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Gymnopus eneficola—species nova from Newfoundland

Ron Petersen,[1] Karen Hughes,[1] Andrus Voitk[2]

Introduction

In the course of a survey of dried collections of *Gymnopus* collected over some years in Newfoundland by AV and Foray Newfoundland & Labrador, a taxon was discovered which did not conform to other known eastern North American or European taxa of the genus.[1,2] Occasional reports of taxa now placed in *Gymnopus* (formerly included in an expansive concept of *Collybia*) include those concerning Nova Scotia,[3] summarized by Gourley,[4] and Michigan,[5,6,7,8] none of which, nor those of the even older publication by Coker and Beardslee[9] circumscribed the proposed species from Newfoundland. In addition to morphological distinctions, DNA analysis also indicated that nuclear ribosomal LSU and ITS sequences from the new taxon did not match those deposited in GenBank <http://www.ncbi.nlm.nih.gov/nucleotide> or sequences in the *Gymnopus* files at TENN.[10] The new taxon is proposed, illustrated and described below.

Materials and methods

Collection of fresh specimens employed typical field techniques. In several cases, photographs were made in the field, and basidiomata were dried and preserved in the fungarium of AV. In situ observation of macroscopic characters by AV have been augmented by deductions from dried material, but chiefly from several photographs, some of which are reproduced here. Microscopic features were observed exclusively from dried basidiomata. Microscopy and "barcoding" (i.e. production of ITS sequences) took place using small bits of dried material. Microscopy was accomplished using an Olympus BX60 microscope under Phase Contrast Microscopy (PhC), using 3% KOH with no stains. Abbreviations: TENN = herbarium, University of Tennessee; Q = spore length divided by spore width; Q^m = mean Q value for all spores measured; L^m = mean length of all spores measured. Colours within quotation marks from Ridgway,[11] and those in parentheses from Kornerup and Wanscher.[12]

Methods for DNA extraction, PCR of the nuclear ribosomal LSU and ITS region and sequencing were carried out as described previously.[13] Sequences were aligned in GCG[14] and a blast search was used to query GenBank. GenBank accession numbers for *G. eneficola* ITS sequences are KJ128262-KJ128268; LSU sequences are KJ189586-189590, and all are listed individually under additional specimens examined. A preliminary analysis of our entire (TENN) LSU *Gymnopus* library showed that *G. eneficola* was most closely related to *G. menehune*, *G. confluens* and *G. biformis*. The closest outgroup was *Gymnopus ramealis*. LSU sequences from collections of these species were used for parsimony analysis to demonstrate placement of *G. eneficola* (Figure 10).

Results

Gymnopus eneficola R.H. Petersen, species nova

MycoBank no. 807536

Holotype: Canada, Newfoundland, Pasadena Ski and Nature Club ski trails, coll. A. Voitk, 49° 0' 6" N, 57° 35' 36"W, 41 m ASL, 26.IX.2009, coll no. 09.09.26av13 (TENN 69123) KJ128264, KJ189586.

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Figure 1. Gymnopus eneficola, *basidiomata*. *Type collection from The Pasadena Ski and Nature Park ski trails, Pasadena, NL, 09.09.26av13 (TENN 69128). Note: the camera angle makes stipes look shorter than they are*.

Figure 2. Gymnopus eneficola, *basidiomata*. *06.08.19av01 (TENN 69126), Humber Village, Newfoundland and Labrador. Note: camera angle makes stipes of lying basiomata seem shorter than in real life*.

Etymology: En = "N;" ef = "F" (NF = abbreviation for Newfoundland); + -icola = dwelling in or preference for.

