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STUDIES ON FOUR SPECIES OF CERATOCYSTIS, WITH A DISCUSSION ON FUNGI CAUSING SAP-STAIN IN BRITAIN

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THE blueing or sap-stain of timber is of great economic importance to foresters and timber merchants. This defect has been studied in detail in Sweden, Germany, and the United States. In Sweden, Lagerberg, Lundberg, and Melin (1928), and later Melin and Nannfeldt (1934), have shown that several species of Ascomycetes, usually classified as *Ceratostomella*, and many species belonging to different genera of Fungi Imperfecti are responsible for blueing of conifers. In the United States, Hedgcock (1906), Rumbold (1929, 1931, 1936, 1941), Davidson (1935, 1944), and Verrall (1939) among others have given a complete account of fungi that cause sap-stain. In Britain, however, little attention has been paid to the work of sap-stain fungi. In papers dealing with the blueing of coniferous timber, blueing is simply mentioned as caused by *Ceratostomella pilifera* Fr., but Münch has shown that so-called *C. pilifera* is made up of a number of different species, and of these *C. pini* Münch has been supposed to be the chief organism responsible for blueing conifers in Britain. The author, however, has not been able to isolate this fungus from numerous specimens of blued wood, but has isolated a number of other fungi. Among these, *Ceratocystis piceae* (Münch) Bakshi (syn. *Ceratostomella piceae* Münch) and *Leptographium lundbergii* Lager. & Melin, isolated from the gallery of the ambrosia beetle (*Trypodendron lineatum*) as well as from blued pine boards, have been discussed elsewhere (Bakshi, 1950*a*). Inoculation experiments with these two fungi have shown that *L. lundbergii* is a strong blueing agent, whereas *C. piceae* is only a weak one. The present paper deals with three more staining fungi, *Ceratocystis coerulescens* (Münch) Bakshi, a new record in Britain, *C. longirostellata* sp. nov., and *C. galeiformis* sp. nov. Life-histories of these fungi are here described, together with that of *C. wilsoni*, a proposed new species of *Ceratocystis*, which does not stain sap wood.

Nomenclature

A short nomenclator of *Ceratostomella* Sacc., *Ceratocystis* Ell. & Hals., and other allied genera is given below:

1. *Ceratostomella* Sacc. (Saccardo, 1878) with the two species *C. vestita* Sacc. and *C. lejocarpa* Sacc. as cotypes.

2. *Ceratocystis* Ell. & Halst. (Halstead and Fairchild, 1891) with type species *C. fimbriata* Ell. & Halst. (monotypic).

3. *Rostrella* Zimm. (Zimmermann, 1900) with type species *R. coffeae* Zimm. (monotypic).

4. *Endoconidiophora* Münch (Münch, 1907) with type species *E. coerulescens* Münch (monotypic).

5. *Linostoma* v. Höhnel (von Höhnel, 1918) with type species *Ceratostomella pilifera* (Fr.) Winter (monotypic).

6. *Ophiostoma* H. & P. Syd. (H. & P. Sydow, 1919) proposed as a new name for *Linostoma* v. Höhn., since *Linostoma* Wall. (Wallich, 1831) was already given to a genus of flowering plants (Thymeleaceae). There it carries forward the same type, viz. *C. pilifera* (Fr.) Winter.

7. *Grosmannia* G. Goid. (Goidànich, 1936) with type species *G. serpens* G. Goid. (monotypic), which was established to include species of *Ophiostoma* with *Leptographium* conidia. Siemaszko (1939) did not adopt the genus *Grosmannia* since *Ophiostoma ips* Rumbold (*Grosmannia ips* (Rumb.) Goid.), which was transferred to *Grosmannia* by Goidànich, has *Graphium* as well as *Leptographium* conidia. *Ophiostoma* is now known to have conidial stages of the type *Chalara*, *Leptographium*, *Graphium*, *Cladosporium*, and *Cephalosporium* (Melin & Nannfeldt, 1934; Siemaszko 1939) and thus I do not consider the genus *Grosmannia* acceptable.

The type species of *Ceratocystis*, *Rostrella*, and *Endoconidiophora* all have endoconidia, and of these, the names *Ceratocystis fimbriata* and *Rostrella coffeae* are here regarded as synonyms. Both Saccardo's original species of *Ceratostomella*, on the other hand, namely, *C. vestita* and *C. lejocarpa*, have persistent asci and monostichous spores, which, incidentally, can become 3-septate. Their asci arise from the wall of the perithecium as in all Sphaeriaceae. In contrast to these characters, the species in the remaining genera cited above have deliquescent asci with unicellular spores arranged irregularly within them. The asci in this group appear as a confused mass and in later stages of their development lie freely within the perithecium embedded in mucilage. Hence the generic name *Ceratocystis* Ell. & Halst. is considered by Mr. E. W. Mason as the most acceptable for species of the latter group and *Ceratostomella* Sacc. is retained for species of the former group.

1. CERATOCYSTIS COERULESCENS (Münch) Bakshi in *Trans. Brit. mycol. Soc.*, **33**, p. 114, 1950.

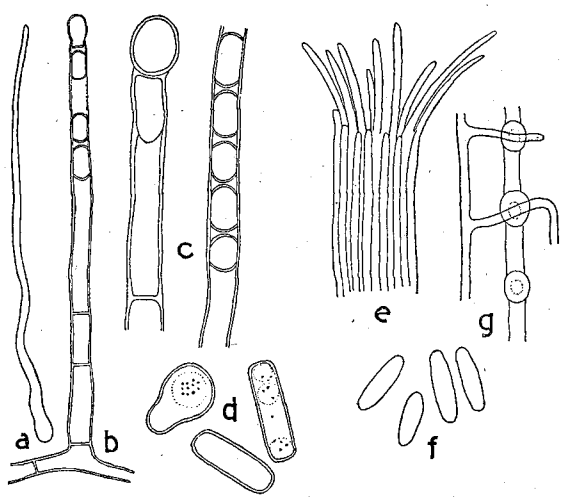
Syn. *Endoconidiophora coerulescens* Münch (Münch, 1907).

Ophiostoma coerulescens (Münch) Nannf. (Melin and Nannfeldt, 1934).

This species was first isolated from spruce in Germany and described by Münch (1907) under the name of *Endoconidiophora coerulescens*. It has been recorded from Sweden by Lagerberg, *et al.* (1928) from living blazed spruce trunks, and from pine and spruce timbers. It had also been reported from the United States by Davidson (1935, 1944) and Verrall (1939) as one of the most important fungi causing stain of hardwood logs and lumber in the South. Davidson (1935) also isolated *E. coerulescens* from *Pseudotsuga taxifolia*. His isolates of the fungus from conifers were identical with those from hardwoods in microscopical and macroscopical characters, but they differed from one other in the substratum and in the fact that the isolates from conifers, like the European *E. coerulescens*, had an odour of amyl-acetate in culture, while those from hardwoods had a musty odour. These differences finally led Davidson (1944) to raise the status of his isolates from hardwoods to a new species, *Endoconidiophora virescens*.

My isolates were obtained along with *C. piceae*, from several specimens of blued pine, collected near Gatehouse of Fleet, Kirkcudbrightshire, Scotland. No fructifications were observed on the specimens, and the cultures were obtained by aseptically transferring slivers of blued wood into agar. The characters of the fungus so obtained agree with those described for *E. coerulescens*. The following account gives its life-history in culture on agar and on wood, and some observations on its behaviour when monoascospore and monoconidial cultures are mated.

