Amer. J. Bot. 63(6): 838–844. 1976.

STRUCTURE AND DEVELOPMENT OF CLEISTOIODOPHANUS CONGLUTINATUS GEN. & SP. N. (ASCOBOLACEAE)[1]

JOSE L. BEZERRA[2] AND JAMES W. KIMBROUGH
Department of Botany, University of Florida, Gainesville 32611

ABSTRACT

Cleistoiodophanus represents a new coprophilous genus of the tribe Iodophaneae in the Ascobolaceae (Pezizales). The only species thus far discovered, *C. conglutinatus*, is described and illustrated. Aspects of its cytological development are described from cultures obtained from apothecia found on sheep dung near Gainesville, Florida. Plasmogamy occurs in acogonial coils, two or three cells of which give rise to ascogenous hyphae. Ascogonia are quickly enclosed by vegetative hyphae and the ascocarp continues in a cleistohymenial development. Unlike *Iodophanus* and related genera, the *excipulum* remains intact even after spore maturation and the asci push through the epihymenial regions to release spores. The asci are characteristically thickened at their apices, diffusely amyloid, and somewhat saccate. The asci are predominantly 8-spored, but have been found with four or 16 spores per ascus. A previously undescribed *Oedocephalum* imperfect stage was induced in culture.

IN A SEARCH for coprophilous discomycetes in Gainesville, Florida, a fungus with light colored, roundish apothecia was found to form abundantly on sheep dung when placed in a moist chamber. Cultures exhibiting an *Oedocephalum* imperfect stage were easily established from fragments of apothecia transferred to agar media. Microscopic examination of squash mounts and cryostat sections of apothecia revealed a typical discomycete hymenium, but the asci remained covered by the excipulum even when fully developed. The color and external morphology of the apothecium, the textura globulosa of the medullary excipulum, the nature of the asexual stage together with the diffusely amyloid reaction of the asci, and the large, ellipsoidal and hyaline ascospores related the fungus very closely to *Iodophanus*. However, the cleistohymenial ascoma remaining permanently closed, the saccate, stalked and thick-walled asci, the smooth ascospores (present only in *I. difformis*), and the different type of ascogonia eliminated *Iodophanus* as a genus to accommodate the present species. Thus a new genus is proposed for this unique Discomycete:

Cleistoiodophanus gen. n.

Apothecia coprophila, gregaria vel cespitosa, sese cleistohimenialiter evolventia, occulsa, autem, manentia, albo colore praedita; excipula ectalis cyanophilica, cellulas rectangulares vel quadratas habens; excipula medularis cyanophilica, cum textura globulosa; asci generaliter octospore, diffuse amiloides, large clavati, cum parietibus crassis in juventute, pedicelati; ascospori uninucleati, hyalini, ellipsoides, laeves, perisporium mucilaginosum ostentantes; paraphyses ramosae, hyalinae, septatae, multinucleatae.

Momentum conidiale: *Oedocephalum* Preuss.

Species typus: *C. conglutinatus* sp. n.

Derivation: Greek, kleistos = closed, plus *Iodophanus*, a closely related genus.

Apothecia gregarious to cespitose, developing cleistohymenially, remaining closed, light colored; ectal excipulum of textura prismatica, cyanophilic, of rectangular or quadratic cells; medullary excipulum cyanophilic, of textura globulosa; asci usually 8-spored, diffusely amyloid, broadly clavate, thick-walled in youth, stalked; ascospores uninucleate, hyaline, ellipsoid, smooth, a mucilaginous perispore present; paraphyses branched, hyaline, septate, multinucleate; conidial stage: An *Oedocephalum* Preuss. Coprophilous.

Cleistoiodophanus **conglutinatus** sp. n.
(Fig. 1–32).

Apothecia gregaria vel cespitosa, sese cleistohimenialiter evolventia, primo globosa, deinde irregulariter lobato-sulcata, occlusa, autem, manentia, colore carneo vel albo-flavo praedita, obscuriora post maturitatem, leviter tomentosa, 0.5–3.0 mm diam; excipula ectalis, 20–35 μm crassa, cyanophilica, cellulas habens protensas, in 2–4 straturis, rectangulares vel quadratas, angulatas, multinucleatas, 6.0–22.0 × 6.0–10.5 μm, cum textura prismatica, haec cellulae sunt lateraliter com-

[1] Received for publication 25 June 1975.