English diagnosis: Basidiomata collybioid or marasmioid. Pileus white when young, remaining so or mellowing to cream color; lamellae subdistant, adnate with slight tooth, white when young, mellowing to off-white by maturity; stipe terete when young becoming compressed or grooved by maturity, lightly vestured above, more strongly so downward, disappearing into a felty mycelial mass binding substrate. Pileipellis a modified dryophila structure with erect pileal hairs. Cheilocystidia 30-40 × 8-13 µm, variously contorted, lobed and roughly coralloid. Caulocystidia abundant, hyphal, downward long and branced. Basidiospores 7.5-9 × 3.5-5 µm (Q^m = 2.32; L^m = 8.30 µm), ellipsoid, slightly flattened adaxially, thin-walled, hyaline, smooth, inamyloid.

Description

Macroscopic

Basidiomata collybioid (Fig. 4), marasmioid,

Figure 3. Gymnopus eneficola *basidiomata*. *05.10.12av05 (TENN 69121), Humber Village, Newfoundland and Labrador.*

Figure 4. Gymnopus eneficola, *basidiomata*. *MR3-016 (TENN 69120), 2011 Faculty Foray, Main River, Deep Section. Photo: Roger Smith.*

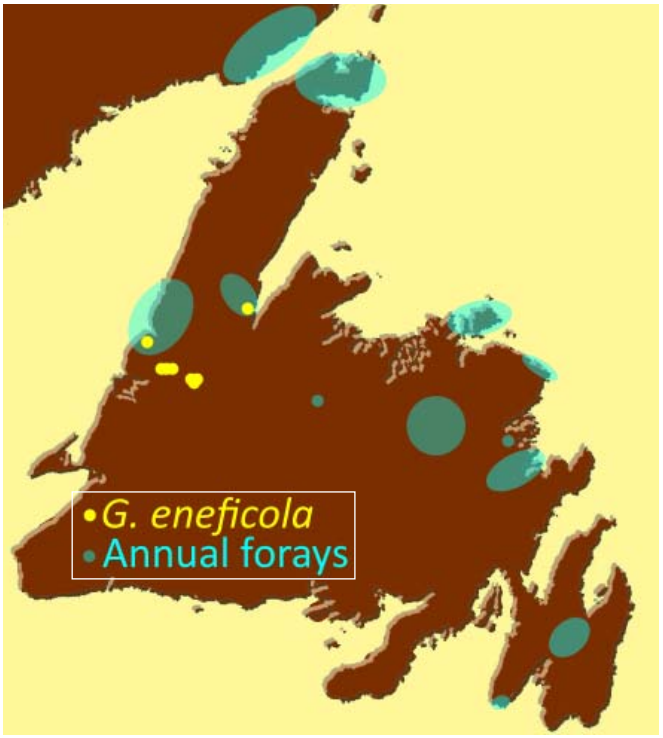

Figure 5. Known distribution of Gymnopus eneficola. *Limitation to the west coast region of the Island likely reflects the location of AV in that area. However, it is noteworthy that annual forays in other areas have not collected it.*