Cultures on malt-agar. The fungus is fast-growing, the radial growth being 7 cm. in ten days at 22° C. in the dark. The mycelium is at first colourless but pigmentation appears in two to three days. The mycelium is mostly appressed



TEXT-FIG. 1. **Ceratocystis coerulescens:** (*a*) germinating ascospore. ×285; (*b*) endoconidiophore with endoconidia in chains. ×480; (*c*) ditto. ×875; (*d*) endoconidia. ×875; (*e*) top of perithecial neck with crown of cilia. ×875; (*f*) ascospores. ×1550; (*g*) penetration of a hypha through bordered pits in tracheid wall. ×285.

to the substratum and produces finely branched, sparse, radiating hyphae in the advancing zone. Strong odour of pear-drops develops in young cultures but dies out with age.

In ten-day-old cultures the aerial mycelium is sparsely developed (Pl. I, fig. 1) and is light grey. The pigmentation, in general, consists of shades of Andover Green (Ridgway, 1912), Slate-Olive, Lincoln Green, Storm Grey to Castor Grey. The mycelium being scanty, a Petri-dish culture is more or less transparent and the undersurfaces of colonies have the same shades of colour as the aerial mycelium. With age, the grey shades of the aerial mycelium deepen to Deep Greyish Olive and Dark Greyish Olive with the earlier shades of green still persisting.

Conidia are produced endogenously within conidiophores. The latter are thick-walled (Text-figs. 1*b*, *c*), 126μ long on the average, 6·6μ broad at base, and 4·8μ at top; the base is 2–6, usually 3–4, septate by transverse walls, but the terminal portion is continuous. Conidia collect at the apices of conidiophores (Pl. I, fig. 3) and these give a powdery appearance to cultures (Pl. I, fig. 2); they (Text-fig. 1*d*) are thick-walled, elongate, hyaline, 6·6–33·0×2·5–6·0 (average 12·3×4·1)μ.

Perithecia appear abundantly (Pl. I, fig. 2) in cultures four to five days old and mature after eight to ten days. They are black. Their ventral diameter and height of the bulb vary from 116·2–208·6 μ, and 140–238 μ, the averages being 157·8 μ and 177·5 μ, respectively. The bulb of the perithecium is covered with long unbranched hairs (Pl. I, fig. 4); these are brown at the base and hyaline at the tip; they are thick-walled, continuous or transversely septate, and may be up to 336 μ long, 4 μ broad at base, and 3·5 μ at tip. The perithecial neck is black except at the top where its colour fades to light brown and its apical cells finally become colourless; it is 536·2–814·8 (average 656·7) μ long, 29·4 μ broad at the base, and 9·0 μ at the top. When mature, some of the hyaline tip cells of the neck open out to form a crown of cilia, 7–11 in number (Text-fig. 1*e*); these are thin-walled, hyaline, non-septate, 8·3–25·0 (average 21) μ long, 2·0 μ broad at base, and 1·0 μ at tip.

When a perithecium is ripe, its ascospores collect at the top of the neck, embedded in mucilage, in the form of a white, shining globule: this globule turns light yellow with age. The ascospores are hyaline, elongate, thin-walled, 1-celled (Text-fig. 1*f*), 5–6·7 × 1·6–1·8 (average 5·8 × 1·7) μ; they germinate usually by a single germ-tube (Text–fig. 1*a*). When, however, a ripe perithecium is placed in a drop of water on a slide, the spores are discharged in the form of a tendril; this tendril retains its form, since the mucilage, in which the spores are embedded, is immiscible with water.

The hyphae, when young, are thin-walled, hyaline with hyaline contents, 3·7–7·0 μ broad; their walls, however, soon turn greenish-brown, and finally, in old cultures, the hyphae become brown and thick-walled.

The fungus produces numerous dark brown to black sclerotia, (Pl. I, fig. 3) on the average, 80 μ in diameter.

On wood. Sterile pieces of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) were successfully inoculated with *C. coerulescens*. The fungus grows readily and produces a greyish-green aerial mycelium with abundant conidia, perithecia, and sclerotia (Pl. I, fig. 5). Blue stain appears in three weeks, and is more severe on pine than on spruce. Within two weeks after the appearance of the stain the pine becomes completely blued. Penetration of the fungus into the wood is moderate. Hyphae are abundant in the pith-rays (Pl. I, fig. 6) and sometimes completely fill the cell-cavities; they are dark brown and 5–7 μ broad. They often pass into the tracheids but are not so abundant there. Penetration between adjoining tracheids occurs through the bordered pits (Text-fig. 1*g*).

Homothallism. The fungus is homothallic and, in some single ascospore cultures, produces conidia, sclerotia, and perithecia. Not all such cultures develop perithecia though they produce conidia and sclerotia.

In an endeavour to find out why some of the monoascospore cultures did not produce perithecia, they were mated together and an interesting phenomenon of mutual aversion between cultures was seen. This feature was also observed when monoascospore and monoconidial cultures, which developed perithecia, were mated together. The matings were divided into the following classes:

1. Monoascospore cultures obtained from the same perithecium.
3. Monoascospore cultures obtained from different perithecia.
3. Different monoconidial cultures.
4. Pairing of the same monoascospore culture.

5. Pairing of the same monoconidial cultures.
6. Pairing of a monoascospore and a monoconidial culture.

In all the series the cultures show mutual aversion to one another. In the mutually averting strains their submerged mycelia stop growing towards each other and a narrow region between them remains comparatively free from hyphae (Pl. I, fig. 7). The aerial mycelia, on the other hand, may closely approach each other, but no fusion takes place. This phenomenon was observed on malt-agar, potato-dextrose agar, and oat-agar, irrespective of the depth of the media, the distance at which the inocula were placed, and age of cultures. The line of aversion cannot be due to a staling of the medium, since each culture, when grown alone, develops well on all of them. It may be due to the secretion of some toxin by the averting mycelia, as suggested by Cayley (1923). This toxin appears to be secreted from the young hyphal tips, and this probably explains why aversion takes place even when pieces of mycelia from the same monoascospore and monoconidial cultures are mated. This mutual antagonism cannot be due to the odour of pear-drops in the cultures, since the line of aversion persists in old cultures after the odour has disappeared.

This peculiar form of antagonism between monospore mycelia of the same fungus was observed by Dodge (1920) in *Ascobolus magnificus*, when monospore strains of the same sex, combined together, would not meet. He observed that, when two strains of opposite sex were plated together, the mycelia met at the centre of the culture, where there appeared to be, for a brief period, a slight antagonism; then the hyphae from either side grew freely across the line of meeting and formed ascogonia and antheridia. Cayley (1923) observed the same phenomenon in *Diaporthe perniciosa* Marchal. As a result of her extensive investigations she concluded that, in that fungus, aversion occurs between monomycelia isolated from related host plants, whether of the same variety, of different varieties, or of different species, but that no aversion occurs between monomycelia isolated from widely different hosts. She observed the presence of averting strains from different perithecia on the same host, and this she explained as due to multiple infection from two or more different sources. She found that no aversion occurred between monoascospore mycelia from the same perithecium.

