Cordyceps variabilis and the genus Syngliocladium

Kathie T. Hodge[1]

> *Department of Plant Pathology, Cornell University, Ithaca, New York 14853*

Richard A. Humber

> *Plant Protection Research Unit, USDA-ARS, Tower Rd., Ithaca, New York 14853*

Chris A. Wozniak[2]

> *NCSL, USDA-ARS, P.O. Box 5677, State University Station, Fargo, North Dakota 58105*

Abstract: *Cordyceps variabilis* has long been erroneously reported to be a pathogen of beetle larvae; studies of newly collected and herbarium specimens revealed that *C. variabilis* attacks fly larvae, particularly those of the family Xylophagidae (Diptera). An emended description of *C. variabilis* is provided and a lectotype designated. The anamorph of *C. variabilis* is reported here for the first time, and is attributed to the hyphomycetous genus *Syngliocladium*. An allied species, *S. tetanopsis* sp. nov. is reported as a pathogen of larvae of *Tetanops myopaeformis*, the sugar beet root maggot (Diptera: Otitidae). The taxonomy of the genus *Syngliocladium* is briefly reviewed, and the generic concept emended.

Key Words: Clavicipitaceae, entomopathogen, Hypocreales, *Sorosporella*, teleomorph

INTRODUCTION

Species of *Cordyceps* Fr. are obligate pathogens of arthropods (insects, spiders), hypogeous fungi (*Elaphomyces* spp.), or other clavicipitaceous fungi (*Cordyceps* and *Claviceps* spp.). Well over 400 species have been described in *Cordyceps*, some of which can be expected to be eventually relegated to synonymy, but it is also apparent that many species have yet to be described. Kobayasi, the foremost modern expert on the genus, accepted some 280 species (1982). Many

species were briefly described based on one or a few fruiting bodies and have never been fully characterized. Mycologists are not necessarily good entomologists, and host records for insect pathogens are often inaccurate or limited to general categories. Given the deficit in teleomorph studies, it is hardly surprising that the anamorphs of *Cordyceps* species have only rarely been characterized, despite their potential utility in elucidating infrageneric relationships.

Known anamorphs of *Cordyceps* species belong to hyphomycetous genera including *Akanthomyces*, *Beauveria*, *Desmidiospora*, *Hirsutella*, *Hymenostilbe*, *Mariannaea*, *Metarhizium*, *Nomuraea*, *Paraisaria*, *Paecilomyces*, *Tolypocladium* and *Verticillium* (Evans and Samson, 1984, 1987; Gams, 1971a; Hodge et al., 1996; Liang, 1991; Liang et al., 1991; Mains, 1950; Petch, 1932b, 1935; Samson, 1974; Samson and Brady, 1983; Shimazu et al., 1988). Mycoparasites growing on *Cordyceps* stromata have sometimes been mistaken for anamorphs. This seems to have been the case for putative anamorphs recorded as *Stilbella* species by Kobayasi (1941); many of these have been recognized as species of *Polycephalomyces* Kobayasi by Seifert (1985). We believe that some putative anamorphs referred to *Sporothrix* may also be mycoparasites growing on *Cordyceps* stromata.

In this paper, the anamorph of *C. variabilis* Petch is characterized for the first time, a correction is made as to the nature of the host, and an emended description of *C. variabilis* is presented. The anamorph is assigned to the genus *Syngliocladium* Petch, which is emended herein, and a similar fungus occurring as a pathogen of the sugar beet root maggot, *Tetanops myopaeformis* Röder (Diptera: Otitidae), is described as a new species.

MATERIALS AND METHODS

Fresh specimens of *C. variabilis* were collected from rotting wood at various sites in New York. Refer to *Specimens examined* for locations and accession information. Larvae of *T. myopaeformis* infected with an unknown fungus were collected during the course of field screening for biocontrol agents of *T. myopaeformis* by CAW starting in the summer of 1994. Color terms are those of Kornerup and Wanscher (1967), and size ranges represent averages that are in some

cases circumscribed by minimum and maximum measurements.

Fungal cultures.—Cultures were obtained from superficial mycelium and from hyphal bodies within cadavers of *T. myopaeformis,* and from superficial mycelium, hyphal bodies, and part ascospores of *C. variabilis.* Observations of growth and morphology were taken from cultures on oatmeal agar (OA: a filtered aqueous solution of 30 g/L rolled oats cooked for 30 min, 1.5% agar), modified oatmeal agar (mOA: an aqueous suspension of 30 g/L rolled oats cooked for 30 min, 1.2% agar, supplemented after autoclaving with 3 mL/L extra virgin olive oil and 30 mg/L cholesterol), malt extract agar (MEA, Difco[3]), and Sabouraud's dextrose agar with yeast extract (SDAY: 40g/L dextrose; 20 g/L bacto-peptone; 20 g/L yeast extract; 1.5% agar). All cultures have been deposited in the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF; Plant Protection Research Unit, U.S. Plant, Soil, and Nutrition Laboratory, Tower Rd, Ithaca, New York 14853) and are referred to hereafter by their ARSEF accession numbers.

Cultures of the *Synglioicladium* anamorph of *C. variabilis* included in the present study are ARSEF 4080 (from mycelium on surface of host bearing stroma of *C. variabilis.* NEW YORK: Hamilton Co., Raquette L., Long Point. *Xylophagus* sp. larva (Diptera: Xylophagidae) in wood, 29 July 1993 *K.T. Hodge kth13*); ARSEF 5365 and 5366 (from mycelium on surface of host bearing stroma of *C. variabilis.* NEW YORK: Danby, Michigan Hollow State Forest. *Xylophagus* sp. larva (Diptera: Xylophagidae) in wood, 3 Aug. 1996 *K.T. Hodge kth50a, kth50b* (CUP 63882)); ARSEF 5367 (from mycelium on surface of host bearing stroma of *C. variabilis.* NEW YORK: Danby, Michigan Hollow State Forest. *Xylophagus* sp. larva (Diptera: Xylophagidae) in wood, 3 Aug. 1996 *K.T. Hodge kth51a* (CUP 63883)); and ARSEF 5414–5429 (single ascospore isolates from *C. variabilis.* NEW YORK: Danby, Michigan Hollow State Forest. *Xylophagus* sp. larva (Diptera: Xylophagidae) in wood, 3 Aug. 1996 *K.T. Hodge kth50a,* (CUP 63882)).

