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## New species and mating system reports in *Gymnopus* (*Agaricales*) from Costa Rica

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Three new species of *Gymnopus* are described from material collected recently in Costa Rica. *Gymnopus cylindricus* and *G. alnicolus* are placed in sect. *Vestipedes*. A self-cross of the new morphospecies *G. pseudolodgeae* revealed a unifactorial mating system. The same result was obtained in the taxonomically related *G. lodgeae* and *G. omphalodes*. *Gymnopus omphalodes* is proposed as a new combination. A single biological species is reported for *G. omphalodes* in central and southern Costa Rica.

**Key words:** collybioid fungi, Talamanca Mountains, *Tricholomataceae*, type specimen.

### Introduction

Antonín *et al.* (1997) suggested dividing *Collybia sensu lato* into three genera, namely *Collybia*, *Gymnopus*, and *Rhodocollybia*, all of which have now gained acceptance at the genus rank (Hughes *et al.*, 2001; Mata, 2002; Moncalvo *et al.*, 2002). Currently, *Gymnopus* represents mushrooms mainly from *Collybia* sections *Vestipedes*, *Levipedes*, *Subfumosae*, and *Iocephalae* (*sensu* Singer, 1986). Accordingly, newly described species from different geographic locations have been placed in *Gymnopus* (Halling, 1996; Antonín and Noordeloos, 1997; Desjardin *et al.*, 1997, 1999; Ortega *et al.*, 2003).

A list of mating systems of several taxa in *Gymnopus* was compiled by Petersen (1995, as *Collybia*). With the exception of *G. subnudus*, reported as unifactorial by Murphy and Miller (1993), all reported taxa seem to exhibit bifactorial-mating systems. Mating studies in basidiomycetes are important in elaborating biological species concepts that may provide better insight in morphotaxonomy, as demonstrated in the work by Vilgalys and Miller (1983, 1987) with the *G. dryophilus* complex.

The purpose of this paper is to report new species placed in *Gymnopus* collected in montane oak forests in Costa Rica and to report on mating systems from several species.

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## Materials and methods

### *Morphological observations*

Portions of pilei of dried specimens were re-hydrated in an aqueous solution of 95% ethanol, then sectioned and placed in 3% KOH. Sections were observed under phase contrast microscopy, or with Congo Red and phloxine under bright field microscopy. Melzer's reagent was used to test for dextrinoidity and cotton blue for cyanophilous reaction of the basidiospores (Largent *et al.*, 1977).

Representative material and type specimens of taxonomically related *Gymnopus* species were studied and compared to each new species and appear fully cited in material examined after a species description. Colour photographs for several of the species described in this paper can be found in Mata (2002).

### *Terminology*

HT = Holotype, IT = Isotype, NT = Neotype; IKI = Melzer's reagent, TFB = Tennessee field book number. Herbarium acronyms are according to Holmgren (Holmgren *et al.*, 1990). Colours in alphanumeric codes in parentheses are from Kernerup and Wanscher (1978). Basidiospore measurements and statistics are as follow: n = total number of spores measured,  $\bar{x}$  = arithmetic mean of spore length and spore width for all spores measured; Q = spore length divided by spore width, indicated as a range of variation in n spores measured;  $\bar{Q}$  = arithmetic mean of Q-values. Single-basidiospore isolate (SBI) = a culture resulting from the germination of a single basidiospore harvested from spore prints from basidiomata from nature. Morph or morphological species = collections of basidiomata which differ sufficiently in morphology (macro- and/or micro-) to have been described as separate taxa. Biological species (i.e. intersterility group) = collections of one or more morphs which exhibit sexual intercompatibility in pairings among SBIs. Sexual compatibility = ability of hyphae of two monokaryon isolates to anastomose, resulting in nuclear migration (uni- or bilateral) and a dikaryon mycelium exhibiting clamp connections (in some other fungal groups, dikaryon hyphae do not exhibit clamp connections, but the definition applies to *Gymnopus*). Partial compatibility = in a given pairing experiment, less than 100% compatibility among SBIs (a numerical value only, not a measure of anastomoses or nuclear migration).

## ***Cultures***

When possible, SBIs were obtained using techniques described by Gordon and Petersen (1991) and Petersen *et al.* (1999). Basidiospores failed to germinate in several specimens of several morphological species, while others collected by one of us (Halling) were not processed for culturing. All cultures were maintained on malt extract (Difco 1.5%) agar (Difco Bacto 2.0%) medium (MEA) in double distilled water (ddH<sub>2</sub>O). Cultures are stored using the agar-disc-in-water method described by Burdsall and Dorworth (1994). Tester strains are deposited in ATCC.

## ***Mating studies***

SBIs were examined to eliminate clamp-bearing (i.e. dikaryon) isolates. A mating system was determined by pairing at least 10 SBIs in all possible combinations. Intercollection sexual compatibility tests consisted in pairing four or eight SBIs from different collections of the same morph.