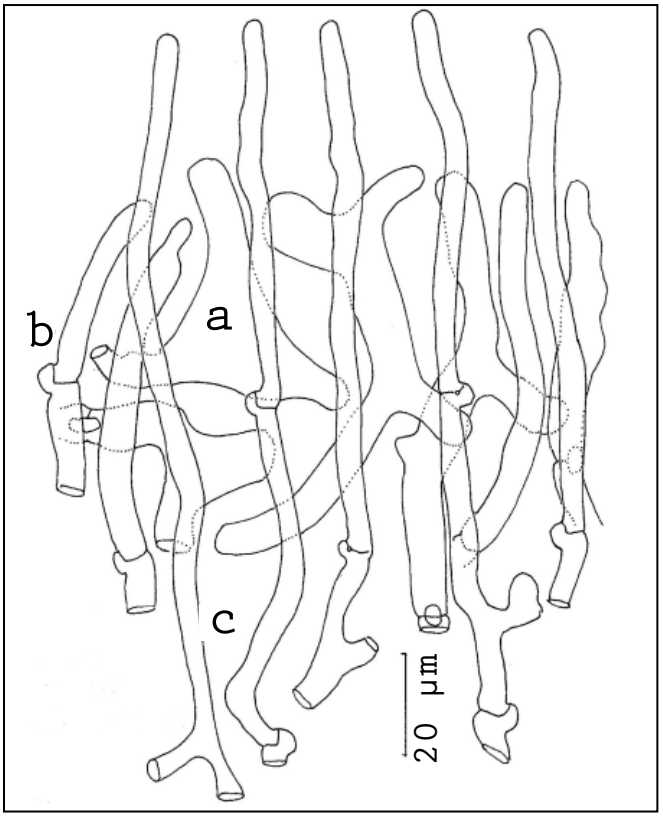

Figure 6. Gymnopus eneficola*: Cells of pileipellis.* ***a****) Cells of pileipellis surface [09.09.26av13 (TENN 69128)].* ***b****) Repent terminal cells of pileus surface [09.09.26av13 (TENN 69128)].* ***c****) Erect slender hyphal tips ("pileal hairs") [06.09.02av01 (TENN 69127)].*

especially sect. *Globulares*; (i.e. adult pileipellis smooth, with pileus diameter/stipe length 1:1-1:1.5; Figs. 1, 2), when young sometimes mycenoid (pileus diameter/stipe length 1:4-1:2) or hygrophoroid (Fig. 3), solitary, gregarious or cespitose in small groups of 2-3 (Fig. 1), with obviously vestured stipe (Fig. 3) and stipe base disappearing into felty-arachnoid hyphal mass involving minute bits of substrate (Fig. 2, right; 4). **Pileus** (Figs. 1-3) 11-24 mm broad, thin, convex when young (Fig. 1) and then with suggestion of an umbo, expanding and flattening through development, finally nearly applanate with thin, downturned but not inrolled margin (Figs. 1, right; 2, left), sometimes with a suggestion of central depression, smooth under lens, matte or occasionally plushy as a peach (60×), appearing polished in small areas especially in age, not striate, not hygrophanous, white when fresh (Fig. 1, center; 3), sometimes with very slight blush of "pinkish buff" (6A3) over disc (Fig. 1, left), slowly mellowing to cream-colored in age (Figs. 2, 4) from margin inward. **Lamellae** subdistant, adnate with slight tooth, ventricose, seceding in drying, sinuate, thickish, white when young (Figs. 1, 3), discoloring slightly over time through "pale cinnamon pink" (5A2) or "tilleul buff" (7B2) to "cinnamon buff" (6B4) or "cinnamon" (6B5) in age and drying; lamellar edge entire or slightly eroded (Figs. 1, 2) or undulate but not serrate; lamellulae in three ranks. **Stipe** (Fig. 2) 30-55 × 1.5-5 mm broad, initially terete (Figs. 1, 3), often becoming grooved or compressed (Fig. 2, right; 4, right), equal through upper portion, slightly expanded in lowest portion and there covered with loosely felty to sublannose mycelium (Figs. 2, 3, 4) and disappearing into and binding superficial substrate (Fig. 2), white when fresh becoming pallid creamy gray on drying, perhaps tan where chafed; vesture of upper stipe appearing frosted or minutely granular (Figs. 2, 3), downward vesture becoming thinly felty, then substrigose and finally at base densely arachnoid to lannose, especially when young (Figs. 3, 4), vesture easily crushed or removed where chafed (Fig. 2, right; 4, left); 3% KOH applied to lower stipe surface (dry) with off-white sublannose vesture = dull orange-brown (no evidence of green or citrine).

Habitat, phenology and distribution

"Mixed woods," (*Betula, Abies balsamea, Picea, Larix*; sometimes dominated by *Betula*, and no record without *Betula*), fruiting mid-August through early Novermber. At the moment, only known from a limited area of the Island of Newfoundland in the Canadian province of Newfoundland and Labrador (Fig. 5).