Cultures of *Synglioicladium tetanopsis* sp. nov. included in the present study were ARSEF 4972 (from hyphal bodies inside cadaver of *T. myopaeformis* larva. NORTH DAKOTA: Red River Valley, 6 June 1995. *C.A. Wozniak* (CUP 64915)); ARSEF 5497 (ex type culture (FStlA8–2294; NRRL 21854), from 3rd instar larva of *T. myopaeformis.* NORTH DAKOTA: Pembina Co., St. Thomas. 1 Aug. 1994. *C.A. Wozniak* (CUP 64913)); and ARSEF 5577 (from hyphal bodies inside cadaver of 3rd instar larva of *T. myopaeformis.* (FHi1A11–295; NRRL 21853). NORTH DAKOTA: Red River Valley, 6 June 1995. *C.A. Wozniak*).

Specimens.—Herbarium specimens of *C. variabilis* were obtained on loan from North American herbaria (CORT, CUP, DAOM, FH, MICH, TRTC). Specimens were examined for the presence of the anamorph on the host body, and the hosts were identified where possible. A slide made from the holotype of *S. aranearum* Petch (ex Araneida, St. Leonard's Forest, Horsham, Sussex, U.K. 31 May 1931) was examined through the kindness of H. C. Evans and the keeper of K; the type specimen of *S. intricatum* Petch (on larva of *Phyllopaga anxia,* Apple Hill, Ontario, Canada. 1934) was also borrowed from K.

Pathogenicity assay.—The ability of ARSEF 5497 and ARSEF 4080 to infect *T. myopaeformis* larvae was tested in a bioassay. Ten third instar larvae of *T. myopaeformis* from field collections were placed in 125 g of autoclaved coarse sand in a 9-cm petri dish. A saline (0.85% w/v) spore suspension collected from mOA plate cultures of ARSEF 5497 or ARSEF 4080 was added to the sand to yield 3×10^5 viable spores per dish, based on hemocytometer counts and fluorescein diacetate staining. Saline solution was added to 7 mL total per dish; saline alone was used as a control for estimation of natural background infection. After 7 da at 24 C, larvae were removed from the sand, rinsed in sterile distilled water, and placed on 9 cm Whatman (Kent, UK) no. 1 filter discs in glass petri dishes wrapped with Parafilm M (American Can Co., Neenah, Wisconsin, USA). Discs were moistened weekly with sterile distilled water to prevent desiccation of the insects. Larvae showing external hyphal growth were transferred to separate dishes so that infection rates do not represent secondary infections among larvae. Forty larvae were used per treatment (5497, 4080, and control), and the experiment was performed three times. Cadavers were inspected for the development of externally sporulating mycelium.

RESULTS

Cultural characteristics.—Cultures isolated from hyphal bodies, mycelium from the host surface, and single part ascospores of *C. variabilis* yielded the same hyphomycetous fungus in culture. Cultures isolated from *T. myopaeformis* cadavers yielded a similar hyphomycetous fungus which differed in key characters as outlined below.

Pathogenicity to T. myopaeformis.—Strains isolated from *T. myopaeformis* were observed to be infective to 3rd instar larvae of *T. myopaeformis*; 85% of inoculated larva were killed by the fungus 30 da after inoculation. Infected larvae typically died within 21 da, developed an orange coloration and eventually produced synnemata similar to those that developed in axenic culture on OA and mOA. The potential of these strains as biological control agents of *T. myopaeformis* will be explored in future investigations.
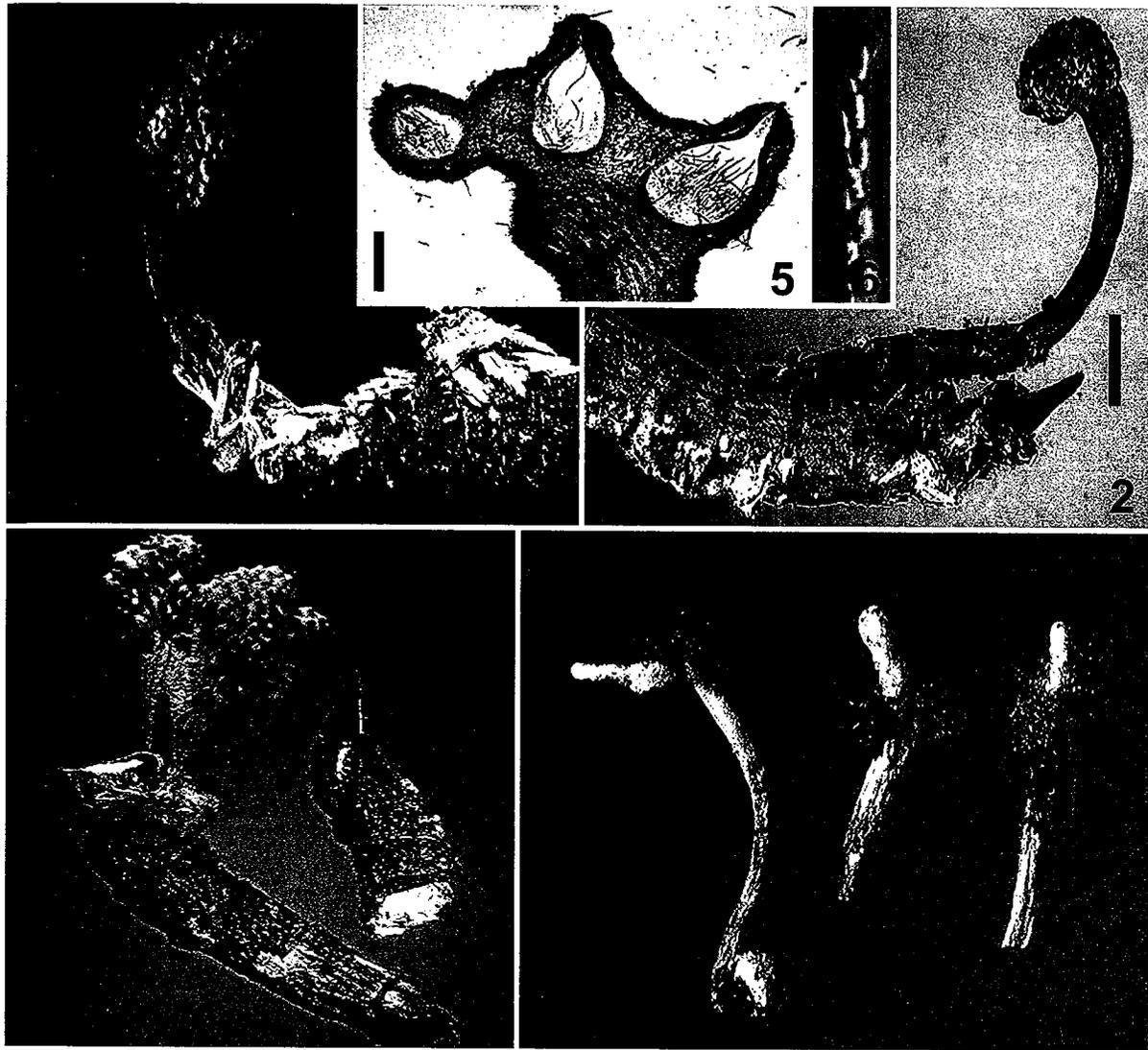
FIGS. 1–6. *Cordyceps variabilis.* 1. Typical stroma arising from anterior end of larva of *Xylophagus* sp. (CUP 47800), bar = 2 mm. 2. Capitate stroma arising from anterior end of larva of *Xylophagus* sp. (CORT 7736), bar = 2 mm. 3. Robust stroma arising near midpoint of larva of *Xylophagus* sp. (the anterior portion has broken off) (TRTC 4344), bar = 2 mm. 4. Small stromata on unidentified dipteran larvae (hosts removed) (FH 9861), bar = 1 mm. 5. (inset) Section of stroma (FH 10169), bar = 50 μm. 6. (inset) Ascus containing part ascospores. (CUP 63882), bar = 10 μm.