[2] Permanent address: Department of Mycology, Universidade Federal de Pernambuco, Recife, PE, Brazil.

The authors wish to acknowledge the assistance of Dr. F. B. Leal (Universidade Federal de Pernambuco, Brazil) in supplying the Latin diagnosis, and that of Dr. Meredith Blackwell and Dr. Gerald Benny for darkroom assistance.

Florida Agricultural Experiment Station Journal Series No. 5955.

pressae et externe hyphis hyalinis circumdatae, quae apotheciis aspectum tomentosum offerunt; excipula medularis cyanophilica, cum textura globulosa et cellulis ellipsoidibus, 15.0–27.0 × 13.5–15.0 μm; asci octospori, aliquando 4 vel 16 sporos habentes, diffuse amiloides, large clavati, in apice rotundi, cum parietibus crassis in juventute, pedicelati, (83.0–) 90.0–120.0 × 25.0–30.0 (–37.5) μm; ascospori uninucleati, distichi vel tristichi, hyalini, large ellipsoides, in polis rotundi, laeves, ad maturitatem parietes tenues ostentates, 15.0–19.0 × 9.0–13.5 μm, cum perisporio mucilaginoso; paraphyses hyalinae, ramosae, septatae, multinucleatae, leviter in apice dilatatae, 3–6 μm diam.

Momentum conidiale: *Oedocephalum* Preuss: conidiophori sese ab hyphis repentibus elevantes, tanquam rami laterales, erecti, simplices, cylindrici, septati, hyalini, 37.0–135.0 × 4.5–7.5 μm; septi, 2–5, non constricti, primus in conidiophori basi formatus et postremus in basi vesiculae; vesicula terminalis, aliquando intercalaris, pyriformis, denticulata, 17–21 × 11–16 μm; denticuli minuti, aliquando inconspicui, 5–16, 1 μm long; conidia subglobosa vel ellipsoidia, botryosa, singularia, leviter verrucosa, 8.5–13.5 (–15.0) × 6.0–9.0 μm, ut apicum denticulorum dilatationes formata, 1–2 tubis germinativis propere germinantia.

In arietis faecibus. Typus: *J. L. Bezerra*, Gainesville, Florida, America septentrionalis (FLAS F-49579).

Apothecia gregarious to cespitose, developing cleistohymenially, globose at first, becoming irregularly lobulate-sulcate but remaining closed, flesh-colored to light yellow, darker on drying, finely tomentose, 0.5–3.0 mm in diam; ectal excipulum 20–35 μm thick, cyanophilic, of 2–4 layers of elongate, rectangular or quadratic, angular, multinucleate cells, 6.0–22.0 × 6.0–10.5 μm, a textura prismatica, laterally compressed and externally covered by hyaline hyphae giving a tomentose appearance to the apothecia; medullary excipulum cyanophilic, of textura globulosa, of globose to ellipsoid cells, 15.0–27.0 × 13.5–15.0 μm; asci 8-spored, sometimes 4 or 16-spored, diffusedly amyloid, broadly clavate, rounded above, thick-walled in youth, stalked below, (83.0–) 90.0–120.0 × 25.0–30.0 (–37.5) μm; ascospores uninucleate, bi- or tri-seriate, hyaline, broadly ellipsoid, rounded at the tips, smooth, thin-walled at maturity, 15.0–19.5 × 9.0–13.5 μm, a mucilaginous perispore present; paraphyses hyaline, branched, septate, multinucleate, slightly inflated at the tip, 3–6 μm in diam.

