OBSERVATIONS ON GYMNOASCACEAE. VIII. A NEW SPECIES OF ARTHRODERMA.

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CURREY (1854) illustrated and briefly described a fungus which he did not recognize and which he tentatively regarded as new to science. He considered it to be "almost identical" with *Trichoderma*, but stated that it differed in the jointed nature of the hyphae around the spores and in the color of the spores, which were "brilliant yellow" The fungus occurred on dead leaves and sticks lying on the ground in a wooded area in moist, almost muddy, conditions. He did not fully describe this fungus, although if it was described as a new genus in the future CURREY suggested that *Arthroderma* would be an appropriate name. BERKELEY (1860) followed the suggestion of CURREY and described this fungus as a new genus and species, *Arthroderma curreyi*.

BENJAMIN (1956) reviewed the subsequent errors of mycologists who came to regard *A. curreyi* as representing the perfect stage of *Ctenomyces serratus* EIDAM. These two species are now regarded as distinct representatives of the Gymnoascaceae (KUEHN, 1958).

In the spring of 1954 the writer observed fungus growth consisting of white tufts on some material in a moist chamber. This material, feathers of a robin, had been collected in the vicinity of Urbana, Illinois. When the tufts of fungus growth on the feathers were examined under the microscope it was apparent that they represented the ascocarps of a Gymnoascaceous fungus, similar to, if not identical with, *Arthroderma curreyi*. The fungus was isolated in pure culture and grew well on YpSs, Sabouraud's and potato dextrose media. However, the ascosporic stage was not observed in pure cultures.

Within a few weeks another strain of this same fungus was isolated from an owl pellet which also had been placed in a moist chamber. This second strain also grew well in pure culture but did not form ascospores on any media upon which it was cultured. Since 1954 these two strains have been maintained in culture, with no apparent production of the ascosporic stage.

Recently, DAWSON & GENTLES (1959) described the perfect stage of *Keratinomyces ajelloi*. Their illustrations and description were such to suggest that they possibly were concerned with a species of *Arthroderma*. Thus, it appeared possible that *K. ajelloi* was the imperfect stage of a new species of *Arthroderma*, and in a personal communication I suggested this theory to DAWSON. She kindly sent me mating strains of her isolates, and an examination of that material revealed nothing to alter my early impression that *K. ajelloi* was an *Arthroderma*. However, it is not my intention to elaborate upon further studies of DAWSON'S strains at this time.

Nevertheless, the stimulation afforded by the report of DAWSON & GENTLES caused a re-examination of available cultures of *Arthroderma* and related forms. During this study material from the original cultures (inoculated April, 1954) of the two supposedly non-fruiting strains from robin feathers and owl pellet was examined and was found to contain abundant ascospores. New sub-cultures transferred from the original cultures also proved to be ascosporic even after repeated transfer. Although the characteristics of the perfect stage of these two strains seemed to be identical to those of *Arthroderma curreyi*, they could be differentiated from *A. curreyi* on the basis of the accessory spore phase. Based on such differences a new species of *Arthroderma* is proposed, represented by the two strains from Illinois.

Arthroderma tuberculatum n. sp. [1]

Cleistotheciis globosis aut sub-globosis, aggregatis, saepe plus aut minus continuis, cremeo-albis ad cremeo-luteis, 435—970 μ diam. appendiculis inclusis. Hyphis peridii hyalinis, septatis, crasso-parietalibus, asperulatis, cum cellulis biscoctiformibus; cellulis asymmetricis vel symmetricis, elongatis, ca. 4.0 μ latis ad centrum, 6.6—7.0 μ latis ad fines, 10—27 μ longis; appendiculis non-numerosis, angustis, aequalibus, hyalinis, septatis, appendiculis cellularum peridi terminalibus, spiralibus, stricte volutis 10 μ latis, 60 μ longis. Ascis hyalinis, ovoideis, 3.9—4.7 X 4.4—5.5 μ, octosporis. Ascosporis luteis, compresso-oblatis, 1.1—2.0 X 2.5—3.0 (3.5) μ, levibus. Aleuriosporis cremeis, lateralibus raro terminalibus, pedicellatis, semi-sessilibus vel sessilibus, subglobosis, ovoideis raro ellipticis, 8.8—14.3 X 13.2—18.7 (24.2) μ, in aestate tuberculiformibus vel papilliformibus.