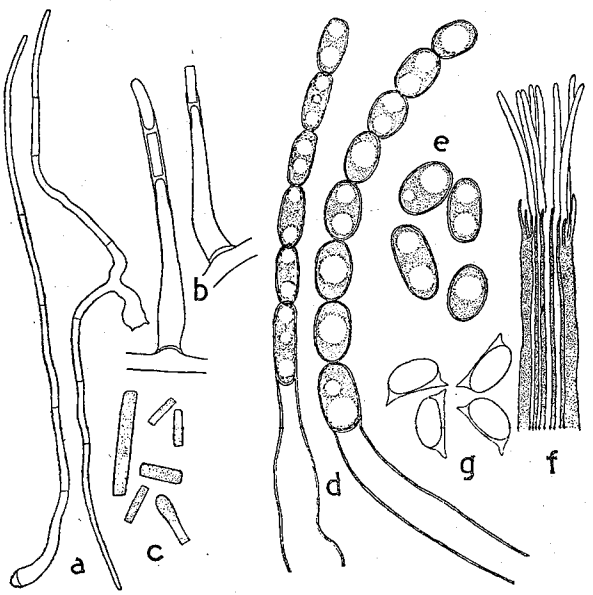
In the present investigation aversion was found between all possible matings. Since, however, the fungus was isolated only from one host and from one locality, it would be interesting to observe the pairing of monoascospore and monoconidial cultures obtained from different hosts collected from widely separated localities.

2. CERATOCYSTIS WILSONI sp. nov.

Isolation. This fungus was isolated, along with *Ceratocystis longirostellata* from felled logs of oak, *Quercus* sp., collected near Blairgowrie, in Perthshire, Scotland. The perithecia of both species were growing abundantly on the bare surface of the wood. The perithecia of *C. wilsoni* could be distinguished from those of *C. longirostellata* by the comparatively long neck of the latter. The spore globule at the top of the perithecial neck, when mature, is yellow in both species, but is oval in *C. wilsoni* and globular in the other species. There was a blackish-grey stain on the surface of the wood, and this stain, as will be shown

later, was caused by *C. longirostellata*. The following account gives the life-history of *C. wilsoni* in culture.

Cultures on malt agar. The fungus is fast-growing, and makes a radial growth of about 6 cm. in ten days at 22° C. in the dark. Young cultures exhibit varied characters; in some the aerial mycelium is scanty (Pl. II, fig. 8), in others well developed. A strong odour of pear-drops develops in young cultures but fades with age and dies out in old cultures. Coloration develops in about a week: around the inoculum, shades of Cream Buff (Ridgway, 1912) and Warm Buff, which later deepen to Light Ochraceous Salmon and Pale Ochraceous Buff and



TEXT-FIG. 2. **Ceratocystis wilsoni:** (*a*) germinating ascospores. ×65; (*b*) endoconidiophore on aerial mycelium. ×875, with (*c*) endoconidia. ×875. (*d*) endoconidiophore on substratum hyphae. ×875, with (*e*) endoconidia. ×875; (*f*) top of perithecial neck with crown of cilia. ×480; (*g*) ascospores. ×1550.

finally to lighter shades of Avellaneous; towards the periphery of the cultures, shades of Warm Buff deepen to Cinnamon Buff.

Conidia are produced endogenously within conidiophores which can be distinguished into two types, based on their size, shape, and mode of origin. Conidiophores of the first type arise from substratum hyphae (Text-fig. 2*d*) and bear conidia in chains. These conidia (Text-fig. 2*e*) are thick-walled, oval or cylindric, with hyaline, granular contents, each with 1–4 vacuoles, 7·3–13·7×3·5–8·9 (average, 9·8×5·5) μ. Conidiophores of the second type appear abundantly on aerial mycelium; they develop laterally from main hyphae (Text-fig. 2*b*) and are cut off from the latter by a transverse septum at the base; occasionally a second septum may appear in the upper portion. They are thin-walled with bases 4·5 μ and apices 2·0 μ broad on average. The apex of the conidiophore, which is closed when young, opens at maturity to liberate the endoconidia which fall away as soon as they are discharged. Conidia are thin-walled, cylindric, with hyaline, granular contents (Text-fig. 2*c*), 4·3–15·5×1–2·5 (average 8·9×1·7) μ.

Perithecia appear abundantly (Pl. II, figs. 8, 9) when the cultures are four to five days old and mature about a similar time later. When young, the base of the perithecium is round and transparent (Pl. II, figs. 10, 11) and has a greenish tinge. The neck is also transparent, except at its base, where the colour is light brown. A characteristic feature of the perithecium is the presence of a collar at the base of the neck (Pl. II, fig. 12) which is laid down very early in the development of the perithecium by a group of small thick-walled cells at the region.

As the perithecium develops, its bulb elongates and often bulges to one side and is sometimes flattened. At maturity the perithecium becomes dark brown to black. Its neck either grows out straight from the perithecial bulb, or more usually makes an angle with it. The bulb of the perithecium is covered with ventral hairs, which are thick-walled and dark brown at the base and thin-walled and colourless at the tip, they are 0–3 septate by transverse walls, 65μ long, and 1·4μ broad on average. In addition to the ventral hairs, short, conical, thick-walled bristles are present on the aerial portion of the bulb. They are 11–36μ long, 7–15μ thick at the base, tapering at the apex to 1·6–2·6μ. The bristles are also present on the collar of the neck (Pl. II, figs. 10, 11). The ventral diameter and height of the perithecial bulb vary from 190·4–245μ and 224–359·8μ, averaging 218·8μ and 316·7μ respectively. The perithecial bulb gradually tapers at the top, so that the collar stands out prominently. The diameter of the collar varies from 94–113 (average 100·7)μ. The neck is 730·8–896·0 (average 760·3)μ long, 39·2–51·8 (average 48·3)μ broad at base, and 14·0μ broad at the top. When mature, the hyaline tip cells of the neck open out and stand erect, giving a frayed appearance (Pl. II, fig. 12; Text-fig. 2*f*). The breadth of the canal in the neck varies from 8·3–9·3μ. The number of cilia at the ostiole varies from 2–14; they are 1-celled, thin-walled, hyaline, 34–40·7 (average 33·5)μ long, 2–2·7μ broad at base, 1·3–2μ at the apex.

When the perithecium is ripe the ascospores collect at the ostiole embedded in mucilage; this spore mass is at first globular and hyaline, but soon becomes oval and yellow. Occasionally, the spores are discharged in a tendril from perithecia on agar, and are always so discharged when a ripe perithecium is placed in water on a slide. The ascospores are hyaline, 1-celled, thin-walled, and oval with a brim (Text-fig. 2*g*). They measure, with brim, 6·0–7·6×3–3·8 (average 6·5×3·3)μ, and without brim 4·5–5·8×2·5–3·2 (average 5·1×2·8)μ. Germination in water takes place usually by one germ-tube which emerges through the brim (Text-fig. 2*a*).

Sclerotia develop abundantly. They are globular, dark brown, and 80μ in diameter on average.

Young hyphae are thin-walled, hyaline, with slightly granular contents, and about 2–5μ broad. Older hyphae are thick-walled, brown, with hyaline or brownish granular contents and up to 8μ broad.

The fungus was unable to infect sterile pieces of Scots pine and Norway spruce.

Heterothallism. The fungus is heterothallic and a single ascospore culture produces only conidia and sclerotia. Perithecia develop only when mycelia of two opposite strains meet (Pl. II, figs. 13, 14). Numerous pairings between eight monoascospore cultures revealed the presence of only two strains.

The nearest relative of the fungus is *Ceratostomella paradoxa* Dade (Dade,

1928) from which *C. wilsoni* differs in many important characters such as its perithecial measurements, appendages at the base of the perithecium, collar at the base of the neck, and cilia at the ostiole; the smell of pear-drops in culture is common to both species. The ascospore in *C. wilsoni* possesses a brim which is absent in *C. paradoxa*.

