

Studies on the Taxonomy of the *Myxobacterales*

III. *Chondromyces* and *Stigmatella*

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Classification of the species of the genus *Chondromyces* Berkeley and Curtis is considered in light of the proposal that the genus be divided into two genera: *Stigmatella* Berkeley and Curtis (family *Cystobacteraceae*), with tapered vegetative cells and encapsulated myxospores (microcysts); and *Chondromyces* (family *Polyangiaceae*), with cylindrical vegetative cells and similar myxospores. The latter genus, as redefined, would include *Chondromyces crocatus* (the type species), *Chondromyces apiculatus*, *Chondromyces pediculatus*, *Chondromyces catenulatus*, and *Chondromyces lanuginosus*. Two species are recognized as belonging to the genus *Stigmatella*: *Stigmatella aurantiaca* (the type species) and *Stigmatella erecta*.

In a previous paper (14), the family *Polyangiaceae* Jahn was redefined to include only those sporangial myxobacters with cylindrical vegetative cells and similar myxospores. Sporangium-forming myxobacters with tapered vegetative cells were placed in a new family, *Cystobacteraceae*. It has been noted (12, 13, 15, 17) that the organisms presently placed in the genus *Chondromyces* Berkeley and Curtis (*Bergey's Manual*, 7th ed., p. 854-891) include organisms of both types. It was suggested that *Chondromyces* (family *Polyangiaceae*; reference 14) be retained for those with cylindrical cells (13). *Chondromyces aurantiacus* (Berkeley and Curtis) Thaxter and *Chondromyces brunneus* Krzemieniewska and Krzemieniewski, having tapered cells, were placed in the genus *Stigmatella* Berkeley and Curtis (family *Cystobacteraceae*), the type species of which is *Stigmatella aurantiaca* Berkeley and Curtis.

The present paper is concerned with the classification of five species of *Chondromyces* which were not attended to in the above mentioned considerations. The relationship of the genus *Synangium* Jahn to *Chondromyces* is also discussed.

MATERIALS AND METHODS

Cultures and specimens. The media and procedures referred to here were described in detail elsewhere (13).

The following were organisms examined in pure culture (strain designations beginning with M are those of the University of Windsor; ATCC strains are

from the American Type Culture Collection): *Chondromyces crocatus* [M38(ATCC 25193), M204, M120]; *Chondromyces apiculatus* (M6, M32); *Chondromyces pediculatus* (M118); *Stigmatella aurantiaca* [M340, M341, "cylindrica" M343, "media" M84, M88, M15 (ATCC 25190), M34]; *Stigmatella erecta* [M26 (ATCC 25191), M27 (ATCC 25192), M162].

The following were specimens from the Thaxter collection, Farlow Herbarium, Harvard University: *S. aurantiaca* (acc. no. 4473-4479); *C. apiculatus* (acc. no. 4471, 4480-83; type = 4481); *Chondromyces catenulatus* (acc. no. 4517 = type, 4518, 3405); *C. crocatus* (acc. no. 4469, 4470, 4484-86, 2466, 6055, 5168, 601 = neotype); *Chondromyces thaxteri* (*Chondromyces lanuginosus*; acc. no. 4494 collected by J. H. Faull); *Chondromyces sessilis* (acc. no. 4505 = type); *C. pediculatus* (acc. no. 4524, 4525 = type); and *Chondromyces erectus* (*Podangium erectum*; acc. no. 4560).

RESULTS AND DISCUSSION

For the purposes of this study, a total of 16 pure cultures were identified as belonging to the genus *Chondromyces* Berkeley and Curtis according to *Bergey's Manual* (7th ed., p. 854-891), by reference to the original literature and when possible by comparison with herbarium specimens. Those identified as *C. apiculatus* (Fig. 6 and 7) and *C. pediculatus* (Fig. 8 and 9) resembled *C. crocatus* (Fig. 1-3) in having cylindrical vegetative cells and myxospores of similar morphology. Furthermore, the herbarium specimens of *C. catenulatus* (Fig. 4 and 5), *C. lanuginosus* (*C. thaxteri*; Fig. 10),