Cleistothecia spherical to sub-spherical, creamy-white to creamy-yellow, distinct but often clustered into large aggregates, 435—970 μ diam. appendages included; each ascocarp with one ascigerous mass forming a yellow spherical central region about 2/3 the diam. of the ascocarp. Peridium composed of a network of branched, anastomosed septate, almost hyaline hyphae composed of thick-walled, densely

[1] The writer acknowledges with appreciation the assistance of Dr. TIBOR BENEDEK in the preparation of the Latin Description.

asperulate, usually dumb-bell shaped cells; cells symmetrical or asymmetrical, elongate, about 4.0 μ wide at narrow waist and 6.6—7.0 μ wide at the enlarged ends, and 10—27 μ long. Infrequently, peridial hyphae terminating in a slender, short, curved, smooth hyphal segment, or terminating in a thin - walled, hyaline, septate, spiral appendage coiled in a spring-like manner with 2—3 turns measuring about 10 μ wide and up to 60 μ long; peridial hyphae usually not terminating in appendages. Asci hyaline, ovoid, 3.9—4.7 X 4.4—5.5 μ, 8-spored, the wall evanescent, ascospores conglomerate for only a short time, then separating. Ascospores bright yellow, smooth, flattened-oblate, often with noticeably flattened sides, 1.1—2.0 X 2.5—3.0 (3.5) μ. Imperfect phase represented by cream colored, lateral or rarely terminal, pedicellate, short-pedicellate or sessile, subglobose, ovoid or rarely elliptical aleuriospores measuring 8.8—14.3 X 13.2 —18.7 (24.2) μ and borne singly, covered at maturity with tuberculations or papillations of variable size and shape; pedicels 2.2 μ diam., and 0.3-12.0 μ long; aleuriospores numerous on aerial hyphae. Racquet mycelium present. Vegetative hyphae hyaline, 2.2—5.0 μ diam.

Colonies on YpSs agar in 7 days at 28° C 2—3 cm. diam., more or less restricted, initially hyaline, then tawny and dense or thick; abundant aleuriospores present; minute white cottony tufts present, representing fruiting areas of young ascocarps; reverse in shades of pale yellow; colonies odorless. In 16 days colonies attain 5—6 cm diam., mostly creamy to tawny, more or less restricted and low growing, but with cottony, white, pleomorphic overgrowths rather general in certain areas of the colony; colorless, small droplets of exudate infrequently present; ascogenous areas of minute, white cottony tufts scattered, not representing the dominant colonial character, but easily distinguished macroscopically; colony not furrowed; faint, slightly moldy to sweet or yeasty odor present; reverse in creamy to buff shades. In 21 days faint moldy-sweet odor remaining; colonies 6—7 cm diam., and similar to the 16 day old colony except ascogenous tufts not white, but similar to and blending with cream colored colony; white, pleomorphic overgrowths present; ascocarps not abundant but with many mature ascospores. No change in appearance of 28 day old colony.

Colonies on Czapek's medium in 7 days at 28C 2—3 cm diam., very sparse and thin, colorless, aerial growth lacking; aleuriospores absent. In 21 days colonies cover the surface of medium in Petri dish, thin, no aerial growth; surface mycelium consisting of aleuriospore-bearing hyphae; perfect stage completely absent.