Microscopic

Pileipellis composed of generally repent, radially oriented hyphae 4-10 µm diam, often broadly "free-form" (i.e. individually resembling cells of a "dryophila structure;" Fig. 6a) firm- to thick-walled (wall -0.7 µm thick), not or hardly ornamented (ornamentation very vague bands under PhC but not raised or producing profile calluses, not visible in BF), frequently branched, conspicuously clamped, hyaline (PhC); terminal cells of pileipellis hyphae (Fig. 6b) 35-80 × 4-7 µm, single or in processes of 2-4 cells with clamp connections at septa, usually simple, occasionally tibiiform or substrangulate near terminus, firm-walled (wall -0.5 µm thick), hyaline (PhC). Occasional slender branches from pileipellis hyphae erect ("pileal hairs," Fig. 6c), -225 × 2-3(-4.5) µm, hyphal, unbranched, not necessarily arising at a clamp connection, usually internally clamped, firm-walled, hyaline. Hyphae of pileus trama 4-8 µm diam, firm-walled, frequently branched, frequently clamped, easily disarticulated, never sculptured. Lamellar trama generally longitudinal; tramal hyphae 4-7.5 µm diam, thin-walled, usually constricted at septa, clamped. **Subhymenium** a tight tissue of tortuous/branched hyphae 2-2.5 µm diam, bearing basidia on subsymbodial branches. **Basidioles** (Fig. 7a) 29-31 × 5-7 µm, digitate when young, becoming clavate/fusiform, clamped, thin-walled; contents homogeneous. **Basidia** (Fig. 7b, c) 23-30 × (7-)9-10 µm, broadly to bulbo-clavate, 2-4-sterigmate (sterigmata – 11 µm long when 2, -5 µm long when 4), clamped; contents homogeneous. **Pleurocystidia** not observed. **Cheilocystidia** (Fig. 7d,e) very locally common, overall occasional, (25-)30-40(-60) × 8-13(-17) µm, usually arising with basidia, hyaline, occasionally rising in subhymenium or outer trama, free-form with irregular lobes and curved branches, clamped. Stipe surface hyphae 4-11 µm diam, adherent, thick-walled (wall -1.5 µm thick), refringent (PhC), seldom but prominently clamped, hyaline. **Caulocystidia** from stipe apex (Fig. 8a) of two types: 1) a solid turf of short (-70 × 3-4 µm) hyphal tips arising as side branches and termini of stipe surface hyphae, firm- to thick-walled (wall -0.7 µm thick), internally clamped; and 2) a tangle of common longer (-175 µm long × 3-4 µm diam) hyphae, similar to shorter caulocystidia, branched 1-3 times at conspicuous clamp connections, hyaline, internally clamped