Technical description

Mycelium fast growing, with aerial mycelium abundant in some cultures, young hyphae colourless becoming light brown with age, odour like pear-drops; young hyphae hyaline, thin-walled, 2–5 μ broad, old hyphae brown, thick-walled, up to 8 μ broad; conidia endogenous, of two kinds: (*a*) borne in chains within conidiophores arising from substratum mycelium, endoconidia thick-walled, oval or cylindric, hyaline, vacuolate, 7·3–13·7 × 3·5–8·9 (average 9·8 × 5·5) μ, and (*b*) borne within conidiophores arising from aerial hyphae, conidiophores thin-walled, 4·5 μ broad at base, 2·0 μ at top, endoconidia thin-walled, cylindrical, hyaline, 4·3–15·5 × 1–2·5 (average 8·9 × 1·7) μ; perithecia abundant, bulb round when young, elongate on maturity and becoming compressed; ventral hairs of two kinds: (*a*) long, thick-walled and brown at base, thin-walled and colourless at tip, 65 μ long, 1·4 μ broad on average, and (*b*) bristle-like, conical, thick-walled, brown, 11–36 μ long, 7–15 μ broad at base, 1·6–2·6 μ at tip; perithecial bulb, 190·4–245 (average 218·8) μ in diameter, 224–359·8 (average 316·7) μ in height; neck with a collar at base, neck 730·8–896·0 (average 760·3) μ long, 39·2–51·8 (average 48·3) μ broad at base, 14 μ at top; cilia 2–14, fimbriate, 1-celled, thin-walled, hyaline, 34–40·7 (average 33·5) μ long, 2–2·7 μ broad at base, 1·3–2 μ at tip; ascospores hyaline, oval, 1-celled, with a brim, 6·0–7·6 × 3–3·8 (average 6·5 × 3·3) μ with brim; sclerotia globular, brown, 80 μ in diameter.

Fungus heterothallic.

On *Quercus* sp. Blairgowrie, Perthshire, Scotland.

Ceratocystis wilsoni Bakshi, sp. nov.

Mycelium celeriter crescens, ut 'amyl-acetate' odoratum, in juventute hyalinum, in maturitate brunnescens. *Conidia* endogena, unicellularia, hyalina, dimorphica; alia crasso-tunicata, ovata, 7·3–13·7 × 3·5–8·9 μ; alia tenui-tunicata, cylindrica, 4·3–15·5 × 1–2·5 μ. *Perithecia* copiosa, hirsuta, nigra, ventre 190·4–245 μ lato, 224–359·8 μ alto; rostello basi collare exhibente, 730·8–8·96 μ longo, 14–51·8 μ lato: ostioli filamentis 2–14, unicellularibus, tenui-tunicatis, hyalinis, 30–40·7 μ longis, 1·3–2·7 μ latis. *Asci* evanidi. *Ascosporae* hyalinae, unicellulares, ovoideae, et mire marginatae, 6·0–7·6 × 3–3·8 μ. *Sclerotia* brunnescentia, ±globosa, 80 μ lata.

Fungus heterothallicus, in truncis *Quercus* sp. Blairgowrie, Perthshire, Scotland.

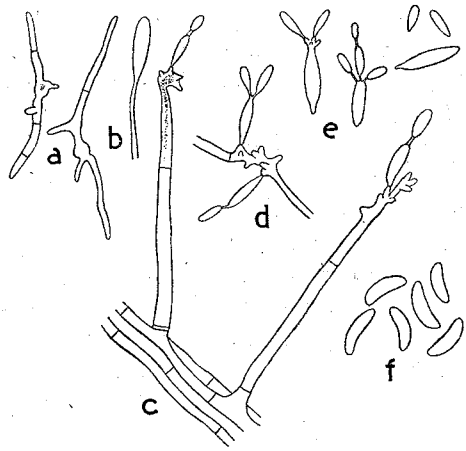
3. CERATOCYSTIS LONGIROSTELLATA sp. nov.

The habitat of the fungus has already been described under *C. wilsoni*. The following account gives the life-history of the fungus in culture.

On malt-agar. The fungus is slow growing, and the radial growth is about 1·5 cm. in ten days at 22° C. in the dark. In ten-day-old cultures, the mat, in general, is appressed and sodden, with an indistinct and sodden advancing zone. Aerial mycelium is generally abundant in the region round the inoculum. Strands

of hyphae, up to 4 mm. long, appear at places, and these become abundant with age (Pl. III, fig. 15). The bases of these strands of hyphae are agglutinated and black, but towards the top they branch repeatedly and the ultimate branches become colourless.

Pigmentation develops in about a week. The colour of the mycelium on agar is Blackish Mouse Grey (Ridgway, 1912) which gradually pales to Dark Mouse Grey, with a greenish tinge in some cultures towards the periphery. With age, the colour deepens to Blackish Green Grey, Dusky Green Grey, Castor Grey, and Dusky Ivy Green. Colour of the medium on the undersurface is Dusky



TEXT-FIG. 3. **Ceratocystis longirostellata:** (*a*) germinating ascospores. × 285; (*b*) conidium borne terminally on a hypha. × 875; (*c*) conidiophores bearing conidia on denticulate apices. × 875; (*d*) conidia on denticulate apices on ordinary hyphae. × 875; (*e*) conidia bearing secondary and tertiary conidia. × 875; (*f*) ascospores. × 1550.

Green Grey to Dull Greenish Black (2) fading to Castor Grey and Storm Grey towards the periphery.

Conidia are borne exogenously on the aerial mycelium. In the early stages a conidium is borne singly at the apex of a hypha (Text-fig. 3*b*). Later, short, unbranched, 2–4 septate hyphae arise laterally from the aerial strand of mycelium (Text-fig. 3*c*) and conidia are borne in groups of 6–8 on denticulate apices at the ends of the hyphae. The term 'conidiophore' can be loosely applied to them. They are 40–66 μ long, 2·5 μ broad at base, and 2·0 μ at apex. Conidia are also borne in groups on denticulate apices on ordinary hyphae (Text-fig. 3*d*). Primary conidia may bear secondary and tertiary conidia (Text-fig. 3*c*, *d*, *e*). Conidia are elongate, 1-celled, hyaline, 4·7–15·7 × 1·3–2·5 (average 8·3 × 2·0) μ.

Perithecia appear abundantly (Pl. III, fig. 15) when the cultures are about seven days old, and mature after a similar time. The bulbs of young perithecia are transparent, light brown, and pseudoparenchymatous (Pl. III, fig. 16). The longitudinal hyphae composing the neck converge at the tip leaving a small ostiole (Pl. III, fig. 16). When mature, the bulb of the perithecium turns black and is covered with numerous ventral hairs, which are thick-walled and usually unbranched 10·0–133·0 μ long, 2·0–2·3 μ broad; they are dark brown at the base and colourless at the tip. The bulb of the perithecium is more or less globular;

the ventral diameter and height vary from 135·8–210·0 (average 178)μ and 123·2–203·0 (average 165·2)μ, respectively. The neck is 1405–2921 (average 2126)μ long, 21·0–30·8 (average 25·6)μ broad at the base, and 11·2–15·4 (average 13·1)μ at the top. Frequently a perithecium with two necks can be seen in cultures.

When mature the hyaline tip cells of the neck open out to form the crown of cilia (Pl. III, fig. 17, 18). The number of cilia at the neck is very large and difficult to count, but the highest number recorded was 56 and the lowest 34. Cilia are unbranched, thin-walled, hyaline, 5–7 septate by transverse walls, 33–83 (average 63)μ long, 2·3–2·7μ broad at the base, tapering to a point at the apex.

At maturity the ascospores collect at the ostiole in the form of a globular, colourless drop which soon becomes milk-white and later turns yellow. Ascospores are hyaline, 1-celled, kidney shaped, thin-walled (Text-fig. 3*f*), 4·3–5·2$\times$ 1·3–1·7 (average 4·7–$\times$1·5)μ. They germinate with more than one germ-tube (Text-fig. 3*a*).