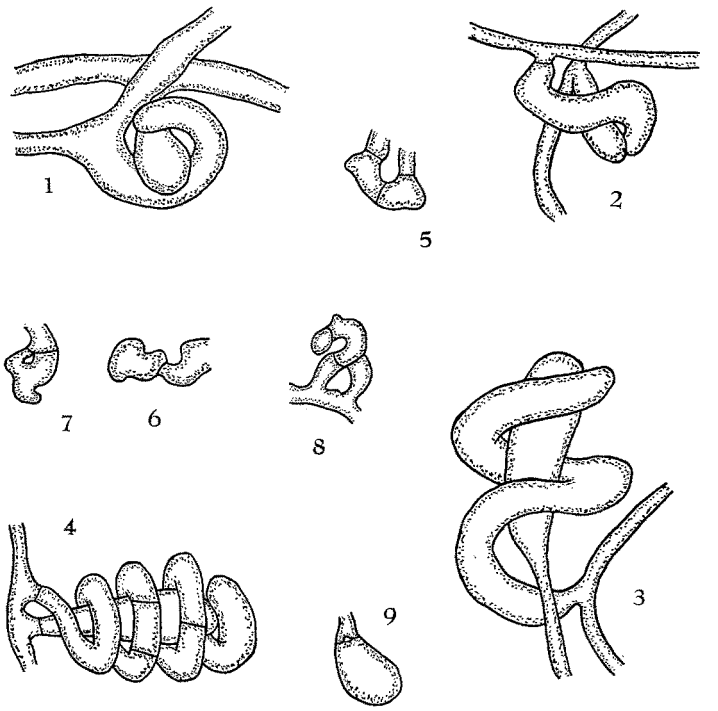
Colonies on potato dextrose agar at 28 C in 7 days 2—3 cm diam., with many minute, white tufts; colony thinner than on YpSs, with tawny shades lacking; earthy or moldy odor pronounced; reverse the color of the medium. In 16 days colonies almost covering surface of the medium, 7—8 cm diam.; colonies white and thin, with white tufts representing aleuriospore-bearing hyphae; odor not

pronounced; reverse colorless. In 21 days colonies covering surface of 9 cm diam. Petri dish; colonies white and thin, reverse colorless; odor faint; ascogenous tissue absent. In 30 days perfect stage absent.

Colonies on Sabouraud's maltose agar in 7 days at 28 C 2—3 cm diam., appearing as on YpSs medium, hyaline, then tawny;abundant aleuriospores present; infrequent white cottony ascogenous tufts present; growth more or less restricted and dense; odor absent; reverse distinctly yellow, more so than on YpSs medium. In 16 days colonies 5—6 cm diam., as on YpSs agar except slightly less pleomorphic growth present; moldy-sweet odor as on YpSs medium; colony restricted and thinner than on YpSs; reverse often streaked with yellow and, in general, creamy-yellow in color. In 21 days colonies 6—7 cm diam., similar in appearance to 16 day colony, and also similar in appearance to colony on YpSs medium except for the yellow color reverse and for less prevalent pleomorphism; perfect stage not abundant; no mature ascospores present. Ascospores present in 29 day old colonies.

The type strain, isolated from robin feathers, has been deposited in the culture collection of the Northern Utilization Research and Development Division, Peoria, Illinois.

Development of ascospores took place in the following manner. The gametangial initials, of which there were few per colony, consisted of slender, lateral branches which arose on the same (Fig. 4) or different parent hyphae (Fig. 1—3). The slender ascogonium coiled about the slender, straight, club-shaped antheridium in a tight (Fig. 4) or loose (Fig. 3) coil. The initials were delimited from the parent hypha by septa (Fig. 4) and soon thereafter the ascogonium became divided into several cells by formation of cross walls (Fig.4). From these cells bulges appeared (Fig. 5) which soon developed into short ascogenous hyphae. Croziers were produced on the ascogenous hyphae but these grew out to form secondary croziers (Fig. 6—8) and no asci were formed at this early stage. Croziers were detected in 9 day old cultures incubated on YpSs medium at 28 C. At this time cleistothecia had become delimited, however, although the peridial hyphae were distinct, the dumb-bell shaped cells were not mature. Each developing ascocarp had only one pair of gametangial initials within it, so that all ascospores in one cleistothecium originated from one pair of initials. Cleistothecia from 9 day old cultures enclosed tightly packed, twisted or coiled ascogenous hyphae and many croziers were in evidence. As the ascogenous hyphae elongated with successive crozier formation, the peridial hyphae became differentiated from vegetative hyphae located at random in the vicinity of the gametangial coil. After about one week of further growth of ascogenous hyphae and enlargement of the cleistothecium, asci were formed from penultimate cells of croziers in great numbers throughout the central areas within the cleistothecium. As the ascospores matured this central area became lemon yellow in color as seen under the microscope.