Conidial stage, an *Oedocephalum* Preuss: conidiophores arising as lateral branches from repent hyphae, erect, simple, cylindrical, septate hyaline, 37.0–135.0 × 4.5–7.5 μm; septa in number of 2–5, non-constricted, the first one formed at the base of the young conidiophore and the last one at the base of the ampulla; ampullae terminal, sometimes intercalary, pyriform, denticulate, 17–21 × 11–16 μm; denticles minute, sometimes inconspicuous, varying in number from 5–16, 1 μm long; conidia subglobose to ellipsoid, botryose, solitary, slightly verrucose, 8.5–13.5 (–15.0) × 6.0–9.0 μm, formed as blown-out ends of the apex of the denticles, germinating readily by 1–2 germs tubes.

Derivation: Latin, *conglutinatus* = united firmly together, referring to the arrangement of the apothecia.

Holotype: On sheep dung, Veterinary Science Farm, University of Florida Campus, Gainesville, Alachua County, Florida, Jan. 18, 1973, J. L. Bezerra (FLAS-49579).

MATERIALS AND METHODS—*Culture and development*—Pellets of sheep dung were collected on January 18, 1973, at the Veterinary Science Farm, University of Florida Campus, Gainesville, Florida. The material was placed in a moist chamber at room temperature and apothecia of the fungus were detected six days after incubation (Fig. 16). Squash mounts and cryostat sections were made of the apothecia and examined directly in water or stained in cotton blue (Korf, 1973), 5 % aqueous Congo red (Kimbrough, 1969), and Melzer's Reagent (Korf, 1973). Nuclear staining was done by the HCl-Giemsa method as described by Furtado (1968) and by the aniline-blue method of Tu & Kimbrough (1973). Cultures were established by washing some apothecia in sterile distilled water and placing apothecial fragments on agar media. After completion of the studies, the dung pellets bearing apothecia were dried at 60 C and accessioned to the University of Florida Mycological Herbarium.

OBSERVATIONS—*Cultural characteristics*—The fungus develops slowly and forms scanty mycelium on mycological agar (MA), potato-dextrose-agar (PDA), cornmeal-malt-yeast extract-agar (CMMY), V-8 agar, V-8 agar plus dung extract, and dung extract-oatmeal-agar (DOA). Conidia are formed abundantly on all testing media but only sexual initials, and immature apothecia are produced.

Mycelium—The vegetative hyphae are consistently branched and multinucleate. Woronin bodies are observed near the septa.

Conidiophores and conidia—The conidiophores arise as small protuberances from the repent hyphae. After a period of elongation, an apical swelling develops at the tip of the developing conidiophores which gives rise to the ampulla (Fig. 3). Thornlike denticles then form over the ampulla (Fig. 3). Conidial formation is initiated by apical swelling of the denticles (Fig. 2, 3) and