Hyphae, in young cultures, are thin-walled with hyaline, granular contents, and are 1·9–3μ broad. With age, they become thick-walled and light brown. Hyphae with clavate cells are present.

On wood. The fungus readily infects various species of conifers and successful inoculations were made on Scots pine, Norway spruce, and Japanese larch (*Larix kaempferi*). Mycelium is sparse on the surface of the wood but conidia and perithecia develop abundantly. The fungus causes a blackish-grey discoloration on all the different species of wood.

The fungus is homothallic and a single ascospore produces conidia and perithecia.

C. longirostellata agrees closely with *Ceratostomella capillifera* Hedgcock (Hedgcock, 1906), but they differ in cultural characters and in conidial and perithecial measurements. The neck in the present species is much longer and the number of cilia at the ostiole greater than in *C. capilliferum*. The natural hosts of the two fungi also differ.

Technical description

Mycelium slow growing, appressed, with long weak aerial strands developing sporadically, at first hyaline, later turning blackish grey with a greenish tinge; young hyphae thin-walled, hyaline, 1·9–3μ broad, older hyphae thick-walled, brown; conidia in groups on denticulations that are formed on the apices of hyphae or conidiophores, the latter 40–66μ long, 2·5μ broad at base, 2μ at top; the primary conidia elongate, narrowed at the end to which they are attached to projections, bear secondary and tertiary conidia; all conidia 1-celled, thin-walled, hyaline, 4·7–15·7$\times$1·3–2·5 (average 8·3$\times$2·0)μ; perithecia develop abundantly, the bulb when young, transparent, light brown, pseudoparenchymatous, becoming black on maturity, hirsute, ventral diameter 135·8–210·0 (average 178)μ, ventral height 123·2–203·0 (average 165·2)μ; neck hyaline to light brown when young, black when mature, 1405–2921 (average 2126)μ long, 21·0–30·8 (average 25·6)μ broad at base, 11·2–15·4 (average 13·1)μ at top, frequently with two necks, ostiole with 34–56 cilia, 63μ long, 2·3–2·7μ broad at base, tapering to a point at tip; spore globule white at first, yellow with age, asco-

spores hyaline, 1-celled, thin-walled, kidney-shaped, 4·3–5·2 × 1·3–1·7 (average 4·7 × 1·5) μ.

Fungus homothallic.

On *Quercus* sp. Blairgowrie, Perthshire, Scotland.

Ceratocystis longirostellata Bakshi, sp. nov.

Mycelium tarde crescens, in juventute hyalinum, in maturitate atrocinereum. *Conidiophora* apice denticulata. *Conidia* ad formam in genere *Cephalosporio* vel in genere *Cladosporio* constructa, elongata, unicellularia, hyalina, 4·7–15·7 × 1·3–2·5 μ. *Perithecia* copiosa, in juventute brunnescentia, ex pseudoparenchymate composita, in senectute nigra, hirsuta; ventre 135·8–210 μ lato, 123·2–203·0 μ alto; rostello 1405–2921 μ longo, 11·2–30·8 μ lato; ostioli filamentis 34–56, septatis, tenuitunicatis, hyalinis, 33–83 μ longis, basi 2·3–2·7 μ latis, apice acutatis. *Ascosporae* hyalinae, unicellulares, tenuitunicatae, 4·3–5·2 × 1·3–1·7 μ.

Fungus homothallicus, in truncis *Quercus* sp. Blairgowrie, Perthshire, Scotland.

4. CERATOCYSTIS GALEIFORMIS sp. nov.

Isolation. The fungus was isolated, along with *Ceratocystis autographa* Bakshi (Bakshi, 1950*b*), from the bark of *Larix kaempferi* collected in the forest at Blair Atholl in Perthshire, Scotland. The standing trees had been killed over a wide area in the forest and were infested with bark-beetles, *Hylurgops palliatus* and *Dryocoetes autographus*, and the ambrosia beetle, *Trypodendron lineatum*. Mycelia, conidia, and perithecia of *C. galeiformis* and *C. autographa* developed under the loosened bark, especially in the insect galleries. The discharged spores from the perithecium, in *C. galeiformis*, were held at the top of the neck in the form of a tendril (Pl. III, fig. 21). In making cultures of the fungus, aseptic removal of the spore tendril to agar was not relied upon, since the mucilage, in which the spores were embedded, was liable to be contaminated. In such cases the perithecial bulb was broken open by means of a sterile needle and the contained spores served as the inoculum for polysporous cultures. The following account gives the life-history of the fungus in culture.

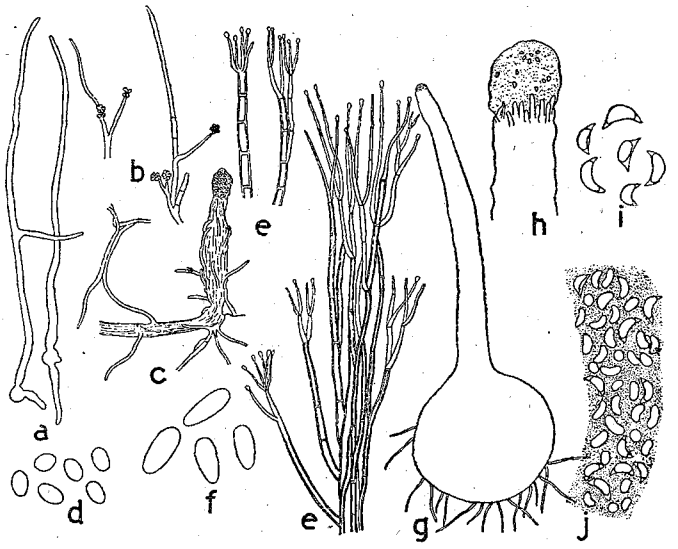
On malt agar. The fungus is slow growing, and the radial growth is about 1–3 cm. in ten days at 22° C. in the dark. The mat is appressed (Pl. III, fig. 19) and presents a yeasty appearance. Young colonies are colourless but pigmentation appears in about ten days and consists of shades of Vetiver Green (Ridgway, 1912) in general. The aerial mycelium assumes a grey colour. With age, various shades of Chaetura Black, Natal Brown, Deep Olive, Grey, and Slate Olive appear.

Conidia may be borne on ordinary mycelium or on special conidiophores of the *Leptographium* and *Graphium* types. In early stages conidia are borne in heads usually at the end of hypha (Text-fig. 4*b*) or a collection of hyphae (Text-fig. 4*c*). These conidia are 1-celled, oval (Text-fig. 4*d*), hyaline, 2·2–3·1 × 1·5–2 (average 2·7 × 1·7) μ.

In later stages the conidia are formed on conidiophores, where the main hyphal branch is penicillated (Text-fig. 4*e*). The cells of the hyphae at the base of this complex branch system are dark brown, but the ultimate branches are hyaline. Each of the ultimate branches cuts off a conidium at the apex. The conidia are

collected in a drop of sticky mucilage, which remains milk white for a long time but gradually become light brown and finally dark brown with age. In extreme cases the individual branches of *Leptographium* may become loosely united to form a compound fascicle, and this is the *Graphium* stage of the fungus (Pl. III, fig. 20). Conidia from the *Leptographium* and *Graphium* heads are 1-celled, hyaline, elongate (Text-fig. 4*f*), 4–5·2 × 1·9–2·2 (average 4·65 × 2·0) μ.