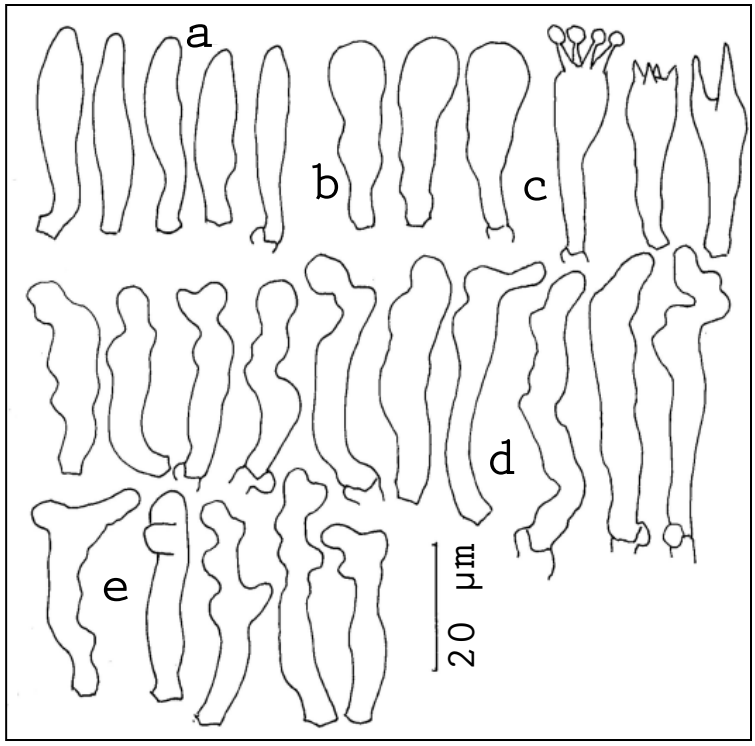

Figure 7. Line drawings of G. eneficola *hymenial structures.*

***a**) Basidioles [09.09.26av13 (TENN 69128)].*
***b**) Immature basidia [09.09.26av13 (TENN 69128)].*
***c**) Mature basidia (note 4- and 2-sterigmate individuals) [09.09.26av13 (TENN 69128)].*
***d**) Cheilocystidia [09.09.26av13 (TENN 69128)].*
***e**) Cheilocystidia [07.11.07av01 (TENN 69125)].*

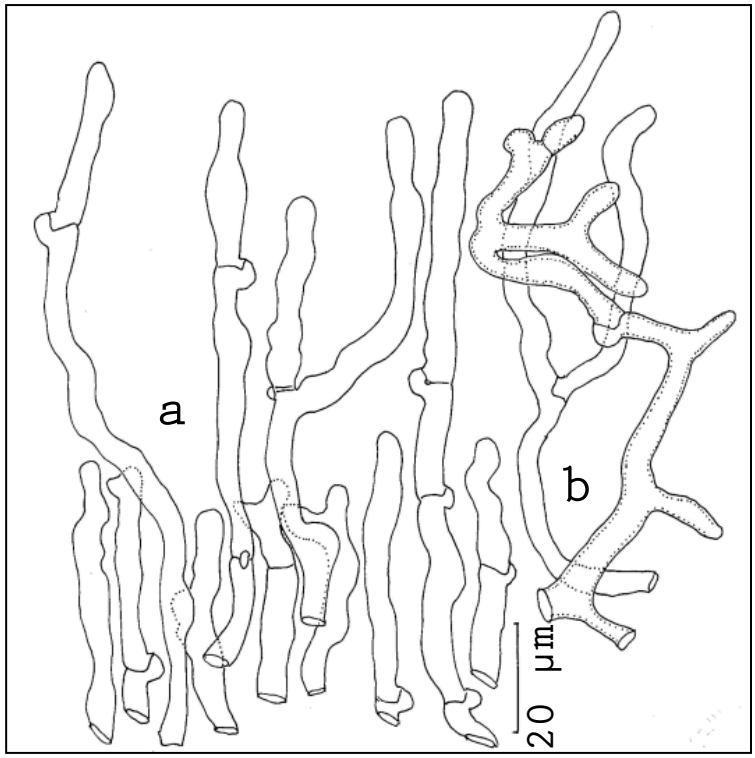

Figure 8. Line drawings of Gymnopus eneficola *caulocystidia.*

***a**) Short caulocystidia with longer individuals from upper stipe [09.09.26av13 (TENN 69128)].*
***b**) caulocystidia from lower portion of stipe [06.09.02av01 (TENN 69127)].*

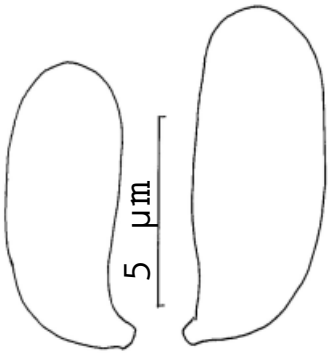

Figure 9. Line drawings of G. eneficola *basidiospores [06.09.02av01 (TENN 69127)].*

