

## A new species of *Lentinula* (Agaricales) from Central America

Juan L. Mata and Ronald H. Petersen

Department of Botany, University of Tennessee, Knoxville, TN 37996–1100, U.S.A.

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*Lentinula aciculospora* sp. nov. is described from a single Province in Costa Rica. Stature and habit of basidiomata of this species are similar to some other *Lentinula* taxa (i.e. *L. edodes*), but the elongate-cylindrical shape of basidiospores is a distinctive character of *L. aciculospora*. The mating system of *L. aciculospora* is tetrapolar as also found in other species of the genus, but this species is genetically isolated from *L. boryana*, and all morphological variants of *L. edodes*.

Key Words—Agaricales; biological species; Costa Rica; *Lentinula*; mating systems.

*Lentinula* Earle has become the object of recent research, especially with the application of molecular studies (Nicholson et al., 1997; Hibbett, 1992; Hibbett et al., 1995, 1996, 1998) to sort out Old and New World taxa. Among Old World populations, including the cultivated *L. edodes* (Berk.) Pegler, several distinguishable morphological variants occur, which while sexually intercompatible (Mori et al., 1974; Shimomura et al., 1992) produce identifiable RFLP signatures (Nicholson et al., 1997) and definite phylogenetic ITS-based lineages (Hibbett et al., 1998). These Old World taxa have received much attention because of their economic importance (Chiu et al., 1999; Hibbett and Donoghue, 1996; Royse and May, 1987) along with morphological and molecular revisions to assess their taxonomic circumscription (Molina et al., 1992; Pegler, 1975, 1983).

*Lentinula boryana* (Berk. & Mont.) Pegler and *L. guarapiensis* (Speg.) Pegler are the two morphospecies reported in the New World (Pegler, 1983), the latter known only from its type collection (Pegler, 1983). Until recently, only *L. boryana* was thought to fruit in subtropical and tropical America (Guzmán et al., 1997; Pegler, 1983), but preliminary genetic data, supported by morphological (Petersen et al., 1998) and phylogenetic analysis (Hibbett et al., 1998; Hibbett, 1999), show a third species also to be present. In recent field trips to Central America, basidiomata from the suspected third *Lentinula* have been collected and will be reported in a subsequent paper. The purpose of this paper is to describe a fourth species of *Lentinula* in the New World, this one known only from the Talamanca Mountains in Costa Rica.

### Materials and Methods

**Morphological observations** Sections of dried specimens were re-hydrated in 95% ethanol and water, then placed in 3% KOH, observed in phase contrast, or with Congo Red and phloxine with bright field microscopy.

Melzer's reagent was used to conduct amyloidy tests.

**Terminology** Colors in quotation marks are from Ridgway (1912) and alphanumeric codes in parentheses from Kornerup and Wanscher (1978). Basidiospore measurements and statistics are as follows: Spore length  $\times$  spore width is given for all measured basidiospores in terms of their range of variation;  $x$ =range of the minimum and maximum arithmetic means of spore length by spore width for all spores measured;  $Q$ =spore length divided by spore width in any spore, indicated as a range of variation in  $n$  spores measured;  $Qx$ =arithmetic mean of  $Q$ -values;  $n$ =total number of spores measured; numbers in parentheses=number of collections and specimens studied; **M**=specimens used for morphological examination; and **P**=collections/cultures used for pairings. As of this moment, the name *Armillaria raphanica* Murrill is applied temporarily to those collections made in the Gulf Coast region of North America and Puerto Rico, belonging to a distinct mating complex (i.e. biological species) from *L. boryana*, and also well-defined by micromorphological characters and ITS sequences (Hibbett, 1999; Petersen et al., 1998). Ongoing studies of appropriate type specimens will reveal the most priorable name for this taxon and an appropriate combination will be adopted.

**Cultures** Culture techniques followed those described by Gordon and Petersen (1991). Spore deposits were obtained from fresh basidiomata placed on the inside of a tilted sterile Petri dish containing malt extract agar (MEA, 1.5% Difco malt extract, 2% Difco bacto-agar, 1 $\ell$  distilled water). Germinating spores were harvested and individually transferred to fresh MEA plates. Full grown mycelium was inspected for absence of clamp connections to ascertain monokaryon state. Small agar blocks with mycelium were placed in small vials (4 ml cap) containing sterile water for long-term storage at room temperature.

**Self-cross** Eleven single-basidiospore isolates (SBIs) from collection 56421 (TENN) (*L. aciculospora*) were

paired in all combinations. Subsequently, rows and columns of the resulting grid were adjusted to reveal patterns of compatibility.

**Inter-collection pairings** In all cases, four SBIs from collection 56421 (TENN) (*L. aciculospora*) were paired with four SBIs of collections from *A. raphanica*, *L. boryana* and all the morphological variants of *L. edodes*. In all cases, pairings totaled four.

**Material studied** *Lentinula aciculospora*: COSTA RICA, Prov. San José, Co. Pérez Zeledón, C.A.T.I.E. Experimental Forest of Villa Mills, 9°33'03"N, 83°40'56"W; 2880 m, 22.VI.95, coll. RHP, no. 7901 (TENN 53813) **M**; same location, 10.VI.97, coll. R.E. Halling, no. REH 7717 **M**; same location, 30.VI.98, coll. RHP, no. 9447 (TENN 56421, HOLOTYPE) **M**, **P**; same location, 9.VII.98, coll. J.L. Mata, no. 10026 (TENN 56738) **M**; same location, 20.VI.99, coll. J.L. Mata, no. 10410 (TENN 58105) **M**; Prov. San José, Co. Pérez Zeledón, Cuerricí Biol. Station, 9°33'17"N, 83°40'04"W, 2560 m, 21.VI.99, coll. J.L. Mata, no. 10418 (TENN 58112) **M**. *L. boryana*: COSTA RICA, Prov. San José, vic. Cerro Chonta, 9°41'35"N, 83°56'19"W, 16.VI.95, coll. RHP, no. 7829 (TENN 53819) **P**; MEXICO, Est. México, vic. Valle de Bravo, 6.VII.96, coll. M. Rodríguez, no. 8436 (TENN 55265) **M**, **P**. *L. edodes*: JAPAN, Pref. Tochigi, vic. Lake Chuzenjiko, 27.IX.89, coll. I. Izawa, no. 2306 (TENN 48220) (*edodes* morph) **P**; NEPAL, Ilma, V.95, coll. H.R. Bhandary, no. HRB 7683 (spore print only) (*edodes* morph) **P**; AUSTRALIA, New South Wales, Watagan Mountains, Boarding House Dam, 21.V.91, coll. RHP, no. 3577 (TENN 50062) (*lateritia* morph) **P**; NEW ZEALAND, North Island, vic. Lower Hutt, Wainuiomata, Orongorongo Trailhead, 25.V.94, coll. A.S. Methven, no. 7563 (TENN 53718) (*novaezealandiae* morph) **P**. *A. raphanica*: UNITED STATES, Florida, Gadsden Co., Torreya State Park, 30°34'35"N, 84°56'57"W, 30.V.97, coll. K.W. Hughes, no. 9156 (TENN 54887) **M**, **P**; Commonw. Puerto Rico, Luquillo Nat. Forest, El Verde Biol. Station, 18°19'19"N, 65°49'12"W, 9.VI.98, coll. E. Lickey, no. 9564 (TENN 56477) **P**.

## Results

### Description of the new species

*Lentinula aciculospora* Mata & R. H. Petersen, sp. nov. Figs. 1–5

Pileus 20–150 mm latitudo, primo convexus, deinde plano-convexus, demum depressus; superficies laevis, primo vinoso-spadicea, dein ochraceo-salmonea, vel ochraceo-aurantiaca, squamulis adpressis, pyramidalis, late dispositis ad marginem pilei, caryophyllaceo-bubalinis; margo semper decurvatus, caryophyllaceo-cinnamomeus; velum partiale plerumque appendiculatum, cortinoideum, laete caryophyllaceo-cinnamomeum. Contextus latus, albus, vel laete caryophyllaceo-bubalinus. Lamellae primo adnatae, adnexescentes, approximatae, exalbidae vel vinoso-bubalinae, aliquando vinaceo-marginatae. Stipes 30–70 × 10–20 mm, cylindricus, basi obclavata vel clavata, squamulis numerosis, subannularibus, laceratis, rubiginosis vel luteo-brunneis

vestitus; consistentia solida, tenax. Odor et sapor vix sensibilibus.

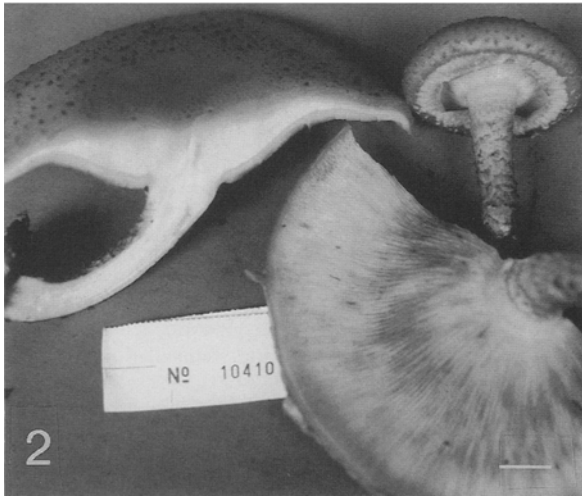
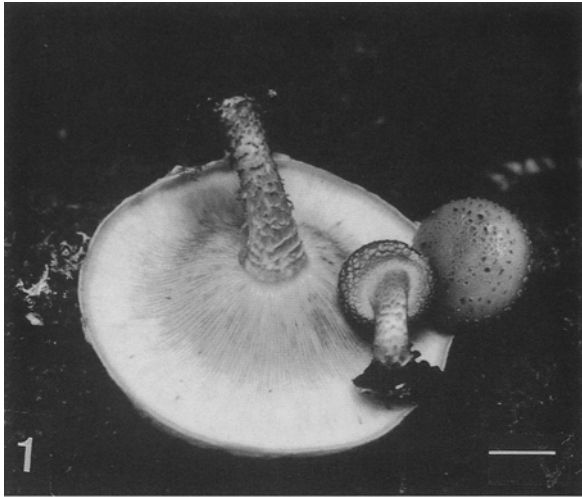
Basidiosporae 5.6–8.8 × 1.6–2.8 μm, cylindricae vel subreniformes, hyalinae, inamyloideae, laeves. Cheilocystidia vermiformia, anguste clavata vel anguste sphaeropedunculata, aliquando apice lobata, hyalina, laevia. Pleurocystidia et caulocystidia absentia. Trama lamellae subregularae. Pileipellis repens. Trama pilei 500–100 μm crassa, intertexta, lacunosa. Hyphae fibulatae, tenui- vel crassitunicatae, hyalinae, inamyloideae.

Habitatio: gregarius, in ligno *Quercus* spp.

*Holotypus*: Costa Rica, Prov. San José, no. 56421 (TENN).

Morphology: Pileus 20–150 mm broad, convex at first, later broadly convex to plano-convex, finally depressed; surface dry, smooth or with scattered, appressed, ±pyramidal squamules over disc, becoming more widely spaced and suberect to erect towards pileus margin; disc surface 'claret brown' (9E8) at first, 'ochraceous salmon' (6A6), 'amber brown' (6D8), to 'ochraceous orange' (6B7), outward 'tawny' (6C6, 6C7) to 'orange cinnamon' (7B6); margin always inrolled, 'pinkish cinnamon' (7B5), 'Hay's russet' (8D7), 'walnut brown' (7D6), 'pinkish buff' (6A3). Partial veil mostly appendiculate, heavily cortinoid to pholiotoid, 'light pinkish cinnamon' (7A2). Pileus flesh 10–15 mm thick at center of disc, solid, white to 'pale pinkish buff' (6A2), with subtle line between pileus and stipe flesh thick, white to abruptly 'warm sepia' (7F6) at base. Odor and taste negligible. Lamellae 2–6 mm deep, adnate at first, becoming adnexed, not seceding, close to crowded, hardly ventricose, off-white at first, 'pinkish buff' (6A3) to 'vinaceous buff' (9B2), bruising slowly and sometimes appearing marginate to 'pinkish buff' (6A3, 5A3), 'avellaneous' (7B3) or 'vinaceous russet' (8D4). Stipe 30–70 × 10–20 mm, cylindrical, expanded to obclavate at base; surface dry, beset with numerous subannulate, lacerate scales; surface 'pinkish cinnamon' (7A2) to 'tawny' (6C6); scales, 'Hay's russet' (8D7) to 'walnut brown' (7D6); consistency tough (Fig. 1, 2).

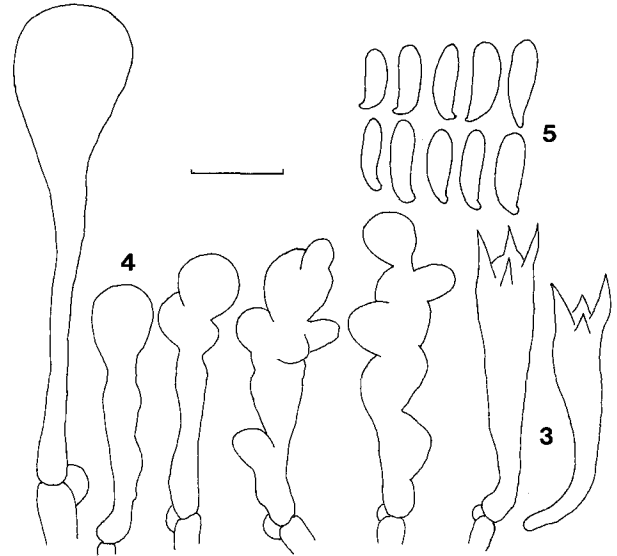
Epicutis ± 20–50 μm thick; hyphae 3–6 μm broad, yellow tan in mass, hyaline singly, repent (except in erumpent warty scales), compacted, interwoven, undifferentiated; thin- to thick-walled (wall up to 0.7 μm thick). Hyphae in scales erumpent, tightly packed, ending in weathered or senescent hyphal fragments. Subpellis 100–250 μm thick; hyphae 3–16 μm broad, hyaline, thick-walled (wall up to 1.5 μm thick), tightly interwoven (except in frequent lacunae). Pileus trama 500–1000 μm thick, loosely interwoven, but generally radially oriented, lacunose; hyphae 6–12 μm broad, thick-walled (wall 1.5–2.5 μm thick), pale straw in mass, hyaline singly, inamyloid. Lamellar trama subregular to tightly interwoven in mediostratum; hyphae 2–4 μm wide, thin- to thick-walled (wall up to 0.5 μm thick), pale straw in mass, hyaline singly; lateral strata irregularly parallel, less tightly packed, abruptly differentiated into subhymenium; hyphae hyaline, more or less thin-walled. Subhymenium rudimentary, pseudoparenchymatous.



Figs. 1, 2. Basidiomata of *Lentinula aciculospora* 58105 (TENN). Scale bar approx. 2 cm.

Hymenium composed solely of basidia and basidioles. Basidia 18–25 × 4–7 μm (n=25), narrowly clavate to subcylindrical, with clamp connections, four-sterigmate; sterigmata slender, small (Fig. 3). Cheilocystidia 16–34 × 4–10 μm (n=20), collybioid, vermiform to narrowly clavate or narrowly sphaeropedunculate, somewhat inflated at least apically, often gnarled or terminally bluntly lobed; wall thin, smooth (Fig. 4); in some cases, lamellar margins sterile. Pleurocystidia absent. Stipitipellis composed of loosely entangled, short or branched hyphae 4–10 μm broad, forming floccons; medullary layer strictly parallel; medullary hyphae 3–12 μm broad, tightly compacted, thin- to thick-walled (wall up to 1.5 μm thick), straw to yellowish brown in mass, hyaline singly, inamyloid. Caulocystidia absent.

Basidiospores white in mass, 5.6–8.8 × 1.6–2.8 μm [x=6.4–7.7 × 2.2–2.5 μm; Q=2.29–4.00, Qx=2.97; n=104(4/5)], cylindrical to subreniform, hyaline, inamyloid, thin-walled, smooth, easily collapsed; hilar appendix small, obscure; contents usually bivacuolate,



Figs. 3–5. Microscopic structures of *Lentinula aciculospora* 56421 (TENN, HT). 3. Basidia. 4. Cheilocystidia. 5. Basidiospores. Scale bar equals 10 μm.

sometimes homogeneous (Fig. 5).

Habitat gregarious, on fallen oak wood. Vegetation: *Quercus costaricensis* Liebmann and *Quercus* spp.

Etymology: *acicularis* (Lat., adj.), narrow, referring to the shape of the basidiospores.

*Holotype*: Costa Rica, Prov. San José, no. 56421 (TENN); with cultures under no. 9447.

**Self cross** When 11 SBIs were paired in all combinations, a tetrapolar mating system was revealed (Fig. 6). Isolates 4\*, 8=A1B1; 1\*, 2, 5, 6, 10=A2B2; 9\*=A2B1; 3\*, 7, 11=A1B2. Subordinate mating types were as-

|         | A1B1 |   | A2B2 |   |   |   | A2B1 |    | A1B2 |   |    |
|---------|------|---|------|---|---|---|------|----|------|---|----|
|         | 4*   | 8 | 1*   | 2 | 5 | 6 | 10   | 9* | 3*   | 7 | 11 |
| 4*      | ■    | - | +    | + | + | + | +    | -  | -    | - | -  |
| A1B1 8  | -    | ■ | +    | + | + | + | +    | -  | -    | - | -  |
| 1*      | +    | + | ■    | - | - | - | -    | -  | -    | - | -  |
| 2       | +    | + | -    | ■ | - | - | -    | -  | -    | - | -  |
| A2B2 5  | +    | + | -    | - | ■ | - | -    | -  | -    | - | -  |
| 6       | +    | + | -    | - | - | ■ | -    | -  | -    | - | -  |
| 10      | +    | + | -    | - | - | - | ■    | -  | -    | - | -  |
| A2B1 9* | -    | - | -    | - | - | - | -    | ■  | +    | + | +  |
| 3*      | -    | - | -    | - | - | - | -    | +  | ■    | - | -  |
| A1B2 7  | -    | - | -    | - | - | - | -    | -  | -    | ■ | -  |
| 11      | -    | - | -    | - | - | - | -    | -  | -    | - | ■  |