TAXONOMY

Cordyceps variabilis Petch, Trans. Br. Mycol. Soc. 21: 44. 1937 (25 Oct.) emend. K. T. Hodge & Humber.

FIGS. 1–6

= *Cordyceps viperina* Mains, Mycologia 29: 674. 1937 (1 Dec.).

= *Cordyceps ithacensis* Balazy & Bujak., Mycotaxon 25: 11. 1986.

=? *Cordyceps ferruginosa* Kobayasi & Shimizu, Bull. Natn. Sci. Mus., Ser. B (Bot.) 6: 139. 1980.

=? *Cordyceps asyuensis* Kobayasi & Shimizu, Bull. Natn. Sci Mus., Ser. B (Bot.) 6: 138. 1980.

LECTOTYPE. NEW YORK: Ithaca, on larva of *Xylophagus* sp., 25 July, 1916, *G.H. Duff* (FH 6132A)

Anamorph. Syngliocladium sp.   FIGS. 7–9, 14–15

Parasitic on larvae of Diptera, particularly of wood-inhabiting Xylophagidae; host discolored burnt sienna [7–8(D8)] as a result of fungal infection. Host cadavers packed with cream-colored hyphal bodies. Hyphal bodies frequently peanut-shaped, 10–35 μm long, sometimes irregularly subglobose, not particularly thick-walled, and containing large, refractive lipid droplets. Host body often bearing orange (6B8)
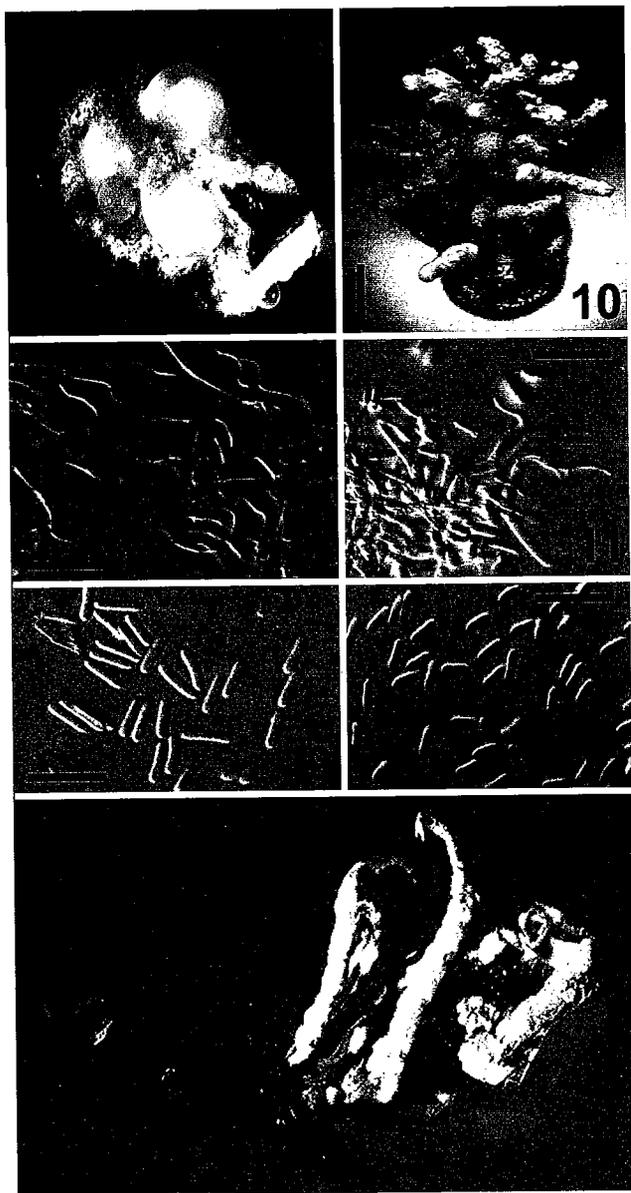
FIGS. 7–13. *Syngliocladium* anamorph of *C. variabilis* (left) and *Syngliocladium tetanopsis* sp. nov. (right). 7–9. Cultural morphology of *Syngliocladium* anamorph of *C. variabilis* (ARSEF 4080). 7. One-mo-old colony on SDAY, bar = 5 mm. 8. Conidiogenous cells, bar = 20 μm. 9. Conidia, bar = 20 μm. 10–12. Cultural morphology of *S. tetanopsis* (ARSEF 4972). 10. Six-wk-old colony on MEA (ARSEF 4972), bar = 5 mm. 11. Conidiogenous cells, bar = 20 μm. 12. Conidia, bar = 20 μm. 13. *S. tetanopsis* on wild-collected larva of *Tetanops myopaeformis* (CUP 64914), bar = 1 mm.

patches of mononematous *Syngliocladium* anamorph, especially at the intersegmental membranes.