so terminal cells of long caulocystidia 17-27 µm long. Caulocystidia from stipe midsection (Fig. 8b) -240 × 3-4 µm, often branched near origin, hyphal, firm- to thick-walled (wall -0.7 µm thick), seldom clamped and almost never near cystidial apex; when branched, one branch consistently short and less developed; turf of shorter caulocystidia characteristic of upper stipe present only as scattered individuals. Basal mycelium inward organized into tissues of adherent hyphae 3-4 µm diam, firm- but not thick-walled, shearing into plates indicating adherent surfaces; outward hyphae disorganized, 3-5 µm diam, similar to caulocystidial hyphae, conspicuously clamped, firm-walled, hyaline, but macroscopically slightly discolored toward pinkish cinnamon, involving small bits of debris including well-decayed leaves, tiny twigs or petioles and occasionally flattened needles, probably of *Abies*. **Basidiospores** (Fig. 9) (7-)7.5-9 × (3-)3.5-5 µm (Q = 2.00-2.73; Q^m = 2.32; L^m = 8.30 µm), ellipsoid, somewhat flattened adaxially, thin-walled, hyaline, inamyloid; contents apparently homogeneous.

Phylogeny

LSU sequences place *G. eneficola* well within the *Gymnopus* clade (clade Omphalotaceae, not Marasmiaceae in the Agaricales). Neither LSU nor ITS sequences from collections of *G. eneficola* match any GenBank sequences more closely than 92%; the closest ITS blast match was to GenBank accession GU234141, *Gymnopus alkalivirens* from the Svalbard. An abbreviated LSU phylogram is shown in Figure 10.

Discussion

From notes and photos accompanying specimens, habitat might be inferred as decaying deciduous leaves and small twigs, favoring *Betula*. Most specimens were collected as *G. confluens*, also fruiting in such habitat, with which they are similar in vestured stipe, which is usually compressed or grooved, and presence of a basal mycelial mass which binds minute bits of litter and probable substrate. The extent of the basal mycelium is unknown at present (whether as widespread sheets as those of typical of *G. confluens*), but lamellar spacing (as a function of number), height and thickness and spore dimensions all differ substantially.

Basidiomata of *G. eneficola* might be mistaken for *Collybia tergina* (Fr.) Lundell if Halling[1] is used, because the stature of basidiomata and spore dimensions are similar. However, under *C. tergina*, Lundell and Nannfeldt[15] described an organism with smaller spores [5.5-7(-8) × 2.5-3.5 µm], "stem … especially lax and almost flabby," stipe "cylindrical and smooth, except for the base, which in some specimens is slightly villose," and "in almost perfect agreement with Fries's taxon" as illustrated in Fries. When Fries's[16] illustration and description are examined, his *Agaricus* (*Marasmius*) *terginus* exhibits a tan, striate pileus (in one individual quite pale but with tan disc) and tan stipe with no evidence of vesture. The sum of all these characters indicates an organism quite different from *G. eneficola*.

When the key to European *Gymnopus* species by Antonin & Noordeloos[2] is followed, a pivotal couplet concerns strong odor of basidiomata, a feature not observed for the new species. Choices which follow the strong odor lead to mismatched character fields, but if the choice of little or no odor is followed, the key permits several more choices until finally *G. oreadodoies* is reached. Because basidiomata of *G. eneficola* resemble those of the *Globulares* section of *Marasmius, G. oreadoides* is a tempting choice. Numerous discrepancies are found, however, when characters of *G. eneficola* are compared to those of *G. oreadoides*, as follows: 1. the pileus of *G. oreadoides* is described as "probably hygrophanous" (the accompanying photo clearly shows this); 2. pileus "pale cream color when young" (the accompanying photo shows strongly convex, neutral brown pileus of young basidiomata and distinctly ochraceous disc when mature); 3. stipe "fistulose, elastic, twisted… turning orange-ochraceous, finely pubescent, glabrescent; 4. spores "5.5-7.5(8.5) × 3.0-4.0 µm … Q = 1.9-2.1;" 5. caulocystidia… "20-50 × 3.5-7.5 µm, forming a dense layer all over the stipe surface." The line drawing accompanying the description illustrates these characters, with only short caulocystidia shown, together with encrusted pielpellis hyphae. Finally, "*Gymnopus strictipes* (Peck) Halling" is reported as similar to *G. oreadoides,* but Peck's species is a member of *Marasmius* sect. *Globulares*.