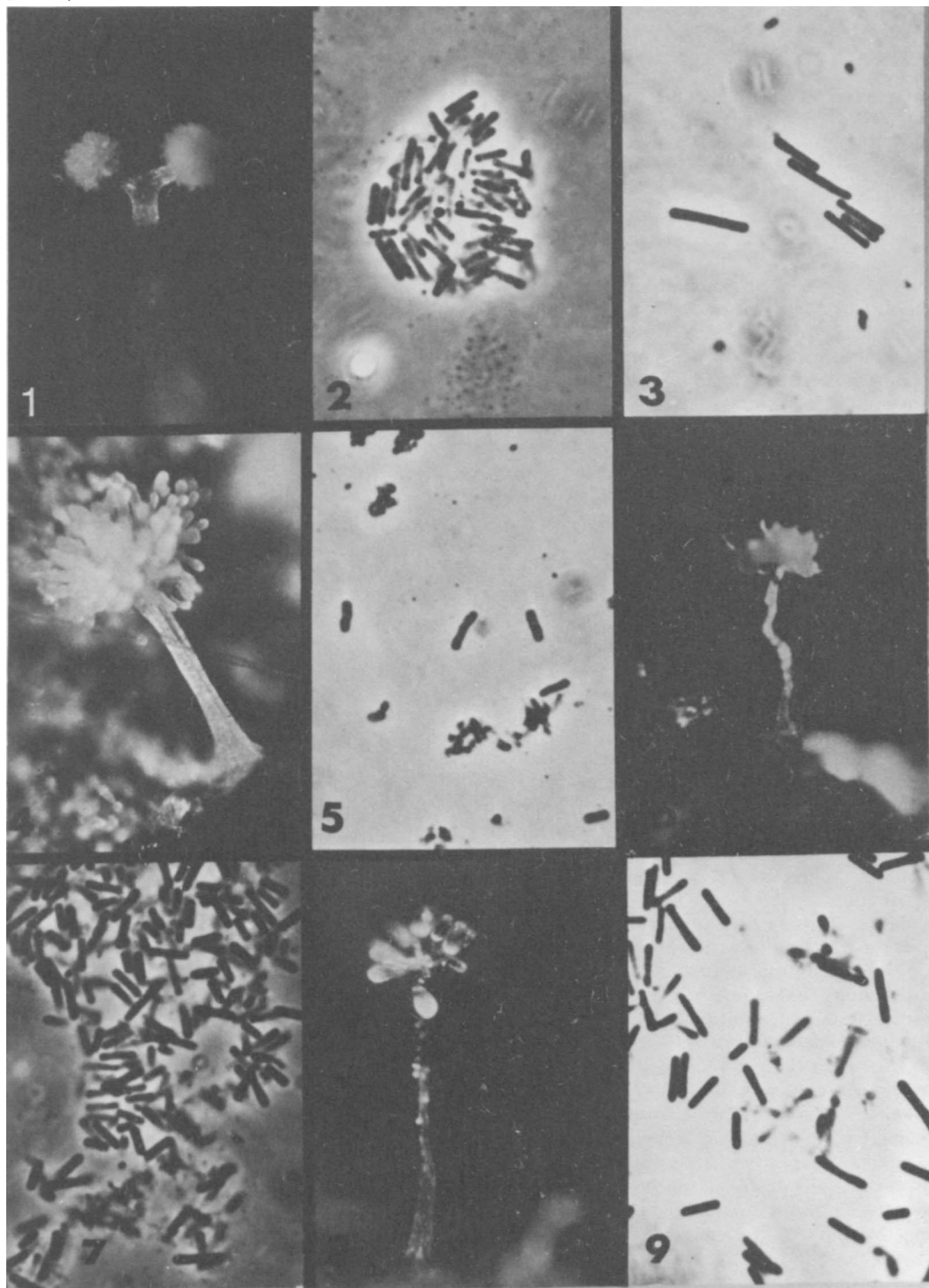


Fig. 1. *Chondromyces crocatus*, fruiting body. $\times 90$. Fig. 2. *Chondromyces crocatus*, myxospores. $\times 1,300$. Fig. 3. *Chondromyces crocatus*, vegetative cells. $\times 1,300$. Fig. 4. *Chondromyces catenulatus*, fruiting body. $\times 120$. Fig. 5. *Chondromyces catenulatus*, myxospores. $\times 1,200$. Fig. 6. *Chondromyces apiculatus*, fruiting body. $\times 100$. Fig. 7. *Chondromyces apiculatus*, myxospores. $\times 1,300$. Fig. 8. *Chondromyces pediculatus*, fruiting body. $\times 90$. Fig. 9. *Chondromyces pediculatus*, myxospores. $\times 1,300$.

and *C. sessilis* (Fig. 11a, 11b, and 12) were also found to have *Chondromyces*-type resting cells.

The remaining isolates were members of the genus *Stigmatella* Berkeley and Curtis. That is, they had tapered vegetative cells and much-shortened, phase-dense or refractile, rigid myxospores (microcysts).

As will be noted by an examination of Table 1, the morphological differences between *Stigmatella* and *Chondromyces* are correlated with a number of cultural and biochemical differences, although within the two groups the cultural and biochemical characteristics are rather uniform. As a consequence, morphological characteristics remain of primary importance in distinguishing species.

Chondromyces. Before giving a detailed consideration of the characteristics of the species of *Chondromyces*, it is necessary to consider the genus *Synangium* Jahn and its relationship to *Chondromyces*.

Thaxter (21) described an organism whose irregular sporangia were arranged in sessile rosettes or tufts, which he named *C. sessilis*. Kofler (7) later reported a stalked organism, *C. lanuginosus*, in which the sporangia were fused in the form of discoid or spherical clusters with each sporangium bearing an apical tuft of hairs. Shortly thereafter, Faull (4) gave the name *C. thaxteri* to what was undoubtedly the same organism. Jahn (5), however, recognized all three as distinct species and erected the new genus *Synangium* to contain them. Krzemieniewska and Krzemieniewski (11), treating Jahn's species as mere varieties, subsequently concluded that there was but one species, *Synangium sessile*.