Figs. 1—9. *Arthroderma tuberculatum*. 1. Origin of gametangia. A slender lateral branch, the ascogonium, coiling about the shorter antheridium which arises as a lateral branch from another hypha. 2—3. Later stages in gametangial relationship. 4. Gametangia arising from the same parent hypha. The ascogonium has become septate. 5. Early stage in the formation of ascogenous hyphae from cells of the ascogonium. 6—8. Stages in development of croziers and their growth into secondary croziers. 9. Early stage in the formation of an ascus. All figures X 1727.

Aleuriospores usually are so short pedicellate as to appear sessile (Fig. 14). Young, immature chlamydospores are smooth-walled, but as they mature they develop decidedly rough walls within 9 days on YpSs agar at 28 C. Germination of the aleuriospores was observed by making dilution plates on malt extract agar. Within 18 hours almost all of the aleuriospores had germinated, with one or two germ tubes per spore. The pattern of origin of the germ tubes was quite variable with the tubes originating on opposite ends of the spore, or close together toward one end. In the 18 hour old cultures germ tubes averaged about 20 μ long, and were branched one or more times. The clavate aleuriospores characteristic of *Arthroderma curreyi* were never encountered in either strain of *A. tuberculatum*.

Asci and ascospores of *Arthroderma curreyi* RSA 186 were similar in size, shape and color to those of *A. tuberculatum*. However, ascocarps of *A. curreyi* were usually smaller and rarely, if ever,

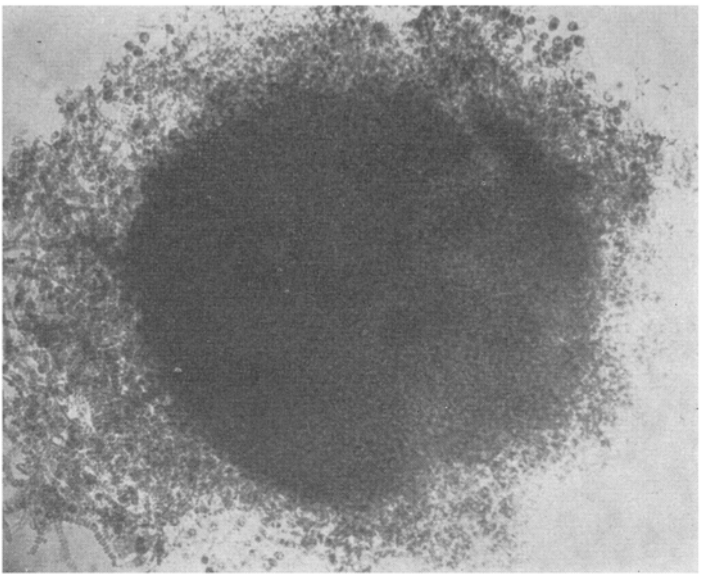


Fig. 10. Mature ascocarp X 143.