Perithecia develop rarely in cultures. The bulb of the perithecium is globose, black, and is covered with thick-walled, unbranched ventral hairs (Text-fig. 4*g*),



TEXT-FIG. 4. **Ceratocystis galeiformis:** (*a*) germinating ascospores. × 330; (*b*) conidia of *Cephalosporium* form at the end of hyphae. × 65; (*c*) do., but borne at the end of a collection of hyphae. × 65; (*d*) conidia from *Cephalosporium* head. × 285; (*e*) conidiophore of *Leptographium* form. × 65; (*f*) with conidia. × 285; (*g*) perithecium from culture. × 65; (*h*) top of perithecial neck. × 285; (*i*) ascospores. × 875; (*j*) a spore tendril. × 875.

brown at base, colourless at tip, and 2–2·5 μ broad. The ventral diameter and height vary from 182–271·6 (average 221·8) μ and 182–273 (average 218) μ, respectively. The neck is black, 539–700 (average 638) μ long, 39·2–60·2 (average 48·6) μ broad at the base, and 15·4–28·0 (average 24·5) μ broad at the top. The ostiole lacks a crown of cilia (Text-fig. 4*h*).

When the perithecium is mature, the ascospores collect at the ostiole in the form of a tendril, milk-white in colour. The ascospores are hyaline, 1-celled, thin-walled, bean-shaped, and possess a brim (Text-fig. 4*i*). They measure 4–5·3 × 2·1–3 (average 4·6 × 2·5) μ with brim and 3·2–4·2 × 1·7–2·1 (average 3·6 × 1·9) μ without brim. The spore germinates by more than one germ-tube (Text-fig. 4*a*).

When a mature perithecium is placed in a drop of water on a slide, the ascospores are liberated in the form of a tendril embedded in mucilage (Pl. III, fig. 22; Text-fig. 4*j*).

Young hyphae are thin-walled, hyaline, septate, 1·3–2·1 μ broad. With age they become thick-walled, 2–5·3 μ broad, and their colour depends upon the

colour of the mat. In green areas the hyphae have brownish-green walls with greenish contents; in brown portions they are light brown to dark brown with brown contents.

On wood. Artificial inoculations on wood show that the fungus develops readily on Scots pine, spruce, and larch. The mycelium is sparse on the wood and bears all the conidial stages as described on agar. The fungus produces a greyish blue discoloration, which is severe on Scots pine but mild on spruce and very feeble or none at all on larch.

Homothallism. The fungus is homothallic and a single ascospore culture produces conidia and perithecia.

The fungus is related to *Ceratostomella ips* Rum. (Rumbold, 1931) but differs from it in cultural characters and conidial measurements. The ascospores are bean shaped with a brim in the present species, while in *C. ips* they are quadrangular-prism shaped with flanges. The measurements of the spores in the two species also differ.

Technical description

Mycelium slow growing, appressed, with aerial mycelium at the inoculum; young colonies white, changing to various shades of green, brown, and black with age; young hyphae thin-walled, hyaline, 1·3–2·1 μ broad, old hyphae thick-walled, brown, or brownish-green, up to 5·3 μ broad; conidial states of the *Cephalosporium*, *Leptographium*, or *Grdphium* types, conidia in *Cephalosporium* heads 1-celled, oval, hyaline, 2·2–3·1 × 1·5–2 (average 2·7 × 1·7) μ, conidia on *Leptographium* and *Graphium* conidiophores 1-celled, hyaline, elongate, 4–5·2 × 1·9–2·2 (average 4·6 × 2) μ; perithecia rare, brown to black, globose, hirsute, ventral diameter 182–273 (average 221·8) μ, ventral height 182–273 (average 218) μ; neck black, 539–700 (average 638) μ long, 39·2–60·2 (average 48·6) μ broad at the base, 15·4–28 (average 24·5) μ broad at the top; ostiole without cilia; ascospores hyaline, 1-celled, thin-walled, bean shaped, with a brim, 4–5·3 × 2·1–3 (average 4·6 × 2·5) μ with brim, 3·2–4·2 × 1·7–2·1 (average 3·6 × 1·9) μ without brim, spore discharge in the form of a tendril.

Fungus homothallic.

On *Larix kaempferi*, under bark, associated with the bark beetles, *Hylurgops palliatus* and *Dryocoetes autographus*. Blair Atholl, Perthshire, Scotland.

Ceratocystis galeiformis Bakshi, sp. nov.

Mycelium tarde crescens, appressum, in juventute hyalinum, in maturitate brunnescens vel nigrum. *Conidia* unicellularia, tenui-tunicata, hyalina, ad formam in genere *Cephalosporio* 2·2–3·1 × 1·5–2 μ, vel ad forman in genere *Leptographio* (vel etiam in genere *Graphio*), elongata, 4–5·2 × 1·9–2·2 μ. *Perithecia* pauca, brunnescentia aut nigra, globosa, hirsuta, 182–273 μ lata; rostello 539–700 μ longo, 15·4–60·2 μ lato; ostioli filamentis multis. *Asci* evanidi. *Ascosporae* hyalinae, unicellulares, fabiformes sed peculiariter marginatae, 4–5·3 × 2·1–3·0 μ.

Fungus homothallicus. In truncis, *Laricis kaempferi* (associated with bark beetles *Hylurgops palliatus* and *Dryocoetes autographus*, Blair Atholl, Perthshire, Scotland.

Discussion

Species of the genus *Ceratostomella* have generally been regarded as an important group among the blueing fungi. In Britain, blueing of coniferous timber has generally been recognized as caused by *Ceratostomella pini*, which is an important blueing agent on conifers in the Continent. References to works on this fungus in Britain (see Bisby and Mason, 1940) show that the authors attributed the blue stain to *C. pini* without systematically identifying the causal organism. MacCallum (1922) stated that she found *C. pini* and *C. piceae* very commonly in and around Edinburgh and thought that they occur all over Scotland. She described *C. piceae* which she isolated from a blued trunk of *Pinus sylvestris*, but in her account no description of *C. pini* appears, and she did not state whether she isolated the fungus in culture. Necks of perithecia in *Ceratocystis* are very brittle so that it is probable that some of the perithecial necks of *Ceratostomella piceae* broke away and MacCallum might have mistaken these apparently short-necked perithecia for those of *C. pini*, which really have short necks. In his account of the successional diseases of Scots pine, Wilson (1928) stated that the plant, in later stages, was attacked by *C. pini*. The fungus was obtained in culture and produced conidia of the *Cladosporium* type. No perithecia developed and the fungus was identified as *Ceratosporella pini*. If the fungus referred to had been *C. pini*, it is interesting to note that it did not produce perithecia in culture, since perithecia developed abundantly in cultures of Münch (1907) and Lagerberg et al (1928). The *Cladosporium* type of conidia is, however, a characteristic of the genus *Ceratostomella* auct., and hence the identification of the species as *C. pini*, on conidial fructifications only, was not confirmatory. Wilson earlier (1922), Brooks (1928) and Muskett, Carrothers, and Cairns (1932) made passing references of *C. pini* causing blueing of pine, but in no case was the fungus brought into culture and identified. These are all the available records of *C. pini* in Britain, and judging from evidence given above, it appears that the fungus, if it exists in Britain, has not yet been correctly identified.

In the present work as well as in an earlier one (Bakshi, 1950*a*), the author has shown that *Leptographium lundbergii* and *Ceratocystis coerulescens*, both new records in Britain, are strong blueing agents on conifers. *L. lundbergii* is a stronger blueing agent than *C. coerulescens*. The former penetrates wood quickly and wood is completely blued in a short time. *C. galeiformis* and *C. piceae* are mild blueing agents. *C. longirostellata*, isolated from oak, has been found to infect conifers where it produced a perceptible blackish grey discoloration.

