

NEW SPECIES OF CERATOCYSTIS FROM CONIFERS

by

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(with 6 figs.)

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In 1958 (1) a species of wood staining fungus from subalpine fir (*Abies lasiocarpa* (HOOK.) NUTT.) in Colorado was reported as the European species *Ceratocystis penicillata* (GROSSMAN) HUNT. More intensive studies during the past two years show that this fungus is not the European species. It is described here as a new species, *C. abiocarpa*. Four other species from more extensive collections in 1962, 1963 and 1964 are also described at this time.

Ceratocystis abiocarpa sp. n.²⁾ Fig. 1, A—C.

Perithecia in culturis recentibus isolatis evoluta, atra, fere superficialia vel ad basim in substrato immersa, magna, globosa, glabra, 180—300 μ in diam.; rostra atra, 600—1200 μ longa, e basi 45—70 μ crassa usque ad apicem subattenuata, usque 75 μ infra ostiolam fere cylindrica, inde ad apicem 20 μ in diam. attenuata; hyphae ostiolaris non visae; ascosporeae in massam hyalinam gelatinosam ostiolarum agglutinatae et plerumque in forma guttulae elongatae circa partem superiorem rostri decurrentes, curvatae, hyalinae, 3.5—4.5 \times 1.2—1.8 μ , sine tunica gelatinosa; conidiophora rara, simplicia, ut rami breves laterales hypharum hyalinarum vegetarum orta; conidia ad apices conidiophorum singula vel caespitosa, hyalina, elongato-ovoidea vel late ovoidea, interdum subcurvata, 4.0—10.0 \times 2.5—5.0 μ .

Perithecia develop on recently isolated cultures, black, nearly superficial or with bases imbedded in substratum, large globose, smooth, 180—300 μ in diameter; necks black, 600—1200 μ long, 45—70 μ thick at base, tapering slightly upward — nearly cylindrical to within 75 μ of ostiole — then often tapering more abruptly to about 20 μ or less at ostiole; no ostiolar hyphae; ascospores

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²⁾ The Latin descriptions were prepared by EDITH K. CASH, formerly Mycologist, National Fungus Collections, Washington, D.C.

forming in hyaline, gelatinous mass at ostiole and usually running down as an elongate droplet around upper part of neck, curved, hyaline, $3.5-4.5 \times 1.2-1.8 \mu$, no gelatinous sheath seen.

Culture white at first, later (10 to 15 days) darkening and substratum finally black and surface mycelium dark gray; growth fast, 80-90 mm diam. in 10 days; Perithecia developing slowly, maturing in 4 to 6 weeks; conidiophores not abundant, simple, short side branches or ordinary hyaline hyphae or slightly modified short or long branches; conidia borne singly or in clusters at tips of conidiophores, hyaline, elongate ovoid to broadly ovoid, usually broader at upper end and narrowed to attachment end, sometimes slightly curved, $4.0-10.0 \times 2.5-5.0 \mu$.

Type specimen, R.W.D. \neq 486, on *Picea engelmannii* PERRY, main stem, cut early in 1963, in close association with bark beetle galleries (*Ips* sp., *Polygraphis rufipennis* KBY., and others), and collected October 22, 1963. Co-Type specimens R.W.D. \neq s 492 and 494 were collected from the same log on November 29, 1963.

Cultures of this species are similar in growth rate (fast growth) to cultures of *C. huntii* ROBINSON (3) but the mycelium is not appressed and with gray surface mat which usually covers the bases of perithecia with only the black necks showing through. Perithecia are similar to the above species but usually with longer necks and ostioles forming more quickly and ascospores collecting in a more liquid mass which runs down around the tip of neck whereas ascospores of *C. huntii* are whiter firmer masses collecting in irregular shapes at the tip or wherever they burst through the neck or perithecium. The ascospores of these two species are somewhat similar in size and shape but those of *C. huntii* have gelatinous sheaths whereas those of *C. abiocarpa* do not. All cultures of *C. huntii* examined have a *Verticicladiella* imperfect stage whereas such a conidial stage has never been proved to belong to *C. abiocarpa* although *V. picea* is often closely associated in host tissue.

In bark of *Abies lasiocarpa* (Hook) NUTT. associated with the bark beetle *Dryocoetes confusus* Sw.; Isolated from adult beetles and from perithecia in bark of dead tree collected in the Roosevelt National Forest, October 24, 1962; Isolated from bark of 10" diam. dead tree along highway 287 in Shoshonie National Forest, Wyoming September 13, 1963; Isolated from standing dead 12" diam. tree on Roosevelt National Forest west of Red Feather Lakes, Colorado, October, 1963; In standing dead trees (cork bark fir) 18" to 30" diam. in Snow Bowl area near Flagstaff, Arizona, July 16, and July 21, 1964.