One or few stromata arising from body of host in rotten wood, varying widely in size (2–24 mm). Stipe round in cross-section, equal, 0.2–3 mm diam, chrome yellow (5A7), furfuraceous, with sterile apex, bearing one or more lateral, or occasionally terminal

fertile cushions in which perithecioid ascomata are immersed, and which are composed of textura intricata with a pseudoparenchymous cortex. Cortex pigmented, 30–45 μm thick, composed of an inner layer of thick-walled parallel hyphae and an outer one of inflated, thick-walled irregular cells with free ends measuring 4.5–7.5 × 3.5–4.5 μm. Perithecia obpyriform, 350–590 × 200–370 μm, immersed perpendicular to the surface. Ostioles darker orange (6C8), protruding slightly above stromal surface and giving the fertile cushion a verrucose appearance. Perithecial walls 12–18 μm thick, with an inner layer of waxy cells about 1 μm wide and an outer one of thick-walled brick-like cells about 1.5 μm wide. Ostiolar canal periphysate, periphyses 11 × 0.5 μm. Paraphyses not observed. Asci cylindric, 210–330 μm long by 6.0 μm wide, with an apical cap 4.5 μm deep, packed with eight filiform ascospores each extending almost the full length of the ascus. Ascospores becoming multiseptate, dividing at maturity into many cylindric part ascospores (5.0–) 8.5 (–10.0) × (1.5–) 2.0 (–3.0) μm, with thick, refractive walls. Distal ends of terminal part ascospores tapered.

Anamorph sometimes produced in sparse patches or tufts on the surface of the host or on immature stromata. Conidiogenous cells in monoverticillate whorls or pairs. Conidiogenous cells enteroblastic, (10.0–) 14.0 (–19.2) × 2.5 μm, with subcylindric base and abruptly narrowing to form a short, tapering neck without a conspicuous periclinal thickening or collarette. Conidiogenous cells often strongly hooked or bent; producing ameroconidia in copious slime. Orange granules often present on the hyphal cell walls. Conidia subcylindric, on average (8.0–) 10.2 (–12.4) × (1.9–) 2.6 (–3.1) μm (n = 100), often slightly constricted in the middle and sometimes slightly curved. Ascospores germinating after one mo on MEA. Colonies developing very slowly in culture on OA, mOA, SDAY, and MEA, reaching only 7 mm diam after 2 wk on SDAY. Colonies on OA composed of zones of white mycelium which become orange as copious phialoconidia are produced in slime balls that unite to form large macroscopically visible droplets. On OA and mOA occasionally producing blunt orange synnemata (1 × 8 mm) with copious conidiogenesis and slime production. Not forming chlamydospores in the host nor on artificial media.

Known distribution. Belize, Canada (Nova Scotia, Ontario), China, USA (Illinois, Maine, New York, North Carolina, Pennsylvania, Tennessee, Washington, Wisconsin) (Liang et al., 1995; Mains, 1937, 1939, 1958).

Specimens examined. (Specimens that include the anamorph are marked with an asterisk) ILLINOIS: McLean
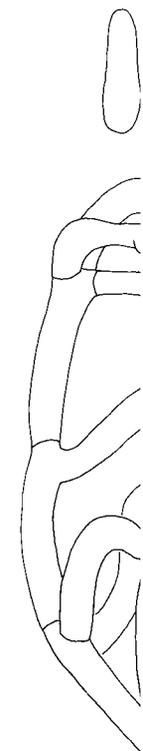
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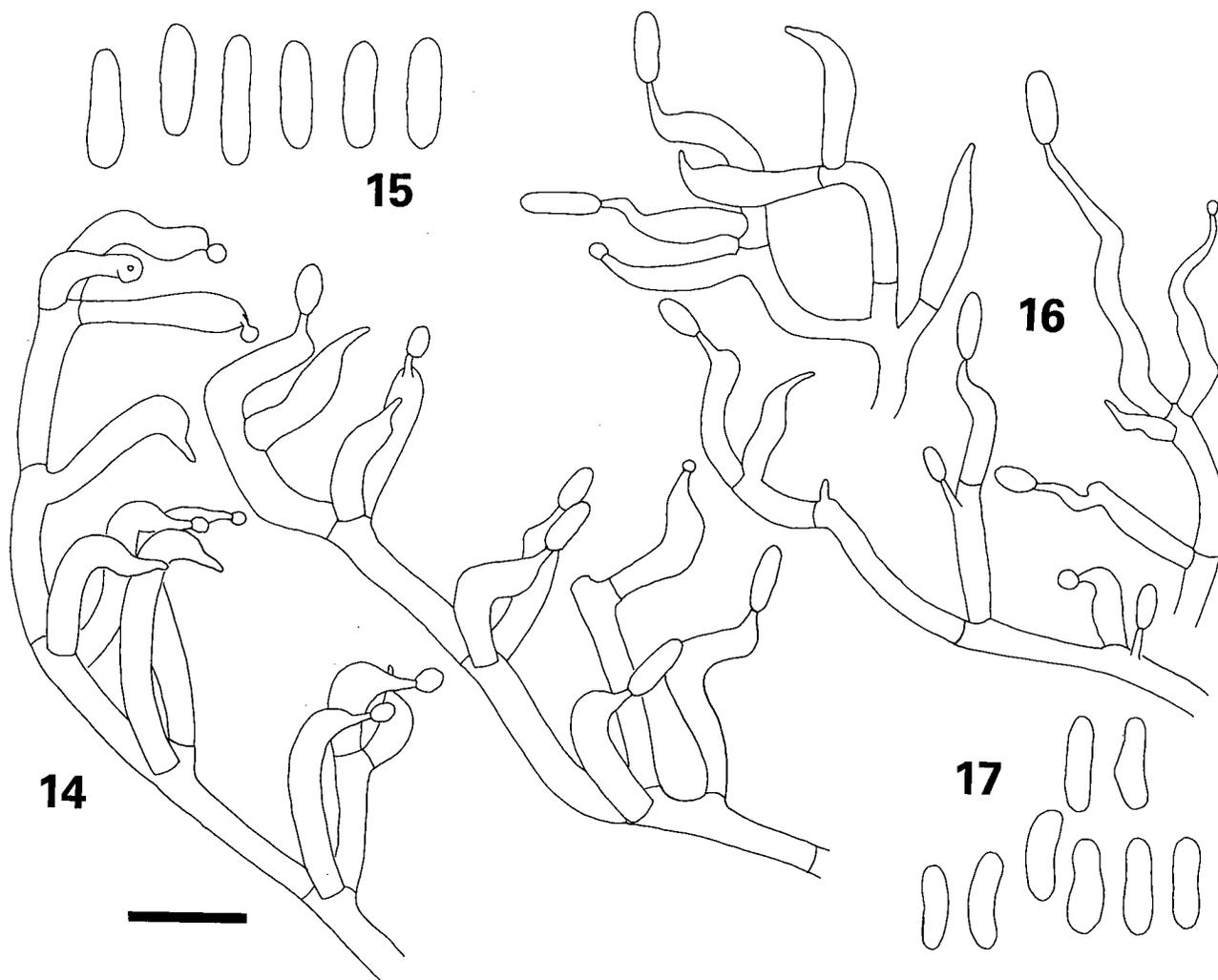
FIGS. 14–17. *Syngliocladium* anamorph of *C. variabilis* (left) and *Syngliocladium tetanopsis* sp. nov. (right). 14–15. *Syngliocladium* anamorph of *C. variabilis* (ARSEF 4080). 14. Conidiophores. 15. Conidia. 16–17. *S. tetanopsis* (ARSEF 4972). 16. Conidiophores. 17. Conidia. Bar = 10 μm.