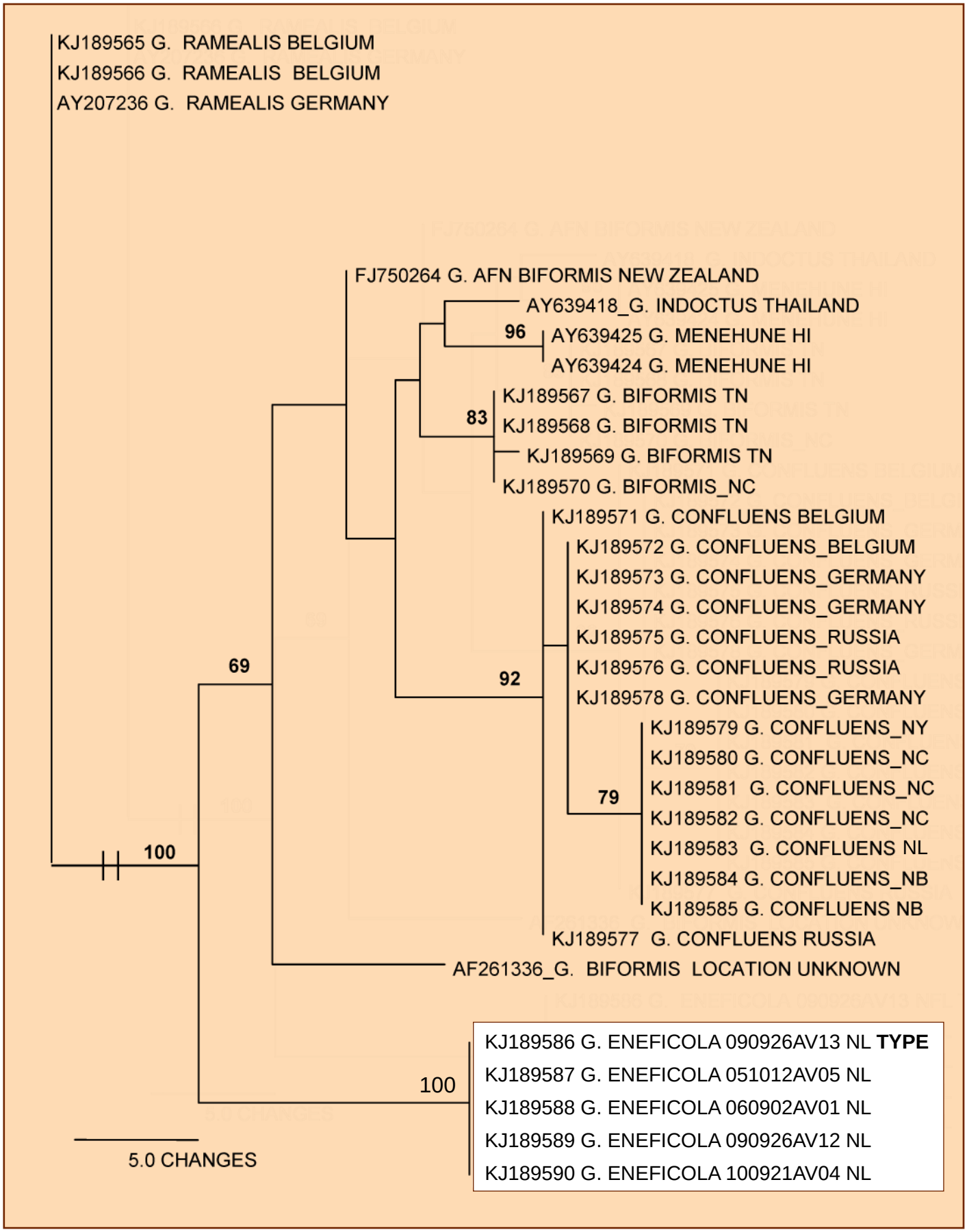

Figure 10. Parsimony analysis of nuclear ribosomal LSU sequences for Gymnopus *collections related to* G. eneficola. *The figure represents one of 1000 most parsimonious trees of length 74. Origin of collections indicated by country name in full or abbreviation for state/province if from the USA or Canada, appearing after the species name. Bootstrap support greater than 50% is given to the left of the supported node.*