Summary

Four species of *Ceratocystis* are described in this paper, of which three are proposed as new species and the fourth is recorded for Britain for the first time. The generic name *Ceratocystis* has been adopted as an earlier synonym of *Endoconidiophora*. The life-histories of the fungi are described on agar and on wood.

The phenomenon of mutual aversion between monoascospore and monoconidial mycelia in *C. coerulescens* has been observed. This fungus as well as *C. longirostellata* and *C. galeiformis* have been found to be homothallic. *C. wilsoni* is a heterothallic species. The role of *C. pini* in the blueing of conifers in Britain

is considered as doubtful. On the other hand, *Leptographium lundbergii* isolated from blued pine boards as well as from ambrosia beetle galleries has been found to be a severe blueing agent. *C. coerulescens* is a moderately strong and *C. piceae* and *C. galeiformis* are mild blueing agents. *C. longirostellata* produces a blackish grey discoloration on wood.

The author is grateful to Dr. Malcolm Wilson under whose guidance the work was carried out in Edinburgh. His sincere thanks are due to Professor Sir W. W. Smith for the Latin diagnosis of the new species and to Mr. E. W. Mason for suggesting the generic name *Ceratocystis* as the appropriate one to use and for advice on its nomenclature. All the cultures described in this paper have been deposited at the Commonwealth Mycological Institute, Kew; Forest Products Research Laboratory, Princes Risborough; and Botany Department, Edinburgh University.

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Plate I

Ceratocystis coerulescens (Münch) Bakshi

Fig. 1. 15 days old culture on agar. (reduced.)

Fig. 2. Do. enlarged, showing perithecia. (× 3.)

Fig. 3. Do., showing sclerotia and endoconidiophores with liberated endoconidia at their apices. (× 80.)

Fig. 4. A mature perithecium in water, with spore tendril. (× 78.)

Fig. 5. Culture on Scots pine, 2 months old. White spore globules on top of perithecial necks can be seen. (× 3.)

Fig. 6. Longitudinal radial section of Scots pine inoculated with *C. coerulescens*, showing hyphae in the rays. (× 115.)

Fig. 7. Pairing of different monoascospore cultures from different perithecia showing mutual aversion.

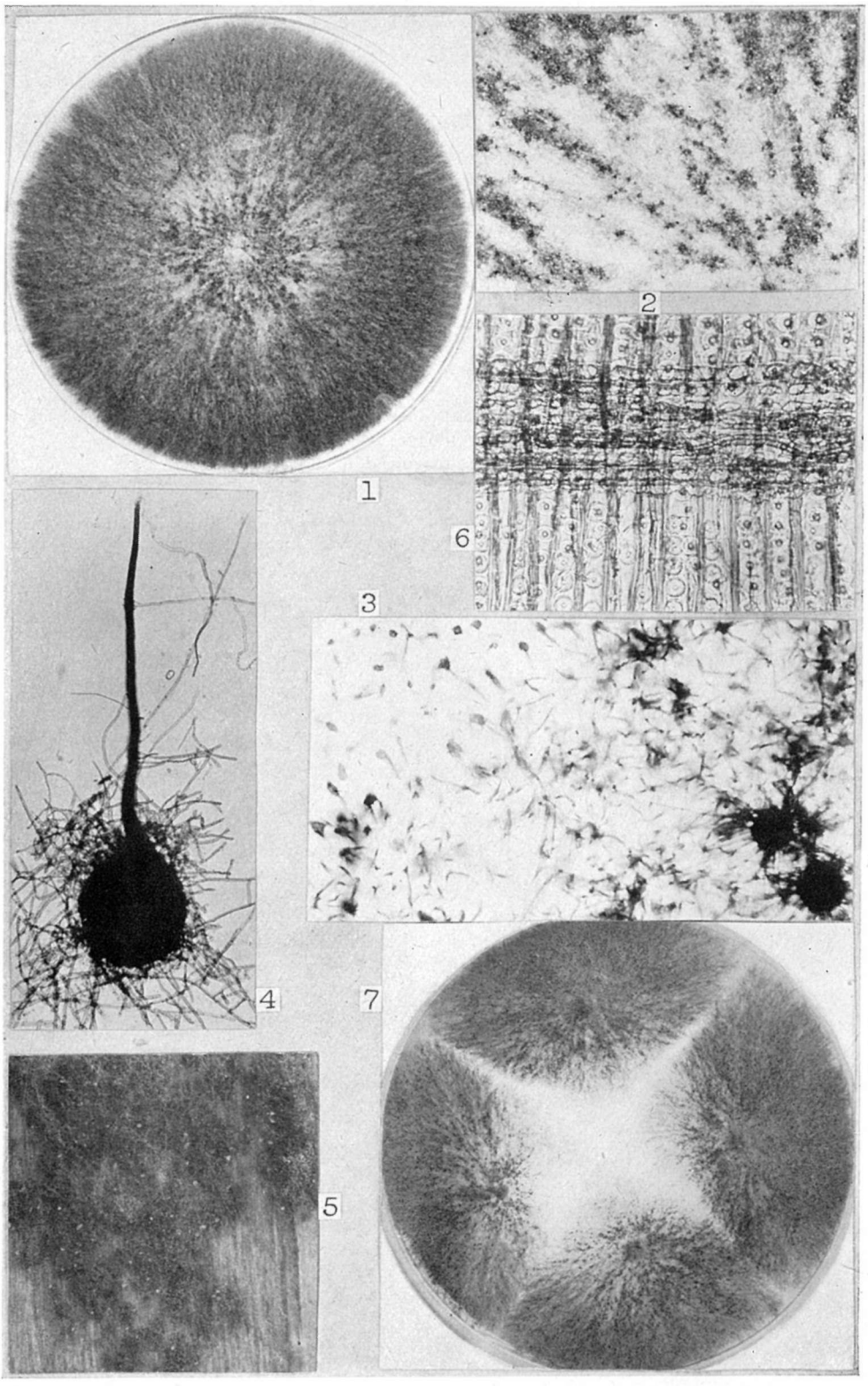
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Plate II

Ceratocystis wilsoni sp. nov.

Fig. 8. 3 weeks old culture on agar. (reduced.)

Fig. 9. Do. enlarged, showing perithecia and spore globules at ostioles. (×4.)

Fig. 10. Young perithecium. Note bristles which are on the upper surface not in contact with agar. (×124.)

Fig. 11. Do. The lower surface of perithecium is focused. Bristles are out of focus. (×124.)

Fig. 12. A mature perithecium. Note the collar at the base of the neck. (×58.)

Fig. 13. Pairing of two monoascospore cultures of different strains. Perithecia formed at line of union.

Fig. 14. Do., the line of union magnified. (×3.)