Co., Larva (Diptera: Xylophagidae) in wood, July–Aug. 1970, *C. H. Runde* (K. A. Harrison 9944, MICH); Champaign Co., Brownfield Woods. *Rhacicerus nitidus* Johnson larvae (Diptera: Xylophagidae), 3 July 1973, *D. W. Webb* (ARSEF); MAINE: Kittery Point, 1895, *R. Thaxter.* (FH 2843*, syntype of *C. variabilis* Petch); Kittery Point, 20 Aug. 1920, *R. Thaxter* (FH 6138*, syntype of *C. variabilis* Petch); York, Chris Swamp, *R. Thaxter* (FH 6146*, syntype of *C. variabilis* Petch); NEW YORK: Ithaca, on *Xylophagus* larva (Diptera: Xylophagidae), 25 July, 1916, *G.H. Duff* (FH 6132A, herein designated LECTOTYPE of *C. variabilis*); Hamilton Co., Raquette L., Antler's Camp. *Xylophagus* larva (Diptera: Xylophagidae) in wood, 1 Aug. 1995, *T. J. Baroni* (CORT 7736*); Danby, Michigan Hollow State Forest, Finger Lakes Trail. *Xylophagus* larva (Diptera: Xylophagidae) in decorticated wood of *Tilia americana,* 3 Aug. 1996, *K. T. Hodge kth50* (CUP 63882;* Living cultures ARSEF 5365, 5366, 5414–5429); Danby, Michigan Hollow State Forest, Finger Lakes Trail. *Xylophagus* larva (Diptera: Xylophagidae) in decorticated wood of *Tilia americana,* 3 Aug. 1996,

K. T. Hodge kth51 (CUP 63883;* Living culture ARSEF 5367); Danby, Michigan Hollow State Forest, Finger Lakes Trail. *Xylophagus* larva (Diptera: Xylophagidae) immature stromata in decaying wood, 3 Aug. 1996, *K. T. Hodge kth52* (CUP 63884*); Alpine, Lost Gorge. *Xylophagus* larva (Diptera: Xylophagidae) in wood, 15 July 1964, *T. Plowman* (CUP 47800); Danby. *Xylophagus* larva (Diptera: Xylophagidae) in wood, 8 Aug. 1971, *Paul E. Powell* (CUP 52353); Ithaca, Coy Glen. *Xylophagus* larva (Diptera: Xylophagidae) in wood, 30 Sept. 1982, *John (Jin-sheng) Hu* (CUP 60118); Enfield, Connecticut Hill Wildlife Management Area. *Xylophagus* larva (Diptera: Xylophagidae) in wood, 10 July 1994, *David Price* (CUP 63314); Essex-Hamilton Co., Catlin L. Larva (Diptera: Xylophagidae) in wood, 19 Aug. 1934, *A. H. Smith 387* (MICH,* holotype of *C. viperina* Mains); NORTH CAROLINA: Swain Co., Great Smoky Mountains National Park, Indian Creek. Larva (Diptera) in wood, 2 Sept. 1939, *A. H. Smith 10834* (MICH); Swain Co., Great Smoky Mountains National Park, Indian Creek. Larva (Diptera) in wood, 5 Sept. 1937, *A. H. Smith 7393* (MICH);

Swain Co., Great Smoky Mountains National Park, Indian Creek. Larva (Diptera) in wood, 14 Aug. 1938, *A. H. Smith 10169* (MICH); PENNSYLVANIA: Bucks Co., State Game Land #157. Larva (Diptera: Xylophagidae) in rotten wood, 1 Aug. 1997, *Judith Fuchs* (CUP 64917*); TENNESSEE: Burbank, 7 Aug. 1886, *R. Thaxter* (FH 6144,* syntype of *C. variabilis* Petch); Sevier Co., Great Smoky Mountains National Park, Coon's Cove. Larva (Diptera) in wood, 8 Aug. 1938, *A. H. Smith 9861* (MICH); WASHINGTON: Whatcom Co., Baber L. Larva (Diptera: Xylophagidae) in wood, 2 Sept. 1941, *E. B. Mains* (MICH*); Skagit Co., Rocks Port, Maple Creek Forest Camp. Larva (Diptera: Xylophagidae) in wood, 15 Aug. 1941, *A. H. Smith 16166* (MICH*); Jefferson Co., Hoh R. Larva (Diptera: Xylophagidae) in wood, 30 June 1939, *A. H. Smith 14687* (MICH); WISCONSIN: Blue Mounds, 8 July 1905, *R. A. Harper.* (FH*, syntype of *C. variabilis* Petch); CANADA. ONTARIO: Haliburton Co., University of Toronto Forest. Larva (Diptera: Xylophagidae) in wood, 13 Sept. 1968, *D. Malloch* (DAOM 136432); Haliburton Co., University of Toronto Forest. *Xylophagus* larva (Diptera: Xylophagidae) in poplar wood, 13 Sept. 1968, *D. Malloch* (TRTC 45496); Temagami F.R., Round Lake. *Xylophagus* larva (Diptera: Xylophagidae) in moss, 2 Sept. 1932, *H. S. Jackson* (TRTC 4344); Lake Temagami. Larva (Diptera: Xylophagidae) in wood, 26 Aug. 1936, *A. H. Smith 4185* (TRTC, paratype of *C. viperina* Mains); NOVA SCOTIA: Colchester Co., Earlton Rd. Larva (Diptera: Xylophagidae) in wood, 26 Aug. 1931, *L. E. Wehmeyer 1213a* (MICH, paratype of *C. viperina* Mains); BELIZE. El Cayo District, Cohune Ridge. Larva (Diptera: Xylophagidae) in wood, 13 July 1936, *E. B. Mains 3837* (MICH, paratype of *C. viperina* Mains).

Commentary. The epithet given by Petch (1937) to this species aptly reflects its extreme variability in size and in the aspect of the fertile portion of the stromata (FIGS. 1–4). Among the specimens examined, the height of the stroma varied from 2–24 mm, and the perithecial "cushion" formed one or more lateral patches on the stipe, exposing a sterile apex, or less frequently assumed a capitate form (FIG. 2). *Cordyceps viperina* was described by Mains (1937) the same year, and was later recognized by him to be a synonym of *C. variabilis* (Mains, 1958), a conclusion supported by our own observations of type material. Both species were based on North American material. *Cordyceps variabilis* has also been reported from China by Liang et al. (1995), and from the Democratic Republic of Congo by Moureau (1961); those specimens were not examined in the present study.