A careful comparison of Thaxter's description of *C. sessilis* with those of Faull's and Kofler's organisms does not at all suggest the conclusion that they are identical. On the contrary, our observations of *C. lanuginosus* (= *Synangium lanuginosus* = *C. thaxteri*; Fig. 10) and *C. sessilis* (Fig. 11a, 11b) from the Thaxter collection confirms that they are not at all alike. From the author's observations of the specimens of *C. sessilis* which were available (Thaxter apparently observed it only once), this organism, although undoubtedly a chondromycete, should not have been regarded as a new species. Indeed, it might well be a variant of any of the other recognized species of *Chondromyces*.

On the other hand, *C. lanuginosus* is certainly a distinct species. The question is whether it is necessary to assign it to a separate genus. Its most distinctive characteristic is the fusion of its sporangia. Both Kofler and Faull remarked

on its close resemblance to *C. apiculatus*. Faull, after a careful study of the ontogeny of the fruiting bodies of *C. lanuginosus*, concluded that the fruiting bodies differed from those of *C. apiculatus* only in the final stages of development. A close relationship between the two is also suggested by the not infrequent occurrence in *C. apiculatus* of fruiting bodies with fused sporangia. We agree with Faull, therefore, that the genus *Chondromyces* constitutes a close evolutionary series in which *C. lanuginosus*, although undoubtedly the most advanced species, is not sufficiently different from the other species to warrant placing it in a separate genus.

Chondromyces Berkeley and Curtis 1874, 64.

(Objective synonym: *Myxobotrys* Zukal 1896, 346.)

Vegetative cells are cylindrical, untapered rods with bluntly rounded ends.

Sporangia borne singly or in clusters on simple or branched stalks.

Myxospores lack capsules and resemble vegetative rods.

Vegetative swarms etch, erode, and penetrate agar media. Vegetative slime does not adsorb Congo red dye.

Aerobic.

Temperature range, 18 to 37°C; optimum 28 to 30°C.

Guanine plus cytosine content of species examined is 69 to 70 moles per cent by T_m determinations.

Type species: *Chondromyces crocatus* Berkeley and Curtis 1874, 64.

Descriptions of Species of *Chondromyces*

Chondromyces crocatus Berkeley and Curtis 1857 and 1874, 64. (See reference 1, Fig. 70a and 313.)

(Objective synonym: *Myxobotrys variabilis* Zukal 1896, 340.)

Vegetative cells (Fig. 3) cylindrical with blunt, rounded ends, 1.1 to 1.4 μm by 3 to 12 μm .

Sporangia broadly spindle-shaped, conical, or nearly spherical, 10 to 25 μm by 15 to 30 μm , straw-colored initially, finally becoming golden-yellow or orange. Sporangia borne in spherical clusters on usually branched stalks up to 700 μm or more in height (Fig. 1). Stalks orange to brown, striated, often spirally twisted, tunneled internally with few cells within. Irregular forms with ramifying branches and few sporangia or with secondary fruiting structures arising from sporangia germinating in situ may be observed in culture.