Fig. 6. Self cross of *Lentinula aciculospora* 56421 (TENN, HT) showing a tetrapolar mating system. The sign—means no clamp connections observed, flat contact reaction; + means clamp connections present. SBIs with an \* have been selected as tester strains.

signed based on  $9 \times 1/2/5/6/10 = \text{"flat"} = \text{common-A}$ . Within-mating type flat contact zone morphologies (i.e.  $1/2/5/6/10 \times 1/2/5/6/10$ ) rendered "flat" observations of  $9 \times 1/2/5/6/10$  less credible, however. "Flat" contact zone morphology was indistinct, with a clear but ill-defined crevasse. No barrage morphologies were observed.

**Culture characters** Growth rate averaged 1 cm radius in 6 wk. Colonies on MEA were white, radially combed, sparsely silky, becoming more aerial toward the colony margin. Aside from occasional "picket fence" hyphae and some mild lethal reactions, little hyphal differentiation was noted.

**Intercollection pairings** In pairing experiments with all collections listed above, all examined pairings of *L. aciculospora* with *A. raphanica*, *L. boryana* and all morphological variants of *L. edodes* were incompatible. In some instances, "non-self" reactions were noted.

## Discussion

Habit and stature of basidiomata of *L. aciculospora* resemble those of other species in the genus, and are similar in size to the morphological variants of *L. edodes* (Pegler, 1983), but notably larger than those of *L. boryana* or *A. raphanica*. Basidiomata of *L. aciculospora* are darker in color than those reported for the morphospecies *L. lateritia* (Berk.) Pegler and nearer to those of the *L. edodes* morphospecies (Pegler, 1983). *Lentinula boryana* and *A. raphanica* present pinkish buff to yellowish white colors in their pilei, separating them from *L. aciculospora* in the New World.

*Lentinula aciculospora* shares the following microscopic characters with other taxa in the genus: 1) undifferentiated pileus epicutis; 2) loosely interwoven pileus trama consisting of thin- to thick-walled hyphae; 3) clamp connections in all tissues; and 4) absence of pleurocystidia. However, the distinctive cylindrical shape of the spores in *L. aciculospora* separates it from all others. Q spore ratio of 2.97 for *L. aciculospora* is larger than those of examined collections of *A. raphanica* and *L. boryana* 2.15 and 1.91 respectively (Mata et al., unpub. data), and larger than those reported by Pegler (1983) for taxa of the Old World. Cheilocystidia in *L. boryana*,  $19\text{--}40 \times 5\text{--}14 \mu\text{m}$ , are broadly clavate to sphaeropedunculate and larger than those in *L. aciculospora* (Mata et al., unpub. data). Cheilocystidia of *L. aciculospora* are similar in shape and size to those of *A. raphanica*,  $17\text{--}28 \times 3\text{--}10 \mu\text{m}$ , but *L. aciculospora* lacks caulocystidia (Mata et al., unpub. data).

All collections thus far have been gathered within a high altitude forest region in the Talamanca Mountain Range, all on logs of probably *Quercus* spp., and all at the same season of the year. *Lentinula boryana* has been collected in several localities of the Talamanca Mountains whereas *A. raphanica* has been collected there once (Mata et al., unpub. data). In all cases, depauperate, rain-soaked basidiomata of *L. aciculospora* were also found, and it was concluded that basidiomata easily became waterlogged and rapidly degenerated, a condition not

readily observable with other *Lentinula* there.

Basidiospores of other *Lentinula* taxa germinate rapidly (within 24 h) on MEA, and colonies grow rapidly. In *L. aciculospora*, germination occurs in 7–10 d, and colonies are slow-growing compared to congeneric taxa. All taxa within *Lentinula* appear to exhibit a tetrapolar mating system, so it is not surprising to find this in *L. aciculospora*. Nevertheless, *L. aciculospora* appears to be genetically isolated from all other *Lentinula* taxa used in this project. The only taxon reported in the genus but not used in this project is *L. guarapiensis*, a species only known from its type specimen (Nicholson et al., 1997; Pegler, 1983).

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