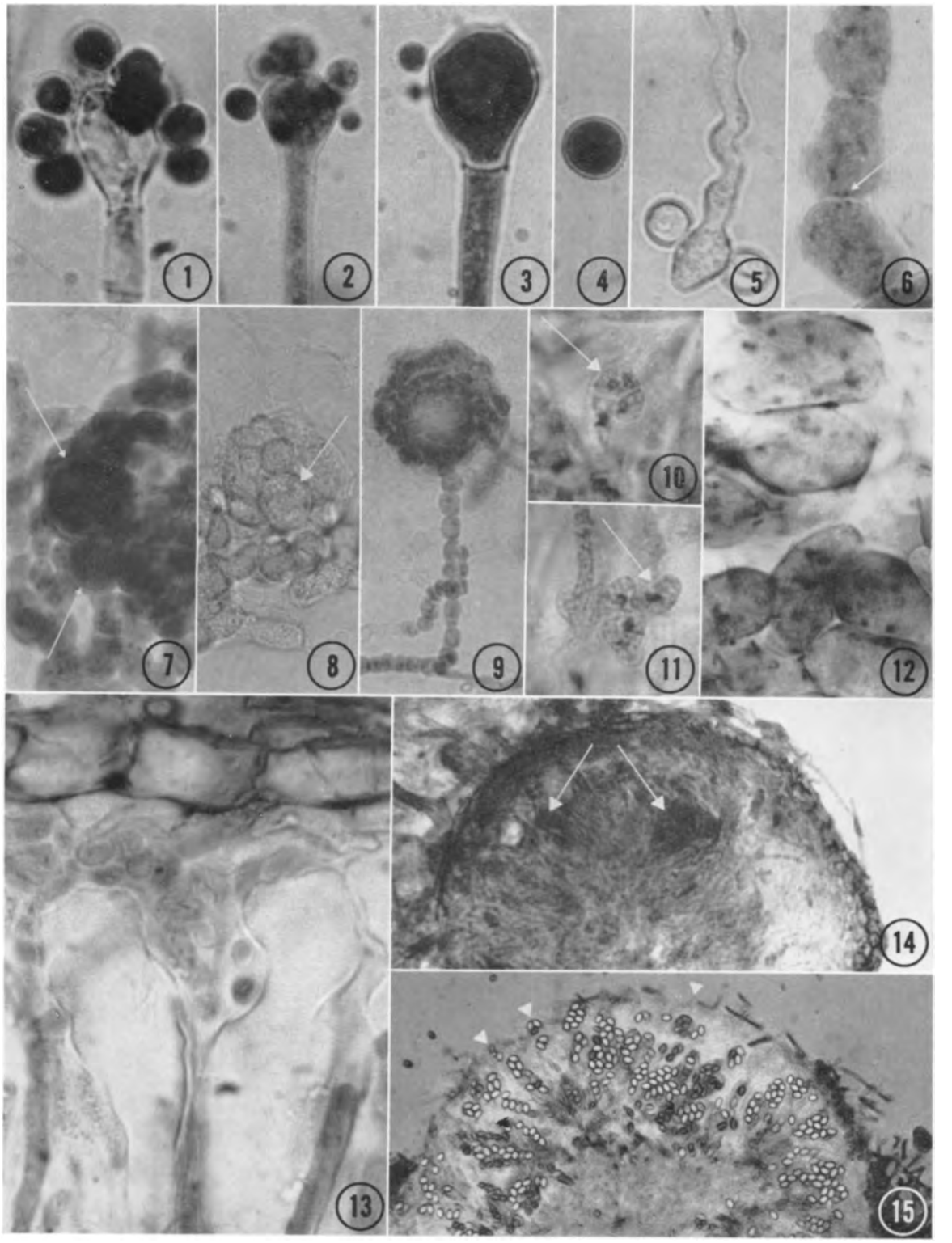

Fig. 1–15. *Cleistoiodophanus conglutinatus.* **1.** Mature conidia attached to an empty ampulla. × 1,000. **2, 3.** Conidiophores bearing an ampulla with developing conidia. × 1,000. **4.** Mature conidium with rough epispore. × 1,000. **5.** Three conidia, the middle one with a germ tube. × 1,000. **6.** Multinucleated and inflated vegetative cells undergoing transformation into ascogonial cells. Note the Woronin bodies (arrow). × 1,000. **7.** Two priviledged ascogonial cells (arrows) surrounded by investing hyphae. × 1,000. **8.** An ascogonial initial (arrow) sur-

continues with the migration of cytoplasm and nuclei into the developing conidia. When maturation is completed, the conidia become surrounded by a thick, slightly verrucose outer wall (Fig. 4), and the ampulla and denticles appear empty (Fig. 1). Mature conidia are arranged in a clustered fashion around the ampulla (Fig. 1). Just before germination, the conidia swell and form usually one, sometimes two, protrusions which elongate into germ tubes (Fig. 5).

Ascogonium—The ascogonium arises by swelling and beading of vegetative hyphae in which the cytoplasm becomes condensed (Fig. 6). The ascogonial cells become more rounded at the time ensheathing hyphae start to encircle the ascogonium. The ensheathing hyphae divide and produce a mass of cells around the ascogonial initials (Fig. 7, 8). No antheridia or male elements were observed fusing with the ascogonium. Usually two or three ascogonial cells can be observed within the mass of cells (Fig. 7, 8). Ascogenous hyphae begin to grow out from the multinucleate ascogonia. The ascogenous hyphae start to branch and form croziers at their ends (Fig. 10, 11).

Ascocarp development—The development of the apothecia is cleistohymenial. The mass of cells around the ascogonial initials becomes the archicarp (Fig. 9, 14). As the archicarp increases in size and the ascogenous system grows, an excipulum develops which is made up of multinucleate subglobose cells (Fig. 12). The external cells of the excipulum become elongated, angular to quadratic (Fig. 13), and those of the basal excipulum remain subglobose. Multinucleate, branched, filamentous paraphyses outgrow the asci and are found lying between the top of the asci and the excipulum (Fig. 17). Frozen sections showed that the excipulum encloses the asci and paraphyses even after sporogenesis is completed; the ascocarp will not separate to expose the hymenium but small fractures will form through which the ripening asci push to liberate the ascospores (Fig. 15). Apothecia remain globose and tightly clustered (Fig. 16).

Asci—The two haploid nuclei of the penultimate cell of the crozier fuse together giving rise to the ascus mother cell with a prominent diploid nucleus containing one or two nucleoli (Fig. 18). As the young ascus increases in size the first meiotic division takes place and a meiotic spindle is formed, generally parallel to the long axis of the ascus (Fig. 19, 20). These meiotic processes result in two vertically arranged nuclei (Fig. 21). The second division is synchronous and the succeeding four nuclei occupy the middle of the ascus (Fig. 22). Mitotic divisions of the tetrad are synchronous (Fig. 23). The nucleolus reappears after each division (Fig. 22). Peculiarities during spore formation sometimes lead to the incorporation of two nuclei into one spore, resulting in four-spored asci (Fig. 24). Occasionally an additional mitotic division gives rise to the formation of sixteen spores inside one ascus (Fig. 25). Ascal tips are initially thick-walled (Fig. 26), but become thin with inconspicuous areas of dehisence at maturity (Fig. 27).

Spores—Each ascus usually forms eight uninucleate ascospores (Fig. 30). The ascospores are somewhat thick-walled when young (Fig. 29), but become thin-walled and remain smooth at maturity. The outer perisporic layer is mucilaginous and may be slightly wrinkled in the mature spore (Fig. 28).

Paraphyses—The paraphyses appear in very early stages of ascal development, are multinucleate, septate, branched, hyaline, and arise from cells which support the croziers (Fig. 31, 32).

DISCUSSION—*Cleistoiodophanus* possesses the most important features attributed to the Ascobolaceae by Korf (1973): ascospores very thick-walled when young, egguttulate, uniformly uninucleate, hyaline and asci diffusely blue in iodine. The cleistohymenial development of *Cleistoiodophanus* is matched only by *Iodophanus, Ascobolus* and *Saccobolus*, among the Ascobolaceae. However, while *Cleistoiodophanus* apothecia always remains closed, those of the other genera open at maturity.

Ascogonial ontogeny in *C. conglutinatus* is very similar to that of *Iodophanus granulipolaris* (Milam, 1971); however, it differs in some aspects. The ascogenous hyphae are of the pleurorhynque type (forming croziers) similar to the species mentioned above and to *Lasiolobus ciliatus* (Conway, 1975). As in *I. granulipolaris*, the ascogonial coil elongates and yields "privileged" cells that give rise to ascogenous hyphae which become surrounded by investing hyphae. The ascogonial coil does not fuse with male elements.

←

rounded by investing hyphae. × 500. **9.** Archicarp. × 250. **10.** Crozier with a dikaryotic penultimate cell (arrow) prior to karyogamy. × 1,000. **11.** Croziers, one with a diploid penultimate cell (arrow) after karyogamy. × 1,000. **12.** Multinucleate, rounded ectal cells of the excipulum in a squash mount. × 1,000. **13.** Longitudinal section through periphery of the ascocarp showing empty asci, and paraphyses covered by quadratic ectal cells of the excipulum. × 1,000. **14.** Longitudinal section through an archicarp with undifferentiated asci (arrows). × 250. **15.** Cross section of an ascocarp showing mechanism of ascospore dehiscence through breaks in the excipulum (arrow heads). × 100.

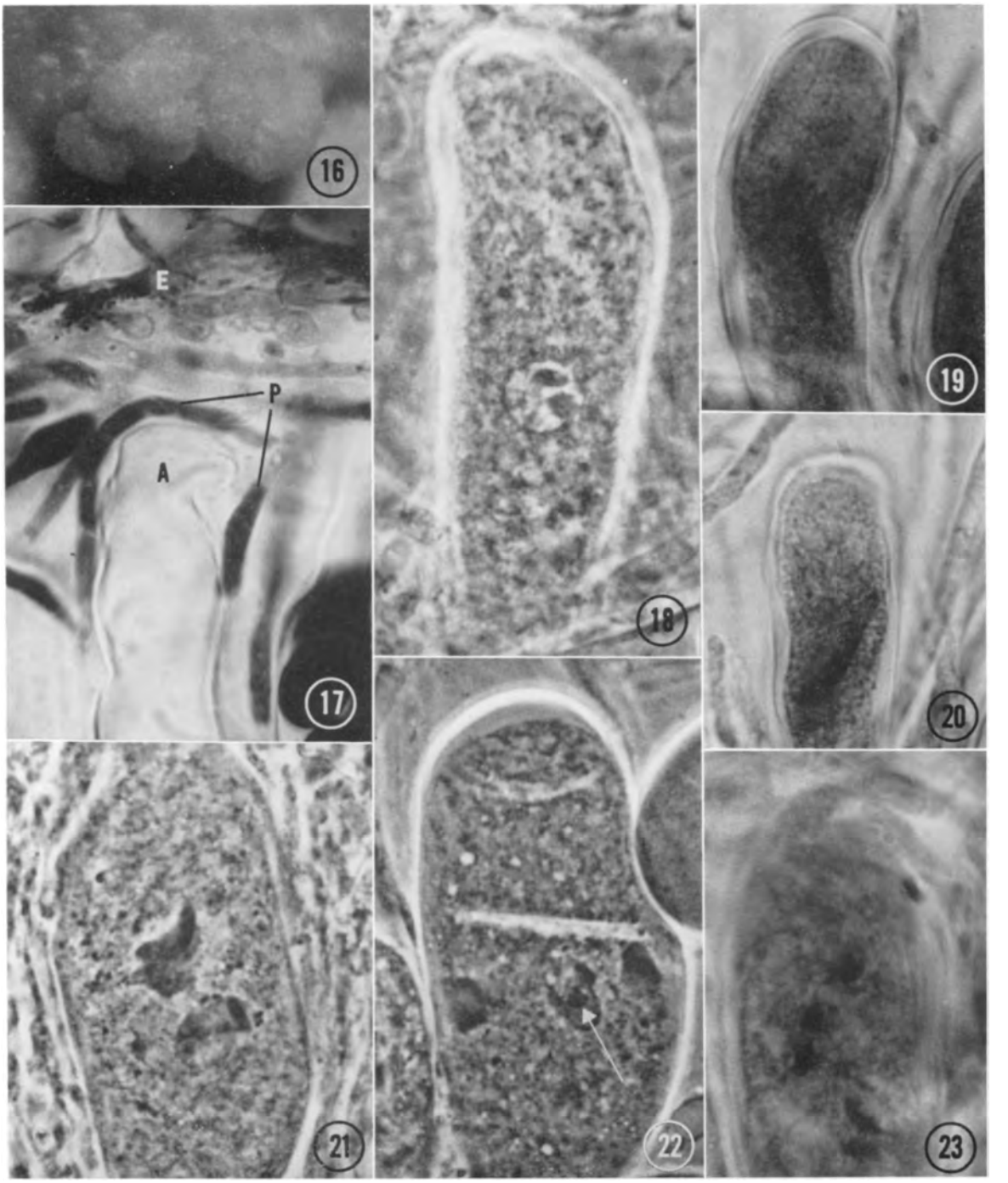

Fig. 16–23. *Cleistoiodophanus conglutinatus.* **16.** Typically confluent ascocarps on dung. × 10. **17.** Paraphyses (P), ascal tips (A), and excipular cells (E). Note paraphyseal apices lying between ascal tips and excipular cells. × 1,000. **18.** Young ascus containing a large diploid nucleus with two nucleoli. × 1,000. **19, 20.** First meiotic division. Note spindles oriented vertically or obliquely in relation to longitudinal axis of ascus. × 1,000. **21.** Two nuclei resulting from the first meiotic division. × 1,000. **22.** Three nuclei resulting from the second meiotic division (the fourth nucleus is out of focus and is not shown). Note the reappearance of the nucleolus (arrow). × 1,000. **23.** Five nuclei resulting from the mitotic division after meiosis (the three other nuclei are out of focus and not shown). × 1,000.

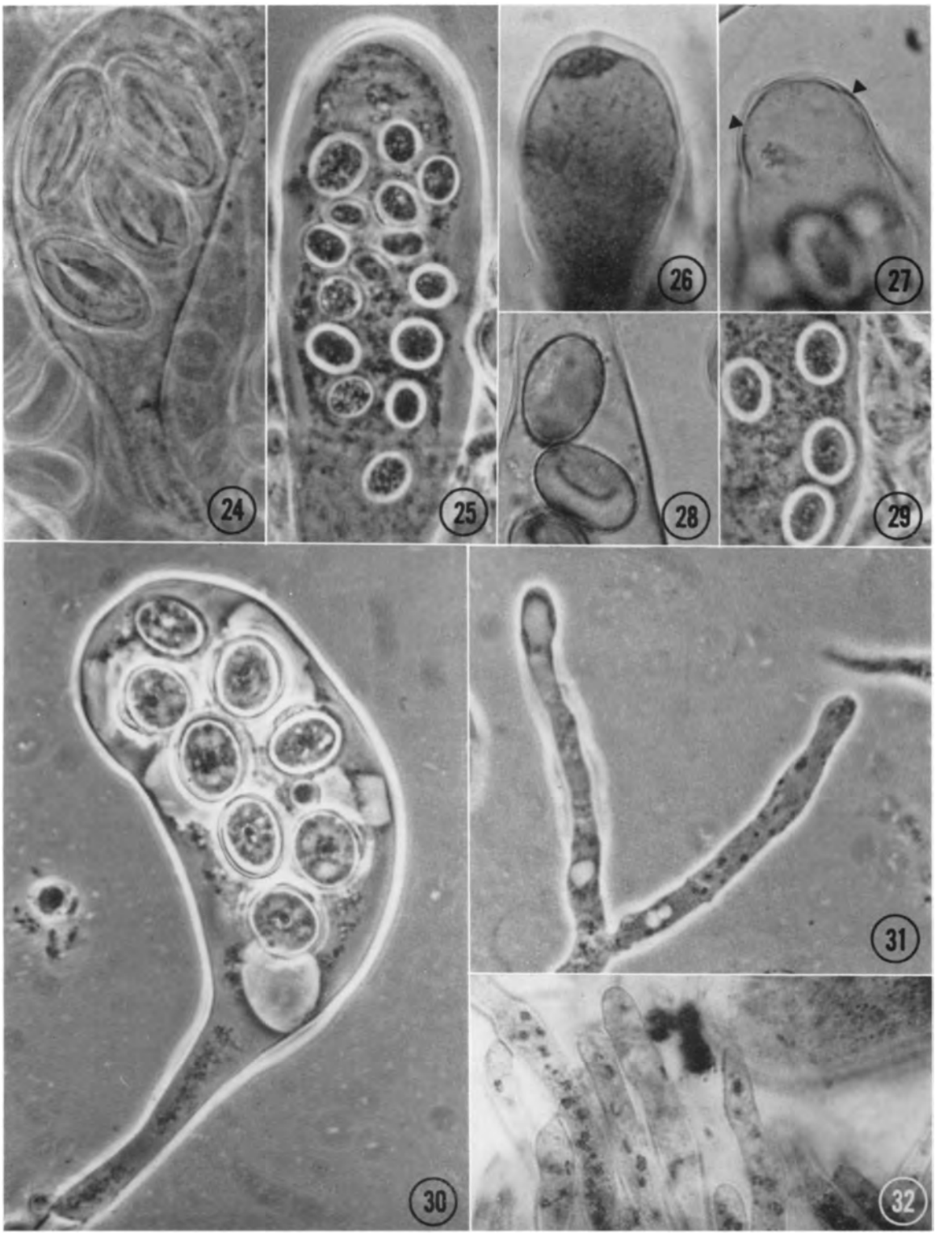

Fig. 24–32. *Cleistoiodophanus conglutinatus*. **24.** Ascus with four large ascospores. **25.** Ascus with 16 small ascospores. **26.** Tip of young ascus showing thick apical wall. **27.** Tip of mature ascus showing thin apical wall with lines of dehiscence (thinner areas at arrow heads). **28.** Mature ascospores with thin, wrinkled walls. **29.** Immature ascospores with thick walls. **30.** Ascus with eight uninucleate ascospores. **31.** A branched, multinucleate paraphysis. **32.** Several simple, multinucleate paraphyses. All × 1,000.

The trichogyne-like structures seen in *Iodophanus granulipolaris* were not observed in *C. conglutinatus*. Crozier formation, ascus development, and ascosporogenesis in *C. conglutinatus*, to the extent that they were studied in this work, did not differ significantly from *I. granulipolaris*. The nuclear condition of the excipular cells and paraphyses is also identical in both species. Despite the fact that the apothecia of *Cleistoiodophanus* do not open at maturity, the apothecial development is the same as in *Iodophanus*. Therefore, we think that both genera should be placed in the Ascobolaceae.

The development of conidiophores and conidia in *C. conglutinatus* is basically the same as in *Iodophanus carneus* (Pers. per Pers.) Korf, in Kimbrough & Korf, as reported by Jeng (1971). The conidial measurements, however, differ for *I. carneus* and for *Iodocephalum glomerulosum* (Bull. ex Harz.) Sacc. *Iodophanus carneus* has small, globose, smooth (?), 5.6–7.0 μm diam conidia (Jeng, 1971) and *O. glomerulosum* has