In bark and insect galleries (*Ips politrons*?) of *Picea engelmannii* west of Red Feather Lakes, Colorado, October 22, 1963 and November 29, 1963; On Carson National Forest, near Taos, New Mexico, June 16, 1964; Near Ski Basin N.E. Santa Fe, New Mexico, July 10, 1964; Along Ski Lift below Snow Bowl near Flagstaff, Arizona, July 21, 1964; Near McNary, Arizona, August 4, 1964 Red Feather

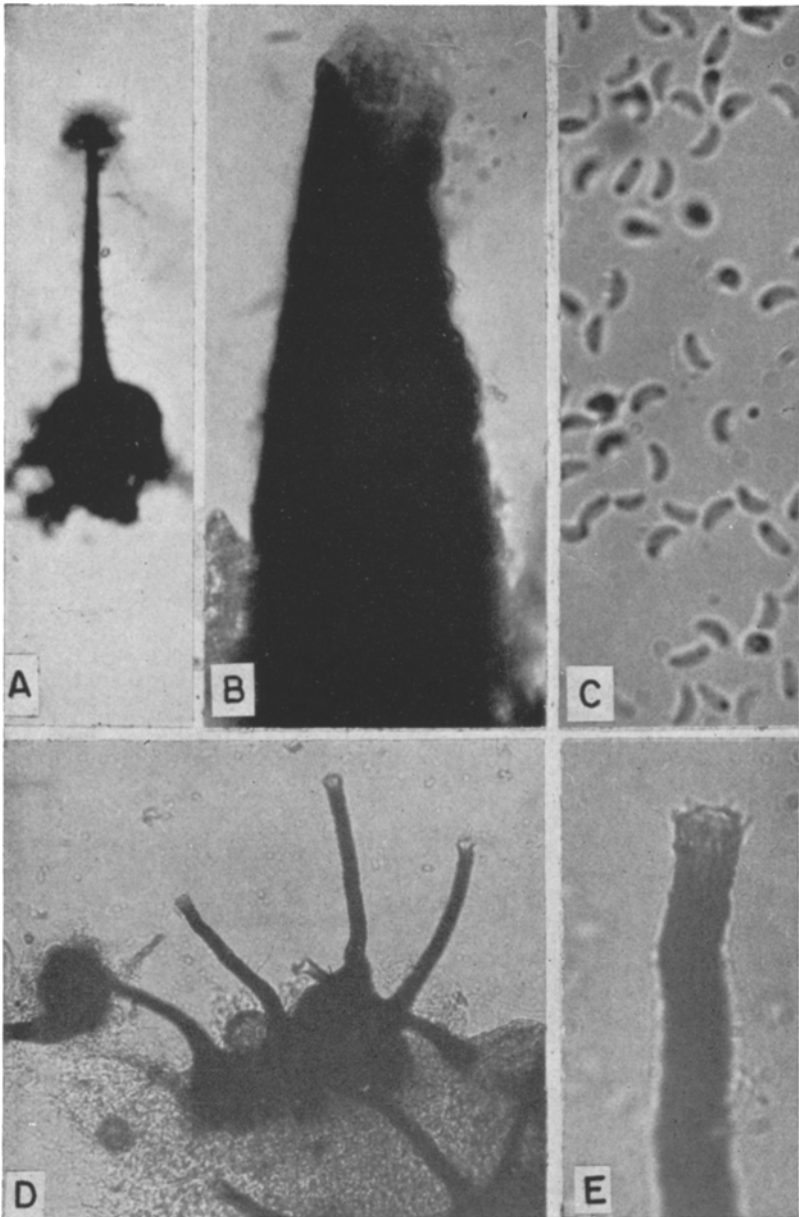


Fig. 1. A-C, *Ceratocystis abiocarpa*. A, Perithecium, $\times 65$; B, Tip of neck showing abrupt taper below ostiole, $\times 670$; C, Ascospores, $\times 1500$. D & E, *Ceratocystis nigrocarpa*. D, Group of perithecia, $\times 150$; E, Tip of perithecial neck (ostiole), $\times 670$.

Lakes area, Colorado, September 16 and 22, 1964. (These last two were identified from cultures isolated from mature *Ips* species in Eng. spruce).

Ceratocystis nigrocarpa sp. n. Fig. 1 D & E & Fig. 2 A—C.

Perithecia atra, parva, 50—80 μ in diam., sphaerica; rostra atra, 120—160 μ longa, e basi 15—25 μ usque ad apicem 10—12 μ in diam. attenuata, ad apicem leniter expansa et subinfundibuliformia, fibrillis ostiolaribus carentibus vel tubercula pauca brevia hyalina circa labrum ostioli expansi efformantibus; ascosporae parvae, hyalinae, curvatae vel lunatae, 3.0—4.0 \times 1.0—1.3 μ , ad apicem rostri in guttulam globosam liquidam pallidem brunneam adhaesae; conidiophora simplicia ut rami breves laterales hypharum vegetarum hyalinarum orta; conidia abunda, per hyphas vel ad apices caespitosa, hyalina, late ovoidea, 3.0—5.0 \times 1.5—3.0 μ .

Perithecia black, small 50—80 μ in diameter, spherical; necks black, 120—160 μ long, 15—25 μ in diam. at base to 10—12 μ diam. at tip, tip proper expanding slightly to appear somewhat funnel-shaped; ostiolar filaments absent or very short hyaline and curved outward, (Fig. 1, E); Ascospores small, hyaline, curved or crescent shaped, 3.0—4.0 \times 1.0—1.3 μ , held at tip of neck in spherical liquid droplet very light brown in color.

Culture hyaline appressed at first about 30 mm diameter in 10 days (intermediate growth rate), gradually turning light gray to dark gray as perithecia develop but no dark color in the mycelium or substratum remaining appressed except for few aerial white strands, surface often yeast-like; perithecia starting to form in about two weeks but maturing slowly, fully matured in 5 to 6 weeks; conidiophores simple, as short side branches of ordinary hyaline hyphae; conidia attached along or hyphae clustered around tips of hyphal branches, hyaline, broadly ovoid, 3.0—5.0 \times 1.5—3.0 μ abundant.

Type specimen, R.W.D. \neq 152 prepared from culture isolated from perithecia on a 12 inch diameter *Abies* sp. log, infested with *Scolytus* sp. and flat headed borers, near McCall, Idaho, July 15, 1962; from perithecia in 36" diam. Douglas Fir log (recently cut) near McCall, Idaho; from pupating larvae of *Dendroctonus brevicomis* LEC. in Ponderosa pine log, collected by R. H. SMITH near Placerville, California, August 10, 1962; collected by R. LYONS and R.W.D. near Westfall Ranger Station, near Oakhurst, California, from *D. brevicomis* in Ponderosa pine log, August 15, 1962; from small perithecia under bark of hemlock log on ground about one year, White Mountain National Forest, New Hampshire, collected by AL SHIGO and R.W.D., July 30, 1963.

This species is variable in that some isolates have short ostiolar hyphae and others do not. There is also slight variation in size of ascospores but growth rate, size of perithecia, and cultural characteristics are very similar.

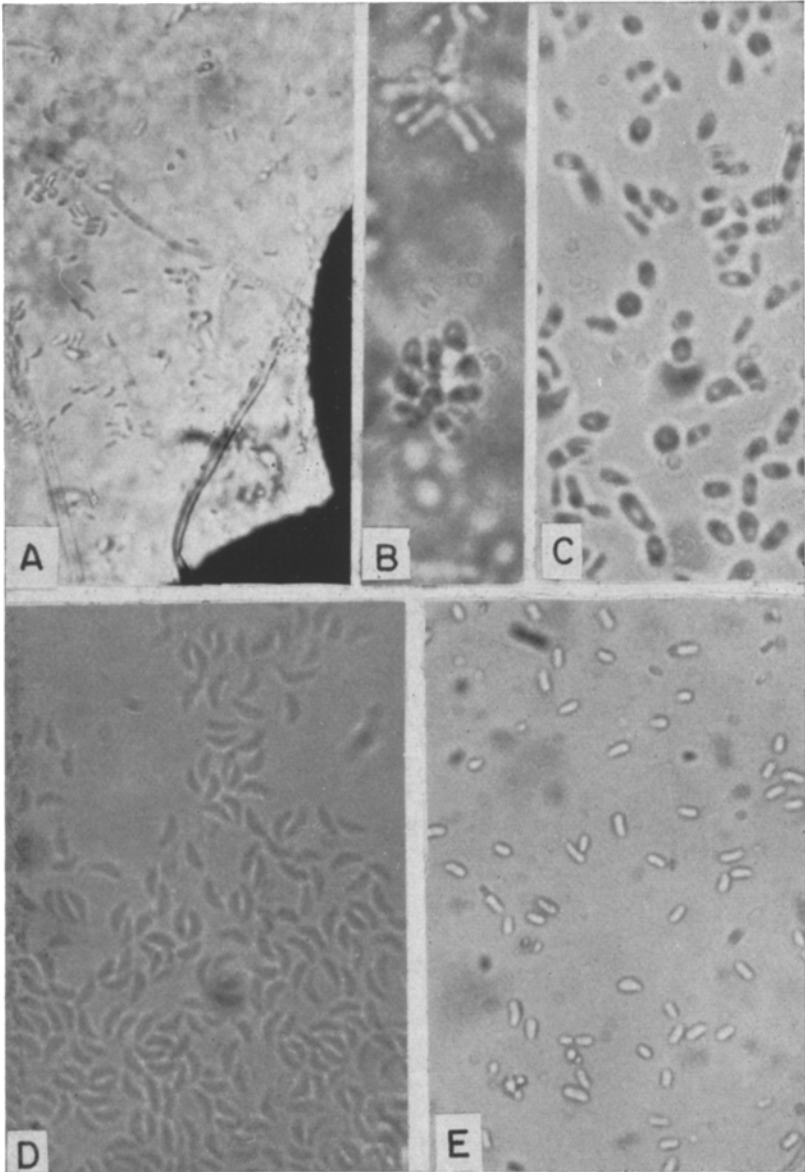


Fig. 2. A-C, *Ceratocystis nigrocarpa*. A, Ascospores, $\times 670$; B, Conidial clusters at tip of conidiophores, $\times 1500$; C, Conidia, $\times 1500$. D & E, *C. leuconcarpa*. D, Ascospores, $\times 1500$; E, conidia, $\times 670$.

Ceratocystis leucocarpa sp. n. Fig. 2, D & E; Fig. 3, A—E

Perithecia alba, globosa, 140—200 μ in diam.; rostra alba, 500—800 μ longa, mediocriter robusta, e basi 80—90 μ in diam. usque 50—65 μ quidam infra ostiolum subattenuata, interdum tuberculis hypharum brevibus obtusis tecta; seatae ostiolorum hyalinae, lanceolatae, e basi 2.0 μ in diam. ostilum obtusum versus attenuatae, aut in fimbriis aut circa ostiolum irregulariter confertae, 15—30 μ longae; asci parvi, ovoidae, 4.0—5.0 \times 5.0—8.0 μ , octospori; ascospores in guttulis albis globosis ad apicem rostri conglobatae, hyalinae, leniter curvatae, 2.0—3.0 \times 1.0—1.2 μ ; conidiophora e hyphis ramosis simplicibus composita; conidia cylindrica usque elongato-ovoidae, ad apicem unum subattenuata, hyalina, 3.0—6.0 \times 1.0—2.5 μ .

Perithecia white, globose, 140—200 μ diam.; necks white, 500—800 μ long, fairly stout, 80—90 μ diam. at base tapering slightly to 50—65 μ just below ostiole, sometimes covered with short blunt hyphal protuberances; ostiolar spines hyaline, lanceolate, 2.0 μ diam. at base and tapering to blunt point, (either as ostiolar fringe or irregularly clustered around and below ostiole), 15—30 μ long; asci small ovoid 4.0—5.0 \times 5.0—8.0 μ , eight-spored, ascospores collecting in a white globose droplet at tip of neck, hyaline, slightly curved 2.0—3.0 \times 1.0—1.2 μ ; Conidiophores simple hyphal branches; conidia cylindrical to elongate-ovoid, narrowed slightly at one end, hyaline, 3.0—6.0 \times 1.0—2.5 μ .

Culture white, slow to medium growth; perithecia white, developing in about 2—3 weeks, maturing in 3 to 5 weeks; hyphae hyaline 3.0—6.0 μ diam.; Usually growing with dark mycelial species.

Type specimen prepared from culture R.W.D. \neq 215. This white perithecial species was first observed in *Ips* sp. beetle galleries in bark of sugar pine (*Pinus lambertiana* DOUGL.) in southern Oregon. The perithecia were imbedded in the bark with their white necks protruding into the galleries. They were closely associated with black perithecial species such as *C. montia* and no pure culture was separated from this bark specimen. However, cultures from attempted isolations were mostly *C. montia* with the white perithecia developing along with those of *C. montia*, the white necks protruding above the substratum in striking contrast to the black mycelium and perithecia.

Later, an apparently pure culture was isolated from stained sapwood of *Pinus ponderosa* LAWS. which has been killed by *Dendroctonus*. It seems probable that more normal growth and fruiting may occur in mixture with the other species.

(No. 215) collected from dead *Pinus lambertiana* broken over by wind and infested with *Ips* sp., near Grant's Pass, Oregon, August 4, 1962; from ponderosa pine logs infested by *Ips* sp. cut in late 1961 at Red Feather Lakes, Colorado and collected