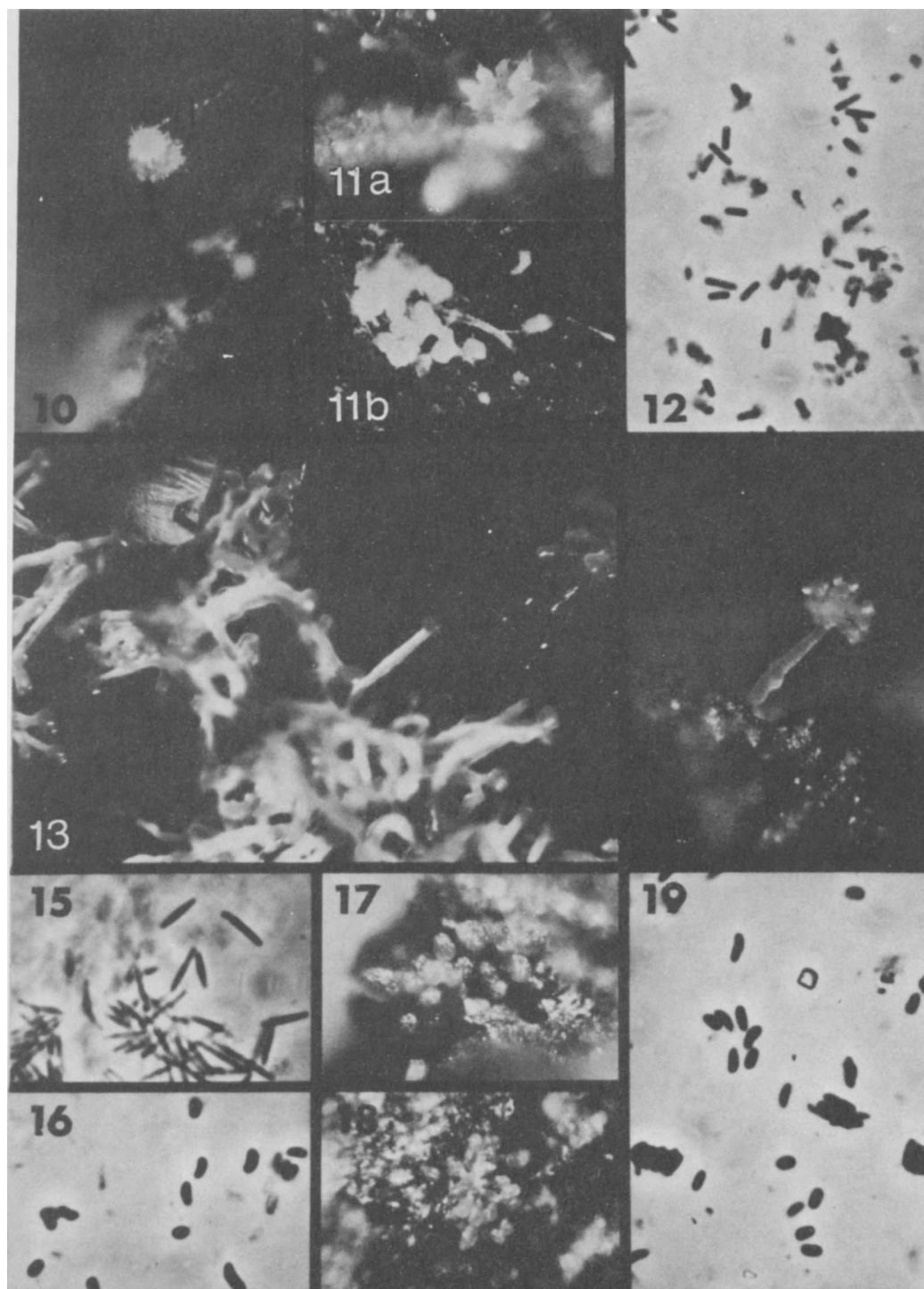


Fig. 10. *Chondromyces lanuginosus*, fruiting body. $\times 120$. Fig. 11. *Chondromyces sessile*, fruiting bodies. $\times 120$. Fig. 12. *Chondromyces sessile*, myxospores. $\times 1,100$. Fig. 13. *Stigmatella aurantiaca*, fruiting bodies on agar. $\times 100$. Fig. 14. *Stigmatella aurantiaca*, fruiting body on bark. $\times 90$. Fig. 15. *Stigmatella aurantiaca*, vegetative cells. $\times 1,200$. Fig. 16. *Stigmatella aurantiaca*, microcysts. $\times 1,400$. Fig. 17. *M 162* sporangia on bark. $\times 100$. Fig. 18. *Podangium erectum* herbarium specimen on bark. $\times 100$. Fig. 19. *Stigmatella erecta* microcysts. $\times 1,300$.

TABLE 1. *Physiological characteristics of isolates*

Organism	Growth medium	Liquid culture	Agar etched	Congo red	Aesculin	Urease	Neomycin	Kanamycin
<i>Stigmatella aurantiaca</i> M341	Sp	+	—	+	+	+	—	+
<i>Stigmatella aurantiaca</i> M340	Sp	+	—	+	+	+	+	+
<i>Stigmatella "cylindrica"</i> M342	Sp	+	—	+	+	—	+	+
<i>Stigmatella "media"</i> M84	Sp	+	—	+	+	+	—	+
<i>Stigmatella "media"</i> M85	Sp	+	—	+	+	+	+	+
<i>Stigmatella "media"</i> M34	Sp	+	—	+	+	+	+	+
<i>Stigmatella "media"</i> M15 (ATCC 25190)	Sp	+	—	+	+	+	+	+
<i>Stigmatella erecta</i> M26 (ATCC 25191)	Sp	+	—	+	+	+	+	+
<i>Stigmatella erecta</i> M27 (ATCC 25192)	Sp	+	—	+	+	+	+	+
<i>Podangium erectum</i> M162	Sp	+	—	+	+	+	—	+
<i>Chondromyces crocatus</i> M38 (ATCC 25193)	SpE	—	+	—	—	—	—	—
<i>Chondromyces crocatus</i> M204	SpE	—	+	—	—	—	—	—
<i>Chondromyces crocatus</i> M120	SpE	—	+	—	—	—	—	—
<i>Chondromyces apiculatus</i> M6	SpE	—	+	—	—	—	—	—
<i>Chondromyces apiculatus</i> M36	SpE	—	+	—	—	—	—	—
<i>Chondromyces pediculatus</i> M118	SpE	—	+	—	—	—	—	—

Myxospores (Fig. 2) lack capsules and differ little from vegetative cells except for the presence of conspicuous refractile granules at one or both ends, 1.0 to 1.3 μm by 3 to 6 μm .

Vegetative colonies on most media are initially translucent, almost colorless, later becoming yellowish-orange, and heaped at the periphery to form a "front" which is particularly conspicuous in contact with masses of living eubacteria. The underlying agar is pitted, eroded, and penetrated by columns of vegetative cells. Congo red is not adsorbed.

Growth on ECM agar (13) is poor with lysis generally limited to the immediate area of the colony. Lysis of living bacteria requires direct contact. Cultivated in pure culture on complex media containing enzymatically hydrolyzed protein and Mg^{++} . Growth is stimulated by, and initially requires, an extract from eubacterial cells.

Nitrate is not reduced. Catalase and oxidase negative. Hydrolyzes starch, Tween 80, indoxyl acetate, ribonucleic acid, deoxyribonucleic acid, gelatin, and casein. Does not hydrolyze urea, aesculin, or cellulose. Agar digestion not detected.

Aerobic.

Temperature range, 18 to 37 C; minimum, 18 C; optimum, 28 to 30 C.

Antibiotic sensitivity (discs): Resistant to neomycin (10 μg), kanamycin (10 μg), penicillin (10 units). Inhibited by streptomycin (5 μg), tetracycline (10 μg), chloramphenicol (10 μg), and erythromycin (5 μg).

Streptomycete-like odor produced is different from that of myxobacters with tapered cells.

Source and habitat: First observed on decayed melons from South Carolina. Later found by Thaxter (19) on old straw from Ceylon. Commonly found on dung in contact with soil, on bacterial streaks inoculated with soil, or on bark.

Neotype: Acc. no. 601, Thaxter collection, Farlow Herbarium, Harvard University.

Reference strain: Windsor M38 (ATCC 25193).

Chondromyces apiculatus Thaxter 1897, 405.

Vegetative rods are cylindrical with blunt, rounded ends, 1.1 to 1.4 μm by 3 to 14 μm .

Sporangia (Fig. 6) straw-colored to bright orange or brownish-orange, variable in form and size, cylindrical to broadly turnip-shaped, 25 to 40 μm by 35 to 50 μm , with colorless, pointed, frequently branched, apical appendages up to 35 μm long. Pedicels absent or up to 30 μm long, colorless. Sporangia borne in spherical clusters of 60 or more, although usually fewer in number than in *C. crocatus*. Stalk seldom branched, up to 700 μm in height, diameter of 15 to 40 μm , longitudinally striated, tunnelled, and without internal cells. Forms without stalks, with basally fused sporangia, or with large, irregular, solitary sporangia are common. Secondary fruiting body formation as in *C. crocatus* or sporangium formation at tips of appendages is often observed.

Myxospores similar to vegetative cells, 1.0 to 1.3 μm by 3 to 6 μm (Fig. 7).

In all other characteristics, it is identical to *C. crocatus*.

Source and habitat: Originally isolated from antelope dung from Liberia. Commonly observed on rabbit dung in contact with soil.

Guanine plus cytosine content is 69.3 moles per cent by T_m determination.

Type: Acc. no. 4481, Thaxter Herbarium, Harvard University.

***Chondromyces pediculatus* Thaxter 1904, 410.**

Vegetative cells cylindrical with blunt, rounded ends, 1.1 to 1.3 μm by 3 to 16 μm .

Sporangia (Fig. 8) pale-yellow to orange, when dry orange-red, nearly spherical to long cylindrical, club-shaped or pyriform, usually broader and truncate at distal end, surface rough, 25 to 40 μm by 35 to 60 μm . Borne in groups up to 60 in umbel-shaped heads on slender pedicels 20 to 40 μm long. Stalk unbranched, up to 750 μm in height, straited, sometimes twisted, colorless initially, becoming orange to brown when dry.

Myxospores lack capsules, resemble vegetative cells, 1.0 to 1.2 μm by 3 to 7 μm (Fig. 9).

Vegetative colonies orange to reddish-orange and resembling those of *C. crocatus*. Congo red is not adsorbed.

Cultivated on dung pellet agar and living bacteria. Growth is slow. Obtained once in pure culture on *Escherichia coli* extract-enriched Casitone- Mg^{++} agar.

Cellulose is not hydrolyzed. Urease negative.

Source and habitat: Originally isolated on goose dung from South Carolina. Obtained on rabbit dung in contact with soil.

Type: Acc. no. 4524, Thaxter collection, Farlow Herbarium, Harvard University.

***Chondromyces catenulatus* Thaxter 1904, 410.**

Rods in rising spore mass, 1.0 to 1.3 μm by 4 to 6 μm . Cells in vegetative colonies not described.

Sporangia (Fig. 4) light-yellow to orange, fusiform, long elliptical or irregular in shape, dimensions 18 μm by 20 to 50 μm , united in chains up to 300 μm long which may be branched once or twice. Sporangia separated by shrivelled, membranous isthmuses. Stalk simple, orange to rust-colored, up to 400 μm in height, broad at base, tapering above, and several times cleft into swollen, tapering parts, each bearing one or several sporangial chains.

Myxospores are nonrefractile cylindrical rods with blunt, rounded ends, 1.2 to 1.4 μm by 3 to 6 μm (Fig. 5).

Cultivated only on original source, not reported in pure culture.

Source and habitat: Decaying poplar wood from New Hampshire.

Type: Acc. no. 4517, Thaxter collection, Farlow Herbarium, Harvard University.

***Chondromyces lanuginosus* Kofler 1913, 861.**

[Objective synonym: *Synangium lanuginosus* (Kofler) Jahn 1924, 79.]

[Subjective synonyms: *Chondromyces thaxteri* Faull 1916, 226 and *Synangium thaxteri* (Faull) Jahn 1924, 79.]

Vegetative cells cylindrical with blunt, rounded ends 0.9 to 1.0 μm by 3 to 8 μm .

Sporangia (Fig. 10) fused at their bases to form discoid or nearly spherical clusters containing up to 80 sporangia each with an apical tuft of hairs. Diameter of clusters variable (40 to 250 μm) as is the length of the apical hairs (7 to 30 μm). Stalks simple or occasionally branched, bearing from 1 to 30 clusters. Sporangia initially white changing to yellow, light-pink, and eventually orange. Stalks are at first colorless but become yellow. Sometimes the sporangial clusters give rise to secondary stalks which are thinner than the primary ones and which are tipped with smaller clusters.

Myxospores do not differ from vegetative cells, only slightly smaller, 0.6 μm to 1.0 by 2.6 μm .

Cultivation: Grown in laboratory culture on hay (Krzemieniewska and Krzemieniewski 1946, 37). Pure cultures not obtained.

Source and habitat: Found on the dung of herbivores in Canada (Faull 1916, 226) and Austria (Kofler 1913, 861); also found in soil in Poland (Krzemieniewska and Krzemieniewski 1946, 37).

Neotype: Acc. no. 4494 collected by J. H. Faull, Thaxter collection, Farlow Herbarium, Harvard University.

Species incertae sedis

***Chondromyces sessilis* Thaxter 1904, 411.** Vegetative rods not described.

Sporangia (Fig. 11a,b) yellow to reddish-orange, forming a sessile rosette or tuft on the substrate without a clearly differentiated stalk, although a poorly developed stalk is said occasionally to occur.

Sporangia quite variable in shape, irregularly and broadly fusiform, often subapiculate, with

a wrinkled surface, variable in size, coherent at base or more or less completely confluent in irregular masses. Dimensions of sporangia, 18 to 35 μm by 25 to 75 μm ; dimensions of rosettes, 100 to 250 μm .

Myxospores are cylindrical with blunt, rounded ends, 0.8 to 1.0 μm by 3 to 5 μm (Fig. 12).

Source: Rotten wood from Florida. Cultivation not reported. Observed only on natural substrate.

Type: Acc. no. 4505, Thaxter collection, Farlow Herbarium, Harvard University.

Stigmatella Berkeley and Curtis.

The distinguishing features of species of *Stigmatella* employed by the Krzemieniewskis (8–11) seemed arbitrary and not always consistent. However, it was possible on the basis of their descriptions and of the appearance of our isolates on the natural substrates on which they were first encountered to assign tentatively each of our isolates to one of the four presently recognized (*Bergey's*

Manual, 7th ed.) species of *Stigmatella*. Table 2 summarizes the characteristics used by Krzemieniewska and Krzemieniewski to distinguish the species and permits a comparison with those of our isolates. It will be noted that great emphasis is placed on cyst shape, size, color, and the presence or absence of pedicels. The Krzemieniewskis were not very specific about the media or substrates employed, and there is no indication that they observed pure cultures. However, both the nature of the substrate and the presence of contaminants (especially molds) can have a profound effect on precisely those characteristics that the Krzemieniewskis emphasized.

It was not surprising, therefore, that there were differences (Table 1) among the pure cultures identified as *Stigmatella aurantiaca*, *S. media*, and *S. cylindrica* in cultural characteristics, fruiting bodies, and biochemical properties; these were trivial, however, and in any case uncorrelated with their "species" assignments. On the basis of these results, we

TABLE 2. Comparison of isolates of *Stigmatella* with original descriptions

Organism ^a	Color of mature sporangia	Shape of sporangia	Sporangial dimensions	Pedicels
<i>S. aurantiaca</i> (11)	Orange-red to yellow-brown	Almost spherical or elongated	14–54 × 24–72 μm	Present or absent
M340	Orange-red	Oval or spherical	13–40 × 30–50 μm	Absent
M341	Orange	Oval or nearly spherical	20–30 × 30–50 μm	Absent
<i>S. "cylindrica"</i> (11)	Brilliant orange sometimes brownish	Usually cylindrical but often oval or spherical	20–30 × 90 μm	30 μm
M342	Orange	Cylindrical	20–30 × 40–70 μm	40 μm
<i>S. "media"</i> (10)	Orange-red, reddish-orange or light brown	Variable, oval or tapering to base	26–93 × 24–78 μm	40 μm
M84	Bright orangish-red	Oval or spherical	25–40 × 40–50 μm	40 μm
M85	Bright orange-red	Oval or spherical	25–40 × 30–50 μm	40 μm
<i>S. brunneus</i> (11)	Dark chestnut-brown, almost black	Spherical or oval	28–83 × 37–102 μm	30 μm but settling down
M26	Dark chestnut-brown, almost black	Spherical or oval	40–80 × 30–50 μm	20–30 μm settling
M27	Dark chestnut-brown	Spherical or oval	Avg 40 × 55 μm	20–30 μm settling