Petch (1937) recorded the hosts of *C. variabilis* as coleopteran larvae or specifically as members of the coleopteran family Elateridae, despite annotations on some FH labels by Roland Thaxter indicating that the hosts were dipteran larvae. Mains (1937, 1958) described the hosts as larvae of Coleoptera. Because Petch (1937) did not designate a holotype, we have

selected as lectotype his syntype specimen FH 6132A (listed incorrectly in his manuscript as FH 613A).

All of the many specimens examined in this study, including type specimens of both *C. variabilis* and *C. viperina,* were found to occur on larvae of Diptera. Many of these can be readily identified as species of the genus *Xylophagus* (Xylophagidae), others are too small or degraded to determine to family. After death, hosts assume an orange-brown coloration that confers some resemblance to elaterid larvae, and this may be the source of confusion. Xylophagid larvae lack legs, however, and have a sclerotized and variously lobed anal plate that Mains (1937) apparently confused with a beetle head. The larval head capsules of *Xylophagus* spp. [FIGS. 1–3 (top)] are darkly melanized and conical with a narrow longitudinal slit through which the mouthparts can sometimes be seen to project minutely on the ventral side; the bodies are elongate (Stehr, 1991). We have not encountered specimens of *C. variabilis* on hosts other than larval Diptera.

Allies and synonyms of C. variabilis. *Cordyceps corallomyces* Møller (1901) was described from Brazil on a "Kellerassel" (sowbug, Crustacea: Isopoda). It is similar to *C. variabilis* in its part ascospore size, red-orange coloration and lateral perithecial cushion with a distinct cortex. Petch (1935) considered *C. corallomyces* to be a synonym of *C. ainictos* Møller. Kobayasi (1941) reported *C. corallomyces* from Japan on a dipteran larva; his description is referable to *C. variabilis.* Unfortunately Møller's specimens were apparently destroyed in Berlin during World War II (B. Hein, pers. comm.). Given the different host and lack of authentic specimens, we consider *C. corallomyces* a nomen dubium. In the future, specimens collected near the type locality may help elucidate the relationship or possible synonymy between it and *C. variabilis.*

Cordyceps ithacensis Bałazy & Bujakiewicz (1986) was described from a single collection on a wood-inhabiting dipteran larva in the family Erinnidae (=Xylophagidae fide Borror et al., 1989) in the vicinity of Ithaca, New York. It was described as having a small capitate stroma with orange perithecia and an almost furfuraceous cortical layer of cells. Although the holotype could not be obtained from POZN, the illustrations and thorough description provided by the authors, the host family and stage, and the locality of collection indicate that it is certainly a synonym of *C. variabilis. Cordyceps ithacensis* is based on a specimen in which the fertile region occurs terminally on the stroma, a condition that we have occasionally observed in *C. variabilis* (FIG. 2).

Both *C. ferruginosa* Kobayasi & Shimizu and *C. asyuensis* Kobayasi & Shimizu were described as species

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resembling *C. variabilis* in that they produce lateral cushions of perithecia on acicular, furfuraceous stromata (Kobayasi and Shimizu, 1980). Although the authors list the hosts for both as coleopteran larvae, their figures (Kobayasi and Shimizu, 1980, Fig. 23A, 23B, 1983b, Pl. 4, Fig. 3) and also those of Imazeki et al. (1988, p. 583) suggest that the hosts might be dipteran larvae, as reported here for *C. variabilis*. Moreover, the descriptions and illustrations differ little from North American material of *C. variabilis*. Type material must be examined before conclusions regarding the synonymy and relationship of these species can be reached; we were unable to obtain the necessary specimens from TNS. *Cordyceps minutissima* Kobayasi & Shimizu also bears a close similarity to *C. variabilis*, and the small host (which was described as a beetle larva) may also be a dipteran larva (see Kobayasi and Shimizu, 1983b, Pl. 11, Fig. 3).

Moureau (1961) reported on a collection of *C. variabilis* on beetle larvae from the Democratic Republic of Congo. He rejected Mains' (1958) treatment of *C. viperina* as a synonym of *C. variabilis* because the pseudoparenchymous cortex described by Mains (1937) in *C. viperina* was not mentioned by Petch (1937) in his discussion of *C. variabilis*. We have found no evidence that Moureau examined any North American specimens; our own examination of Petch's type specimens indicates that a pseudoparenchymous cortex is present. In 1962, Moureau changed his views on this issue, deciding that *C. viperina* was indeed a synonym of *C. variabilis*. He then went on to erect a new species from the Democratic Republic of Congo, *C. inconspicua* Moureau, which differed from *C. variabilis* only in its lack of a pseudoparenchymous cortex. Unfortunately the holotype of *C. inconspicua* and Moureau's collections of *C. variabilis* were inaccessible due to political unrest in the Democratic Republic of Congo (V. Demoulin, pers. comm.). Without his specimens we are unable to address his changing arguments and remain uncertain regarding the status of *C. inconspicua* and whether *C. variabilis* might occur in the Democratic Republic of Congo.

A number of other *Cordyceps* species produce stromata with lateral, pulvinate fertile portions. They are included by Kobayasi (1982) in *Cordyceps* subgenus *Eucordyceps* Kobayasi, section *Laterales* Kobayasi. Neither of these taxa is nomenclaturally acceptable (Greuter et al., 1994): *Cordyceps* subg. *Eucordyceps* includes *C. militaris*, the type species of *Cordyceps*, and therefore must be referred to by the autonym *Cordyceps* subg. *Cordyceps* (Art. 22.1, 21.3). *Cordyceps* subsect. *Laterales* was validly described by Kobayasi (1941), but was never validly transferred to the rank of section. *Cordyceps purpureostromata* Kobayasi (Ko-

bayasi and Shimizu, 1980) was described from Japan on larvae of Elateridae (Coleoptera). It differs from *C. variabilis* in its purplish coloration, longer part ascospores, and smooth stipe. *Cordyceps clavata* Kobayasi & Shimizu (1980) differs from *C. variabilis* in having perithecia that are partially free and in producing multiple stromata on a coleopterous host. *Cordyceps nigripoda* Kobayasi & Shimizu (1982) differs in its fuscous coloration, lack of a furfuraceous cortex, nondisarticulating ascospores, and its habit on coleopterous larvae. *Cordyceps pentatomae* Koval occurs on adult Hemiptera and lacks a furfuraceous cortex (Kobayasi and Shimizu, 1978). *Cordyceps ramosopulvinata* Kobayasi & Shimizu differs in its branching stromata, shorter part ascospores, and its habit on larvae of Cicadidae (Kobayasi and Shimizu, 1983a). *Cordyceps unilateralis* differs in its typically filiform, dark stromata, nondisarticulating ascospores, and its habit on ants (Hymenoptera: Formicidae) (Kobayasi, 1939). *Cordyceps baumanniana* Henn. and *C. interrupta* Höhn. are poorly known species from Lepidoptera and Coleoptera, respectively. Petch (1932b) considered *C. baumanniana* to be an aberrant specimen of *C. australis*, a capitate species with obliquely embedded perithecia. *Cordyceps interrupta* produces branching, brown, filiform stromata which bear multiple discrete perithecial cushions (Höhnel, 1909).