## **Results and discussion**

### ***Enumeration of taxa***

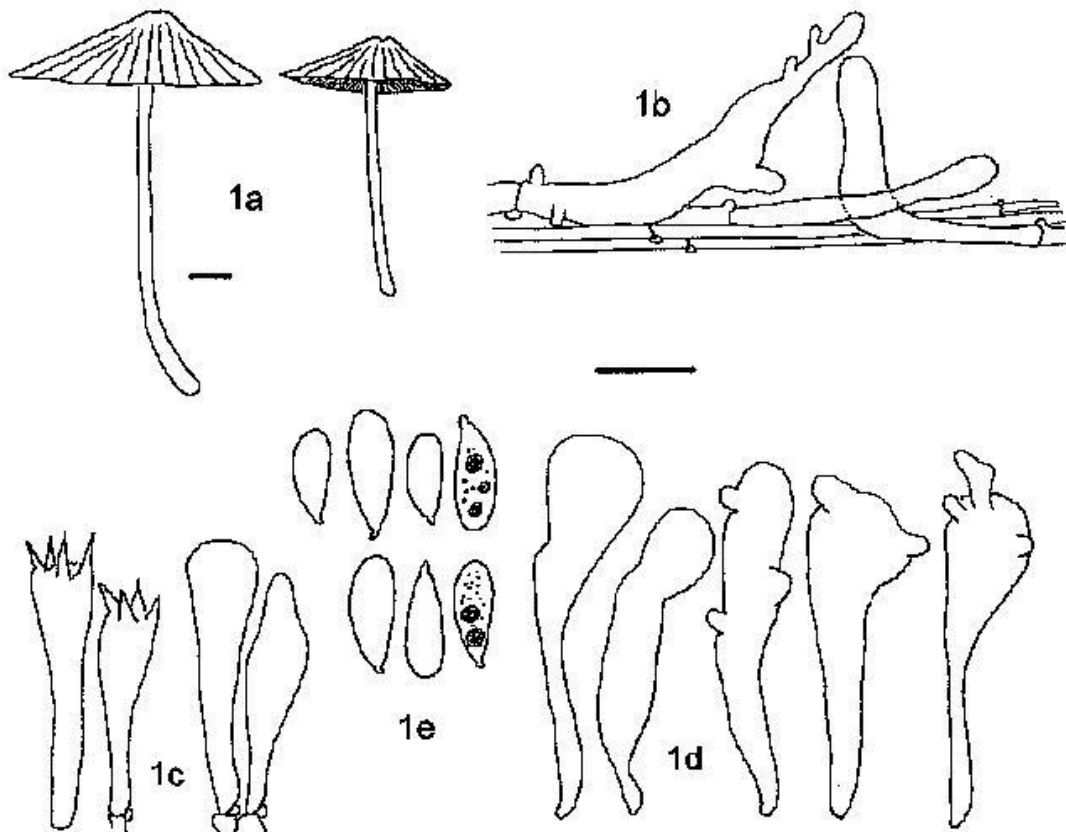
#### ***Gymnopus alnicolus* J.L. Mata & Halling, *sp. nov.* (Figs. 1, 8)**

*Pileo* plano-convexo, fusco, albido in centro; carne concoloram pileo. *Odore et sapore* mitis. *Lamellae* albida ad palidae lilacea. *Stipite* equalis, pubescente, roseo-bruneo ad fusco. In *Alnus* vegetatio. *Basidiosporae* 8.8-13.6 × 3.2-4.8 μm.

***Holotype*** [hic designatus]: COSTA RICA, Cartago, El Guarco, Tapantí, Parque Nacional Tapantí, Macizo de La Muerte, Area de Conservación La Amistad Pacífico, 9° 41' 06" N, 83° 52' 30" W, 2600 m, 6.VII.2001, col. RE Halling, no. 8266 (USJ).

*Etymology*: from *Alnus* spp.; cola (Latin) = dweller

*Pileus* (Fig. 1a, 8) (10-)35-60 mm diam., obtusely convex, campanulate, convex to plano-convex, depressed to sharply depressed on disc; surface moist, hygrophanous, finely striate to sulcate, at first dark brown (7/8F8, 9F5), fading to pale tan to white in depression, with age gray-orange (6B5) to orange-brown (6C-D4); margin at first smooth and even, with age striate-translucent, ± lacerate; context thin, watery gray-brown. *Lamellae* adnate to adnexed, crowded or close to subdistant, less than 1 mm broad, at first white to pallid cream, in age orange-brown (6/7C3), sometimes with a pale lilac tint; margin even, minutely pruinose to fimbriate; lamellulae in three tiers of different lengths. *Stipe* 50-100 × (1-)3-8 mm, equal to slightly broadened towards base, flattened and compressed to cleft; surface dry, with white pubescence, more so at apex, at first pale pink-brown, then concolorous with lamellae at apex,



**Fig. 1.** *Gymnopus alnicolus* (Halling 8266). **a.** basidiomata. **b.** pileus epicutis. **c.** basidia and basidioles. **d.** cheilocystidia. **e.** basidiospores. Bars: a = 1 cm; b-e = 10  $\mu$ m.

brown (7F5) at base, and eventually gray-brown (7D3) or concolorous to pileus overall in age; interior hollow. *Rhizomorphs* and binding mycelium white. *Odor and taste* none to mild. *Chemical reactions* syringaldazine (-), p-Cresol: orange-brown at stipe.

*Habitat:* on leaf litter, under *Alnus acuminata* Humb., Bonp. & Kunth vegetation; gregarious.

*Pileus epicutis* (Fig. 1b) a cutis; hyphae 2-8  $\mu$ m diam., cylindrical, some with diverticulate outgrowths, radially oriented, heavily pigment-encrusted, olive-brown in mass, hyaline singly, orange-brown in IKI; walls thin; terminal cells 16-64  $\times$  3-6  $\mu$ m, mostly cylindrical, some with diverticula, prostrate to erect, singly or in fascicles, common. *Pileus trama* interwoven to  $\pm$  radially oriented; hyphae 4-12  $\mu$ m diam., hyaline, inamyloid; walls thin. *Lamellar trama* irregular to interwoven; hyphae 3-10  $\mu$ m diam., hyaline, inamyloid;

walls thin. *Basidia* (Fig. 1c)  $19-31 \times 5-9 \mu\text{m}$ , clavate; sterigmata four; basidioles  $19-34 \times 5-8 \mu\text{m}$ , cylindrical to clavate, occasionally mucronate or subfusoid. *Pleurocystidia* absent. *Cheilocystidia* (Fig. 1d)  $(19-)-28-45 \times 5-12 \mu\text{m}$ , cylindrical, narrowly to broadly clavate, or subsphaeropedunculate, some flexed, prostrate, collapsing and not easy to see; apex sometimes knobbed or with diverticulate outgrowths. *Stipe* epicutis a cutis; hyphae  $4-14 \mu\text{m}$  diam., parallel, cylindrical, outermost hyphae pigmented like pileus epicutis; walls up to  $0.8 \mu\text{m}$  thick. *Caulocystidia* abundant, cylindrical,  $2-4 \mu\text{m}$  diam., some with diverticulate outgrowths, entangled, some septate. *Clamp connections* present in all tissues. *Basidiospores* (Fig. 1e)  $(6.8-)-7.6-9.6(-13.6) \times 3.2-4 \mu\text{m}$  ( $n = 100/4$ ;  $\bar{x} = 8.6 \times 3.7 \mu\text{m}$ ;  $Q = (1.88-)-2-2.78(-3.11)$ ;  $\bar{Q} = 2.33$ ), narrowly ellipsoid or lacrymoid in side view and in face view, hyaline, inamyloid; walls smooth, thin; contents often multiguttulate.

*Notes:* Basidiomata of *G. alnicolus* are found fruiting under trees of *Alnus acuminata*. The collybioid characters of the basidiomata in combination with radially oriented hyphae in the pileus epicutis and hyaline, inamyloid spores place this taxon in *Gymnopus* sect. *Vestipedes* (*sensu* Antonín and Noordeloos, 1997). Other distinctive features of *G. alnicolus* include a dark brown to orange-brown, striate pileus, white to orange-brown lamellae, and spores with multiguttulate contents. While the specimens from Ecuador are very similar in all morphological characters, a third collection from Costa Rica is a little different. When dried specimens were compared to each other, the Costa Rican material presented larger basidiomata, pilei more sulcate, lamellae appearing more distant and broader, and a stipe vesture denser and beige coloured, than the Ecuadorian material. Most notable is that spore length is larger in the Costa Rican basidiomata while cheilocystidia are similar.

Basidiomata of *G. alnicolus* may be confused in the field with those of *G. bififormis* which typically present chestnut to red-brown pilei, white to cream lamellae, in combination with shorter spores ( $5.6-8 \times 2.4-4.4 \mu\text{m}$ ,  $\bar{x} = 6.6 \times 3.5 \mu\text{m}$ , HT). Microscopically, *G. cylindricus* (see description below) can be distinguished from *G. alnicolus* by the cylindrically shaped cheilocystidia. Basidiomata of *G. confluens* have paler colours and cheilocystidia are more knobbed to nodulose (Halling, 1983; Antonín and Noordeloos, 1997; Mata, 2002). Cheilocystidia and spore shape and dimensions in *G. luxurians* and *G. dichrous* are somewhat similar to those of *G. alnicolus* but basidiomata of the former are much larger in size with paler colours overall, and those of *G. dichrous* have a tuberculate stipe base and strictly lignicolous habit (Halling, 1983; Mata, 2002).

*Material examined:* COSTA RICA, Cartago, El Guarco, Tapantí, Parque Nacional Tapantí, Macizo de La Muerte, Area de Conservación La Amistad Pacífico,  $9^{\circ} 41' 06'' \text{N}$ ,  $83^{\circ} 52' 30'' \text{W}$ , 2600 m, 6.VII.2001, col. RE Halling, no. 8266 (HT: USJ, IT: NY). ECUADOR,

Napo, road from Papallacta to Baeza, 3.8 km E. of Papallacta, 30.IV.1987, col. RE Halling, no. 5224 A (NY, QCA); same location, ± 8-9 Km E. of Papallacta, at Puente Guango, in *Alnus* forest, 30.IV.1987, col. RE Halling, no. 5225, no. 5226, no. 5227 (NY, QCA).

*Gymnopus bififormis* (Pk.) Halling. [1997. Mycotaxon 63: 363.] UNITED STATES, New York, Rensselaer Co., Sandlake, VIII.1902, col. CH Peck, (HT of *Marasmius bififormis*, NY).

*Gymnopus confluens* (Pers.: Fr.) Antonín *et al.* [1997. Mycotaxon 63: 364]. BELGIUM, Namur. Grande Tinémont near Han-sur-Lesse, 26.IX.1974, col. ME Noordeloos, no 7479 (NT of *Agaricus confluens*, L).

*Gymnopus luxurians* (Peck) Murrill. [1916. N. Amer. Fl. 9: 362.] UNITED STATES, Alabama, July 1896, LM Underwood, no. 1787 (HT of *Marasmius luxurians*, NYS).

***Gymnopus cylindricus* J.L. Mata, sp. nov.** (Figs. 2, 9)

*Pileo* plano-convexo, glaber, aurantiaco-brunneus. *Lamellae* adnatae ad adnexas, angustas, confertes, cremicolores. *Stipite* equalis, concolore pileo ad roseo-brunneo. *Basidiosporis* ellipsoides, 8.8-13.6 × 3.2-4.8 µm. *Cheilocystidia* cylindricales, septates.

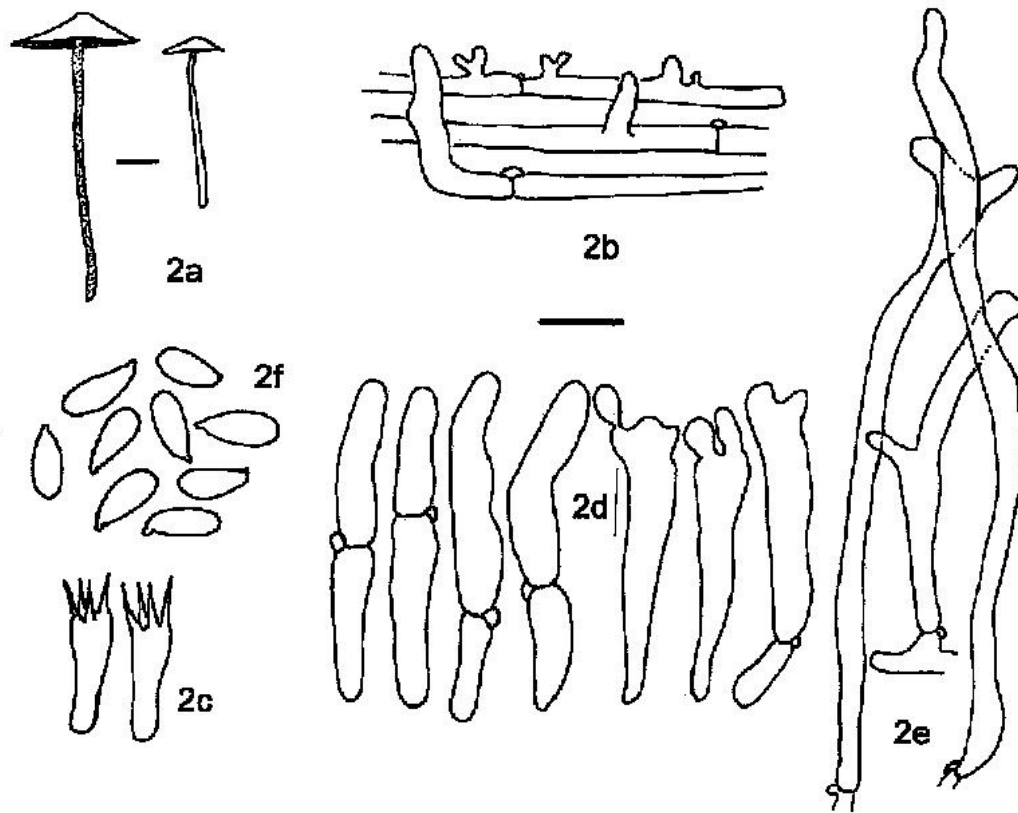
**Holotype** [hic designatus]: COSTA RICA, San José, Dota, San Gerardo de Dota, vic. Hotel de Montaña Savegre, 9° 32' 71" N, 83° 48' 39" W, 2300 m, 19.VI.1999, col. JL Mata, TFB 10402 (TENN).

Etymology: *cylindricus* (Latin) = cylindrical shape of cheilocystidia.

*Pileus* (Figs. 2a, 9) 8-30 mm diam., convex, plano-convex, with a broad and flattened umbo, or centrally depressed; surface glabrous, radially wrinkled, becoming ± translucent outwards, when young brown (6C6) to dark brown (7F8), with age brown (7E8), light brown (7C5), orange-brown (5C7, 6D7) to tan (6C6-6D7), near or at margin always paler than disc, light brown to creamy (4A3); margin curved to plane, striate-crenate, translucent; context thin, concolorous with surface. *Lamellae* adnate to adnexed, very narrow, crowded, at first white, with age off-white (3A2) to cream (3A4, 4A3); margin even; lamellulae in several tiers of different lengths. *Stipe* 40-100 × 1-2(-4) mm, equal, terete or compressed; surface overall white-pruinose, red-brown (8E6, 7E8), orange-brown (6E6) to brown (6D8-6E8), darker than pileus, at apex fading to light brown (7D8); interior hollow; consistency fleshy, brittle. Odor not distinctive or mild; taste not distinctive.

*Habitat*: on leaf litter; solitary to gregarious.

*Pileus epicutis* (Fig. 2b) a cutis; hyphae 2-8 µm diam., cylindrical, frequently with diverticula, pigment-incrusted, straw coloured in mass, orange-brown in IKI, hyaline singly, inamyloid; walls thin; terminal cells occasional, semi-erect to erect, originating at hyphal septa. *Gloeoplerous* hyphae rare, up to 10 µm diam. *Pileus trama* interwoven; hyphae 2-12 µm diam., hyaline, inamyloid; walls thin. *Lamellar trama* subregular to irregular; hyphae 2-10 µm diam., hyaline, inamyloid; walls thin; subhymenium pseudoparenchymatous. *Basidia* (Fig. 2c) 20-29 × 5-8 µm, clavate; sterigmata four; basidioles similarly sized as basidia, clavate to mucronate. *Pleurocystidia* absent. *Lamellar margin*



**Fig. 2.** *Gymnopus cylindricus* (TFB 10402). **a.** basidiomata. **b.** pileus epicutis. **c.** basidia. **d.** cheilocystidia. **e.** caulocystidia. **f.** basidiospores. Bar: a = 1 cm; b-f = 10  $\mu$ m.

sterile. *Cheilocystidia* (Fig. 2d) 24-65  $\times$  6-9  $\mu$ m, cylindrical,  $\pm$  flexed, septate; apex obtuse, lobed, or with minor striangulations. *Stipe epicutis* a cutis; hyphae 4-12(-16)  $\mu$ m diam., parallel, cylindrical, brown in mass, pale yellow singly, inamyloid; walls thin to 1  $\mu$ m thick. *Caulocystidia* (Fig. 2e) up to 170(-300)  $\times$  6(-8)  $\mu$ m, cylindrical, septate, forming an entangled mat; apex obtuse, knobbed or furcate. *Clamp connections* present in all tissues. *Basidiospores* (Fig. 2f) (8.8-)-9.6-11.2(-13.6)  $\times$  3.2-4.0(-4.8)  $\mu$ m (n = 92/5;  $\bar{x}$  = 10.4  $\times$  3.7  $\mu$ m; Q = 2.10-3.63,  $\bar{Q}$  = 2.85) subfusoid, narrowly ellipsoid to subaciculate, in side view, narrowly ellipsoid to cylindrical in face view, hyaline, inamyloid; walls smooth, thin.

*Notes:* *Gymnopus cylindricus* is characterized by the cylindrical shape of the cheilocystidia in combination with narrowly ellipsoid to cylindrical spores. Taxonomic relatives of *G. cylindricus* are *G. biformis*, *G. confluens*, and *G.*

*alnicolus*. Basidiomata of *G. biformis* have similar colours but spores are shorter ( $\bar{x} = 6.6 \times 3.5 \mu\text{m}$ , HT) and cheilocystidia are frequently knobbed, lobed and diverticulate. Basidiomata of *G. confluens* have confluent, more densely pruinose, grayish stipes and lobed cheilocystidia. Basidiomata of *G. alnicolus* also produce narrow elliptical spores but cheilocystidia are clavate to subsphaeropedunculate.

Other tropical to subtropical species similar to *G. cylindricus* are *G. menehune* described from the Hawaiian Islands, *G. subcyathiformis* from México, and *G. collybioides* from Brazil. Both *G. menehune* and *G. subcyathiformis* have clavate cheilocystidia but in the former pilei are distinctly umbilicate and conspicuously striate-sulcate, and in the latter pilei have brown to violet tints. Spore dimensions in the type specimen of *G. collybioides* are much smaller ( $5.6\text{-}7.2 \times 2.4\text{-}4 \mu\text{m}$ ). Basidiomata of all of these morphospecies, including those of temperate range, share a small stature and gregarious fruiting on leaf litter with very similar microscopic elements such as dimensions and shape of basidiospores, cystidia, and pileus epicutis (Mata, 2002).

*Material examined*: COSTA RICA, San José, Dota, Jardín de Dota, 3.5 km W off Inter American Hwy, 9° 42' 42" N, 83° 53' 28" W, 2300 m, 18.VI.1999, col. JL Mata, TFB 10091 (TENN 58086); Finca El Jaular, 9° 39' 39" N, 83° 52' 01" W, 2300 m, 17.VI.1999, col. JL Mata, TFB 10084 (TENN 58024); San Gerardo de Dota, Hotel de Montaña Savegre, 9° 32' 71" N, 83° 48' 39" W, 2300 m, 29.VI.1998, col. RH Petersen & JL Mata, TFB 9664 (TENN 56625); same location 19.VI.1999, col. JL Mata, TFB 10402 (HT, TENN 58097); Pérez Zeledón, Villa Mills, Estación Experimental CATIE, 9° 33' 03" N, 83° 40' 56" W, 2880 m, 30.VI.1998, col. JL Mata, TFB 9671 (TENN 56632).

*Gymnopus confluens* (Pers.: Fr.) Antonín *et al.* [1997. Mycotaxon 63: 364]. BELGIUM. Namur, Grande Tinémont, near Han-sur-Lesse, 26.IX.1974, col. ME Noordeloos, no. 79, (NT of *Agaricus confluens*, L 0053911).

*Gymnopus collybioides* (Speg.) Desjardin *et al.* [1999. Mycologia 91: 175]. BRAZIL. Apiaty, IV. 1888, col. J. Puiggari, no. 2893 (HT of *Clitocybe collybioides*, LPS).

*Gymnopus menehune* Desjardin *et al.* [1999. Mycologia 91: 173.]. UNITED STATES. Hawaii, Hawaii, Puna, MacKenzie State Park on the SE coast, 3.VII.1993, col. DE Desjardin, D Hemmes & M Goldsmith, DED 5866 (IT, NY).

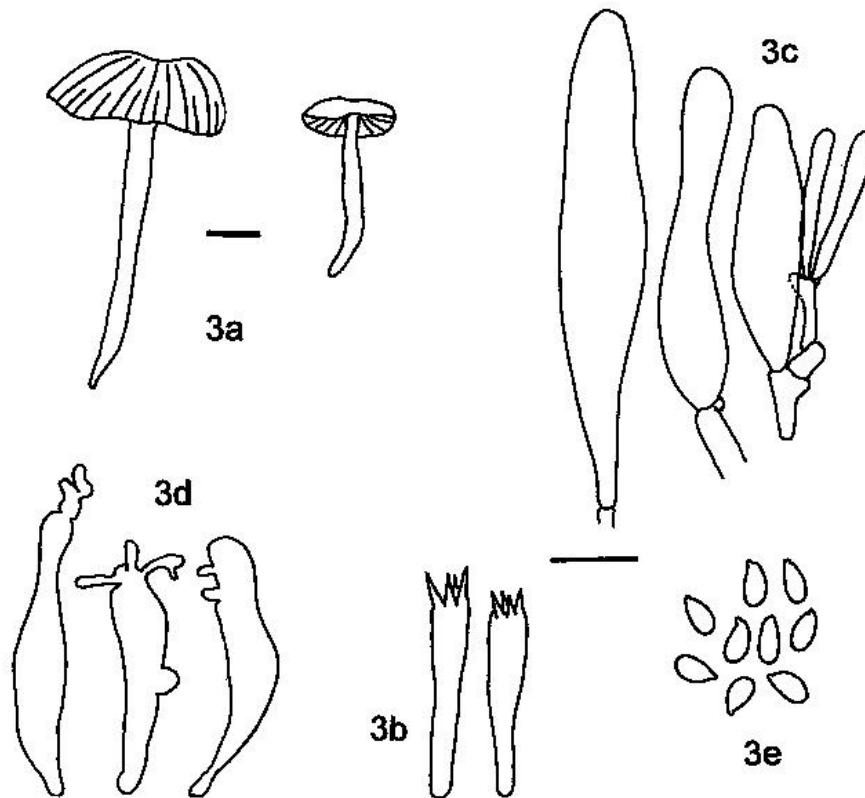
*Gymnopus subcyathiformis* (Murr.) Desjardin *et al.* [1999. Mycologia 91: 175]. MEXICO. Colima, 3-4.I.1910, col. Edna & WA Murrill, no. 615 (HT of *Marasmius subcyathiformis*, NY).

***Gymnopus pseudolodgeae* J.L. Mata, sp. nov.** (Figs. 3, 10)

*Pileo* convexo-umbonato ad plano-depresso; superficies velutinus, sulcatus, porphyreus. *Lamellae* distantes, roseo-fuscae. *Stipite* albidus ad nigricantis vetustatis. *Habito* lignicola. *Pleurocystidia et cheilocystidia* similes ad *Gymnopus lodgeae* cum basidiosporae  $4.8\text{-}6.4 \times 2.4\text{-}3.6 \mu\text{m}$ .

**Holotype** [hic designatus]: COSTA RICA, Puntarenas, Coto Brus, vic. San Vito, Estación Biológica Las Alturas, OTS, on the trail to Cerro Chia, 1520 m, 21.VI.2000, col. RH Petersen & JL Mata, TFB 10493 (TENN).





**Fig. 3.** *Gymnopus pseudolodgeae* (TFB 10493). **a.** basidiomata. **b.** basidia. **c.** pleurocystidia. **d.** cheilocystidia. **e.** basidiospores. Bar: a = 1 cm; b-e = 10  $\mu$ m.

*Etymology:* *pseudo* (Greek) = false, resembling *G. lodgeae*.

*Pileus* (Figs. 3a, 10) 10-30(-40) mm diam., at first convex-umbonate, with age gradually expanding to applanate-depressed; surface finely velvety, strongly hygrophanous, deeply sulcate almost entirely,  $\pm$  translucent, at first red-brown (8E6-7E7), with age tan to light brown; margin at first incurved, with age curved to expanded; context very thin. *Lamellae* adnate,  $\pm$  broad, distant, pink-brown (7B4-2); margin even; lamellulae in two tiers of different lengths. *Stipe* 30-65  $\times$  2-5 mm,  $\pm$  equal, twisted; surface fibrillose, at apex at first off-white to cream or slightly red-brown, with age turning black; interior hollow; consistency fibrous. *Odor* not distinctive; taste mealy to raphanoid.

