

THE LEPTOGRAPHIUM COMPLEX. PHIALOCEPHALA GEN. NOV.<sup>1</sup>

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**Abstract**

Some *Leptographium*-like hyphomycetes are maintained as distinct from that genus on the basis of spore ontogeny, and a new genus, *Phialocephala*, erected for them.

This paper is the first of a series comprising a taxonomic treatment of a poorly known but important group of hyphomycetes, which are often associated with disease in forest trees, and have usually been lumped together under the generic name *Leptographium* Lagerb. and Mel. (1928). This complex of fungi is characterized by the possession of a highly evolved, darkly pigmented, often massive conidiophore, in which a mononematous stipe bears at its apex a sporogenous apparatus consisting of one to several multiplicative series of parallel or slightly divergent metulae. Each metula of one series bears from one to four of the metulae comprising the next higher series, and each of the ultimate metulae bears sporogenous cells in the same ratio. Thus, assuming the multiplication factor of each series to be approximately three, a conidiophore with five series of metulae will bear about  $3^6$  or 729 sporogenous cells. Each sporogenous cell may produce a considerable number of more or less hyaline amerspores, and the products of all the sporogenous cells accumulate round the sporogenous apparatus in a milky drop of slime. A single conidiophore may produce many thousands of spores, which are presumably distributed by the various small animals which come in contact with the spore drop.

It has become clear from examination of the type species of *Leptographium*, *L. lundbergii*, that the sporogenous cells of this species are annellophores, and that this genus therefore belongs to Section III of the experimental classification proposed for the hyphomycetes by Hughes (1953). The fungi considered in this paper, while possessing a conidiophore very similar in construction and general appearance to that of the true *Leptographium*, have been found to produce phialides as their sporogenous cells instead of annellophores. This places these fungi in Section IV of Hughes' scheme, and the erection of a separate genus for such phialide-bearing species becomes necessary.

***Phialocephala* Kendrick gen. nov.**

Fungi imperfecti, hyphomycetes dematiaceae.

Conidiophorae erectae, solitariae vel raro fasciculatae. Stipites mononematei, dematiacei. Apparatus sporogenous obconicus complexus in apice stipitis natus, ex seriebus 1-pluribus metularum, distaliter phialides ferentes. Phialides saepius strophium gaudentes. Phialosporae parvae, unicellulariae,

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saepius hyalinae, ex phialidibus in capitulum mucosum successive extrusae.

*Habitat*: Saepius in ligno et cortico putrescentibus, vel isolata ex ligno condito vel arboribus viventibus.

*Species typica*: *P. dimorphospora* Kendrick sp. nov. (vide infra).

*Other species*: *P. bactrospora* Kendrick sp. nov. (described below).

Fungi imperfecti, dematiaceous hyphomycetes. Conidiophores are darkly pigmented, solitary or occasionally clustered, with a single stipe bearing at its apex a complex sporogenous head. This consists of from one to several multiplicative series of metulae, the ultimate series of which collectively bear numerous phialides. The phialides often possess well-marked collarettes, and produce numerous small, usually hyaline, atherospores, which often become aggregated around the sporogenous head in a drop of slime.

*Habitat*: Often on decaying wood or bark, or isolated from worked wood or living trees.

*P. dimorphospora* has been chosen as the type species because of its unmistakable phialides and its well-differentiated conidiophore.

***Phialocephala dimorphospora* Kendrick sp. nov.**

In sylvestris, **conidiophorae** inconspicuae, erectae, sparsae, raro fasciculatae. **Stipites** mononematei, parietibus crassis, basi fusci, apicaliter pallescentes, 2-5-septati, 19-81  $\mu$  long., 6.7-10.1  $\mu$  crass. ad basas, 4.9-6.7  $\mu$  ad summas. **Caput sporogenum** obconicum complexum, 30-70  $\mu$  long., in apice stipitis natum, ex seriebus 2-5 metularum distaliter ferentes phialides. **Metulae** primariae saepius geminae, pariete crasso, cum stipite concolores, 8.0-16.2  $\times$  3.5-5.4  $\mu$ . Metulae secundariae saepius 3-7, pariete tenui, pallidioribus, 8.0-13.5  $\times$  2.7-3.8  $\mu$ . Tertiariae saepius 15-20, subhyalinae, 6.7-13.5  $\times$  2.4-3.2  $\mu$ . Quaternariae plurimae, hyalinae, 5.4-9.4  $\times$  2.2-3.0  $\mu$ . **Phialides** compactae, hyalinae, subcylindraceae, ad medias crassiores, ad summas strophium elongatum cylindricumque gaudentes. Strophium 3.8-5.4  $\times$  2.3-2.8  $\mu$ . Phialides 10.8-17.5  $\times$  2.5-3.1  $\mu$ . **Phialospora** primaria ovoidea, 3.6-5.4  $\times$  2.3-2.8  $\mu$ , alterae subsphaericae 1.7-2.4  $\times$  1.7-2.4  $\mu$ . Phialosporae laeviae, hyalinae, glutinosae, in catenis pertinacibus extrusae, et in capitulum mucosum congregantes.

In vitro, coloniae lente crescentes, diam. aetate 15 dierum 4 cm in agaro 'malt' dicto, 3.5 cm in agaro 'potato-dextrose' dicto. Vitrum mensibus duobus tectum. Coloniae in ambis solis consimiles aspectu. Mycelium aerium coactile, a 'deep mouse gray'<sup>2</sup> 'dark mouse gray'. Colonia in tergo 'fuscous black'. Margo subdiffusa, minute striata. