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Sexuality in the Kickxellaceae

R. K. Benjamin

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R. K. BENJAMIN

INTRODUCTION

The family Kickxellaceae was established by Linder in 1943 to accommodate three genera of very unusual "conidial" fungi, *Coemansia* Van Tieghem and Le Monnier (1873), *Kickxella* Coemans (1862), and *Martensella* Coemans (1863), the relationships of which had long been in doubt. Linder's paper, based primarily on a study of specimens in the Thaxter collection in the Farlow Herbarium at Harvard, includes characterizations and illustrations of most of the previously known representatives of the family as well as descriptions of several new species. In addition, there is a summary of the history, developmental morphology, and presumed natural relationships of members of the family. In 1952, Raper and Fennell described a fourth genus, *Linderina,* and very recently Meyer (1957) added another, *Martensiomyces.*

The writer's interest in the Kickxellaceae first was aroused in 1950 when Dr. Raper kindly presented him with a transfer of the type and only known species of *Linderina, L. pennispora,* shortly after this fungus had been isolated from soil collected in Liberia. In November, 1952, the author isolated a second member of the family, a species of *Coemansia,* from soil collected near the Rancho Santa Ana Botanic Garden, Claremont, California. An effort since has been made to obtain isolates of representatives of the family, and to date over sixty strains, representing about twenty species, mostly species of *Coemansia,* have been collected from various localities; about three-fourths of these strains have been isolated by the writer—the remainder has been obtained from other collectors. Representatives of five genera of Kickxellaceae are being maintained in culture, and a comparative study of the family is in progress. The purpose of this paper shall be to describe zygospore formation in *Coemansia* and *Kickxella.*

CHARACTERISTICS OF THE KICKXELLACEAE

Vegetative mycelium consisting of delicate, hyaline, branched, thin-walled, regularly septate hyphae; fruiting structures white or light-colored, erect or ascending, simple or branched, septate, relatively thick-walled, giving rise to sessile or stalked, septate or nonseptate fertile branches (sporocladia) bearing unispored sporangiola acrogenously on small, hyaline, ovoid or elongate-ellipsoid cells (pseudophialides); sporangiole wall delicate, persistent; sporangiospores simple, hyaline or yellowish, ovoid, elongate-ellipsoid, or fusoid, usually enveloped in liquid at maturity. All septa of both the vegetative and fruiting hyphae characteristically with median disciform cavities containing colorless biconvex or biumbonate plugs. Zygospores thick-walled, hyaline or yellowish, produced in the substratum from the fusion of similar hyphae.

Most of the known representatives of the Kickxellaceae are saprophytes and commonly are encountered in cultures of soil, dung, dead insects, or other organic debris; a few species are parasites or facultative parasites. The saprobic species usually can be grown on any of several agar substrata such as PDA, CM, CMPY, ME-YE, PAB,

and PAB-DEX[1], but, as might be expected, species differ with regard to which of several media is most favorable for normal development. Often, a given medium will support vigorous vegetative growth, but sporangiophores may not be formed or they may be highly aberrant and nearly or quite sterile. The writer has found PAB or PAB-DEX to be perhaps the best general media for members of the genus *Coemansia;* more species have been found to do better on these media than on any other of those which has been employed.

All members of the family produce a rather delicate, colorless, branched, septate vegetative mycelium which usually remains completely submerged in the substratum. The sporangiophores of *Coemansia* and *Kickxella* typically give rise to one or two germ hyphae produced laterally near the central part of the spore (Pl. 1 *m-n;* Pl. 4 *k-l;* Pl. 8 *d-f*), whereas the germ tubes of *Linderina* (also *Martensiomyces*), although lateral, are produced near the base of the spore (Raper and Fennell, 1952, fig. 19). Spore germination in *Martensella* has not yet been observed by the writer, but it seems probable that it proceeds as in *Coemansia.* The dimensions of the vegetative hyphae vary somewhat depending on the species, but the diameter, which may be relatively uniform or highly irregular, ranges usually from about two to ten microns. Each segment of the hyphae delimited by adjacent cross-walls contains finely granular vacuolate cytoplasm and several small nuclei. The septum of both the vegetative and fruiting hyphae in all members of the family which have been examined is distinctive in that it possesses a usually conspicuous central biconvex or biumbonate thickening (Pl. 8 *g*). This structure, as suggested by Raper and Fennell (1952), is a distinct family character, and in the past it has been noted by several students of these fungi (Van Tieghem and Le Monnier, 1873; Thaxter, 1895; Beer, 1902; Torrey, 1921). In marked contrast to observations published earlier, Linder (1943, p. 51), who studied several species of *Coemansia* in culture, states that the vegetative mycelium of members of the Kickxellaceae remains coenocytic until sporangiophore formation begins to take place. In reality, septa are formed very soon after spore germination (Pl. 1 *m;* Pl. 4 *k-l;* Pl. 8 *f*), and the vegetative mycelium of all members of the group studied here is septate from the beginning.

The hyaline or light-colored, usually yellowish, erect or ascending, often minutely asperulate fruiting structures arise directly from segments of the vegetative hyphae which may or may not be somewhat differentiated morphologically. The sporophores give rise to specialized sporogenous branches, or sporocladia, bearing numerous uni-spored sporangiola produced singly by apical budding on small ovoid or flask-shaped cells (Pl. 1 *d-g;* Pl. 4 *d-h;* Pl. 7 *j-m*) which here are termed *pseudophialides* in order to distinguish them from the phialides characteristic of many of the Fungi Imperfecti. The delicate wall of the sporangiole usually is not distinguishable from that of the sporangiospore except distally where, in most species, it extends beyond the apex of

the spore and forms a more or less conspicuous hyaline projection (Pl. 1 *h;* Pl. 4 *j;* Pl. 7 *n*). The sporangiolous nature of the kickxellaceous "conidium" is demonstrated in the accompanying photographs of germinating spores of *Kickxella* (Pl. 8 *d-e*) which show the upper portion of the sporangiole wall surrounding the germinated spore. In an undescribed species of *Coemansia* in the writer's collection the sporangiole wall is separated from the sporangiospore at the basal as well as the distal extremity of the spore (Pl. 6 *d*). Often, when the sporangiola are shed, the extreme basal portion of the sporangial membrane remains attached to the apex of the pseudophialide as an irregular collar-like projection (Pl. 1 *i;* Pl. 4 *i*).

The sporocladia of *Coemansia* (Pl. 1 *b-c;* Pl. 4 *b-c*), *Kickxella* (Pl. 7 *h-i;* Pl. 8 *i*), *Martensella* (Coemans, 1863, Pl. II, fig. 6), and *Martensiomyces* (Meyer, 1957, figs. 4-8) are more or less elongate, finger-like, septate structures which produce the pseudophialides and spores on one surface. The sporocladium of *Linderina* (Raper and Fennell, 1952, fig. 21), however, differs conspicuously from that found in the preceding genera and is nonseptate, somewhat ovoid, and bears the pseudophialides crowded on the upper, or outer surface. In *Coemansia, Linderina,* and *Martensella* the sporocladia are formed acrogenously, but, by the continued growth of the main axis, become diffusely arranged along the sporophore and appear pleurogenous (Pl. 1 *b;* Pl. 4 *b*); the sporocladia of *Kickxella* are formed simultaneously and are arranged in verticils on the apices of the sporophore branches (Pl. 7 *b, c-i*); the sporocladia of *Martensiomyces* are produced successively and borne in terminal umbels (Meyer, 1957). A more detailed illustrated discussion of the characteristics of all of the known genera of the Kickxellaceae will be given in another paper now in preparation.

<div align="center">SEXUALITY IN THE KICKXELLACEAE</div>

The type genus of the Kickxellaceae still is represented by only one species, *Kickxella alabastrina,* which Coemans (1862) named for the Belgian botanist, Kickx, and the strikingly white color of the fruiting structures. This species was described again in 1867 by Crouan and Crouan under the name *Coronella nivea.* Together with his characterization of *K. alabastrina* Coemans mentioned and gave a vague description of a cleistothecial ascomycete which he found growing in company with his fungus; he stated that he could not demonstrate a connection between the two, but he expressed the opinion that they might represent the conidial and ascosporic phases of a single species. Coemans (1863) also considered that *Martensella* probably belonged to the Hyphomycetes, but strangely enough he did not mention the similarity between this genus and the one which he had described only the year before. Van Tieghem and Le Monnier (1873) also believed, as had Crouan and Crouan (1867) and Roumeguere (1870) before them, that *Kickxella alabastrina* very probably was the imperfect stage of an ascomycete, and they made the same presumption concerning *Coemansia* and *Martensella.* Saccardo (1883), ignoring *Coronella,* created a third name, *Coemansiella alabastrina,* for *Kickxella alabastrina* and reserved the latter name for the supposed perfect stage mentioned by Coemans. Although Coemans had provided "no description worthy of the name" (Linder, 1943, p. 50) for the ascomycete, Saccardo was able to furnish a quite remarkable description of it. Linder (1943) discussed the nomenclatural history and status of *Kickxella* in detail, and the reader is referred to his paper for further information.

In the absence of definitive knowledge of their sexuality, *Coemansia, Kickxella,* and *Martensella* long have been allied with the Hyphomycetes as probable imperfect stages of the Ascomyceteae by many other students of the fungi in addition to those listed above (Van Tieghem, 1875; Costantin, 1888, Lambotte, 1890; Lindau, 1900,

1907; Beer, 1902; Oudemans, 1905; Bainier, 1906; Torrey, 1921; Migula, 1934). De Bary, however, as early as 1866 suggested that *Kickxella alabastrina* might perhaps be a member of the Mucorales, and he reasserted this opinion again in 1884 (De Bary, 1887). Among others who since have considered these fungi as doubtful Zygomycetes are Istvánffi (1895), Thaxter (1895, 1897), Vuillemin (1904), and Zycha (1935).

When Linder placed *Coemansia, Kickxella,* and *Martensella* in the new family, Kickxellaceae, in 1943 he presented, for the first time, evidence of the zygomycetous affinities of the group in the form of a presumably immature zygospore discovered in one of Thaxter's slide mounts of a species of *Coemansia*. From an analysis of Thaxter's rather fragmentary herbarium notes, Linder concluded that this preparation originated from a culture of an undescribed species of *Coemansia* in Thaxter's collection which he named *C. aciculifera*. Linder (1943, p. 53) admitted that further confirmation of sexual reproduction in the Kickxellaceae was needed before the true relationships of the family could be proved. In the course of the present investigation, zygospores have been found in several species of the family. These will be described for only four species at this time, *Coemansia aciculifera, C. braziliensis, C. mojavensis,* and *Kickxella alabastrina.*

COEMANSIA ACICULIFERA Linder.

Farlowia 1(1): 65-66. 1943.

(Plates 1-3)

Colonies on PAB-DEX pale yellow (near *Martius Y:* Maerz and Paul, 1950, Pl. 9, 1-I). Vegetative hyphae colorless, septate, about 3-6 μ wide, with numerous gangliform swellings. Sporangiophores erect or ascending, simple, furcate, or irregularly branched, usually 4-6 mm. high, but reaching as much as 1 cm. in age, minutely asperulate; sporangiophore segments below the nonfertile zone 95-180 μ × 8-12 μ. Sporocladia arranged in a loose spiral on the sporangiophore, 1, rarely 2, per cellsegment; intersporocladial distance 30-120 μ (aver. 80 μ); sporocladial stipe 1-, rarely 2-celled, 18-36 μ (aver. 26 μ) long × 4.5-7.5 μ wide at the middle, often proliferating to produce an additional smaller sporocladium or branch. Sporocladia asperulate, composed of about 7-11 cells (aver. 9) excluding the stipe, 30-50 μ (aver. 39 μ) long × 7-10 μ wide, turned up sharply on the divergent stipe and nearly paralleling the sporangiophore, slightly curved outward, the small, usually sterile, terminal cell recurved and rounded apically. Pseudophialides obovate, flask-shaped, 5-7 μ × 2.2-2.6 μ, arranged on the lower surface of the fertile cells in two more or less transverse rows of 3-7 pseudophialides each. Sporangiola nearly colorless or pale-yellow, elongate fusiform-elliptical, 16-25 μ (aver. 19 μ) × 2 μ, in lateral view one margin convex, the other nearly straight or slightly concave; sporangiole wall projecting

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PLATE 1. *Coemansia aciculifera* Linder. *a.* Habit sketch showing general characteristics of the fruiting structures. × 30. *b.* Upper portion of fruiting branch showing acrogenous formation of the long-stipitate sporocladia and the disposition of the latter singly on each cell of the fertile axis. × 400. *c.* Mature sporocladium showing disposition of pseudophialides and sporangiola on lower surface. × 1080. *d-g.* Four pseudophialides showing successive stages in the acrogenous development of the unispored sporangiole. × 1700. *h.* Three mature sporangiola. × 2000. *i.* Two pseudophialides after the sporangiola have been shed, each with an apical collar-like projection representing the basal portion of the sporangiole wall. × 1700. *j.* Sexual hyphae showing fused gametangia. × 1080. *k.* The same; the immature zygospore beginning to enlarge. × 1080. *l.* Mature zygospore. × 650. *m-n.* Germinating spores. The germ hypha shown in fig. *m.* already has formed a cross-wall. × 900.
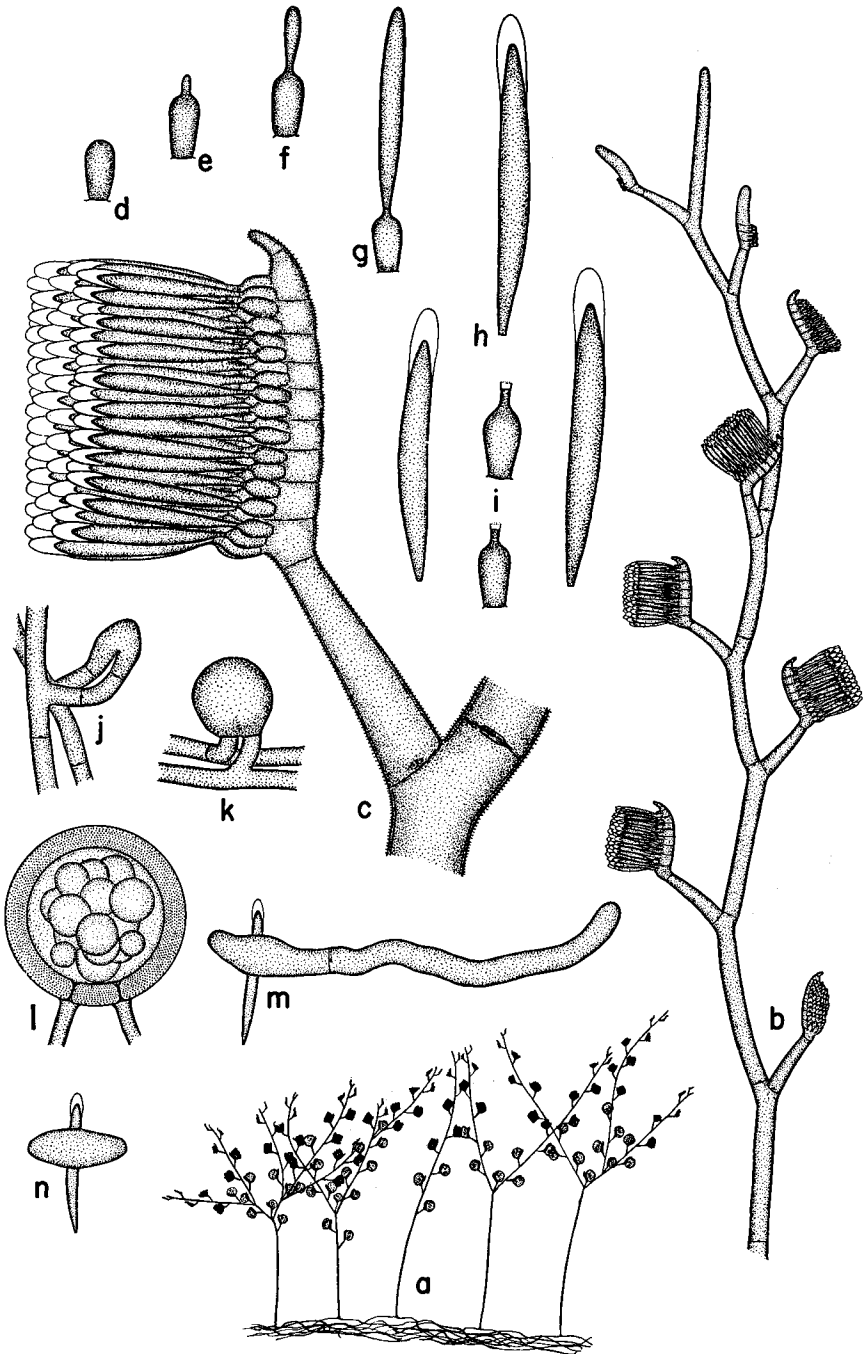
PLATE 1

about 2 μ beyond the apex of the spore as a distally rounded hyaline enlargement having a diameter equal to or slightly exceeding the greatest diameter of the spore. Sporangiospores 14-23 μ (aver. 17 μ) $\times$ 2 μ, tapered abruptly and rounded distally, gradually narrowed and truncate below, enveloped in liquid at maturity. Homothallic. Zygospores developed from the conjugation of two usually short undifferentiated hyphal branches, globose, nearly colorless, thick-walled, smooth, 27-44 μ (aver. 36 μ) in diameter, wall 3.4-6.5 μ (aver. 4.5 μ) thick, containing 5-15 spherical refractive globules 1.5-15 μ in diameter.

Specimens examined.—CALIFORNIA. Monterey County: Castroville, spring, 1956, from soil, collected and isolated by D. H. Ford (RSA Culture 476). Sonoma County: Occidental, spring, 1956, from soil, collected and isolated by D. H. Ford (RSA Culture 477). MAINE. York County: Kittery Point, "from Sphagnum zygospores," date ? (Farlow Herbarium: *R. Thaxter Acc. No. 6404; Type*). Transfers of Nos. 476 and 477 have been deposited in the collections of the following institutions: American Type Culture Collection, Washington, D. C.; Centraal-bureau voor Schimmelcultures, Baarn, Netherlands; Commonwealth Mycological Institute, Kew, Surrey, England. Dried specimens have been placed in the Mycological Collections of the Rancho Santa Ana Botanic Garden.

Except for slightly shorter sporangiola, both of the isolates of *C. aciculifera* from California compare in all respects with the type specimen. The latter consists of a dried agar culture which Thaxter had annotated, without date, simply "from Sphagnum zygospores, Kittery Pt., Me." It is very likely that this material originated from the *Sphagnum* cultures, bearing unknown zygospores, from which Thaxter obtained his *Syncephalis tenuis* (Thaxter, 1897, p. 12), for in the discussion following the description of this species Thaxter states:

"It has made its appearance twice in cultures of Sphagnum on which were zygo-spores of an unknown zygomycete,[2] the orange yellow coherent waxy masses of which are not infrequently found in swampy places on this host, usually at the tip of its axis, occurring more rarely on other substances like decaying wood, etc. These zygo-spores, which are oblong and orange and are produced by budding upward from the point of union of the two gametes as in species of Syncephalis, although they are widely different in color, form, and condition of aggregation from any of the known zygospores of this genus, may possibly be connected with the present species; but as all attempts to cultivate them under test conditions have thus far proved fruitless, and as the same cultures of Sphagnum on which they were growing have also yielded a new Martensella [*Coemansia*] (in my opinion a zygomycete), two species of Mor-tierella, and a peculiar orange-colored Mucor, it is doubtful which, if any, of all of these forms should be connected with them.

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[2]"This fungus corresponds closely to the description of *Endogone xylogena* Schröter."

There is little doubt that "the orange yellow coherent waxy masses" of zygospores actually belonged to *Endogone pisiformis* Link, which Thaxter, in his *A Revision of the Endogoneae* (1922, p. 299), reported as frequent "especially near the tip of Sphagnum" and to which he referred *E. xylogena*, and, therefore, it is very doubtful that Thaxter actually obtained his culture of *Coemansia* "from Sphagnum zygo-spores." It is, however, interesting to note the marked resemblance between the zygospores of species of the Kickxellaceae and certain species of the Endogonaceae.

Regardless of the exact origin of his isolate of *Coemansia*, Thaxter did leave a slide mount, later restored from a dry condition by Linder, which he had labelled simply,

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PLATE 2. *Coemansia aciculifera* Linder. *a-k*. Successive stages in the formation of the zygospore. See explanations in the text. All $\times$ 1300.
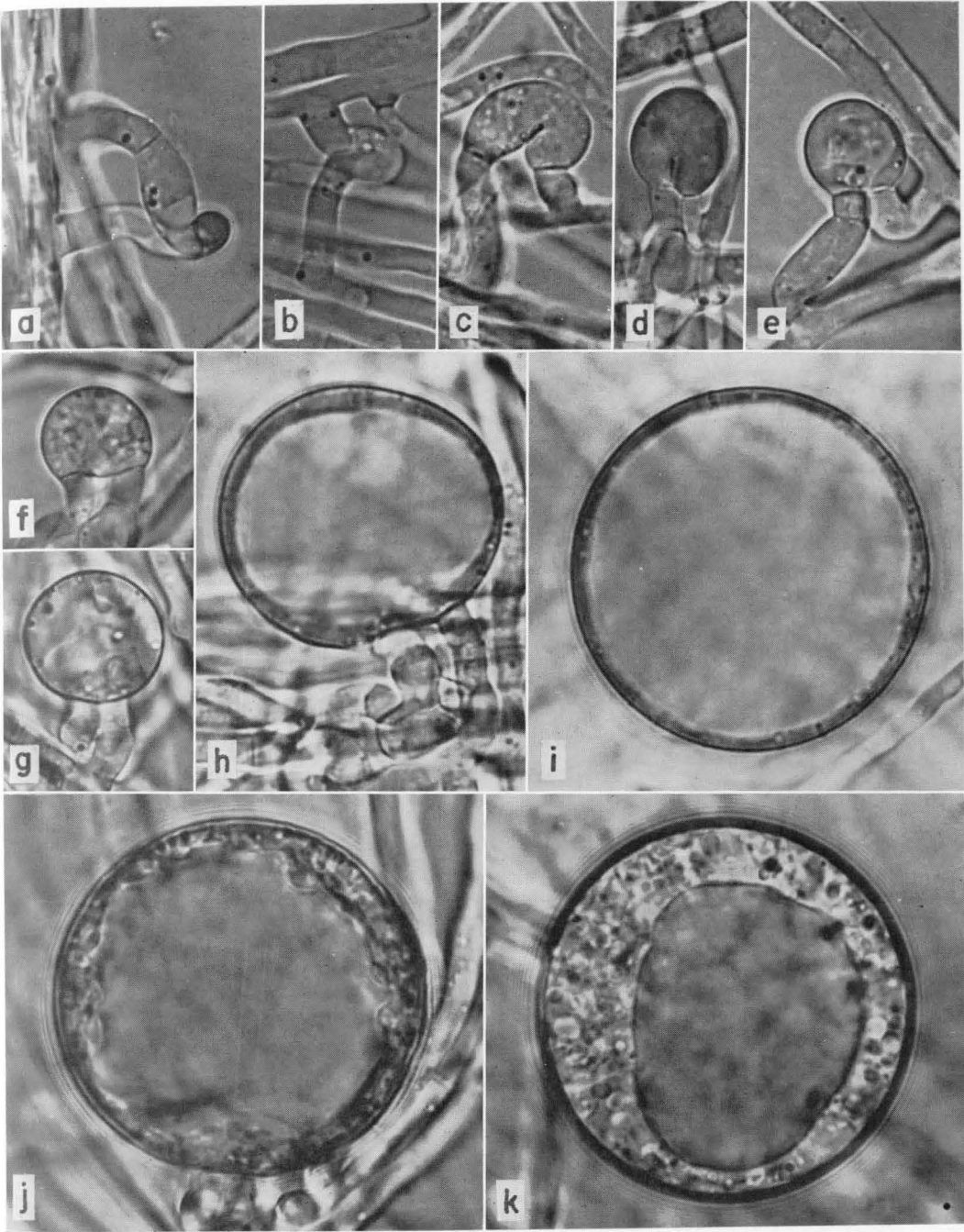
PLATE 2

"An attempt at zygospore formation." This preparation, presumably derived from a culture of the above isolate, included a structure which Linder (1943, Pl. 4, fig. I) interpreted as an immature zygospore. On the basis of this evidence he accepted Thaxter's view that *Coemansia* indeed is a zygomycete and he established the family Kickxellaceae to accommodate this genus as well as *Kickxella* and *Martensella*.

Both of the isolates here referred to *Coemansia aciculifera* readily produce zygospores in pure culture. On PAB-DEX these begin to appear after about a week and are initiated by the apical fusion of two usually relatively short hyphal branches not differing morphologically from the vegetative hyphae from which they arise (Pl. 1 *j;* Pl. 2 *a-b*). Fusion branches regularly originate from neighboring hyphae; this pattern of development is the one normally encountered in cultures derived from single spores, and, since the spores are uninucleate, the species is presumed to be homothallic. A terminal cell or gametangium is delimited in each of the sexual branches by the time of fusion (Pl. 2 *a*) and the incipient zygosporangium develops usually as a lateral outgrowth (Pl. 1 *j;* Pl 2 *b-c*) which enlarges rapidly as a more or less globose thin-walled swelling (Pl. 1 *k;* Pl. 2 *d-h*) until it has attained approximately definitive size (Pl. 2 *i*). At this stage of development the protoplasm of the zygosporangium consists of a thin peripheral layer of granular cytoplasm surrounding a large central vacuole. Apparently a considerable quantity of material moves through the sexual branches into the developing zygosporangium prior to and during the early stages of maturation of the zygote, for as progressive thickening of the zygote wall takes place there is a concomitant increase in the amount and density of the protoplasm and decrease in the size of the central vacuole (Pl. 2 *j-k;* Pl. 3 *a*). When the zygote wall has attained approximately 1/3-1/2 its ultimate thickness, the dense protoplasm which fills the globose body begins to condense into numerous small globules (Pl. 3 *b*), which, through continued coalescence, decrease in number and increase in size (Pl. 2 *c*) until in the mature zygote (Pl. 1 *l;* Pl. 3 *d*) they number about 5-15, range from about 1.5-15 μ in diameter, and are surrounded by a clear nearly homogenous cytoplasm. The smooth nearly hyaline zygote measures about 27-44 μ in diameter and has a wall about 3.4-6.5 μ thick. The thin membrane comprising the zygosporangium is everywhere adnate to the wall of the mature zygote, and the openings in the spore wall through which materials moved by way of the sexual branches into the developing zygote remain in evidence as closed pits traversing the entire thickness of the wall (Pl. 1 *l*). The young zygospore figured by Linder (1943, Pl. 4, fig. I) would appear to have reached a state of maturity approximately equal to the one shown in Pl. 2 *h.*

The method of zygospore formation described above for *Coemansia aciculifera* has been found in several species of *Coemansia* studied here, but a distinctly different pattern of development has been observed in a few others including the following species.

Coemansia mojavensis sp. nov.

(Plates 4-5)

Coloniae in agaro PAB-DEX luteolae; hyphis vegetantibus hyalinis, spetatis, 2-6 μ latis; sporangiophoris rectis, septatis, infra simplicibus, supra in partibus fertilibus simplicibus, furcatis vel trifurcatis, 3-5 mm. altis, minute asperulatis; cellulis sporangiophorum infra partibus fertilibus 95-175 μ × 7-10 μ; sporocladiis spiraliter dispositis, in cellula 4-9 (med. 6); distantia intrasporocladiosa 6-16 μ (med. 10 μ); stipite sporocladii 4.8-7 μ × 3.4-4.4 μ; sporocladiis asperulatis, 18-30 μ (med. 24 μ) × 5-7 μ, divergentibus, leviter sigmoideis, 4-8 (med. 6) cellulis; vesiculis sporigeris ellipsoideis, 4-5.2 μ × 2.2-3 μ; sporangiolis hyalinis vel luteolis, elongate ovaleovatis, 10-15.4 μ (med. 12.2 μ) × 1.8-2.4 μ, leviter arcuatis; sporangiosporis
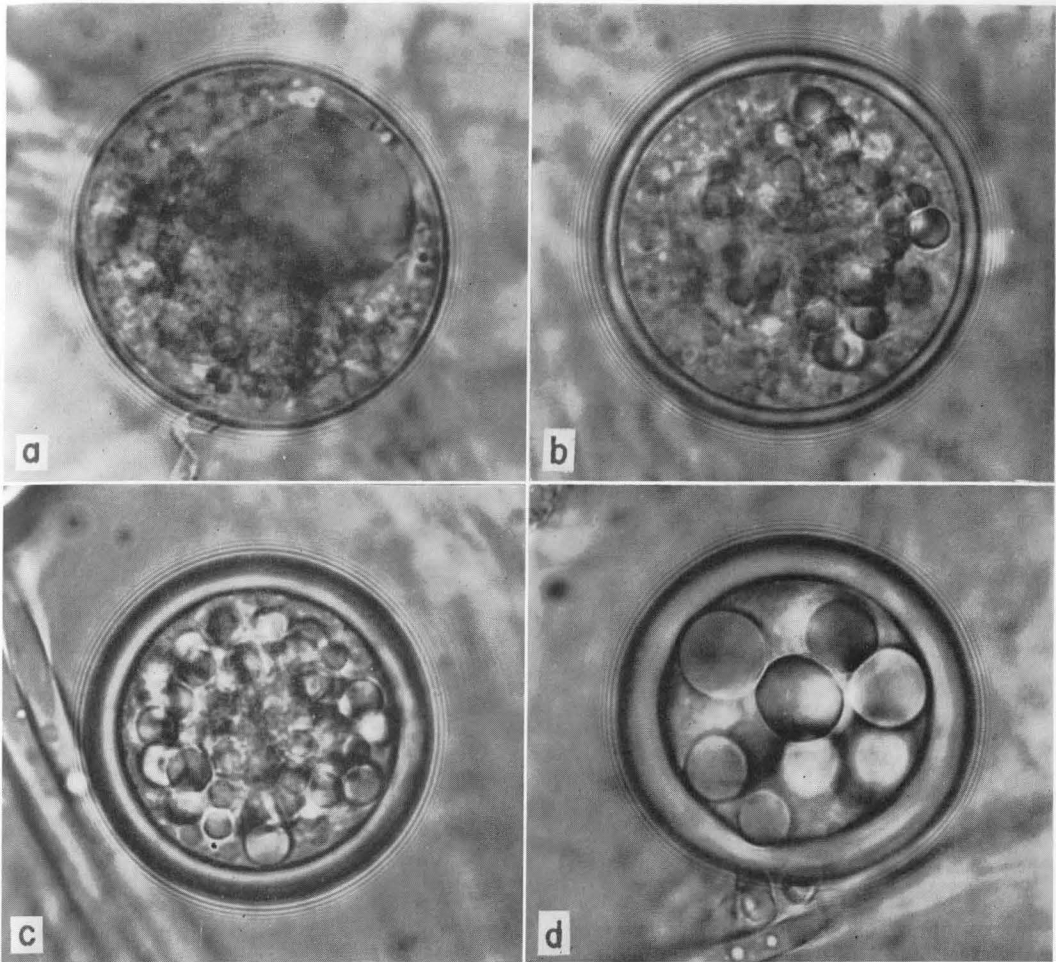
PLATE 3. *Coemansia aciculifera* Linder. *a-c.* Late stages of maturation of the zygospore showing the progressive thickening of the zygote wall and the formation of numerous refractive globules by the gradual coalescence of the densely granular cytoplasm. All × 1300. *d.* Mature zygospore. × 1300.

8.8-13 μ (med. 10.5 μ) × 1.8-2.4 μ, in apice spinosis, ad basim truncatam rotundatis, ad maturitatem in liquido involutis; homothallicis; zygosporis globosis, cum muris crassis, prope hyalinis, levibus, 35-62 μ (med. 48 μ) diam., muris 2.6-8.5 μ (med. 5.8 μ) crassis, globulos multos refractivos 2-20 μ diam. contentis.

Colonies on PAB-DEX pale yellow (near *Marguerite Y:* Maerz and Paul, 1950, Pl. 10, 1-C). Vegetative hyphae colorless, septate, about 2-6 μ wide, often with gangliform swellings. Sporangiophores erect, septate, simple below, unbranched, furcate or trifurcate above in the fertile zone, 3-5 mm. high, minutely asperulate; sporangiophore segments below fertile zone 95-175 μ × 7-10 μ. Sporocladia arranged spirally on the sporangiophore, 4-9 (aver. 6) per cell-segment; intersporocladial dis-

tance about 6-16 μ (aver. 10 μ), rarely up to 30 μ; sporocladial stipe 4.8-7 μ $\times$ 3.8-4.4 μ. Sporocladia asperulate, composed of 4-8 (aver. 6) cells excluding the stipe, 18-30 μ (aver. 24 μ) long $\times$ 5-7 μ wide, divergent, slightly sigmoid, the attenuated, usually sterile, apical cell slightly longer than the fertile cells. Pseudophialides ellipsoid, 4-5.2 μ $\times$ 2.2-3 μ, arranged on the lower surface of the fertile cells in two more or less transverse rows of 2-5 or 6 pseudophialides each. Sporangiola nearly colorless or pale yellow, elongate oval-ovate, 10-15.4 μ (aver. 12.4 μ) $\times$ 1.8-2.4 μ, slightly arcuate, in lateral view one margin convex, the other slightly concave in the distal two-thirds; sporangiole wall projecting about 2 μ beyond the apex of the spore as a tapered, apically rounded, hyaline extension. Sporangiospores 8.8-13 μ (aver. 10.5 μ) $\times$ 1.8-2.4 μ, abruptly attenuated distally to form a spine-like process, rounded and truncate below, enveloped in liquid at maturity. Homothallic. Zygospores developed from the conjugation of a short undifferentiated branch with a vegetative hypha and formed by the enlargement of a cell delimited in the sexual branch, globose, nearly colorless, thick-walled, smooth, 35-62 μ (aver. 48 μ) in diameter, wall 2.6-8.5 μ (aver. 5.8 μ) thick, containing numerous spherical refractive globules 2-20 μ in diameter.

Type.—CALIFORNIA. San Bernardino County: 18 miles north of Vidal, April 9, 1953, isolated from rat dung (RSA Culture 71). Dried specimens have been placed in the Mycological Collections of the Rancho Santa Ana Botanic Garden, Claremont, California. Transfers of the type culture have been deposited in the collections of the following institutions: American Type Culture Collection, Washington, D. C.; Centraalbureau voor Schimmelcultures, Baarn, Netherlands; Commonwealth Mycological Institute, Kew, Surrey, England.

Coemansia mojavensis more nearly resembles *C. braziliensis* Thaxter ex Linder (Linder, 1943) in general habit than any other of the described species of the genus, but it may be distinguished from the latter by its longer more slender sporangiophores, smaller sporocladia bearing slightly larger and morphologically distinct sporangiola, and by the character of its zygospores. A culture of *C. braziliensis* on deposit in the collections of the Northern Utilization Research and Development Division, Peoria, Ill., has been obtained through the courtesy of Dr. C. W. Hesseltine. This strain (NRRL 1566), according to Raper and Fennell (1952), was obtained originally from Linder in 1940 as representing Thaxter's isolate of the species. The sporangiola of this strain measure about 6-10 μ (aver. 8μ) $\times$ 2.6 μ. My own measurements of the sporangiola of the type specimen of *C. braziliensis* (Farlow Herbarium: *R. Thaxter Acc. No. 6398*) gave a value of about 8-11 μ (aver. 9.5 μ) $\times$ 2.2-2.6 μ, whereas Linder, in his description of the species, gives 11-14.5 μ $\times$ 2-2.5 μ; I cannot account for this discrepancy. The sporangiospores of *C. braziliensis*

PLATE 4. *Coemansia mojavensis* sp. nov. *a.* Habit sketch showing general characteristics of the fruiting structures. $\times$ 30. *b.* Upper portion of fruiting branch showing acrogenous formation of the short-stipitate slightly sigmoid sporocladia. The latter are arranged spirally on the fertile axis, each cell of which, in this species, bears an average of about six sporocladia. $\times$ 400. *c.* Sporocladium; the sporangiola not yet fully mature. $\times$ 1360. *d-h.* Five pseudophialides showing successive stages in the acrogenous development of the unispored sporangiole. $\times$ 1700. *i.* Two pseudophialides after the sporangiola have been shed, each with an apical collar-like projection representing the basal portion of the sporangiole wall. $\times$ 1700. *j.* Three mature sporangiola. $\times$ 2000. *k-l.* Germinating spores. Note early formation of cross-walls. $\times$ 900. *m.* Apex of sexual branch which has arisen from one vegetative hypha, fusing laterally with another vegetative hypha. Note cell delimited distally in the sexual branch. $\times$ 780. *n.* Early stage in the development of the zygospore from a cell delimited distally in the sexual branch. $\times$ 790. *o.* Mature zygospore. $\times$ 490.
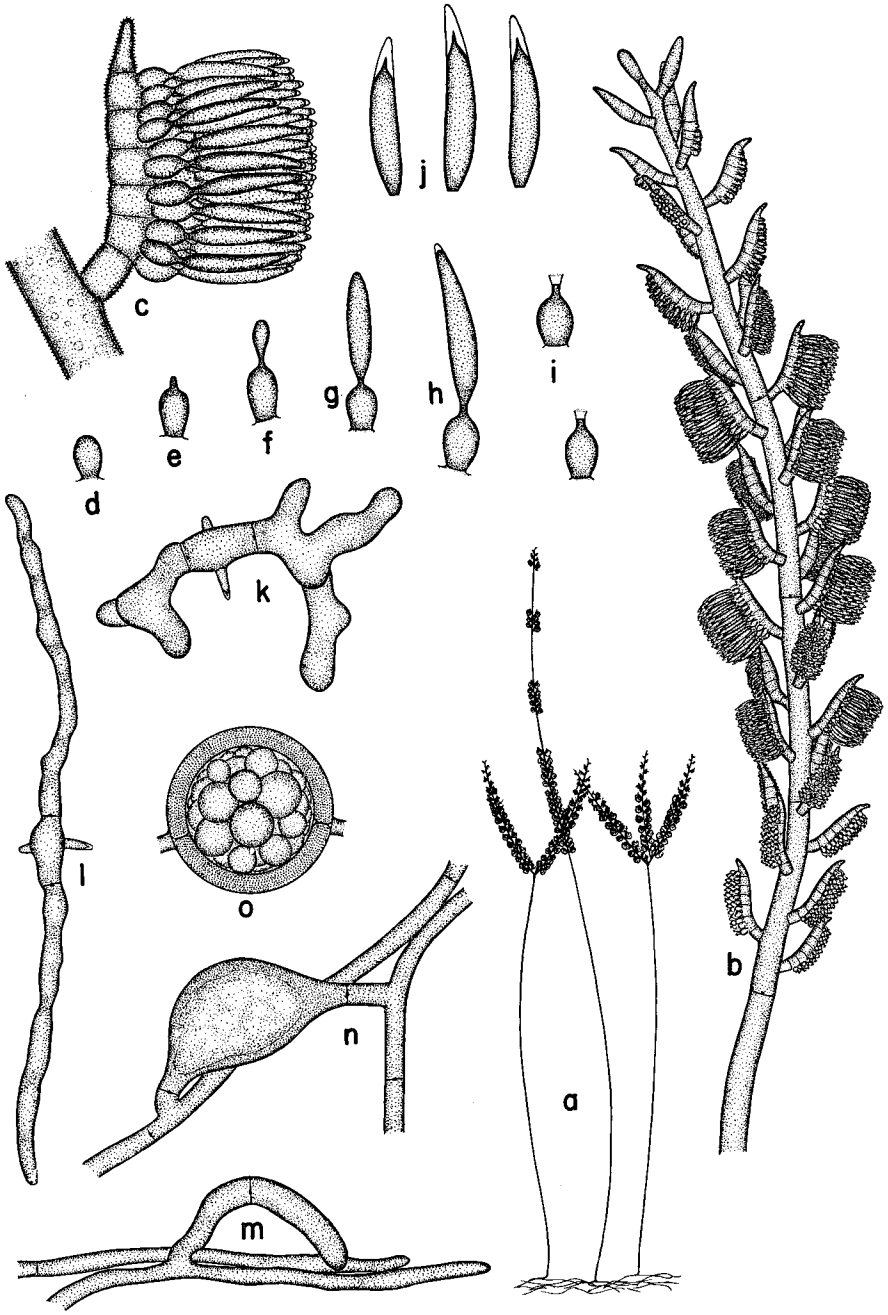
PLATE 4

are elliptical, often slightly more convex on one side, bluntly rounded distally, and abruptly tapered to the truncate base. The sporangiole wall projects about 0.4 μ beyond the apex of the spore.

On PAB-DEX, zygospores of *C. mojavensis* begin to appear in about three to four days. The apex of a usually short undifferentiated hyphal branch contacts another hypha (Pl. 4 *m;* Pl. 5 *a-c*) and fuses with it (Pl. 5 *a*). A cell, delimited in the distal portion of the sexual branch (Pl. 4 *m;* Pl. 5 *a*), undergoes a series of transformations (Pl. 5 *b-f*) exactly like those described above for *C. aciculifera* and produces a smooth, thick-walled, nearly hyaline zygote (Pl. 4 *o;* Pl. 5 *g*) about 35-62 μ (aver. 48 μ) in diameter with a wall 2.6- 8.5 μ (aver. 5.8 μ) thick surrounded by the thin zygosporangial membrane (Pl. 5 *h*). This pattern of zygospore development has been observed in several other species of *Coemansia* in the writer's collection including *C. braziliensis* (Pl. 6 *a-c*). In the latter species an interesting phenomenon, not yet encountered in any other species of the genus, has been observed. Very frequently not only the apical part of the sexual branch but also the cell of the vegetative hypha with which the sexual branch has fused enlarge in their entirety and are incorporated into the zygospore (Pl. 6 *c*) ; this results in anomalous zygospores very irregular in size and shape. In a random sample of 2147 zygospores of *C. braziliensis* examined, 610 or 28.4% were anomalous, whereas 1537 or 71.6% were of the normal usually globose type (Pl. 6 *b*). The zygospores of *C. braziliensis* are pale orange-brown, slightly roughened, and measure about 32-52 μ (aver. 42 μ) in diameter; they have a wall 3.5-8.8 μ (aver. 5.4 μ) thick.

<div align="center">

KICKXELLA ALABASTRINA Coemans.

Bull. Soc. Royale Bot. Belgique. 1: 153-159. 1862.

(Plates 7-8)

</div>

Coronella nivea Crouan and Crouan, Florule du Finistere, p. 12. 1867.
Coemansiella alabastrina (Coemans) Saccardo, Sylloge Fungorum. II: 815. 1883.

Colonies on CMPY white. Vegetative hyphae colorless, septate, relatively uniform at first, about 4-8 μ in diameter, becoming less uniform and often developing vesicular enlargements of more or less irregular size and shape. Sporangiophores erect, septate, minutely asperulate, at first simple, becoming cymosely branched (Pl. 7 *a-b*), about 1-2 mm. high, 12-25 μ or more in diameter, bearing verticils of 9-15 (aver. 12) sporocladia formed simultaneously on apical enlargements (Pl. 7 *b-i;* Pl. 8 *h-i*). Sporocladia arcuate, asperulate, 65-85 μ × 10-13 μ, consisting usually of three cells: the proximal cell about three times as long as the median cell and about equal in length to the more slender, often bifurcate, sterile distal cell (Pl. 7 *b-i;* Pl. 8 *i*). Sporangiola fusiform, 13-17 μ × 4-4.5 μ, wall separated from the sporangiospore only at the extreme apex (Pl. 7 *n*), borne singly on numerous ovoid pseudophialides, 6-6.5 μ × 3.5 μ (Pl. 7 *j-m*), arranged in more or less transverse rows on the upper surfaces of all but the terminal cell of the sporocladium (Pl. 7 *f-i*). Homothallic. Zygospores developed from the conjugation of two undifferentiated hyphal branches, globose or ovoid, nearly colorless, thick-walled, smooth, 23-50 μ (aver. 33 μ) in diameter, wall 3-7 μ (aver. 5 μ) thick, containing numerous smallish refractive globules.

PLATE 5. *Coemansia mojavensis* sp. nov. *a.* Undifferentiated sexual branch showing the fusion of its apex laterally with a vegetative hypha. × 1300. *b-e.* Successive stages in the development of the zygospore from a cell delimited in the distal portion of the sexual branch. × 750. *f.* Slightly immature zygospores. × 560. *g.* Mature zygospore. × 560. *h.* Mature zygospore showing discrete zygosporangial membrane separated from the thick-walled zygote. (Crushed in Hoyer's medium.) × 560.
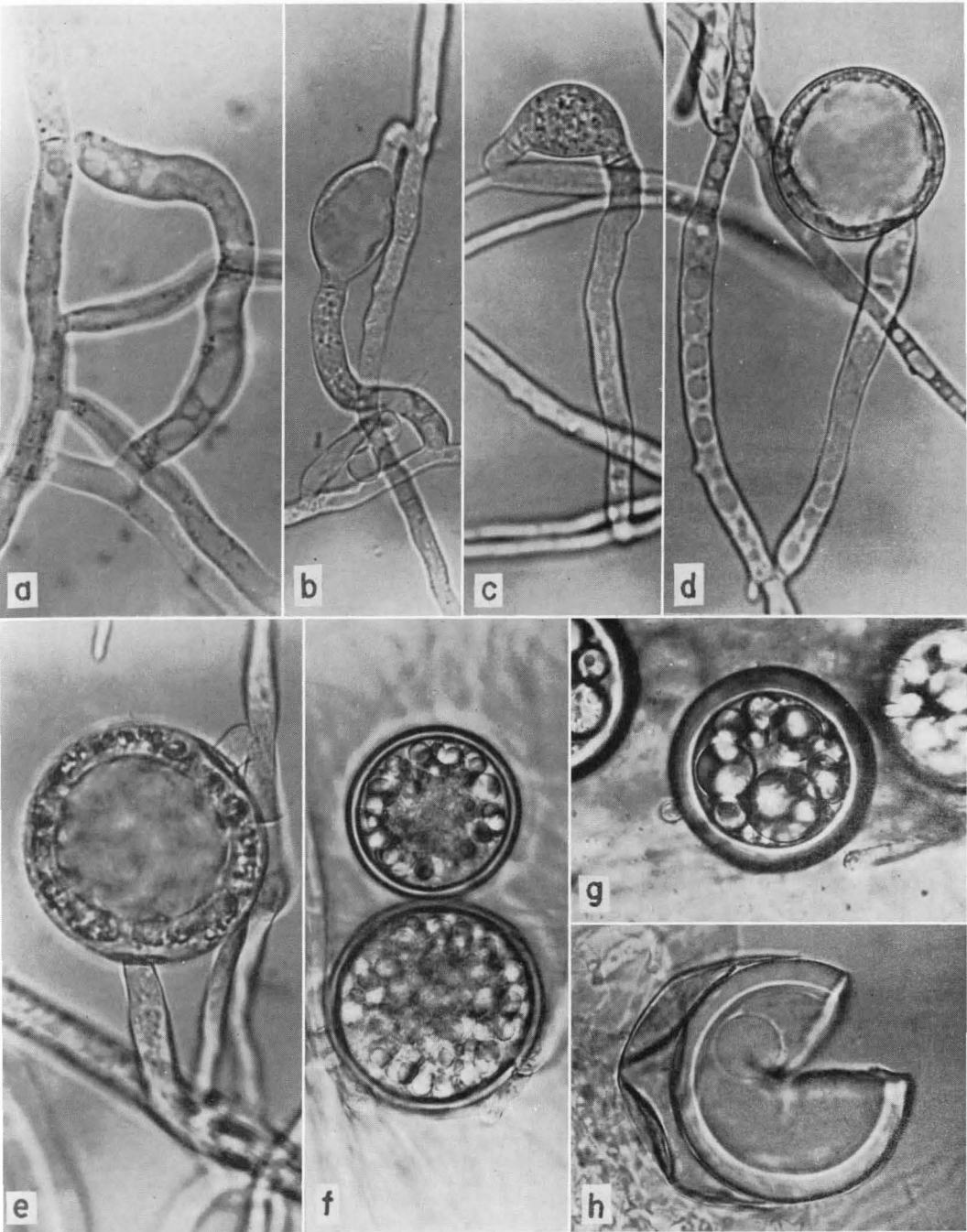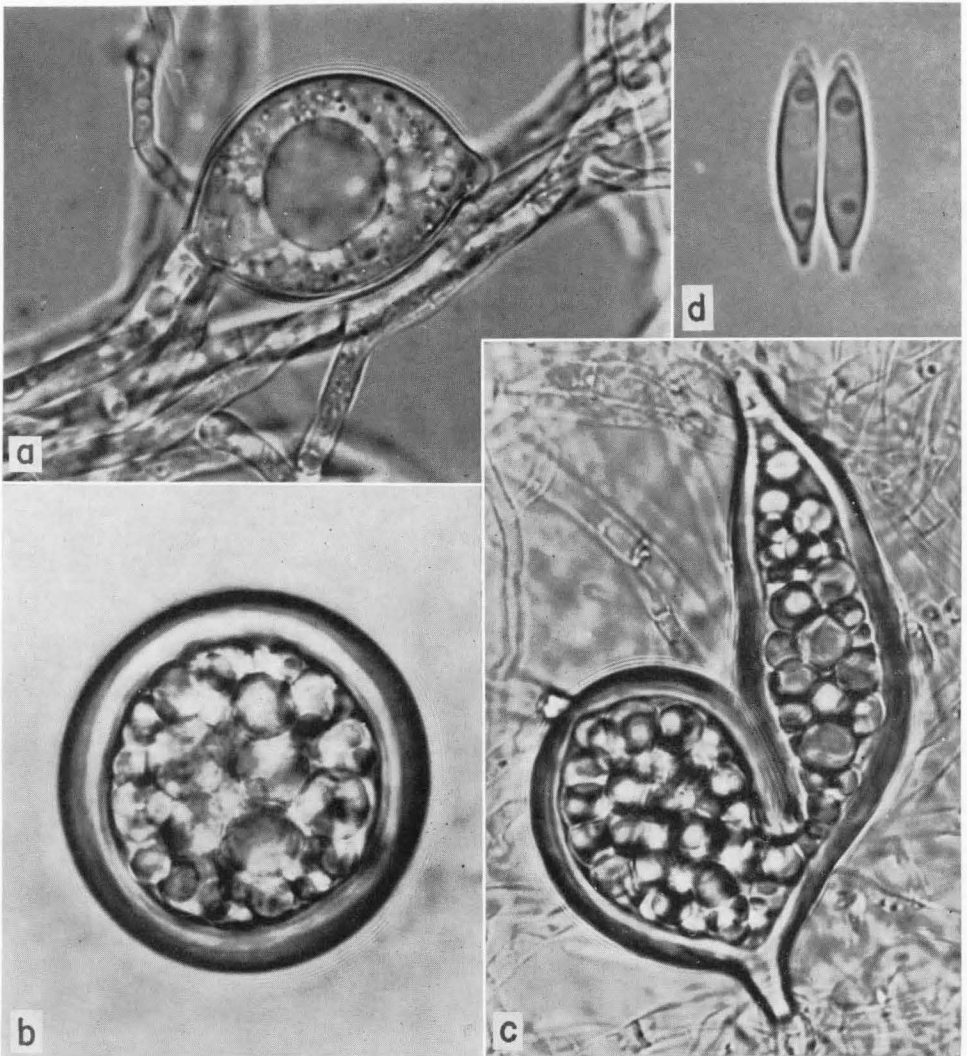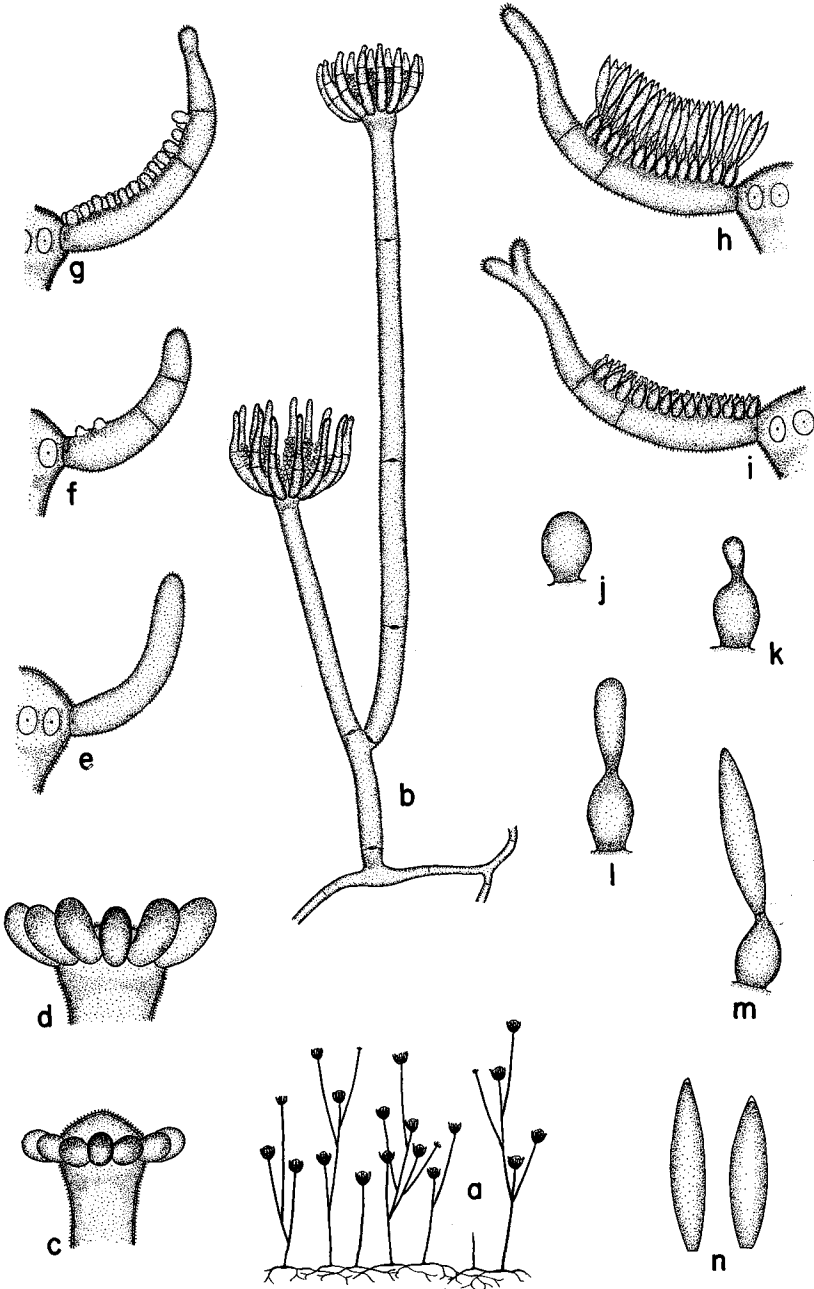
Plate 5

PLATE 6. *a-c. Coemansia braziliensis* Thaxter ex Linder. *a.* Immature zygospore developing from a cell delimited distally in a sexual branch as in *C. mojavensis.* × 1300. *b.* Mature zygospore of normal type. × 1000. *c.* Mature zygospore of anomalous type resulting from the enlargement of not only the distal part of the sexual branch but also the cell of the hypha with which the sexual branch has fused. × 1000. *d. Coemansia* sp. Two unispored sporangiola showing the sporangiospores separated from the basal as well as the apical part of the sporangial wall. × 2330.

PLATE 7. *Kickxella alabastrina* Coemans. *a.* Habit sketch showing general characteristics of the fruiting structures. × 30. *b.* Once branched sporangiophore bearing distal whorls of sporocladia. × 180. *c-d.* Early stages in the development of the sporocladia showing their simultaneous origin in a whorl around the slightly swollen apex of the sporangiophore. × 600. *e-g.* Successive stages in the development of the sporocladium and pseudophialides prior to sporangiole formation. × 600. *h.* Mature sporocladium showing disposition of pseudophialides and spor-

angiola on upper surface. × 600. *i*. Mature sporocladium after sporangiola have fallen away; note furcate apical cell. × 600. *j-m*. Four pseudophialides showing successive stages in the acrogenous development of the unispored sporangiole. × 1360. *n*. Two mature sporangiola. × 1360.

Specimens examined.—CALIFORNIA. Los Angeles County: Evey Canyon, San Gabriel Mts., 4½ miles north east of Claremont, April, 1955, isolated from mouse dung (RSA Culture 352). MAINE. York County: Kittery Point, Aug.-Sept., 1895, on horse dung collected by R. Thaxter (Farlow Herbarium: *R. Thaxter Acc. No. 765*). Transfers of Culture No. 352 have been deposited in the collections of the following institutions: American Type Culture Collection, Washington, D. C.; Centraalbureau voor Schimmelcultures, Baarn, Netherlands; Commonwealth Mycological Institute, Kew, Surrey, England. Dried specimens have been placed in the Mycological Collections of the Rancho Santa Ana Botanic Garden.

Three accounts of studies of *Kickxella alabastrina* have been published since Coemans' original description of it (Van Tieghem and Le Monnier, 1873; Beer, 1902; Torrey, 1921). Van Tieghem and Le Monnier gave the best discussion of the species which has been presented up to now; they claim to have cultured the fungus in horse dung decoction, and they gave a relatively accurate account of spore germination, the development of the vegetative mycelium, and the formation and characteristics of the fructifications. In addition, they described a singular type of "chlamydospore" about which more will be said subsequently. Oddly enough, as pointed out by Torrey (1921), Van Tieghem in 1875, without reference to his and Le Monnier's earlier success in culturing *Kickxella,* concluded that both *Kickxella* and *Coemansia* were obligate parasites of various Mucoraceae.

Beer (1902), working in England, succeeded in growing *Kickxella alabastrina* in hanging drop cultures, but he added nothing of importance to the account of this species given by Van Tieghem and Le Monnier. He encountered chlamydospores in one of his cultures but stated that he was not able to connect the mycelium in which these were formed with that which bore the fruiting structures of *Kickxella.* Beer did not describe these chlamydospores, but his figures of them suggest that they differed from those described by Van Tieghem and Le Monnier. In addition, he described small thin-walled spores borne at the septa of hyphae which he also was not able to connect with the mycelium of *Kickxella;* an examination of his figures of these spore-bearing filaments leads one to suspect that his cultures were contaminated with one of the filamentous yeasts.

Torrey (1921) encountered *Kickxella alabastrina* on zebra dung collected in the Jardin des Plantes in Paris, but, despite repeated attempts to grow the fungus on a variety of media under varying conditions, he did not succeed in obtaining pure cultures. He postulated, in view of the apparent success which Van Tieghem and Le Monnier (1873), Beer (1902), and Thaxter (1895) had enjoyed in culturing this organism, that both a saprophytic, or at least a facultatively parasitic, and an obligately parasitic race of *K. alabastrina* must exist.

Linder did not have an opportunity to study living material of *Kickxella alabastrina,* and his observations were made on dried specimens preserved in the Thaxter collection. This circumstance led him to describe a so-called annular thickening laid

PLATE 8. *Kickxella alabastrina* Coemans. *a.* Immature sporangiophore showing appearance of septa prior to formation of sporocladia. $\times$ 225. *b.* Mature living sporangiole as seen in optical section. $\times$ 1300. *c.* Mature sporangiole mounted in Lacto-phenol with acid fuchsin as seen in optical section. Note the apparent annular thickening on inner wall of spore caused by localized shrinkage of the cytoplasm. $\times$ 1300. *d-e.* Germinated spores; the distal part of the sporangiole wall is shown surrounding the corresponding portion of the germinated spore. $\times$ 1300. *f.* Spore which has given rise to one lateral germ hypha; note early formation of septum. $\times$ 1300. *g.* Septum of sporophore showing median biumbonate thickening. $\times$ 1300. *h.* Whorl of sporocladia as seen from above. $\times$ 340. *i.* The same as seen laterally in optical section. $\times$ 560. *j-k.* Young zygospores initiated by the apical fusion of two undifferentiated hyphal branches. $\times$ 1000. *l.* Immature zygospore of approximately definitive size. $\times$ 1000. *m.* Nearly mature zygospore. $\times$ 1000.
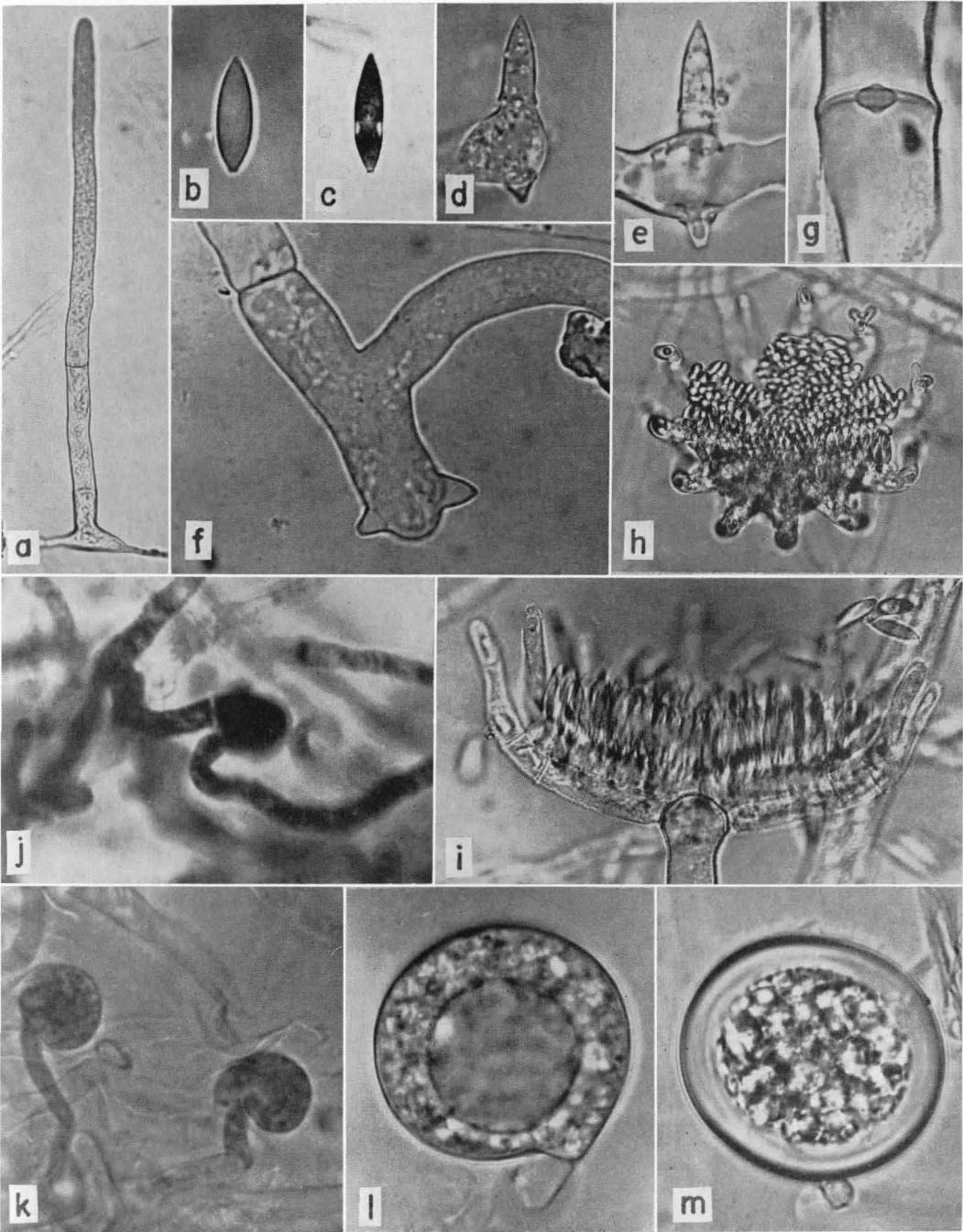
PLATE 8

down internally near the middle of the sporangiospore. From a study of *K. ala-bastrina* using both bright-field and phase-contrast oil-immersion objectives (Leitz apochromatic), the writer has found no evidence of the median annular thickening or incomplete septum described by Linder (Pl. 7 *n;* Pl. 8 *b*). A possible explanation of Linder's observation has been found, however. Lacto-phenol preparations of spores taken from the collection of *K. alabastrina* on deposit at Harvard—the collection studied by Linder—and from dried material of the strain of this species in the author's collection have shown that the spore often appears to possess a more or less median internal annular thickening. This phenomenon, however, appears to be a result of the separation of the spore contents away from the wall due to localized shrinkage of the cytoplasm (Pl. 8 *c*). Linder (1929) used lacto-phenol routinely in the course of his studies of the fungi, and it may be presumed that he used this mounting medium in connection with his work on the Kickxellaceae. It is the opinion of the writer that Linder's interpretation of a pseudoseptate spore in *Kickxella* was based on an artifact. The author has observed a similar phenomenon in lacto-phenol and lactic acid preparations of many species of *Coemansia,* and in no instance has evidence of a pseudoseptum been found in living spores of members of this genus.

Of several substrata employed here for culturing *Kickxella alabastrina,* CMPY has given the most satisfactory results. During the summer following the isolation of the strain of this sepecies cited above, all attempts to culture it in the laboratory at Clare-mont, where the temperature ranged between 25°-30°C. or more, were relatively unsuccessful; growth was slow and sporulation sparse and erratic. When, later, it was grown in a culture chamber at a constant temperature of about 20°C. the species developed rapidly and produced an abundance of normal sporangiophores. Recently it has been observed that excellent growth and sporulation also occurs in the refrig-erator at 6-7°C.

As mentioned earlier, Van Tieghem and Le Monnier (1873) believed, as had Coemans before them, that *Kickxella alabastrina* very probably was the imperfect stage of an ascomycete, but they never succeeded in demonstrating a connection between this species and any of the Ascomyceteae found growing with it in gross culture on dung. The only accessory structures which they observed in their supposedly pure cultures of *Kickxella* were large, globose, hyaline, thick-walled "chlamydospores" containing numerous small globules (Van Tieghem and Le Mon-nier, 1873, pp. 390-391, figs. 134-135). These spores were about 24-30 μ in diameter with a wall about 6 μ thick and appeared to be formed in an ordinary mycelial fila-ment or by small twisted lateral branches. The similarity between the "chlamydo-spores" of *Kickxella* described by Van Tieghem and Le Monnier and the zygospores of *Coemansia aciculifera, C. mojavensis,* and *C. braziliensis* described above is obvious. Until recently, however, the writer had not encountered spores like those described by Van Tieghem and Le Monnier in any of his cultures of *K. alabastrina.* Recalling the discovery by Hesseltine and Anderson (1956) of the low temperature requirement for zygospore formation in *Thamnidium elegans,* the author incubated cultures of *K. alabastrina* at 6-7°C. and "chlamydospores," actually zygospores, formed in abundance in about two weeks.

Zygospore initiation in *Kickxella* has been difficult to observe because it takes place after the hyphae of the vegetative mycelium have become relatively densely inter-twined, but it appears to involve the fusion of similar hyphae as in *Coemansia aciculifera* (compare Pl. 2 *b-e* and Pl. 8 *j-k*). Zygospore maturation proceeds as in *Coemansia* (Pl. 8 *l-m*). The mature spore is approximately globose and in a sample of 100 spores the diameter ranged from 23-50 μ (aver. 33 μ) and the thickness of

the wall from 3-7 μ (aver. 5 μ). Ovoid or slightly irregularly shaped zygospores are not uncommon.

It is apparent that Van Tieghem and Le Monnier actually observed the zygospores of *Kickxella alabastrina* in 1873, but they were looking for an ascomycetous perfect stage and, of course, like other members of the Kickxellaceae, the zygospores of this species have a habit quite unlike that of an orthodox mucoraceous zygospore.

DISCUSSION

As a result of the discovery reported here of the mechanism of zygospore formation in *Coemansia* and *Kickxella,* the true nature of sexual reproduction in the Kickxellaceae finally has been demonstrated. The disposition of the family in the Zygomycetes by Linder (1943) and others (De Bary, 1866, 1887; Istvánffi, 1895; Thaxter, 1897; Vuillemin, 1904; Zycha, 1935) thereby has been confirmed.

The zygospores of all members of the Kickxellaceae observed thus far typically are more or less globose, nearly smooth, relatively thick-walled, hyaline or light colored bodies containing few or many spherical refractive globules of variable size surrounded by a clear homogenous cytoplasm. They bear a close resemblance in structure and manner of formation to those found in the Endogonaceae and Zoopagales. At maturity the zygosporangium proper consists of a thin colorless membrane closely adherent to but readily separable from the zygote wall. Because the vegetative mycelium of all members of the Kickxellaceae typically develops within the substrate, the zygospores usually are completely submerged.

Two distinct patterns of zygospore development are represented by the four species studied here. In *Coemansia aciculifera* and *Kickxella alabastrina* the zygospore is initiated by the fusion of small gametangia delimited apically by two usually relatively short undifferentiated sexual branches. The zygosporangium enlarges as a more or less lateral bud-like outgrowth within which the thick-walled zygote is differentiated. In contrast to the above mechanism of zygospore formation, the zygosporangium of *Coemansia mojavensis* and *C. braziliensis* develops from an intercalary cell delimited in an undifferentiated sexual branch near the point of fusion of the branch with an ordinary vegetative hypha. Here, distinct gametangia are not produced and a mingling of compatible nuclei apparently takes place immediately following the anastomosis of the sexual and vegetative hyphae.

After summarizing the available evidence in support of his placing the Kickxellaceae in the Zygomycetes, Linder (1943, p. 55) presented a phylogenetic scheme in which the three genera of the family known to him fell into two groups derived through *Syncephalis* from *Syncephalastrum. Kickxella* was thought to have evolved from a form like *Syncephalastrum racemosum* Cohn ex Schroeter (1886) through stages similar to those represented by such species as *Syncephalis tenuis* Thaxter, *S. pycnosperma* Thaxter, and *S. wynneae* Thaxter (all Thaxter, 1897), whereas *Coemansia* and *Martensella* were pictured as having had their origin in a species like *Syncephalis reflexa* Van Tieghem (1875).

The writer is not ready at this time to present a detailed discussion of his ideas concerning the phylogenetic relationships of the Kickxellaceae, for very little of the data which he has assembled regarding this and related families has been presented in this paper. Like Linder, however, he is convinced that the Kickxellaceae is a highly derived group and that the family probably has evolved from forms similar to those found at present in the genus *Syncephalastrum* which undoubtedly includes the most primitive modern representatives of what may be termed the merosporangiferous Mucorales.

SUMMARY

The general characteristics of the Kickxellaceae and a history of previous ideas concerning natural relationships in the family are reviewed briefly. The true nature of the zygospore is described for the first time, and the disposition of the family in the Zygomycetes by Linder (1943) is confirmed. Species for which zygospore formation is reported are: *Coemansia aciculifera* Linder, *C. braziliensis* Thaxter ex Linder, *C. mojavensis* sp. nov., and *Kickxella alabastrina* Coemans. The zygospore of these species typically is produced within the substratum; that of *Coemansia aciculifera* and *Kickxella alabastrina* develops from a cell formed by the fusion of gametangia delimited apically in two similar undifferentiated conjugation branches produced by the vegetative mycelium, whereas that of *Coemansia braziliensis* and *C. mojavensis* develops from an intercalary cell delimited in an undifferentiated sexual branch after the apex of the latter has fused with another vegetative hypha. The nearly globose, usually smooth, thick-walled, hyaline or yellowish zygospores are very similar in structure and manner of development to those encountered in such presumably highly derived Zygomycetes as the Endogonaceae and Zoopagales. The Kickxellaceae is believed to be a highly derived group which has evolved from forms similar to those found at present in the genus *Syncephalastrum*.

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