*Habitat* on wood chips of fallen, decomposed log; gregarious.

*Pileus epicutis* a simple cutis; hyphae 2-8  $\mu$ m diam., radially oriented, not branched, diverticulate or knobbed, frequently septate, pigment-incrusted light olive-brown in mass, hyaline singly, inamyloid; walls thin; terminal cells

sub-erect to erect, up to 12  $\mu\text{m}$  long. *Pileus trama* interwoven to  $\pm$  radially oriented; hyphae 2-16  $\mu\text{m}$  diam., hyaline, inamyloid; walls thin to 1.6  $\mu\text{m}$  thick. *Lamellar trama* subregular; hyphae 2-14  $\mu\text{m}$  diam., hyaline, inamyloid; walls thin. *Basidia* (Fig. 3b) 24-33  $\times$  4-6  $\mu\text{m}$ , narrowly clavate; sterigmata four. *Pleurocystidia* (Fig. 3c) 40-80  $\times$  8-12  $\mu\text{m}$ , mostly fusoid ventricose, hyaline, with clamp connections; walls thin. *Cheilocystidia* (Fig. 3d) (16-)32-48  $\times$  6-12  $\mu\text{m}$ , cylindrical, clavate to fusoid, hyaline, inamyloid; apex obtuse diverticulate. *Stipe epicutis* a cutis; hyphae 2-20  $\mu\text{m}$  diam., parallel, hyaline, inamyloid, with clamp connections; walls thin. *Gloeoplerous hyphae* rare, up to 7  $\mu\text{m}$  diam. *Caulocystidia* 24-32  $\times$  5-8  $\mu\text{m}$ , clavate to ventricose, or as small knobs. All tissues inamyloid and with clamp connections present. *Basidiospores* (Fig. 3e) 4.8-6.4  $\times$  2.4-3.6  $\mu\text{m}$  ( $n = 28$ ;  $\bar{x} = 5.6 \times 3.1 \mu\text{m}$ ;  $Q = 1.56-2.29$ ,  $\bar{Q} = 1.82$ ) ellipsoid in side view, obovoid in face view, hyaline, inamyloid; walls smooth, thin.

*Notes:* Basidiomata of this taxon are very similar to those of *G. lodgeae*, but spores, cheilocystidia and pleurocystidia of *G. pseudolodgeae* are considerably smaller. Other species presenting pleurocystidia are *G. peronatus*, *G. subnudus*, and *G. omphalodes* (Dennis, 1951; Pegler, 1983; Antonín and Noordeloos, 1997; Murphy, 1997). Basidiomata of *G. omphalodes* have much paler pilei, bases of stipes become black in age, pleurocystidia are much broader (50-68  $\times$  10-17  $\mu\text{m}$ ) and basidiospores are longer ( $\bar{x} = 7.2 \times 3.3 \mu\text{m}$ ) (Mata, 2002). Basidiomata of *G. subnudus* are brown with ochraceous lamellae and have longer spores (8.9  $\times$  4.2  $\mu\text{m}$ , HT, *teste* Desjardin, 1989). Basidiomata of *G. peronatus* have yellowish lamellae, mucronate pleurocystidia and longer spores ( $\bar{x} = 8.4 \times 4 \mu\text{m}$ , NT).

*Material examined:* COSTA RICA, Puntarenas, Coto Brus, vic. San Vito, Estación Biológica Las Alturas, OTS, on the trail to Cerro Chia, 1520 m, 21.VI.2000, col. RH Petersen & JL Mata, TFB 10493 (TENN 58601).

*Gymnopus lodgeae* (Singer) Mata. [2003. Mycotaxon 86: 313]. COSTA RICA, Heredia, Sarapiquí, near Puerto Viejo de Sarapiquí, OTS-La Selva Biological Station, Research Trail, 23.XII.1979, col. J Lodge, CR 294 (HT of *Collybia lodgeae*, F). San José, Dota, Jardín de Dota, 3.5 km W. off Interamerican Hwy at El Empalme, 9° 42' 52" N, 83° 53' 28" W, 2220 m, 25.VI.2000, col. JL Mata & RH Petersen, TFB 11013 (TENN 58621).

*Gymnopus omphalodes* (Berk.) Halling & J.L. Mata, **comb. nov.**

*Basionym:* *Marasmius omphalodes* Berkeley. Hooker, Journ. Bot. & Kew Misc. 8: 138. 1856.

BRAZIL, Amazonas, Sao Jeronimo, II. 1853, Spruce 131, (HT of *Marasmius omphalodes*, K, *non vide*), *teste* Dennis (1951). COSTA RICA, Alajuela, Grecia, Reserva Forestal de Grecia, Bosque del Niño, 10° 8' 38" N, 84° 14' 62" W, 1750 m, 27.VI.1998, col. JL Mata, TFB 9642 (TENN 56603), TFB 9643 (TENN 56604), TFB 9644 (TENN 56604), TFB 9653 (TENN 56614); same location 8.VIII.1998, col. JL Mata, TFB 10021 (TENN 56733), TFB 10022 (TENN 56734), TFB 10023 (TENN 56735). Cartago, El Guarco, vic. Estrella, 9° 47' 6" N, 83° 57' 57" W, 1700 m, 13.VI.1995, col. RE Halling & RH Petersen, TFB 7687 (TENN 53790); same location, 16.VI.1999, col. JL Mata, TFB 10075 (TENN 58015).

Puntarenas, Coto Brus, Sabalito, vic. La Amistad Lodge, Pizote Trail, 8° 54' 22" N, 82° 47' 40" W, ± 1330 m, 23.VI.1999, col. JL Mata & I González, TFB 10427 (TENN 58121). San José, Dota, Jardín de Dota, 3.5 km W off Interamerican Hwy at El Empalme, 9° 42' 52" N, 83° 53' 28" W, 2220 m, 1.VII.1998, col. RH Petersen & JL Mata, TFB 9677 (TENN 56638); same location, 25.VI.2000, col. JL Mata, RH Petersen, RE Halling & L Norvell, TFB 11012 (TENN 58620); Pérez Zeledón, Hotel de Montaña Savegre, 9° 32' 42" N, 83° 48' 39" W, 2300 m, 19.VI.1999, col. JL Mata, TFB 10404 (TENN 58099); same location, 26.VI.2000, col. JL Mata, RH Petersen, RE Halling & L Norvell, TFB 11021 (TENN 58629).

*Gymnopus peronatus* (Bolt.: Fr.) Antonín *et al.* [1997. Mycotaxon 63: 365.]. UNITED KINGDOM, Yorkshire, Halifax, Elland Park Wood, 7.IX.1996, A Leonard (NT of *Agaricus peronatus*, E).

*Gymnopus subnudus* (Ellis ex Peck) Halling. [1997. Mycotaxon 63: 365.]. UNITED STATES, Louisiana. Baton Rouge Co., E. Baton Rouge, 25.V.1995, col. SC McCleneghan, TFB 9120 (TENN 55747); New Jersey, Newfield, IX-X.1882, col. Ellis, N. Amer. Fungi Exs. No. 909 (HT of *Marasmius subnudus*, NY, *non vide*) teste Halling (1983). North Carolina, Macon Co., Blue Valley, Forest Service Rd 89, 14.VI.1989, col. RH Petersen, TFB 1804 (TENN 48444); Coweeta Hydrological Laboratory, 22.VI.1989, col. RH Petersen, TFB 1818 (TENN 48353); Whitesides Cove Rd, trail from "Summer Chapel", 8.VII.1999, col. RH Petersen, TFB 10338 (TENN 57899).

### ***Mating studies***

Self-crosses with SBIs from specimens representing *G. pseudolodgeae* and *G. lodgeae* resulted in an unresolved unifactorial (i.e. bipolar) system (Figs. 4, 5) where presence of clamp connections between SBI's 7/9/14 × 8/15 in *G. lodgeae*, and SBIs 11/7/1 × 13/2/4/3 in *G. pseudolodgeae* was not constant. An intercollection pairing, n = 8, of SBIs from *G. pseudolodgeae* (TFB 10493) with those from *G. lodgeae* (TFB 11013) resulted in 100% incompatibility (data not shown). These results are matched by nrITS sequence divergence (i.e. 89% similarity) and support the concept of two biological species (Mata, 2002).

The self-cross in *G. omphalodes* revealed a well-resolved unifactorial mating system (Fig. 6). The two mating types A<sub>1</sub> and A<sub>2</sub> resulting in each self-cross were assigned arbitrarily. Intercollection pairings in *G. omphalodes* demonstrate it forms a biological species across the Talamanca Mountains and the Central Volcanic Range of Costa Rica (Fig. 7). High mating compatibility exists within single geographic locations, i.e. Talamanca Mountains and Central Volcanic Range. Conversely, partial compatibility was observed across these two distinct geological units in Costa Rica (Castillo-Muñoz, 1983). Observation of this phenomenon raises the possibility of a cryptic sibling species, but no morphological distinction among voucher specimens could be found and ITS sequences show no significant variation (Mata *et al.*, 2003). Explanations for these mating results fall beyond the scope of this study but

|                |    | A <sub>1</sub> |   |   |   |    |    |   |   | A <sub>2</sub> |    |    |   |
|----------------|----|----------------|---|---|---|----|----|---|---|----------------|----|----|---|
|                |    | 5*             | 6 | 8 | 9 | 10 | 13 | 2 | 4 | 3              | 11 | 7* | 1 |
| A <sub>1</sub> | 5* | ■              | - | - | - | -  | -  | - | - | -              | +  | +  | + |
|                | 6  | -              | ■ | - | - | -  | -  | - | - | -              | +  | +  | + |
|                | 8  | -              | - | ■ | - | -  | -  | - | - | -              | +  | +  | + |
|                | 9  | -              | - | - | ■ | -  | -  | - | - | -              | +  | +  | + |
|                | 10 | -              | - | - | - | ■  | -  | - | - | -              | +  | +  | + |
|                | 13 | -              | - | - | - | -  | ■  | - | - | -              | -  | +  | + |
|                | 2  | -              | - | - | - | -  | -  | ■ | - | -              | -  | +  | + |
|                | 4  | -              | - | - | - | -  | -  | - | ■ | -              | +  | +  | - |
| A <sub>2</sub> | 3  | -              | - | - | - | -  | -  | - | ■ | +              | +  | -  |   |
|                | 11 | +              | + | + | + | +  | -  | - | + | +              | ■  | -  |   |
|                | 7* | +              | + | + | + | +  | +  | + | + | +              | -  | ■  |   |
|                | 1  | +              | + | + | + | +  | +  | + | - | -              | -  | -  | ■ |

Fig. 4. Self cross between 12 SBIs of *Gymnopus pseudolodgeae* (TFB 10493). + = presence of clamp connections; - = absence of clamp connections; \* = tester strains. Isolate numbers and assigned mating types top and side.

|                |    | A <sub>1</sub> |    |   |    | A <sub>2</sub> |   |   |    |   |   |    |
|----------------|----|----------------|----|---|----|----------------|---|---|----|---|---|----|
|                |    | 2*             | 11 | 8 | 15 | 1*             | 3 | 4 | 13 | 7 | 9 | 14 |
| A <sub>1</sub> | 2* | ■              | -  | - | -  | +              | + | + | +  | + | + | +  |
|                | 11 | -              | ■  | - | -  | +              | + | + | +  | + | + | +  |
|                | 8  | -              | -  | ■ | -  | +              | + | + | +  | + | - | -  |
|                | 15 | -              | -  | - | ■  | +              | + | + | +  | + | + | -  |
| A <sub>2</sub> | 1* | +              | +  | + | +  | ■              | - | - | -  | - | - | -  |
|                | 3  | +              | +  | + | +  | -              | ■ | - | -  | - | - | -  |
|                | 4  | +              | +  | + | +  | -              | - | ■ | -  | - | - | -  |
|                | 13 | +              | +  | + | +  | -              | - | - | ■  | - | - | -  |
|                | 7  | +              | +  | + | +  | -              | - | - | -  | ■ | - | -  |
|                | 9  | +              | +  | - | +  | -              | - | - | -  | - | ■ | -  |
|                | 14 | +              | +  | - | -  | -              | - | - | -  | - | - | ■  |