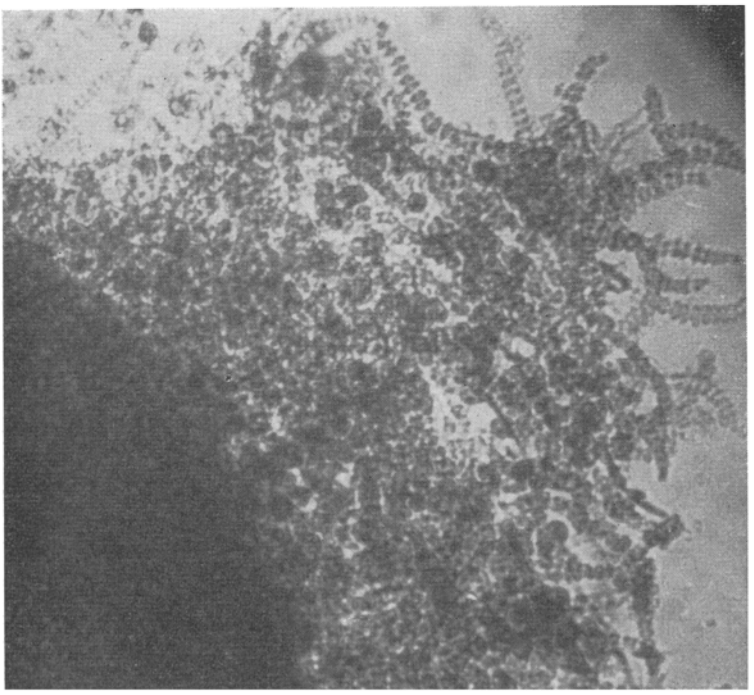


Fig. 11. Peridial hyphae. X 158.

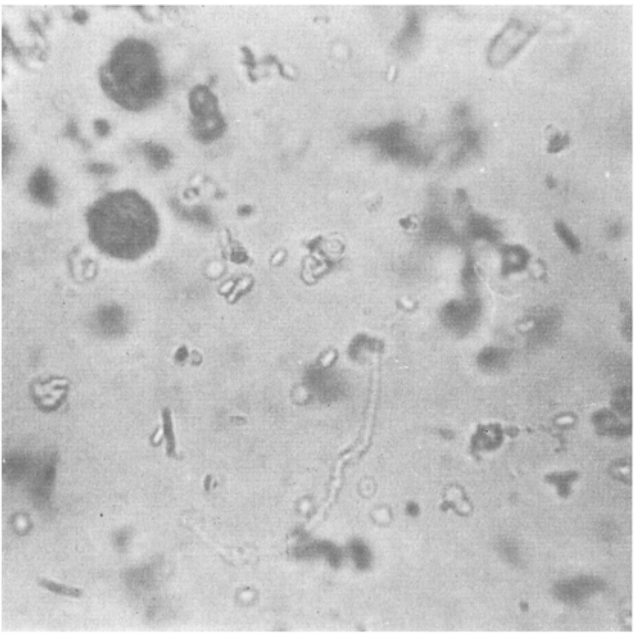


Fig. 12. Ascospores. × 833.

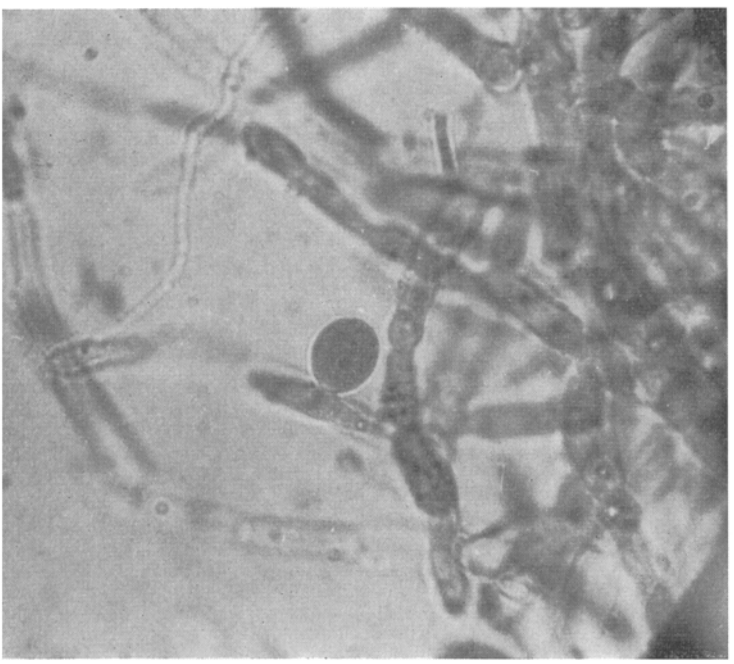


Fig. 13. Young sessile aleuriospore. × 800.