^a Numbers in parentheses refer to references.

conclude that the diagnostic characteristics used by Krzemieniewska and Krzemieniewski to distinguish *S. cylindrica* and *S. media* fall well within the range of variation of *S. aurantiaca*, not only as observed among our isolates and in the Thaxter specimens but also as was previously described (5, 11, 17, 19). We conclude, therefore, that *S. media* and *S. cylindrica* are merely varieties of *S. aurantiaca*.

Reichenbach and Dworkin (17) previously stated the opinion that all of the "species" of *Stigmatella* are varieties of *S. aurantiaca*. We believe, however, that *Stigmatella brunnea* is sufficiently different morphologically to retain it as a separate species. We previously expressed the opinion (15) that *S. brunnea* and *Podangium erectum* are identical. Various authors have cited the close similarity between *P. erectum* and the stigmatellae and in fact the Krzemieniewskis (8) made special reference to the very close resemblance of *S. brunnea* to *P. erectum*. In the present study, isolate M162 was initially identified as *P. erectum* (9) because, when first observed on bark (Fig. 17), it had produced no chondromyces-type fruiting bodies and it appeared to be identical to the Thaxter herbarium specimens (Fig. 18). However, once obtained in pure culture, it was indistinguishable from our cultures of *S. brunnea*.

The name *Cystobacter erectus* Schroeter 1886 antedates *Chondromyces brunneus* Krzemieniewska and Krzemieniewski 1946, and the specific epithet *erectus* has priority over *brunneus*. Consequently, the name *Stigmatella erecta* (basonym: *Cystobacter erectus* Schroeter) is proposed for this organism. The characteristics distinguishing *S. erecta* from *S. aurantiaca* are the greater frequency of monosporangial fruiting bodies, the consistently dark color of the sporangia, the larger average size of the sporangia, and the "settling down" of the stalks at maturity of the former.

Thus, the genus *Stigmatella* is to be regarded as consisting of a range of closely related morphological types with the chondromyces-like *S. aurantiaca* at one extreme and *S. erecta* of the "*Podangium*" morphological type at the other.

Genus *Stigmatella* Berkeley and Curtis 1874, 97.

(Objective synonym: *Polycephalum* Kalchbrenner and Cooke 1880, 22.)

Vegetative cells are rods with tapered ends.

Sporangia borne singly or in clusters on stalked fruiting bodies (the stalks often occurring in groups arising from a common hypothallus).

Myxospores are short, rigid, phase-dense or refractile rods surrounded by a definite slime capsule.

Vegetative colonies do not etch, erode, or penetrate agar media. Congo red is adsorbed.

The minimal nutritional requirements are not known, but the organism is easily cultivated on media containing enzymatically hydrolyzed protein.

Urea is usually hydrolyzed.

Aerobic.

Temperature range, 18 to 37 °C; optimum 30 °C.

Guanine plus cytosine content of species examined is 68.5 to 68.7 moles per cent by T_m determination (16).

Type species: *Stigmatella aurantiaca* Berkeley and Curtis 1874, 97.

Descriptions of Species of *Stigmatella*

***Stigmatella aurantiaca* Berkeley and Curtis 1874, 97.**

[Objective synonym: *Chondromyces aurantiacus* (Berkeley and Curtis) Thaxter 1892, 401].

Vegetative cells are rods with tapered ends, 0.6 to 1.0 μm by 4 to 10 μm (Fig. 15).

On bark (Fig. 14) sporangia are spherical, oval, pear-shaped, or cylindrical; yellowish-orange to bright orange-red or reddish-brown; variable in size, 16 to 70 μm by 25 to 102 μm . Pedicels may be absent at maturity; when present, they are up to 40 μm long. Stalks up to 400 μm long, usually unbranched, sometimes arising from a common origin in a fascicled arrangement, granular, not striated, of hardened slime containing some cells, colorless to the color of the sporangia. Morphology of the fruiting bodies is variable especially in culture; forms (Fig. 13) with very long (to 900 μm) and irregularly branched, white to orange stalks lacking sporangia or tipped by one or few sporangia are often observed on plain agar in the presence of microbial contaminants; archangium-like forms are also common. When induced on Ca^{++} -water agar, the fruiting bodies bear one or few sporangia.

Microcysts, 0.9 to 2 μm by 1.6 to 4.0 μm (average 1.0 μm by 2.8 μm ; Fig. 16).