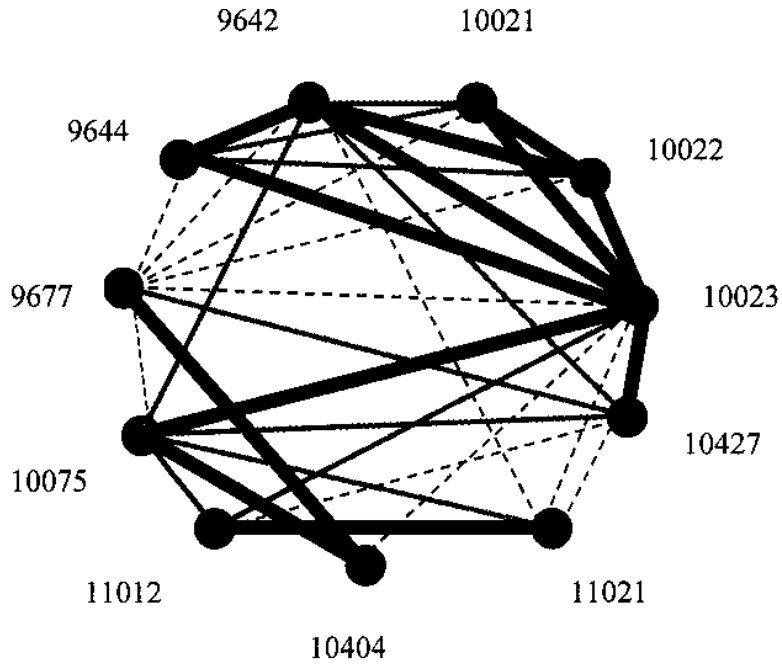
Fig. 5. Self cross between 11 SBIs of *Gymnopus lodgeae* (TFB 11013). + = presence of clamp connections; - = absence of clamp connections; \* = tester strains. Isolate numbers and assigned mating types top and side.

|                |    | A <sub>1</sub> |    | A <sub>2</sub> |    |    |   |   |   |    |    |    |   |
|----------------|----|----------------|----|----------------|----|----|---|---|---|----|----|----|---|
|                |    | 5              | 2* | 3              | 4* | 13 | 1 | 8 | 9 | 10 | 11 | 12 | 7 |
| A <sub>1</sub> | 5  | ■              | -  | +              | +  | +  | + | + | + | +  | +  | +  | + |
|                | 2* | -              | ■  | +              | +  | +  | + | + | + | +  | +  | +  | + |
| A <sub>2</sub> | 3  | +              | +  | ■              |    | -  | - | - | - | -  | -  | -  | - |
|                | 4* | +              | +  | -              | ■  | -  | - | - | - | -  | -  | -  | - |
|                | 13 | +              | +  | -              | -  | ■  | - | - | - | -  | -  | -  | - |
|                | 1  | +              | +  | -              | -  | -  | ■ | - | - | -  | -  | -  | - |
|                | 8  | +              | +  | -              | -  | -  | - | ■ | - | -  | -  | -  | - |
|                | 9  | +              | +  | -              | -  | -  | - | - | ■ | -  | -  | -  | - |
|                | 10 | +              | +  | -              | -  | -  | - | - | - | ■  | -  | -  | - |
|                | 11 | +              | +  | -              | -  | -  | - | - | - | -  | ■  | -  | - |
|                | 12 | +              | +  | -              | -  | -  | - | - | - | -  | -  | ■  | - |
|                | 7  | +              | +  | -              | -  | -  | - | - | - | -  | -  | -  | ■ |

**Fig. 6.** Self cross between 12 SBIs of *Gymnopus omphalodes* (TFB 10023). + = presence of clamp connections; - = absence of clamp connections; \* = tester strains. Isolate numbers and assigned mating types top and side.

help emphasize that genetic barriers may exist within small tropical mountainous regions (Mata and Petersen, 1999).

Murphy and Miller (1993) first reported a bipolar mating system for *G. subnudus*, but Petersen (1995) argued that a tetrapolar component might be present, as in *G. peronatus*. Previously, Gordon and Petersen (1991) justified an infrageneric taxonomy based on genetic and morphological characteristics for certain sections in *Marasmius*, another genus in the *Tricholomataceae*. For the purpose of this work, it is interesting to note that *G. pseudolodgeae*, *G. lodgeae*, *G. peronatus*, *G. subnudus*, and *G. omphalodes* all share similar micromorphological characteristics such as presence of conspicuous pleurocystidia and cheilocystidia coupled with a prevailing unifactorial mating system. ITS-based phylogenetic studies by Mata (2002) and Mata *et al.* (2003) indicate all these morphospecies are placed in a clade within *Gymnopus* separate from those placed in sect. *Vestipedes* (*sensu* Antonín and Noordeloos, 1997). Discovery of these new *Gymnopus* species brings improved resolution to the taxonomy of the genus and may provide complementary information for hypothesis in systematics and evolution.



| Location                                | Collection Number                                   |
|---|---|
| Bosque del Niño, Central Volcanic Range | TFB 9642, TFB 9644, TFB 10021, TFB 10022, TFB 10023 |
| Estrella                                | TFB 9677  |
| Jardín de Dota                          | TFB 10075, TFB 11012                                |
| San Gerardo de Dota                     | TFB 10404, TFB 11021                                |
| La Amistad                              | TFB 10427   |

**Fig. 7.** Recognition grid for intercollection pairings from *Gymnopus omphalodes*. All matings are  $n = 4$  SBIs. Compatibility based on clamp connection formation; **thick line** 75-100% compatibility, **medium line** 25-50% compatibility, **dashed line** no compatibility, **no line** = no pairing done.



**Fig. 8.** Basidiomata of *Gymnopus alnicolus* (Halling 8266). Bar = 1 cm.



**Fig. 9.** Basidiomata of *Gymnopus cylindricus* (TFB 10402). Bar = 1 cm.



**Fig. 10.** Basidiomata of *Gymnopus pseudolodgeae* (TFB 14903). Bar = 1 cm.

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