large, ovoid, smooth, 20–27 (–28) × 8.3–13.5 μm conidia (Gamundi and Ranalli, 1964). Jeng (1971) referred to "smooth conidia" in her description of *I. carneus* despite the fact that she presented an electron microscope photograph of a conidium with warts on the wall which were noted and labelled. We did not attempt to compare the conidial stage of *C. conglutinatus* with all descriptions of *Oedocephalum* reported in the literature because we believed that it was superfluous to describe it as a separate taxon.

LITERATURE CITED

CONWAY, K. E. 1975. The ontogeny of *Lasiobolus ciliatus* (Pezizales, Ascomycetes). Mycologia 67: 253–263.

FURTADO, J. S. 1968. Basidial cytology of *Exidia nucleata*. Mycologia 60: 9–15.

GAMUNDI, I. J., AND M. E. RANALLI. 1964. Estudio sistematico y biologico de las Ascobolaceas de Argentina. I. Nova Hedwigia 7: 517–533.

JENG, R. S. 1971. Studies on the morphology and development of *Iodophanus carneus* (Pers. per Pers.) Korf. *In* Kimbrough & Korf. M.S. Thesis, University of Toronto, Toronto, Canada.

KIMBROUGH, J. W. 1969. North American species of *Thecotheus* (Pezizeae, Pezizaceae). Mycologia 61: 99–114.

KORF, R. 1973. Discomycetes and Tuberales, p. 249–319. *In* G. C. Ainsworth, F. K. Sparrow and A. S. Sussman [ed.], The fungi: an advanced treatise, Vol. IV A. Academic Press, New York and London.

MILAM, J. R. 1971. Cytological development of *Iodophanus granulipolaris* (Pezizazeae, Ascomycetes). M.S. Thesis, University of Florida, Gainesville, Florida.

TU, C. C., AND J. W. KIMBROUGH. 1973. A rapid staining technique for *Rhizoctonia solani* and related fungi. Mycologia 65: 941–944.