Cordyceps michiganensis Mains (1934) is macroscopically similar to *C. variabilis* in its production of chrome yellow, acicular stromata with sterile apices and a furfuraceous layer of cortical cells. In *C. michiganensis* the perithecia are superficial and free, seldom united in groups of more than one or two, and many stromata arise from a single host. No anamorph was observed in specimens present at MICH, and the host in all cases is a coleopteran larva (the host of one specimen (A. H. Smith 63538, MICH) was identified by Dr. J. K. Liebherr (Cornell University) as Carabidae: *Pterostichus* sp.). Although *C. michiganensis* and *C. variabilis* are macroscopically similar and exhibit overlapping distributions, the two species are distinct.

The anamorph of C. variabilis. Petch (1937) reported the presence of "acervuli" on the stromata of specimens from FH on which he found conidia matching in description those figured here (FIGS. 9, 15). He believed, however, that they were produced by a hyperparasitic fungus, and asserted that the anamorph of *C. variabilis* was a *Hirsutella* species which he did not describe. Having examined the same specimens on which Petch based his conclusions, we believe that the conidia described by him are indeed those of the anamorph of *C. variabilis* and that no hyperparasite is present. Furthermore, the conidi-

ogenous cells of the anamorph described below are similar in shape to the typically subulate phialides of *Hirsutella*. In our studies, cultures isolated from hyphal bodies, surface mycelium on the host, and from part ascospores all yielded the same slow-growing hyphomycetous fungus, which is described above as the *Syngliocladium* anamorph of *C. variabilis*.

Syngliocladium tetanopsis K. T. Hodge, R. A. Humber et C. A. Wozniak, sp. nov.

FIGS. 10–13, 16–17

Habitus entomogenus, mononematosus vel synnematosus, conidiophoris super exosceleto hospitis vel in synnematibus crassis efferentibus. Cellulae conidiogenae plerumque monoverticillatae vel geminatae, (10.0–) 12.2 (–13.7) × 2.0 μm, basibus subcylindricis, in collum curtum abrupte decrescentibus; apicibus saepe valde uncinatibus flexibusve; ameroconidiis in muco copioso enteroblastice formantibus. Granula aurantiaca superficiaribus in hyphis et cellulis conidiogenis saepe praesentia. Conidia aseptata subcylindrica, (4.3–) 6.8 (–9.3) × (1.9–) 2.0 (–2.5) μm (n = 100). Chlamydosporae non vidunt. Status teleomorphosus ignotus.

HOLOTYPUS. CUP 64913 (cultura desiccata ex ARSEF 5497).

Entomogenous, mononematous or synnematous, conidiophores produced on the surface of the host and on stout synnemata. Conidiogenous cells frequently in monoverticillate whorls or pairs, (10.0–) 12.2 (–13.7) μm × 2.0 μm, with subcylindric base abruptly narrowing to a short, tapering neck; often strongly hooked or bent; producing ameroconidia enteroblastically in copious slime. Orange granules often present on the hyphae and conidiogenous cells. Conidia one-celled, subcylindric, (4.3–) 6.8 (–9.3) × (1.9–) 2.0 (–2.5) μm (n = 100). Chlamydospores not observed. Teleomorph unknown.

PARATYPES. CUP 64915, Laboratory-infected *T. myopaeformis* larvae. 6 June 1995. *C.A. Wozniak* (source of living culture ARSEF 4972); CUP 64914, *T. myopaeformis*, larvae. NORTH DAKOTA: Red River Valley, summer 1995; CUP 64916, *T. myopaeformis*, larvae. NORTH DAKOTA: Red River Valley near Fargo, summer 1994.

Commentary. *Syngliocladium tetanopsis* was isolated from larvae of *T. myopaeformis* (sugar beet root maggots), an economically important pest of sugar beets. Fungus-infected *T. myopaeformis* larvae were collected from sugar beets in untreated border rows planted as buffer zones in a study of the efficacy of entomopathogenic nematodes as biocontrol agents. The studies were performed in Pembina Co., North Dakota, by CAW beginning in summer 1994. Dead or dying third instar larvae collected in the field were

discolored yellow-tan with darkened cuticular lesions. When held in a moist chamber, white to yellow-orange tufts of sporulating mycelium erupted through the cuticle, and synnemata ultimately developed after 4–5 wk at 25 C. The fungus differs from the anamorph of *C. variabilis* in its smaller conidia, host of origin, and smaller conidiogenous cells.

Syngliocladium Petch, emend. K. T. Hodge & R.A. Humber

Synnematous or mononematous; synnemata either loosely fasciculate and bearing laterally projecting conidiophores with apical clusters of *Gliocladium*-like conidiogenous cells and tending to form upon germination of clusters of chlamydospores (*Sorosporella* synanamorphic state) or stout and comprised of relatively densely packed parallel hyphae, arising directly from stromatic tissues either inside an infected host or from in vitro cultures. Conidiogenous cells (phialides) occurring in clusters, often with a *Gliocladium*-like appearance, or singly; with a swollen or flask-like base narrowing to a usually obvious and often prominent neck that may be straight, hooked (uncinate), or bent out of the axis of the cell base. Conidia one-celled, ovoid to cylindric with rounded ends, hyaline, released successively into apical mucoid droplets, with mucoid droplets often aggregating with those on adjacent phialides into larger mucoid drops or masses.

Chlamydosporic synanamorphic state: species of *Sorosporella* Sorokin. Teleomorphic state (where known): species of *Cordyceps* Fr.

Commentary. Although the two anamorph species discussed here present some problems in generic placement, we feel they are best accommodated in the poorly known genus *Syngliocladium*, as emended above. Their affinities with *Tolypocladium* W. Gams (1971b), *Culicinomyces* Couch, Romney & Rao (1974), and *Paraisaria* R. A. Samson & B. L. Brady (1983) are discussed below.