Vegetative colonies are thin, flat with numerous radiating and concentric ridges, edge more or less definite or thin, filamentous and poorly delimited, light yellow or flesh-colored, occasionally producing a yellowish or brownish discoloration of agar media. Orange aggregates often form in old cultures on 0.1% casitone or *E. coli* media, but fruiting bodies fail to develop. Continued laboratory cultivation

yields variants which produce mucoid colonies but which are unable to form fruiting bodies.

Easily cultivated on agar or liquid media containing 0.1 to 0.2% hydrolyzed protein, starch, and 0.01 M Mg^{+} or on agar media containing dead bacterial cells.

Nitrate is not reduced. Catalase positive, oxidase negative. Hydrolyzes starch (3 days), Tween 80, indoxyl acetate, ribonucleic acid, deoxyribonucleic acid, gelatin, casein, urea, and aesculin.

Aerobic.

Temperature range, 18 to 37 C; optimum, 30 C.

Optimum pH 7.0 to 7.2.

Antibiotic sensitivity (discs): Resistant to penicillin (10 units), sensitive to streptomycin (5 μ g), tetracycline (10 μ g), chloramphenicol (10 μ g), kanamycin (10 μ g), and erythromycin (5 μ g). Response to neomycin is variable.

Source and habitat: Originally observed on lichen. Most commonly observed on bark kept in a moisture chamber. Also from soil inoculated on streaks of living bacteria on filter paper over water agar.

Guanine plus cytosine content is 68.5 to 68.7 moles per cent by T_m determination.

Neotype strain: Windsor M 341.

Stigmatella erecta (Schroeter) *comb. nov.*

[Objective synonyms: *Cystobacter erectus* Schroeter 1886, 170

Chondromyces erectus (Schroeter) Thaxter 1897, 407

Podangium erectum (Schroeter) Jahn 1924, 80.]

[Subjective synonyms: *Chondromyces aurantiacus* var. *frutescens* Krzemieniewska and Krzemieniewski 1927, 91

Chondromyces brunneus Krzemieniewska and Krzemieniewski 1946, 44

Stigmatella brunnea (Krzemieniewska and Krzemieniewski) McCurdy 1969, 1460.]

Vegetative cells are slightly tapering, flexible rods, 0.7 to 0.8 μ m by 5 to 10 μ m.

On bark the sporangia are spherical (Fig. 17, 18), oval or elongated, at first flesh-colored becoming orange-red and finally dark chestnut-brown or almost black at maturity, 30 to 90 μ m by 35 to 140 μ m. Sometimes (especially on rabbit dung) borne singly on opaque white, later yellowish, stalks arising in groups from a common hypothallus. In other forms, several sporangia are arranged in clusters on yellowish-white to orange-red stalks. The sporangia may or may not be borne on pedicels. In both forms, the stalks (and pedicels) commonly wither, depositing the cysts on the substrate; thus, they appear sessile in masses of fifty to

hundreds together. On rabbit dung pellets, the fruiting bodies may appear as archangium-like masses or coralloid accumulations of pinkish-white or salmon-colored, finger-like projections without clear-cut delimitation of sporangia. The tips eventually turn dark brown, the remainder becoming yellowish-white. On Ca^{+} -water-agar, fruiting bodies with one to four sporangia on sparingly branched stalks (30 to 50 μ m by 80 to 200 μ m) are produced.

Microcysts are straight, curved or somewhat fusiform, short rigid, phase-dense or refractile rods, 0.8 to 1.5 μ m by 1.5 to 3.5 μ m (Fig. 19).

Vegetative colonies are at first thin and transparent, later yellow or light flesh-colored with numerous radiating ridges. The edge is thin and indefinite. Continued laboratory cultivation selects variants with yellow or orange mucoid colonies. In old cultures the swarm turns dark brown, and the surrounding medium also becomes darkened.

Easily cultivated on media containing enzymatically hydrolyzed protein, starch, 0.01 M Mg^{+} , and salts. Growth has also been obtained on a medium containing 17 amino acids, thiamine, 0.01 M Mg^{+} , and starch (16). Strictly respiratory, utilizing complex amino acids as energy source.

Nitrate is not reduced. Catalase positive. Oxidase negative.

Hydrolyzes starch (3 days), Tween 80, indoxyl acetate, deoxyribonucleic acid, ribonucleic acid, gelatin, casein, urea, and aesculin. Aerobic.

Temperature range, 18 to 37 C; optimum, 28 to 30 C.

Optimum pH 7.0 to 7.2

Antibiotic sensitivity (discs): Resistant to penicillin (10 units), sensitive to streptomycin (5 μ g), tetracycline (10 μ g), chloramphenicol (10 μ g), kanamycin (10 μ g), and erythromycin (5 μ g). Response to neomycin is variable.

Source and habitat: Obtained on the dung of herbivores placed in contact with soil or bark incubated in a moist chamber.

Guanine plus cytosine content is 68.7 moles per cent by T_m determination (16).

Neotype strain: Windsor M26 (ATCC 25191).

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