

A taxonomic reassessment of *Sclerotinia camelliae* Hara
(=*Ciborinia camelliae* Kohn), with observations
on flower blight of camellia in Japan

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Key Word Index—*Sclerotinia camelliae* Hara; *S. camelliae* Hansen & Thomas; *Ciborinia camelliae* Kohn; Sclerotiniaceae; microconidia; taxonomy; flower blight of camellia; Helotiales.

Summary

Based on studies of recent collections and cultures of *Sclerotinia camelliae* Hara from Japan and comparative study of the holotypes of *S. camelliae* Hara and *Ciborinia camelliae* Kohn, the Japanese and American fungi are determined to be conspecific. Although Hara's epithet has priority, the combination in *Ciborinia*, the most suitable generic accommodation, is preoccupied with *C. camelliae* Kohn. *Ciborinia camelliae* Kohn is therefore the correct name of this species. Observations on the disease caused by *C. camelliae*, based on our studies at Tottori-shi and on the Japanese literature are presented with reference to the original description of the disease in North America. A description of the holomorph, *Ciborinia camelliae* Kohn, is provided.

Introduction

In 1919 Hara described a disease causing flower blight of camellia (*Camellia japonica* L.) and in the same paper described the causal agent of this disease as a new species, *Sclerotinia camelliae* Hara. In 1940, unaware of Hara's work, Hansen and Thomas described a camellia flower blight occurring in California and in the same paper described as new the causal agent, *Sclerotinia camelliae* Hansen & Thomas. Since they failed to provide a Latin diagnosis, the epithet was invalidly published; no holotypic or authentic specimens appear to have been designated or preserved. Later (1946), aware of Hara's earlier research, Thomas and Hansen reported that while Hara's measurements of asci ($120-140 \times 6-8 \mu\text{m}$) and ascospores ($8-11 \times 4-5 \mu\text{m}$) were larger than theirs ($100-125 \times 4.3-5.8$ and $5.3-7 \times 2.5-3.5 \mu\text{m}$, respectively), the fungus from California was "essentially the same" as the fungus from Japan, and therefore a later synonym of Hara's name. From their discussion, it appears that Thomas and Hansen made this decision based on

Hara's published description only, without examining the holotype. Based on comparative study of Hara's holotype and a fresh collection of the causal agent of camellia flower blight from California, Kohn (1979) concluded that the two species were not the same, providing valid publication of the epithet, based on the California collection, as *Ciborinia camelliae* Kohn. Of *Sclerotinia camelliae* Hara, Kohn wrote, "This is either a species of *Moellerodiscus* or of an as yet unnamed genus in the Sclerotiniaceae."

Recently, one of us (E. N.) was able to make large collections of the causal agent of flower blight of camellia in Japan, providing the opportunity to prepare living isolates. Based on our studies of the holomorph, reported here, supported by examination of both Hara's holotype and the holotype of *C. camelliae* Kohn, we conclude, in agreement with Thomas and Hansen, that *Sclerotinia camelliae* Hara and *Ciborinia camelliae* Kohn are synonyms. Hara's epithet has priority in *Sclerotinia*, but the combination in *Ciborinia*, still the most appropriate accommodation for this species, is preoccupied with *C. camelliae* Kohn. If the epithet is ever transferred to another generic accommodation, it is *S. camelliae* Hara which is the appropriate basionym; Kohn was in error for not basing the combination in *Ciborinia* on Hara's name. Accommodated in *Ciborinia*, however, *C. camelliae* Kohn is the correct name of this fungus.

In addition to reassessing the taxonomic and nomenclatural position of *S. camelliae* Hara, it is the purpose of this paper to provide a full technical description of the holomorph, a discussion explaining the accommodation in *Ciborinia*, and a review of the Japanese literature on sclerotial flower blight of camellia.

Materials and Methods

Procedures for histological study follow the protocols of Kohn (1979). The presence of lipids was confirmed using a solution of 0.5 g Sudan Black (Sigma Co.) in 100 ml ethylene glycol; procedures for preparing and using this stain were those outlined in the Mycology Guidebook (1981). Studies of nuclei were made using a 62 $\mu\text{g}/\text{ml}$ mithramycin (Sigma Co.) solution containing 15 mM MgCl_2 (pH 6.8), following the procedure of Franklin et al. (1983); staining time was 12 hr. Dried apothecia were prepared for mithramycin staining in two steps: 1) apothecia were rehydrated in distilled water and sectioned on a freezing microtome at 20 μm thickness, then allowed to air dry on a microscope slide; 2) sections were rehydrated in 70% ethanol for 20 min, then placed in the mithramycin staining solution.

Cultures were grown and maintained on DIFCO Potato Dextrose Agar (PDA), on malt extract agar (MEA), and on Leonian's medium (LM); MEA and LM were prepared following the formulae presented in the Mycology Guidebook (1981). Cultures were grown in the dark at 18°C.

Terminology and abbreviations are explained in Kohn (1979). Color terminology cited in quotation marks is from Rayner (1970). Collections made in the course of these

studies are accessioned at the herbarium of the Tottori Mycological Institute (TMI), with duplicates accessioned at the Plant Pathology Herbarium, Cornell University (CUP); cultural isolates are accessioned in culture storage at TMI.

Description

Ciborinia camelliae Kohn, Mycotaxon 9: 399, 1979. (!!)

=*Sclerotinia camelliae* Hara, Dainippon Sanrin Kaiho (Bull.) 436: 31, 1919. (!!)

= [*Sclerotinia camelliae* Hansen & Thomas, Phytopathology 30: 170, 1940 (not validly

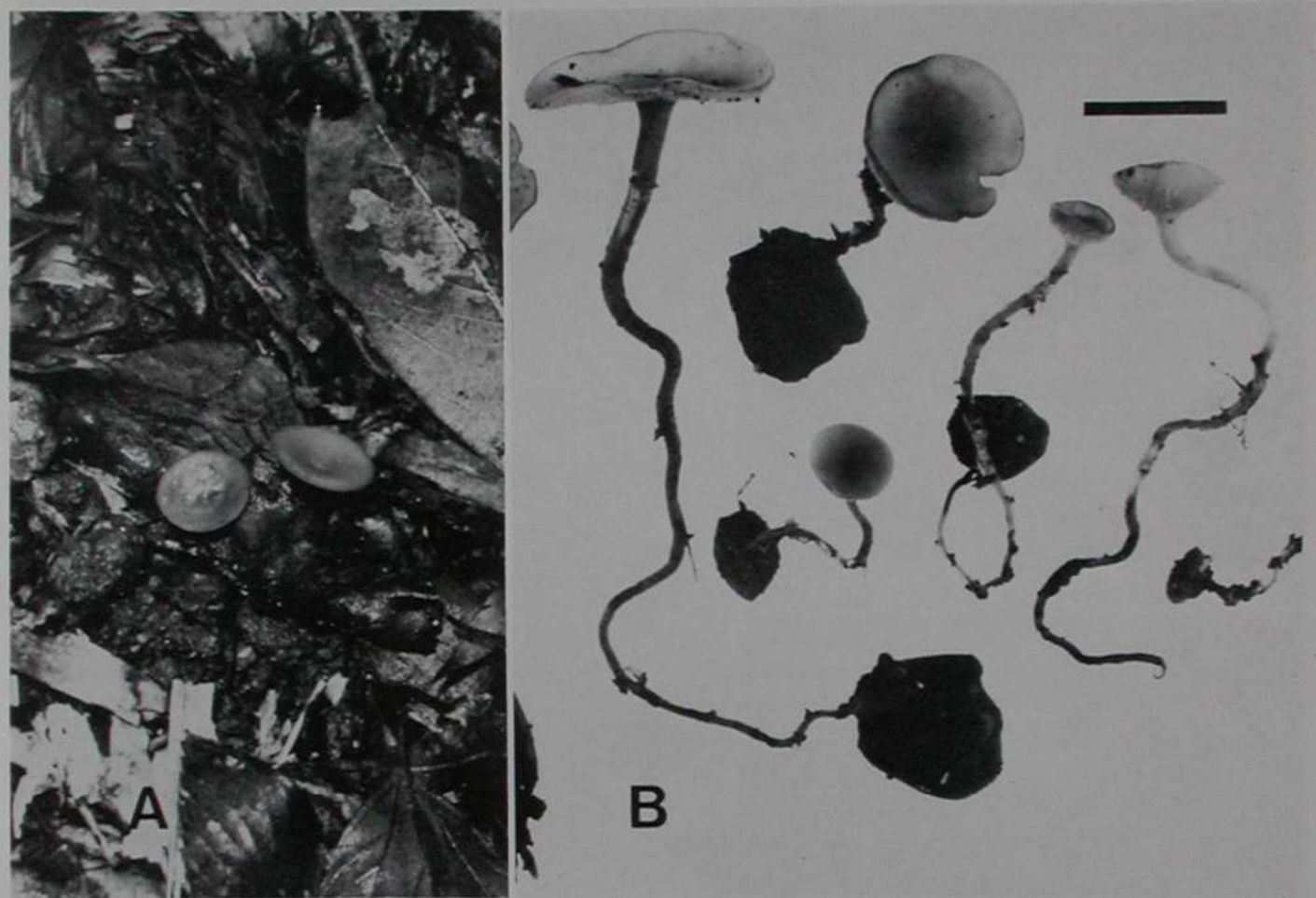


Fig. 1. *Ciborinia camelliae*, TMI-7633. A) Apothecia in habitat among wet, decaying perianths of *Camellia japonica*. B) Sclerotia bearing stipitate-capitate apothecia. Scale: B=1 cm.

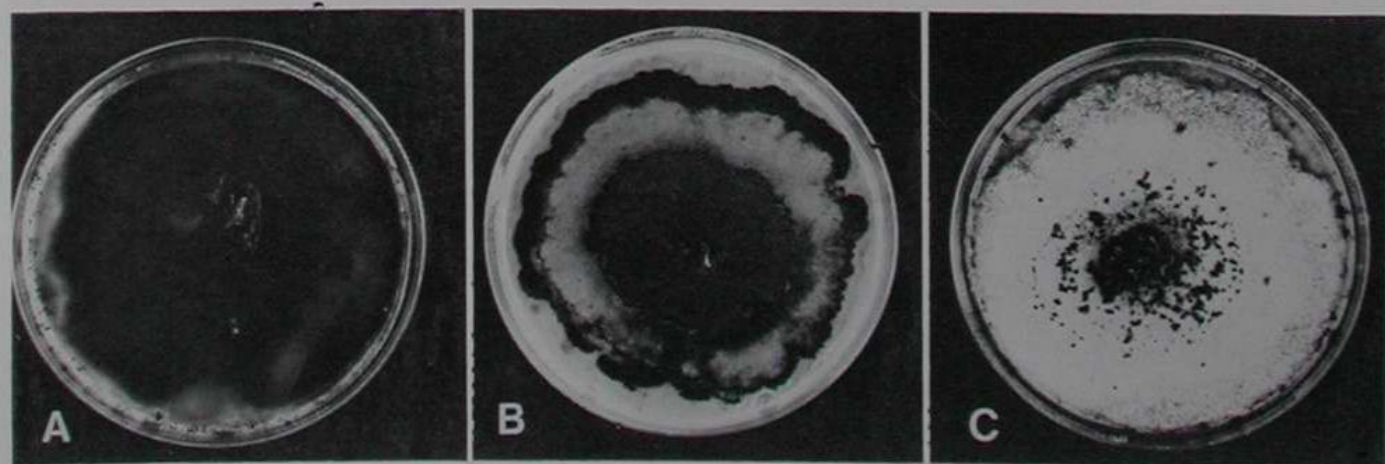
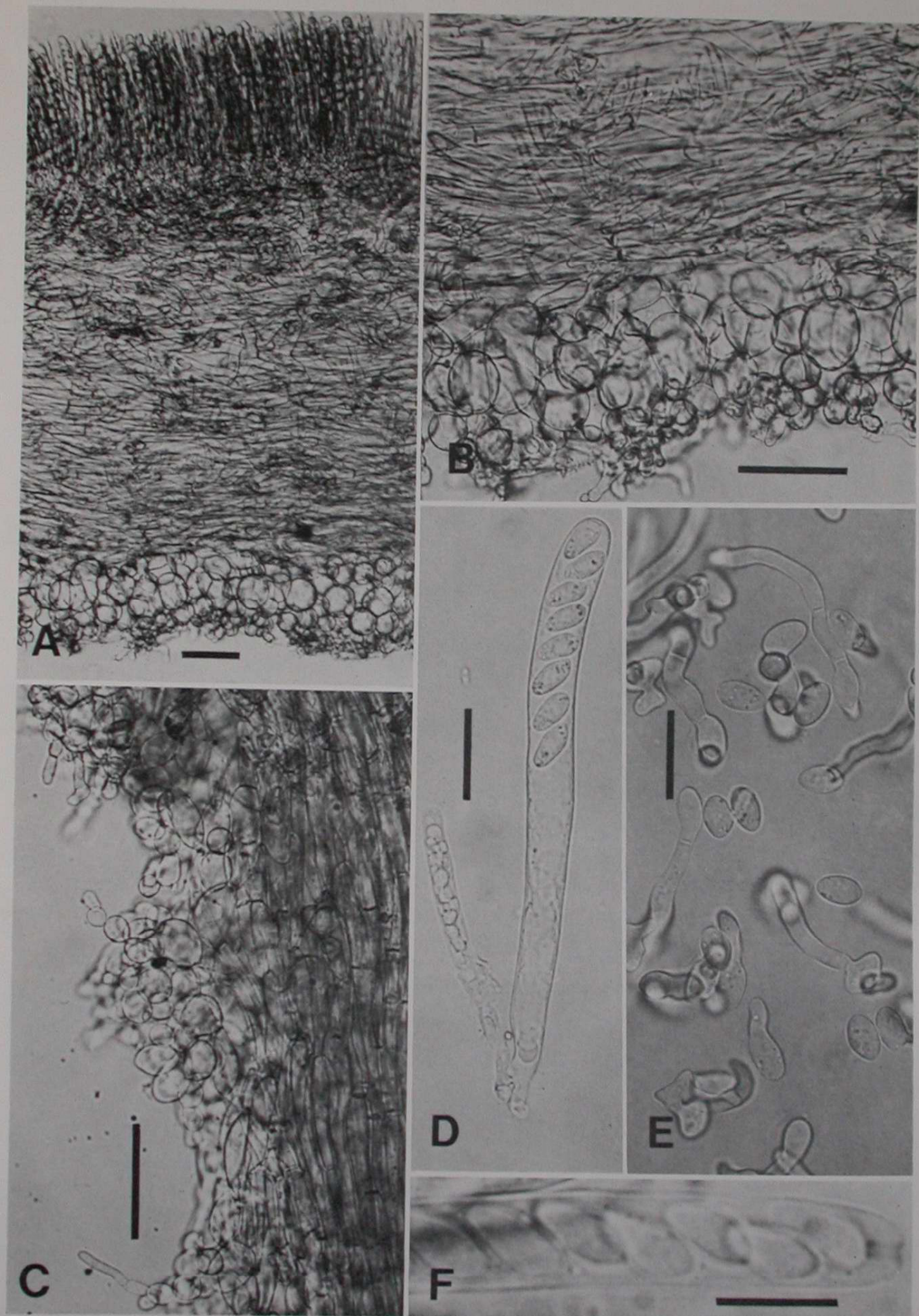
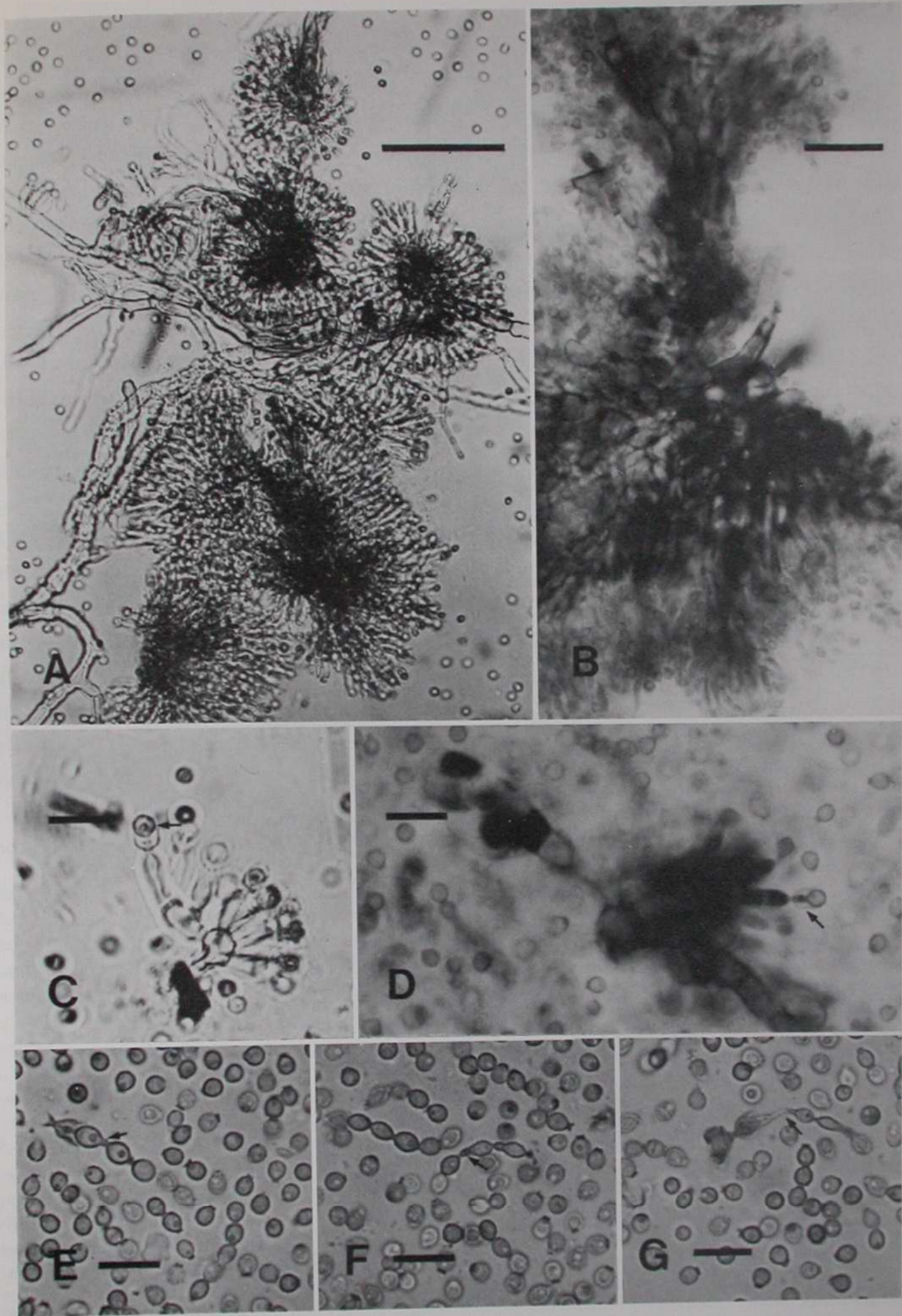


Fig. 2. *Ciborinia camelliae*, TMI-65011-2. A) On malt extract medium. B) On Leonian's medium. C) On potato dextrose agar.





published, no Latin diagnosis).]

= [*Sclerotinia camelliae* Hara forma *macrospora* Yoshimi, *Illust. Jap. Mushrooms* Vol. 5, no. 1027, 1979. (not validly published, no Latin piagnosis).]

Figs. 1, 2, 3, 4, 5.

Sclerotia up to $12 \times 10 \times 2$ mm, determinate, discoid, developing on senescent petals and sepals of *Camellia japonica* L., remaining attached to suscept in or on soil after infected flowers have dropped to the ground; remnants of suscept tissue remaining embedded among the cortical and medullary sclerotial hyphae, dorsal surface convex, ventral surface concave, with black outer rind and white inner context. Sclerotial Medulla of compactly or loosely interwoven, hyaline *textura oblita*, cells 5–7 μm broad, walls thick, gelatinized, 2–3 μm thick; polygonal walls of suscept cells and spiral wall thickenings of suscept vessel elements typically embedded among medullary cells. Sclerotial cortex a well developed zone showing dorsi-ventral differentiation; dorsal cortex a compact zone 20–30 μm wide, of brick-shaped to globose cells, 4–8 μm wide, inner subzone hyaline, outer subzone light brown-walled, walls gelatinized, 1 μm thick; ventral cortex not as compact, 50–70 μm wide, of brick-shaped to globose cells, 4–10 μm broad, inner subzone hyaline, outer subzone light brown-walled, walls 0.5–1 μm thick. Sclerotial rind showing dorsi-ventral differentiation; dorsal rind very compact 4–8 cells wide, of *textura globulosa*, cells 4–8 μm in diam, walls dark brown, carbonaceous, irregularly thickened, 1–3 μm thick; ventral rind less compact than dorsal rind, 4–8 cells wide, of brick-shaped to globose cells arranged more or less in chains perpendicular to the surface axis of the sclerotium, cells 4–7 μm broad, walls dark brown, irregularly thickened, carbonaceous, ca. 1 μm thick, ventral rind giving rise to an irregular layer of light brown-walled, but not carbonaceous, brick-shaped to globose cells.

Apothecia stipitate-cupulate, arising, usually singly, from sclerotia on or in the

Fig. 3. *Ciborinia camelliae*, apothecium and ascospores. A, B, C, D, F, TMI-7633. A) Vertical, median section of flank of receptacle showing hymenium, subhymenium, medullary excipulum, and ectal excipulum. B) Vertical, median section of flank of receptacle showing medullary excipulum, ectal excipulum, and tomentum hyphae. C) Vertical, median section of stipe showing medullary excipulum, ectal excipulum, and tomentum hyphae. D) Young asci and ascospores. E) TMI-7635, germinating ascospores on malt extract agar at 20–23°C, about 20 hrs after discharge. F) Ascus and ascospores in Melzer's reagent; note bluing of ascus pore channel wall. Scales: A=50 μm ; B, C=50 μm ; D, E=20 μm ; F=10 μm .

Fig. 4. *Ciborinia camelliae*, microconidial anamorph, TMI-65010-2 on potato dextrose agar. A) "Spermodochia" of microconidial anamorph. B) Underside of "spermodochium" composed of dematiaceous, barrel-shaped cells supporting microconidiophores and phialides. C) Phialides borne on inflated central cell; arrow indicates microconidium developing within prominent collarette; note scar at phialide apex, the remnant of previous microconidial development. D) Dark-walled phialides from older cultures; arrow indicates prominent collarette. E, F, G, microconidia and phialide apices with attached chains of microconidia showing elongation of microconidia and microconidial appendages (cfr. "disjunctors"). E) Arrow indicates short microconidial appendage at juncture of microconidia. F) Arrow indicates elongation of microconidial appendage and adjoining microconidia. G) Arrow indicates further elongation of microconidial appendage. Scales: A=50 μm ; B=20 μm ; C, D, E, F, G=10 μm .

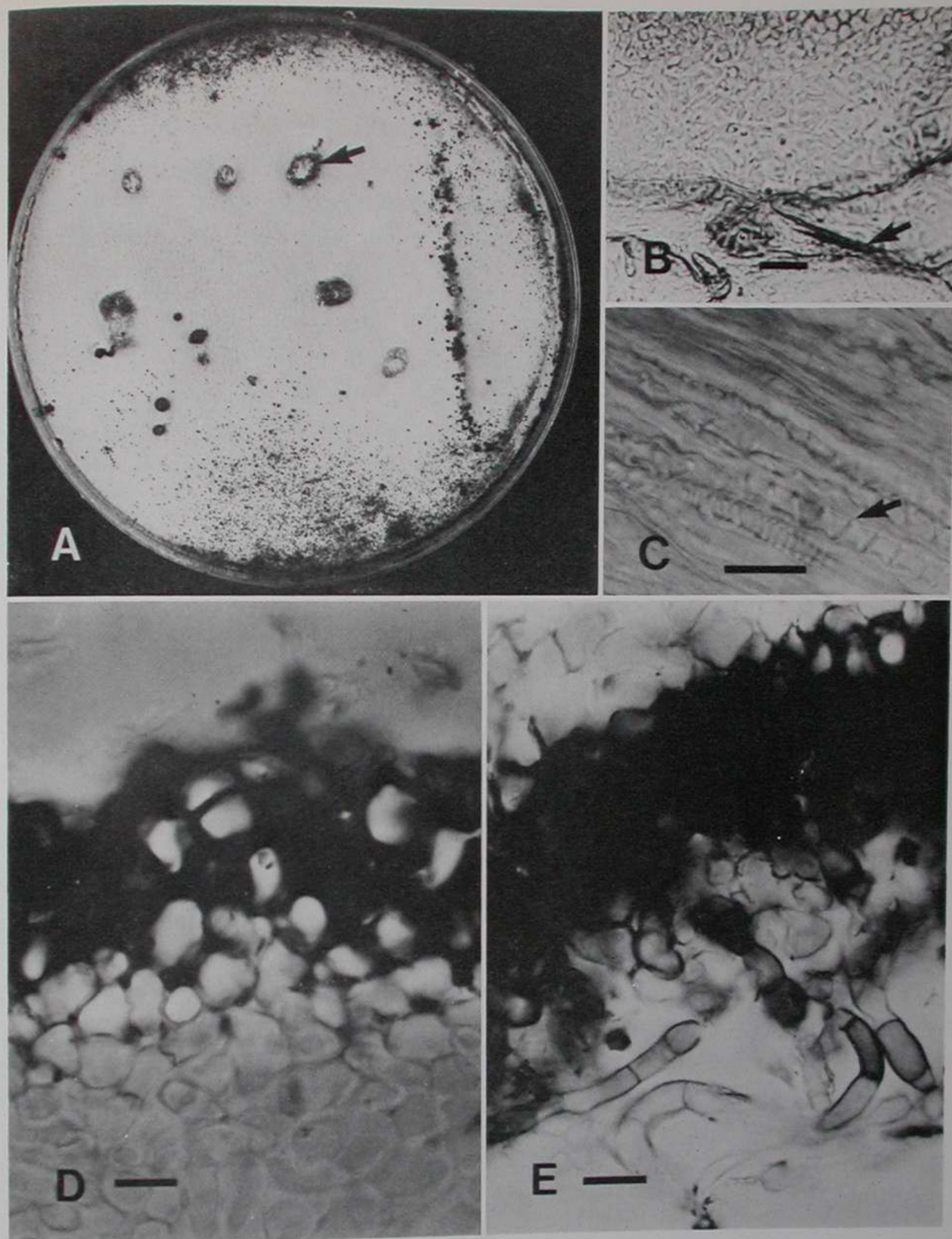


Fig. 5. *Ciborinia camelliae*, sclerotia. A) TMI-65010-1 on potato dextrose agar; arrow indicates sclerotium. B) TMI-7633, longitudinal section showing cortex and medulla; arrow indicates wall of polygonal host cell embedded in sclerotial medulla. C) TMI-7287, longitudinal section showing medulla; arrow indicates spiral wall thickenings of host vessel elements embedded in sclerotial medulla. D) TMI-65010-1, dorsal sclerotial rind and cortex. E) TMI-65010-1, ventral sclerotial rind and cortex; note layer of loose globose cells and tomentum hyphae originating from rind. Scales: B=20 μ m; C=20 μ m; D, E=10 μ m.

soil. Stipe variable in length depending on the depth at which the sclerotium is buried in the soil, 2–100 × 1–2 mm, tapering gradually toward base, flexuous in portions embedded in soil, concolorous with receptacle, surface pruinulose. Receptacle (3–)5–18 mm in diam, cupulate at first, becoming discoid to plano-convex, often with a slight central depression at maturity; hymenium “Buff” to dull “Cinnamon” when young, becoming dull “Umber” to “Dark Brick,” occasionally with irregular black stains at maturity; outer surface concolorous with hymenium, pruinulose, often faintly radially rugulose around the base toward stipe.

Ectal excipulum of stipe a compact zone, 50–60 μm wide, of light brown-walled *textura porrecta* parallel to the stipe axis, cells 2–7 μm broad, thin-walled, smooth or superficially granularly roughened, giving rise to mostly one-celled tomentum hyphae aggregated in fascicles. Medullary excipulum of stipe composed of light brown-walled *textura porrecta*, somewhat interwoven, cells 2–15 μm broad, walls gelatinized, up to 2 μm thick, often superficially granularly roughened. Ectal excipulum of receptacle along flanks 40–75 μm wide [50–70(–100) μm in fresh specimens], of hyaline to light brown-walled *texture angularis* to *textura globulosa*, turning light blue in Melzer's reagent, in two subzones; inner zone 3–4 cells wide, of *textura globulosa* (*textura angularis* in rehydrated material), 10–25(–32) μm in diam, walls refractive, 0.5–1.0 μm thick; outer zone 2–3 cells wide, of smaller globose cells, 5–12 μm in diam, giving rise to one- to multicelled tomentum hyphae, up to 50 μm long and 2.5–6 μm wide, with refractive walls, 1–2 μm thick; at margin composed of light brown-walled, inflated brick-shaped cells and globose cells 5–12 μm broad, arranged in chains perpendicular to the apothecial surface axis, giving rise to tomentum hyphae, with occasional prosenchymatous elements originating from the medullary excipulum, turning out perpendicularly to the apothecial surface axis, walls refractive, 1–2 μm thick. Medullary excipulum of receptacle 30–200 μm wide (–400 μm in fresh specimens, along flanks), in two subzones; inner zone of hyaline to light brown-walled *textura intricata*, 7.5–15(–25) μm broad, walls thin, often superficially granularly encrusted, especially toward margin; outer zone of hyaline to light brown-walled *textura porrecta*, cells 5–7.5(–10) μm broad, walls 1–3 μm thick. Subhymenium 15–35(–60) μm wide, of light brown-walled, compact *textura intricata*, cells 2–5 μm broad, walls 1–2 μm thick, entire zone turning light blue in Melzer's reagent. Asci (100–)120–145 × (5–)6–10.5 μm , cylindrical, arising from croziers, 8-spored, apices generally thin in fresh specimens, thickened in rehydrated specimens (up to 4 μm thick), ascus pore channel wall weakly J+ without KOH pretreatment, with KOH pretreatment strongly J+. Ascospores hyaline, one-celled, ovate to obovate, bi- to multiguttulate, uninucleate, size range for all treatments 7.5–12.5 × 4.0–5.0(–6.0) μm , for fresh ascospores mounted in water (TMI-7633, n=25), range 9–12 × 5–6 μm , \bar{x} = 10.48 × 5.2 μm , SD for length = 0.80, SD for width = 0.29, length : width \bar{x} = 2.0, obliquely uniseriate, germination terminally or laterally unipolar.

Paraphyses filiform, slightly apically inflated, 1–1.5 μm broad (to 3 μm at apices), usually 3-septate, simple or rarely branched below midpoint, hyaline, thin-walled, not exceeding asci.

Appearance in culture (Fig. 2): On PDA: after one wk, surface mycelium white, "felty," in 5–7 concentrically zonate sectors bearing small, wet, black, "spermodochial" pustules, 1 mm in diam; after 4 wk, pustules larger, confluent, some isolates forming discoid, convex sclerotia, 5–8 mm in diam on the agar surface but not superficial, covered with an appressed, hyaline hyphal mat.

Radial growth rate on PDA up to 42 mm per wk; on LM up to 15 mm per wk; on MEA up to 30 mm per wk. All cultures grown at 18°C.

Conidial anamorphs (Fig. 4): No macroconidial anamorphs produced under any of the conditions employed in these studies.

Microconidial anamorph observed in all isolates under all conditions employed. Microconidia catenate, brown-walled, globose to obovate, 2.5–4 μm (for TMI 65010-1, $n=30$, $\bar{x}=3.1 \times 2.13 \mu\text{m}$, SD for length=0.44, SD for width=0.32, measurements made in water), uninucleate, containing a large, partially lipid-containing guttule, with a small basal appendage often bearing two fringes of wall material where the appendage was previously attached to the subtending microconidium; in age, where microconidia are still in chains, both microconidia and appendages becoming elongated and expanded; microconidia not germinating after 4 wk on PDA, MEA, or LM; microconidia borne on discrete phialidic pegs. Phialidic pegs hyaline to brown-walled, ca. 6 μm long, 2–2.5 μm broad, basally septate, each with a prominent, deeply cupulate collarette. Phialides borne in whorls on an inflated central cell, these cells borne on branching clusters of dark brown-walled, barrel-shaped cells, forming "spermodochia," immersed in a water-soluble, black mucilage.

Specimens examined: All collections on fallen flowers of *Camellia japonica* L. ("Tsubaki"). Japan: Honshu: Shizuoka: K. Hara, V. 1913 [TNS-209284 (Holotype of *Sclerotinia camelliae* Hara)]. Tottori: Kokoge, Tottori-shi, E. Nagasawa ("E. N."), 1. IV. 1978 (TMI-6570, duplicate as CUP-61117); S. Kaneko, 1. IV. 1981 (TMI-7286, duplicate as CUP-61118); E. N., 11. IV. 1983 (TMI-7633, duplicate as CUP-61120); Ochidani, Tottori-shi, E. N., 24. IV. 1974 (TMI-7634, duplicate as CUP-61121); E. N. & S. Nagasawa, 11. IV. 1982 (TMI-7287, duplicate as CUP-61119); E. N., 3. IV. 1983 (TMI-7635); Ohe, Funaoka-cho, Yazu-gun, E. N., 26. IV. 1983 (TMI-7636). United States: California: Pomona, M. Stoner, III, 1977 [CUP-58248 (Holotype)].

Cultures: Mass ascospore isolate made by E. Nagasawa ("E. N."), 5 April, 1983 on MEA from TMI-7635 (isolate as TMI-65009); single ascospore isolates made by E. N., 6 April, 1983 on LM from TMI-7635 (isolates as TMI-65010-1, -65010-2); single ascospore isolates made by E. N., 13 April, 1983 on LM from TMI-7633 (isolates as TMI-65011-1, -65011-2, -65011-3).

Notes: Comparison of our Japanese collections with the holotypes of *S. camelliae* Hara and *Ciborinia camelliae* Kohn revealed that the Japanese and American fungi are conspecific. Unfortunately no isolates of the holotype of *C. camelliae* Kohn are viable, but notes on these isolates made during previous studies by one of us (L. M. K.) indicate that cultural characteristics, especially those of the distinctive microconidial anamorph, are comparable with those of our isolates. Cultural characteristics of our isolates also agree with those described by Hansen and Thomas (1940). The sclerotial and apothecial microanatomy of the holotype of *C. camelliae* Kohn, a fairly recent collection preserved in ethanol, is similar to that of our collections. The holotype of *S. camelliae* Hara, on the other hand, is deceptively dissimilar to our recent collections. The Hara specimen has been dried for many years; the globose cells of the ectal excipulum are permanently collapsed, not regaining their original dimensions even when heated in 2% KOH. Consequently this zone appears to be composed of *textura porrecta* or *textura intricata*, rather than the actual *textura globulosa*, with the excipular tomentum hyphae forming a prominent layer, a "pseudo-ectal excipulum." It is probable that Kohn (1979) either did not examine actual sclerotia in Hara's holotype or that there are no longer intact sclerotia in the holotype specimen. We compared ascospore measurements among one of our collections (TMI-7287), the holotype of *C. camelliae* Kohn (CUP-58248) and the holotype of *S. camelliae* Hara (TNS-209284) with the following results ($n=30$): TMI-7287, rehydrated and measured in water, range $7-11 \times 3-5 \mu\text{m}$, $\bar{x}=8.7 \times 3.7 \mu\text{m}$, SD of length=0.9, SD of width=0.6; CUP-58248, preserved in 70% ethanol, measured in water, range $7-10 \times 3.5-4 \mu\text{m}$, $\bar{x}=8.28 \times 3.94 \mu\text{m}$, SD of length=0.68, SD of width=0.22; TNS-209284, rehydrated and measured in water, range $9-10 \times 3-5 \mu\text{m}$, $\bar{x}=8.72 \times 3.92 \mu\text{m}$, SD of length=0.66, SD of width=0.47. Although the treatments of ascospores among the three samples vary so much (age, drying vs. preservation in ethanol) that further statistical comparison is difficult, we feel that the ascospore measurements of the three specimens are similar enough to fall within an appropriate range for a single species. Yoshimi (1979) described, but did not validly publish, a "forma *macrospora*" of *S. camelliae* Hara, with ascospores $12-13 \times 6-8 \mu\text{m}$. Although we have not examined Yoshimi's material, his report suggests that some variation in ascospore size exists within this species; Yoshimi's measurements exceed even our measurements of fresh ascospores, with are usually slightly larger than rehydrated ascospores.

Accommodation of *S. camelliae* in *Ciborinia* Whetzel is the most appropriate placement in the Sclerotiniaceae, in agreement with Kohn (1979). The discoid sclerotia which remain attached to the suscept, the dorsi-ventrally differentiated sclerotial rind, the apothecial ectal excipulum composed of *textura globulosa*, and the production of sclerotia on elements of the perianth, rather than on the androecium or gynoecium, all support accommodation in *Ciborinia*. While other species currently assigned to *Ciborinia* produce sclerotia on leaves and stems of the suscept, we consider the perianth to be sufficiently

foliar in structure to fit the circumscription of *Ciborinia*. The dematiaceous microconidial anamorph with its catenate microconidia, microconidial appendages, and prominent collarettes, all borne in black, mucilaginous pustules, is anomalous for *Ciborinia*. Though all microconidial anamorphs in the Sclerotiniaceae are in need of comparative study, Whetzel (1945) noted that the microconidial anamorphs in *Ciborinia* are hyaline. There is strong morphological resemblance to the hyaline microconidial anamorph of *Septotinia podophyllina* (see Whetzel, 1937), but since no macroconidial anamorph is produced in *C. camelliae*, *Ciborinia* rather than *Septotinia* is the most appropriate generic assignment.

Observations on sclerotial flower blight of camellia in Japan

Sclerotial flower blight of camellia has been described in the Japanese literature by Hara (1919a, 1919b, 1923, 1927) and by Ito (1973). This disease of *Camellia japonica* L. produces symptoms in the spring, at flowering time. The causal agent, *Ciborinia camelliae* Kohn, attacks only flower buds and flowers, causing a blotch, blight, or rot of these organs; heavily infected buds and flowers senesce prematurely and drop. Flowers developing from infected buds are often smaller than those from healthy buds. Infected flowers develop brown blotches which, when they originate at the tips of petals, resemble spots caused by frost damage. The illustrations of infected flowers provided by Hansen and Thomas (1940) accompanying their description of flower blight of camellia occurring in California agree well with our observations of infected flowers in Japan. This disease detracts from the beauty of camellia trees as well as the production of the fruits, but does not appear to be fatal to the suscept as reported by Hara (1919a, 1919b). Most infections occur on buds and flowers still on the tree, but since healthy trees shed the perianth, leaving the pistil on the tree, infection may also occur on perianths on the ground.

According to Nunomura (1980), the sclerotia of *C. camelliae* are formed frequently on sepals of *Camellia japonica*, but we have not observed this at Tottori; careful observation of fallen flowers suggests that sclerotia develop primarily in tissues of the basal portion of petals, in agreement with the observations of Hansen and Thomas (1940) on California material. Yoshimi (1979) reports that sclerotia are also produced on fruits of *C. japonica*. Sclerotia overwinter, producing apothecia the following spring. Since the reports of Hara (1919a, 1919b), the teleomorph has been reported in Japan by Otani (1979, 1983), Yoshimi (1979), and Nunomura (1980). Ascospores are the primary inoculum. Observations on the life history of *Ciborinia camelliae* and the disease cycle of sclerotial flower blight of camellia in Japan agree with the observations based on the North American studies of Hansen and Thomas (1940).

Microconidia are produced on the senescent flowers after they have fallen on the ground; we made abundant collections of perianths covered with "spermodochial" pustules in mid-June. Perianths with this microconidial anamorph are not yet decomposed, still retaining their form.

Yamamoto (1959) and Ito (1973) report the occurrence of this disease on *Camellia sasanqua* Thunb., but without description of the disease on this host.

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