicated by the dark orange-brown hyphae and auxiliary cells recovered from the root zone. Pot cultures were successfully established in Hawaii at the outdoor greenhouse of the National Tropical Botanical Garden, Kauai, Hawaii, using sudan grass as a host. The fungus sporulated well in several of the pots there. When more than 30 pot cultures were attempted in a heated greenhouse with supplemental lighting in Rhode Island, however, sporulation was poor.

Although spores of *S. hawaiiensis* routinely were found free in the soil, a few spores were found inside dead spores of an unknown *Gigaspora* species. Such spore-within-a-spore occurrence has previously been reported from other sand dune sites (Koske, 1984, 1988).

Parasitism of spores of AM fungi is common in Hawaiian dune soils (Koske, 1988), and many of the spores of *S. hawaiiensis* that were isolated from the dunes had been attacked by other soil organisms. Spores that have been parasitized sometimes did not have a typical spore wall structure, and walls 3-6 were difficult to distinguish.

We thank Tim Flynn and Dave Lorence for assistance in collecting soil samples and for identifying plants, J. Błazskowski for providing specimens of *S. armeniaca,* William Theobald and the National Tropical Botanical Garden for generous support for mycorrhiza research in the Hawaiian Islands, Eric Roberts for preparing the murograph, Jim Trappe and Chris Walker for their helpful reviews of the manuscript, and the latter for providing specimens of *S. castanea.*

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