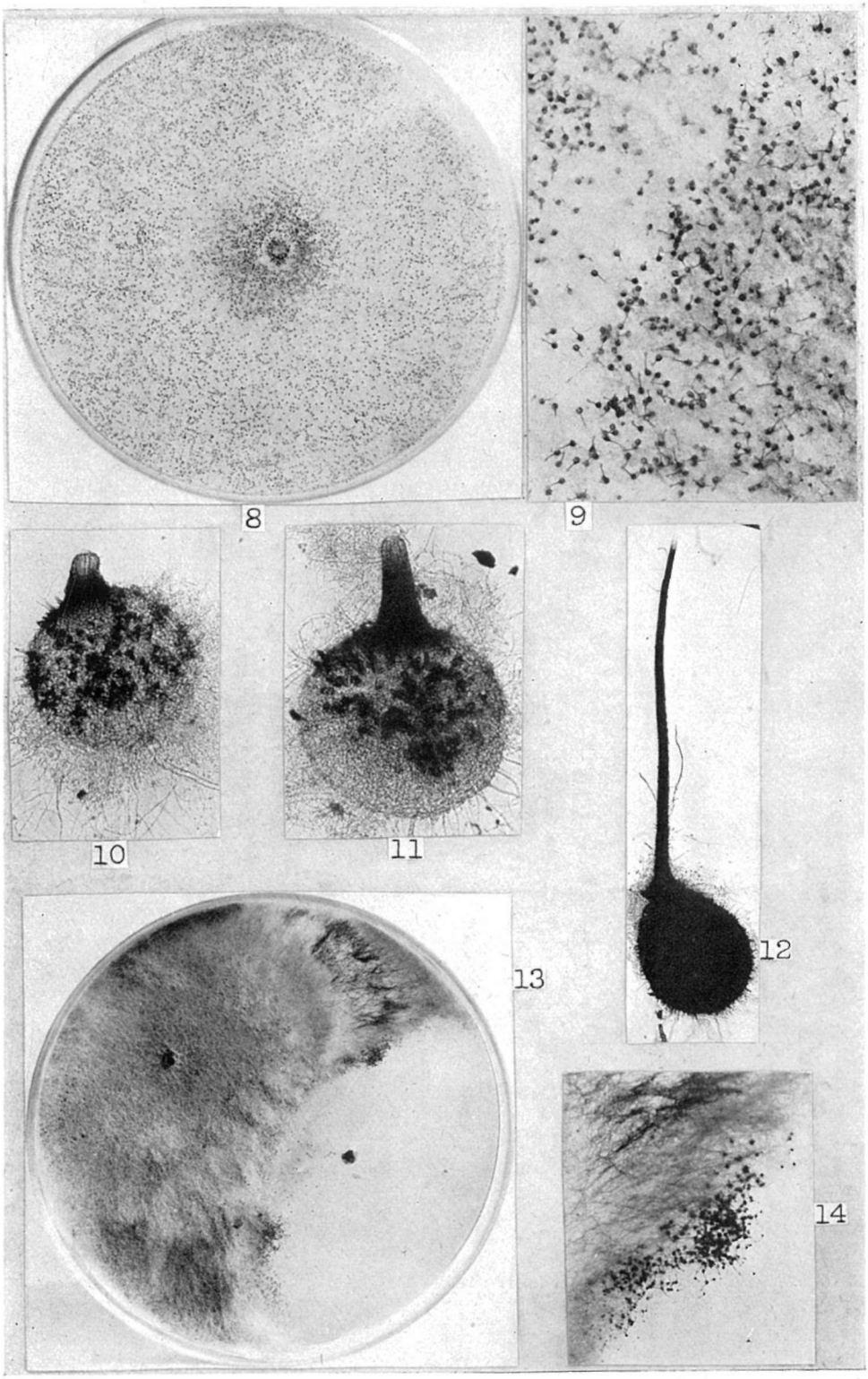
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Plate III

Ceratocystis longirostellata sp. nov. (figs. 15–18) and *Ceratocystis galeiformis* sp. nov. (figs. 19–22)

Fig. 15. 1 month old culture on agar. (reduced.)

Fig. 16. A young perithecium. Note the ostiole. (×220.)

Fig. 17. A mature perithecium. (×48.)

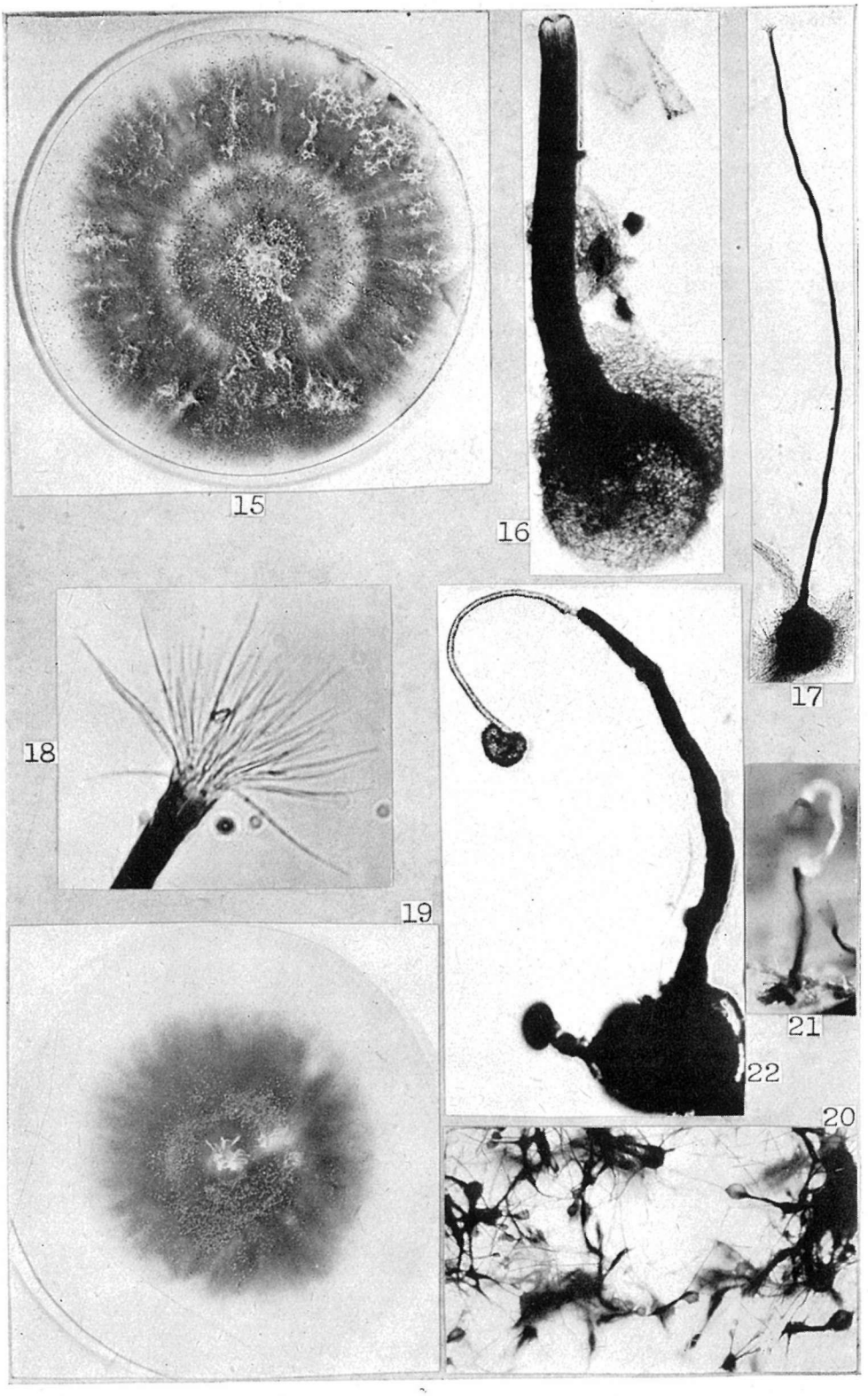
Fig. 18. Top of perithecial neck showing crown of cilia. (×360.)

Fig. 19. 3 weeks old culture on agar. (reduced.)

Fig. 20. Do. magnified, showing conidiophores of *Leptographium* and *Graphium* forms. (×12.)

Fig. 21. Perithecial neck showing spore tendril at the ostiole. Perithecial base hidden in wood frass. Photographed under bark. (×45.)

Fig. 22. A mature perithecium from bark in water with spore tendril. (×110.)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