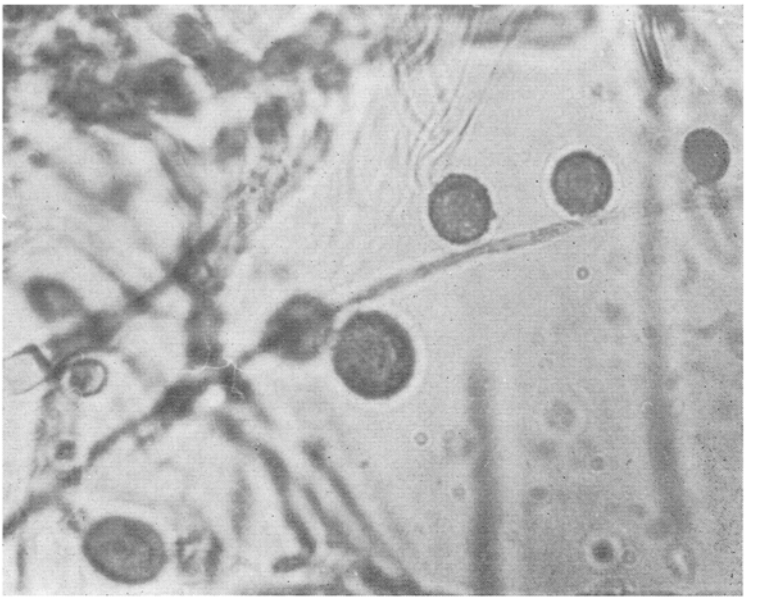


Fig. 14. Immature aleuriospores. × 800.

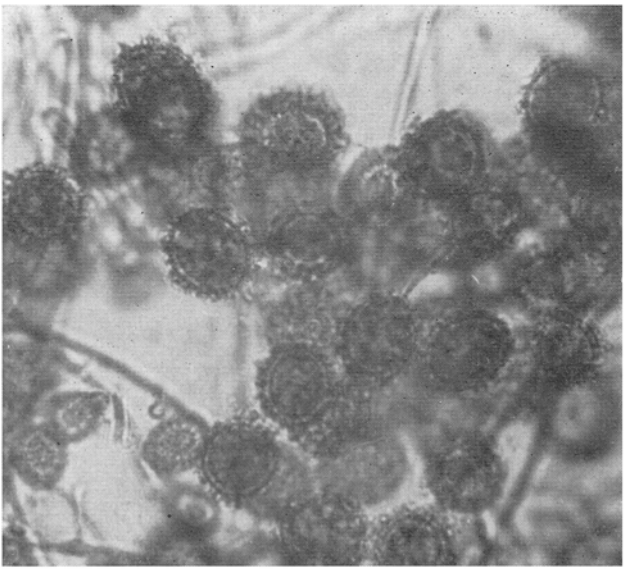


Fig. 15. Mature aleuriospores. × 800.

exceeded 600 μ diam. A culture obtained from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands, as *Ctenomyces serratus*, proved not to represent *C. serratus*. It actually was another strain of *A. curreyi*. This strain did not differ from *A. curreyi* in any important particular. Both cultures formed clavate aleuriospores in abundance but never formed the tuberculate aleuriospores so characteristic of *A. tuberculatum*.

SUMMARY

A new species of *Arthroderma, A. tuberculatum,* is described and illustrated. This new species is represented by two strains isolated from feathers of a robin and from an owl pellet. Although *A. tuberculatum* is similar to the only other known species of the genus, *A. curreyi,* with regard to the characteristics of the perfect stage, the two species may easily be distinguished by virtue of the accessory spore forms. *Arthroderma curreyi* forms small aleuriospores while *A. tuberculatum* produces large, tuberculate aleuriospores. The morphological development of the ascocarp is presented along with other diagnostic characteristics. Asci are formed through the intervention of croziers.

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