The anamorph genus *Syngliocladium* was described by Petch for *S. aranearum* Petch on a spider from England (Petch, 1932a). He described the genus as producing *Gliocladium*-like conidiophores arising laterally from synnemata on the host. The conidia are borne on subulate phialides in copious slime. Petch (1942) later added the species *S. intricatum* Petch (from a coleopterous larva) and *S. cleoni* (Wize) Petch. The status of *Syngliocladium* has been considered dubious by some researchers (Samson and Brady, 1983; Shah and Evans, 1997; Carmichael et al., 1980). Despite Samson and Brady's (1983) assertion that *S. intricatum* is a stilbellaceous fungus not closely allied with other species later included in the genus,

our examination of the type (K; FIG. 19) led us to conclude that Petch's concept of *Syngliocladium* was cohesive. No teleomorphs have previously been reported for *Syngliocladium*, although its slimy phialoconidia, synnematous tendency, and insect pathogenic habit suggest the now confirmed affinity to the entomopathogenic members of the Clavicipitaceae as noted by Speare (1920).

Sorosporella uvella (Krass.) Giard, characterized by the brick-red resting spores that fill the cadavers of its insect hosts, has been shown by Speare (1917, 1920) to have an unnamed synanamorph attributable to *Syngliocladium*. Not all *Syngliocladium* species have *Sorosporella* synanamorphs; we prefer to maintain the two genera as distinct for descriptive purposes and because of their disparate modes of spore ontogeny (Gams, 1982). *Sorosporella* infections of orthopteran insects that have been by reported by Pendland and Boucias (1987), Shah (1993), Shah et al. (1994), Shah and Evans (1997), and Welling et al. (1995), may represent one or more undescribed species, but the simple morphology of *Sorosporella* resting spores makes the taxonomy of the genus problematic. Most *Sorosporella* species that have been studied in culture produce *Syngliocladium* synanamorphs, and these more highly differentiated states are likely to be more diagnostic for taxonomic purposes (Gams, 1982). That these fungi might best be characterized by their *Syngliocladium* state is complicated by the practical consideration that the *Sorosporella* state appears to be more commonly encountered in nature. Studies of host range and cultural morphology are needed to elucidate species in this long-neglected group.

Syngliocladium tetanopsis and the *Syngliocladium* anamorph of *C. variabilis* are morphologically similar and clearly congeneric. *Syngliocladium tetanopsis* differs from the anamorph of *C. variabilis* in its smaller conidia and phialides, host, and cultural characteristics. Whereas *S. tetanopsis* killed more than 85% of inoculated third instar larvae of *T. myopaeformis* (its host of origin), the *Syngliocladium* anamorph of *C. variabilis* killed fewer than 8%. Reciprocal bioassays of *S. tetanopsis* against the host of the *Syngliocladium* anamorph of *C. variabilis* were not performed because cultures of xylophagids were not available.

A slide taken from the holotype of *S. aranearum* Petch, the type species of *Syngliocladium*, was generously loaned by H. C. Evans and the curator of K. Because of its fragile condition (H. C. Evans, pers. comm.), the type specimen itself was not inspected. Compound, *Gliocladium*-like conidiogenous cells were clearly visible; no simple conidiogenous cells were observed in the small synnema fragment mounted on the slide (FIG. 18). The type specimen of *S. intricatum* Petch (K) was examined, and com-
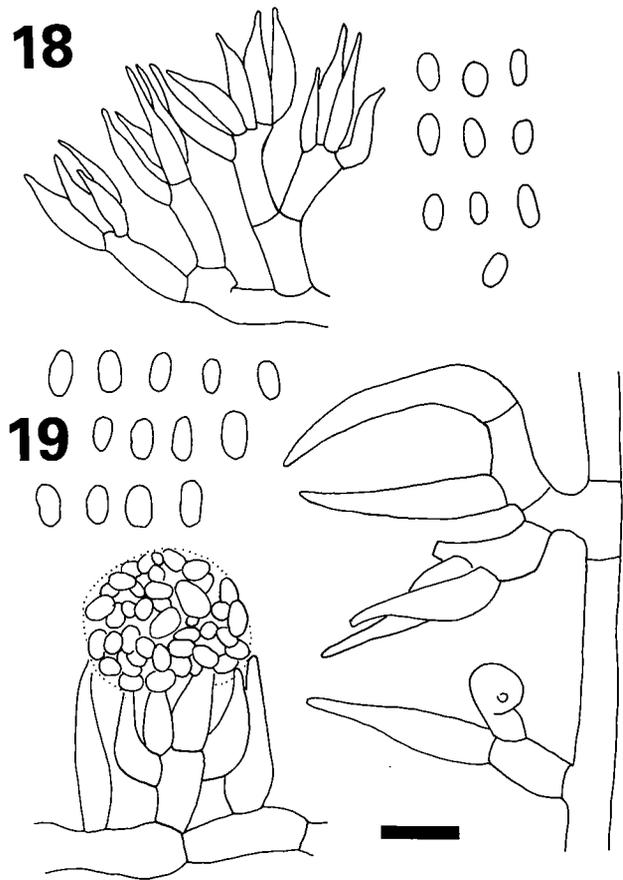
FIGS. 18, 19. Morphology of *Syngliocladium* spp. 18. Conidiophores and conidia of *S. aranearum* (holotype, K). 19. Conidiophores and conidia of *S. intricatum* (holotype, K). Bar = 5 μm.

plex conidiophores as well as simple, frequently hooked phialides were observed (FIG. 19). Similar phialides were illustrated by Speare (1917, 1920) for the *Syngliocladium* synanamorph of *Sorosporella uvella*, and by Pendland and Boucias (1987) for an undescribed *Syngliocladium* sp. from mole crickets (Orthoptera: Gryllotalpidae). In culture, the *Syngliocladium* synanamorphs of undetermined *Sorosporella* spp. (ARSEF 1123, 1856) produced both simple phialides and complex conidiophores. Gams (1982) suggests that synanamorphs that differ in conidiophore complexity but not in conidial ontogeny need not be assigned different generic names. In the above cases the name *Syngliocladium* can be applied to both complex and simple conidiophores. We therefore treat the two taxa presented here as closely allied species of *Syngliocladium* on the basis of their simple, hooked, conidiogenous cells, conidia borne in copious slime, synnema-forming ability, and entomopathogenic habit.

Similar genera. Tolypocladium resembles *Syngliocladium* in its frequently hooked conidiogenous cells