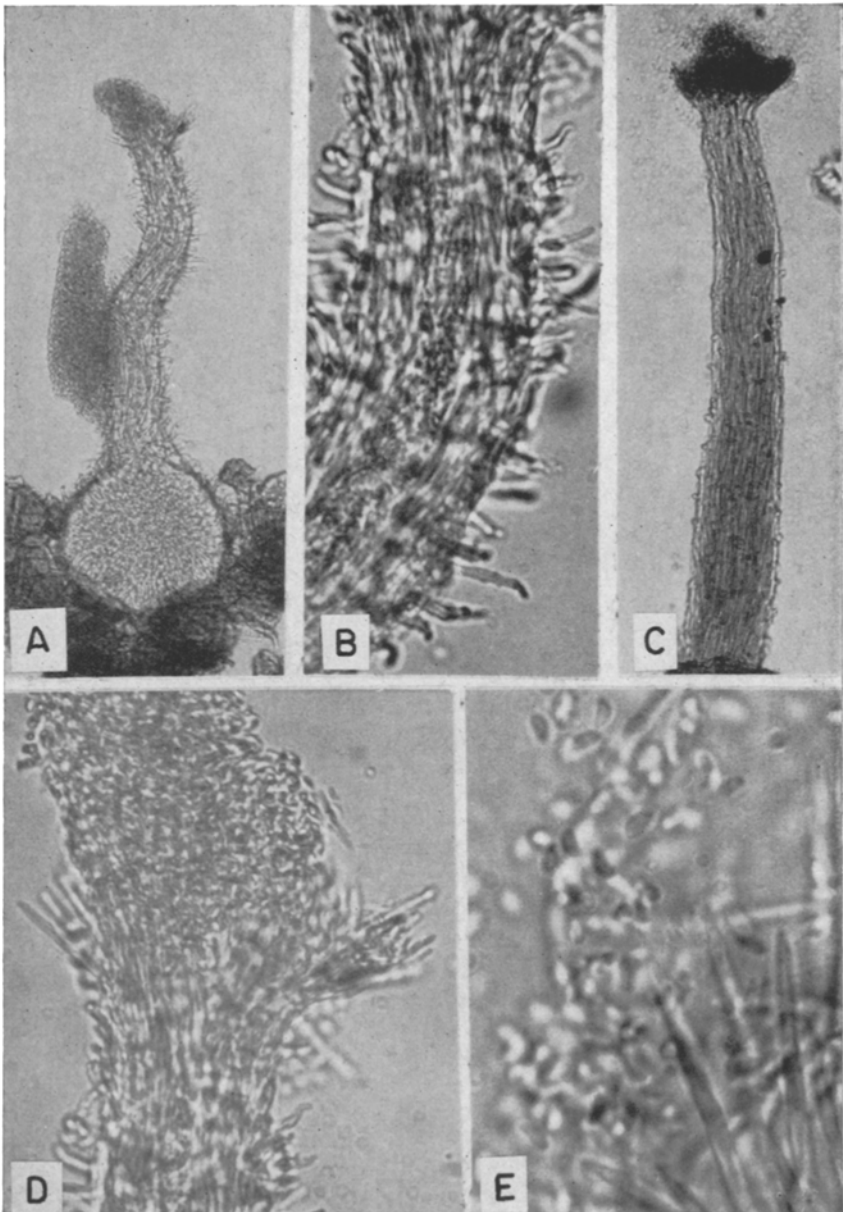


Fig. 3. A-E, *Ceratocystis leucocarpa*. A, Entire perithecium, $\times 150$; B, Part of neck showing short hyphal protuberances, $\times 670$; C, Neck of perithecium with ascospore mass at tip, $\times 150$; D, Ostiolar fringe and ascospore mass, $\times 670$; E, Ostiolar setae and ascospores, $\times 1500$.

September, 1962; from standing dead ponderosa pine containing both *Dendroctonus ponderosae* HOPK. and *Ips* sp. near Fort Collins, Colorado, September 21, 1962; from bark of dead subalpine fir (*Abies lasiocarpa*), Roosevelt National Forest, Colorado, October 24, 1962.

Ceratocystis minuta-bicolor sp. n. Fig. 4, C—H.

Perithecia ex alba ad pallide luteo-brunneam desinentia, plerumque in substrato immersa, parva fere globosa, 60—75 μ in diam.; rostra atra vel atro-brunnea, 95—180 μ longa, e basi 20—30 μ usque ad apicem 13—18 μ in diam. quidam infra ostiolem subattenuata, inde rotundata vel ad ostiolem 10—13 μ in diam. abrupte angustata, subtiliter asperata; hyphae ostiolarum numerosae (10—15) rectae, conum acuminatum supra ostiolem efformantes, 15—25 μ longae, e basi 1.5 μ crassa ad apicem attenuatae, infra pallide brunneae, supra hyalinae; fibrae ascosporae ex ostiole extrudentes et inter apices fibrarum in columna longa araneaosa tenui emergentes; ascosporae hyalinae, filiformes, utrinque aculatae, 17—22 \times 1.1—1.3 μ ; conidia apicaliter in hyphis hyalinis vegetis enata, parva, 2.0—4.0 \times 1.0—1.4 μ , hyalina, cylindro-bacillaria vel apicem unum versus subattenuata.

Perithecia white to very light buff-brown, usually imbedded in the substratum, small, nearly globose, 60—75 μ diam.; necks black to dark brown 95—180 μ long, 20—30 μ diameter at base, tapered slightly to 13—18 μ diameter just below ostiole, then rounded or tapered abruptly to 10—13 μ diam. at ostiole, surface finely roughened; ostiolar filaments numerous (10—15) straight, forming a pointed tepee over ostiole, 15 to 25 μ long, 1.5 μ thick at base and tapered to smaller diameter at tip, bases light brown but hyaline at tips; ascospores filament extruded from the ostiole and emerging between the tips of the filaments in a long narrow column resembling a spider web, filaments usually about 2 spores thick; ascospores hyaline, long narrow filiform and pointed at ends, 17—22 μ long by 1.1—1.3 μ wide. Central part staining with orseillin BB, about 8 μ long with hyaline segments on each end about 5 μ long; Conidia borne at tips of ordinary hyaline hyphae, small 2.0—4.0 \times 1.0—1.4 μ hyaline, cylindrical, rod-shaped to slightly tapered at one end.

Culture white, remaining white except for dark perithecial necks; mycelia met. slightly raised fine cottony tufted to soggy and appressed, with wide appressed marginal zone, margin proper finely fimbriate, medium growth rate. Perithecia developing in about 10 days, maturing in 14 to 20 days, often the black necks are so numerous they color the mat a dark gray, sometimes forming in dense patches with white sectors of culture having few if any perithecia. Conidiophores not conspicuous, merely as hyphal branches with groups of conidia at tips or on short side branches of hyphae, conidia very

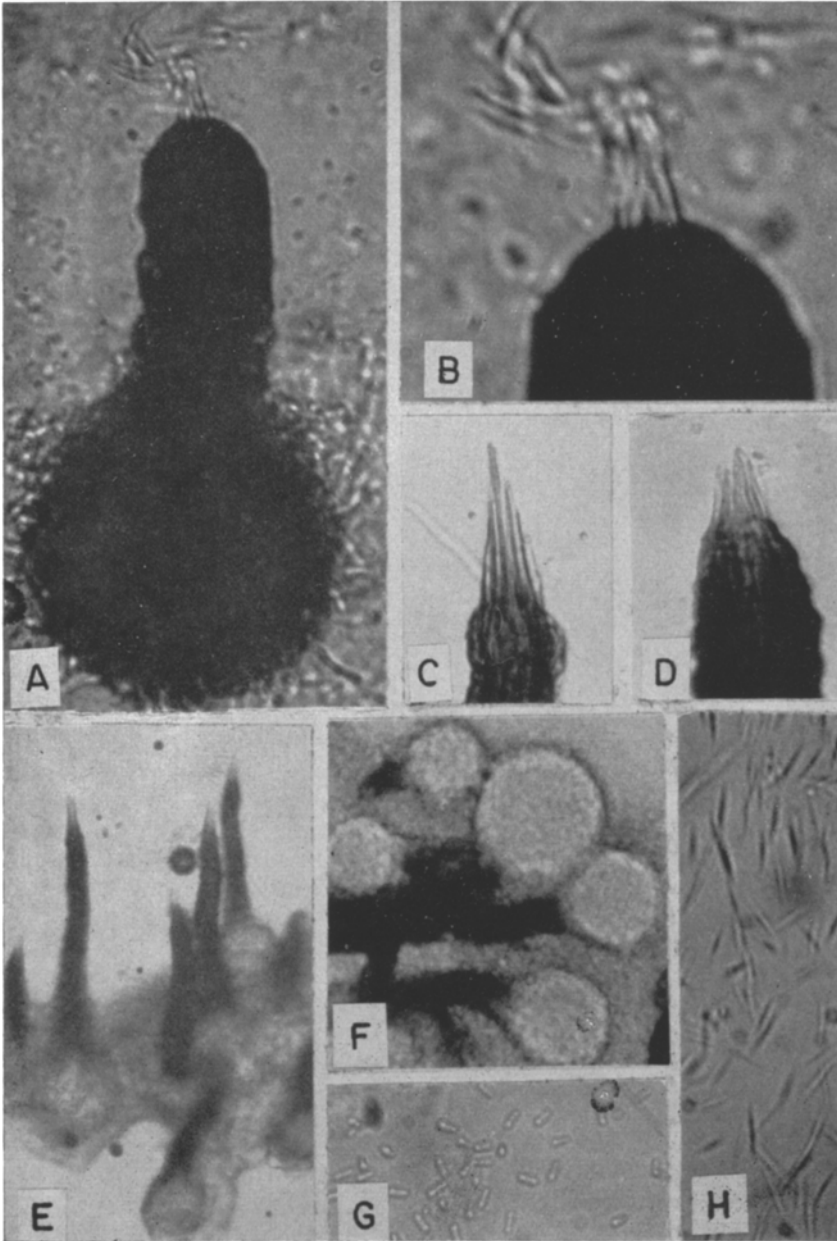


Fig. 4. A & B, *Ceratocystis minuta*. A, Typical perithecium, $\times 670$; B, Tip of neck showing ostiolar filaments and ascospores, $\times 1500$. C-H, *Ceratocystis minutabicolor*. C & D, Tips of perithecial necks showing prominent ostiolar filaments, $\times 670$; E, Group of typical perithecia, $\times 150$; F, Perithecia from another strain, $\times 150$; G, Conidia, $\times 670$; H, Ascospores, $\times 670$.

numerous but small rod-shaped and quite different from the ascospores.

Type specimen prepared from culture R.W.D. ± 247 . Perithecia often present in *Ips* spp. galleries and sometimes isolated from the adult beetles as follows: from ponderosa pine logs invaded by *Ips confusus* (LEC.), collected by R. H. SMITH near Placerville, California, August 10, 1962; From perithecia in ponderosa pine log infested by *Ips oregoni* (EICHN.) cut one year earlier but collected September 10, 1962, on Roosevelt National Forest near Red Feather Lakes, Colorado; From adult *Ips oregoni* in lodgepole pine killed in early 1962, collected October 9, 1962 on Roosevelt National Forest west of Fort Collins, Colorado; From *Ips* beetles in dead standling lodgepole pine, six miles west of Red Feather Lakes, Colorado, on Roosevelt National Forest, September 18, 1963; From young adult *Ips oregoni* and from perithecia in *Ips oregoni* galleries in lodgepole pine collected at Dillon, Colorado, September 27, 1963; also collected in New Mexico and Arizona in July, 1964.

This species is similar to *C. minuta* (SIEM) HUNT in size and shape of perithecia as well as the filiform type of ascospores (Fig. 4, A and B) but has white to light buff-brown perithecia with black necks. Also the necks are somewhat longer, as are astiolar filaments and ascospores than for *C. minuta*. However *C. minuta* is common and widespread in the United States in pines and other conifers infested by *Ips* spp.

Ceratocystis seticollis sp. n. Fig. 5, A—D & Fig. 6, A—F.

Perithecia pallide brunnea, in substrato immersa, interdum ad superficiem et atro-brunnea, 100—150 μ lata, 105—160 μ alta, hyphis laxis hyalinis vel pallide brunneis extus vestita; rostra atro-brunnea, asperata, subbulbosa usque cylindrica, 150—165 μ longa, ad basim 40—50 μ , quidam infra ostiolem 45—60 μ crassa, ad ostiolem abrupte 30—40 μ attenuata; hyphae ostiolaris elongato-lanceolatae, apicem longum conicum brunneum vel atrum efformantes, pernumerose, hyphae exteriores 40—60 μ , interiores usque 140 μ longae, ad bases brunneae et 2.0—2.5 μ crassae, ad apices pallide brunneae vel fere hyalinae, usque 1.0 μ attenuatae; ascosporae lateraliter visae cylindricae sed apicaliter quadratae in aspectu, ad apices rotundatae, hyalinae, tunica tenui gelatinosa utrinque expansa involutae 4.0—5.2 \times 2.5 μ , sine tunica 3.2—5.0 \times 1.1—1.6 μ .

Perithecia light brown and submerged in substratum, (Diamalt 2.5%), sometimes forming on surface of substratum and dark brown, 100—150 μ wide by 105—160 μ high with loose hyaline to light brown hyphae over surface; necks dark brown, roughened on surface and slightly bulbous to cylindrical, 150—165 μ long, 40—50 μ wide at base and 45—60 μ wide just below ostiole, abruptly rounded to 30—40 μ at ostiole; ostiolar filaments long lanceolate forming a long brown to black conical tip to the necks,

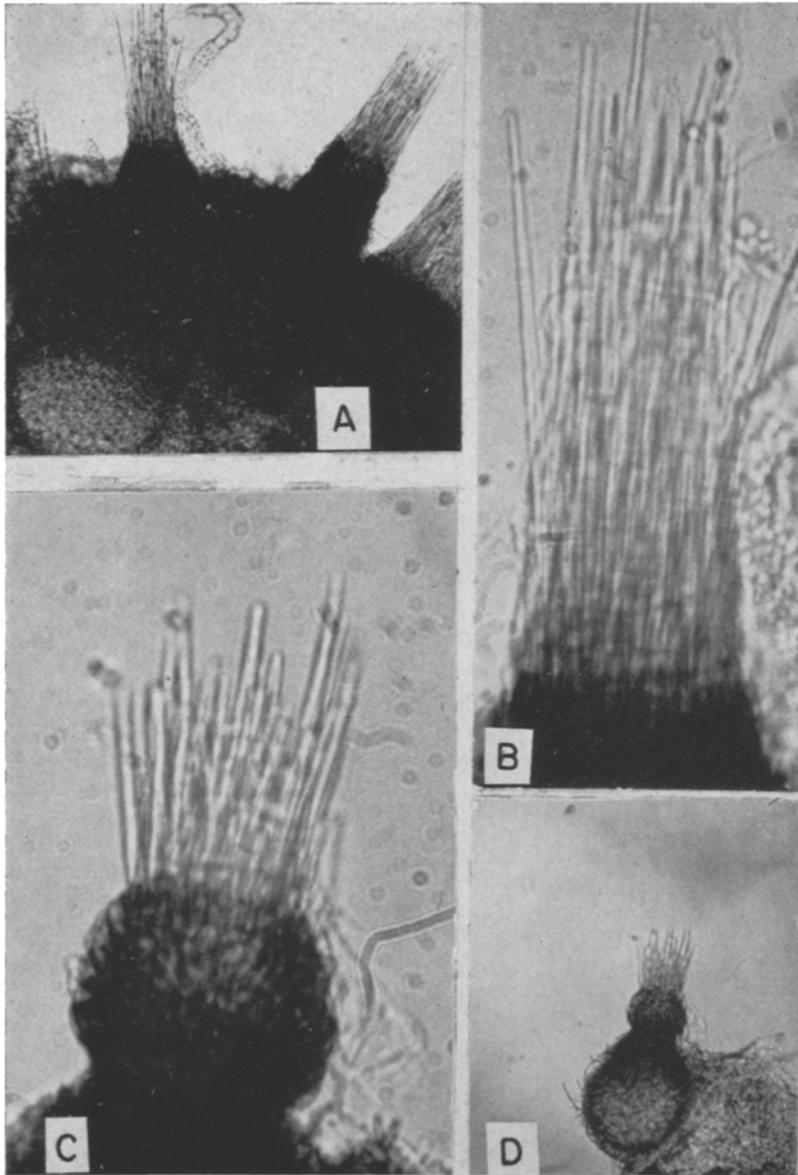


Fig. 5. A-D, *Ceratocystis seticollis*. A, Group of perithecia imbedded in substratum but with short necks protruding, $\times 150$; B, Tip of perithecial neck showing ostiolar filaments nearly mature, $\times 670$; C, Neck of young perithecium with ostiolar filaments developing, $\times 670$; D, Entire perithecium (enlargement shown in C), $\times 150$.

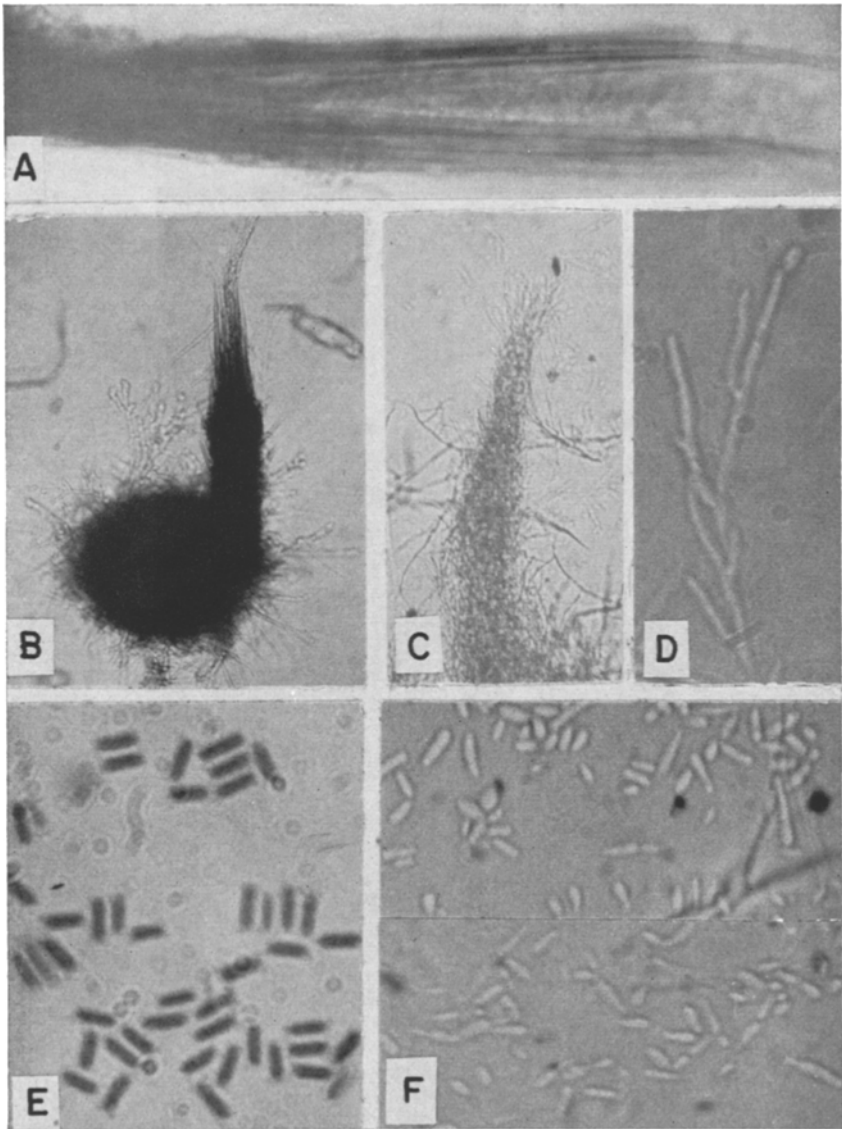


Fig. 6. A-F, *Ceratocystis seticollis*. A, Long, slightly-colored ostiolar filaments from ambrosia beetle tunnel, $\times 670$; B, Mature perithecium, $\times 150$; C, Hyphal strand, $\times 150$; D, Hyphal with conidium, $\times 670$; E, Ascospores stained with orseill in BB, $\times 1500$; F, Conidia (not stained), $\times 670$.

very numerous, outer ones 40—60 μ long, inner central ones up to 140 μ long, brown at the base to light brown or almost hyaline at tip, 2.0—2.5 μ thick at base, tapered to about 1.0 μ at tip; ascospores appearing cylindrical from side view but nearly square from end view, spore proper slightly rounded at ends, hyaline, and 3.2—5.0 \times 1.1—1.6 μ , with slight gelatinous sheath flared out at ends, overall size 4.0—5.2 \times 2.5 μ exuding in rather heavy honey colored tendrils through the ostiolar filaments.

Culture on 2.5% Diamalt agar fine white cottony, medium growth rate (43 mm in 10 days), whip-like hyphal strands forming in central area of mat, perithecia starting to develop as yellow brown submerged globules in 10 to 14 days, maturing in about three weeks old cultures. Brownish stromatic areas forming on surface of substratum or in mat and perithecia often developing in these brown areas in dense mass.

Type specimen R.W.D. \neq 435 prepared from culture isolated from ambrosia beetle galleries in hemlock stump, *Tsuga canadensis* (L.) CARR, on Pack Forest near Warrensburg, New York, July 23, 1963.

This species is a very striking one and quite different from any known to the writer. Black patches sometimes develop with abundant perithecia imbedded in them indicative of stromata. The short necks with long very abundant ostiolar filaments or setae which terminate in a sharp point above the ostiole are the most distinctive characters.

DISCUSSION

The new species of sapwood staining fungi described in this paper were encountered in a preliminary survey. Their specific insect associations have not been worked out in detail. *C. abiocarpha* seems to be closely associated with *Dryocetes* sp. in subalpine fir but abundant perithecial formation of it in Engelmann spruce are usually present in *Ips* sp. galleries. However, spruce *Ips* sp. is also known to be closely associated with the fungus *C. bicolor*.

C. minuta-bicolor and *C. minuta* (SIEM.) HUNT are often associated with *Ips* spp. bark beetles in spruce, subalpine fir, and pines. They have been isolated from mature *Ips* spp. beetles but could also be carried by other closely associated beetles.

C. seticollis was isolated from fruiting, probably perithecia, developed in ambrosia beetle galleries in sapwood of a large hemlock stump. It may therefore be closely associated with such beetles which were not identified.

Summary

Five new species of *Ceratocystis* wood staining fungi are described. The species *Ceratocystis abiocarpha* is common in recently killed subalpine fir (*Abies lasiocarpa* (HOOK) NUTT.), trees or felled logs

in the Central Rocky Mountain area from Wyoming to Northern Arizona and New Mexico. It also occurs commonly in Engelmann spruce (*Picea engelmannii* PERRY) logs infested with *Ips* sp. bark beetles in this same area.

C. minuta-bicolor is widespread in beetle infested logs of spruce, firs, pines and other conifers. *C. nigrocarpa* was isolated only occasionally but is apparently rather widespread in bark beetle infested pine logs. *C. leucocarpa* was isolated only occasionally from beetle infested pine logs and rarely in other conifers. It has been encountered mainly in the Fort Collins, Colorado area and from southern Oregon.

The fifth new species (*C. seticollis*) was isolated only once from ambrosia beetle galleries in sapwood of a hemlock stump in eastern New York state.

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