Superficially, basidiomata are reminiscent of members of *Marasmius* sect. *Globulares* (i.e. *M. cystidiosus*, *M. nigrodiscus*, *M. oreades*) in general stature, lamellar morphology and attachment and pileus shape. Pileipellis construction of *Gymnopus eneficola* is not hymeniform, however, and DNA sequences place the Newfoundland taxon well within the *Gymnopus* clade. Moreover, basidiomata of *Gymnopus eneficola* are macromorpholigally more diminutive, have a greater stipe:pileus ratio and exhibit a vestured stipe at all ages.

At first, ventricose-rostrate immature basidia were thought to be cheilocystidia, being present consistently and abundantly, but they are present on lamellar faces (i.e. among basidia covering the lamellar surfaces) and seem to disappear near the lamellar edge. Once true cheilocystidia were observed, they could be compared to other hymenial structures for shape and size (see Fig. 7). Future caution is recommended. Caulocystidia (Fig. 8) are somewhat unique in distribution, dimensions and structure. The upper stipe appears only "frosted" without magnification, but magnification (750×) reveals a solid turf of short caulocystidia with some long indivduals present. Downward on the stipe, the turf remains but long caulocystidia become more numerous to give a somewhat more hirsute appearance to the naked eye. Both types of caulocystidia are consistently internally clamped so that the terminal cell is considerably shorter than expected. Clamp connections, especially those of basal mycelium, vary from papillate to medallion shapes to individuals with the "hook cell" elongated over the parent hyphae.

At the time of this writing (Jan. 2014), GenBank does not report any nucleotide sequences under the name *Gymnopus oreadoides*. Just as important, ITS sequences from collections of *G. eneficola* do not match any GenBank sequences more closely than 92%. However, with a blast match of 92% percent to a specimen from Svalbard, collections of *G. eneficola* from Newfoundland would be considered a distinct species by any criteria. LSU and ITS sequences of *G. eneficola* have been deposited in GenBank and are listed under additional specimens examined.

Additional specimens examined (all Canada, all Newfoundland, all collected as *G. confluens*): Humber Village, 13 Balsam, 48° 59' 21" N, 57° 45' 18" W, 12.X.2005, coll. A. Voitk, no. 05.10.12av05 (TENN 69121) KJ128263, KJ189567; same location, 7.XI.2007, coll. A. Voitk, no. 07.11.07av01 (TENN 69125) KJ128266; Humber Village, Weldon's Road, 48° 59' 43" N, 57° 44' 15" W, 19.VIII.2006, coll. A. Voitk, no. 06.08.19av01 (TENN 69126); Pasadena Ski Trails, 49° 0' 6" N, 57° 35' 36" W, 26.IX.2009, coll. A. Voitk, no. 09.09.26av12 (TENN 69128) KJ128268, KJ189589; Pasadena Stream Trail, 49° 00' 39" N, 57° 36' 38" W, 21.IX.2010, coll. A. Voitk, no. 10.09.21av04 (TENN 69122) KJ128265, KJ189590; Gros Morne Nat. Park, Stuckless Pond Trail, 49° 25' 55" N, 57° 43' 38" W, 1.XI.2005, coll. A. Voitk, no. 05.11.01av08 (TENN 69124); same location, 2.IX.2006, coll. A. Voitk, no. 06.09.02av01 (TENN 69127) KJ128267, KJ189588; Lower Main River (deep section), Trail 1, 49° 46' 40" N, 57° 53' 03" W, 6.IX.2011, coll. Foray Newfoundland and Labrador, no. MR3-016 (TENN 69120) KJ128262.

Acknowledgments

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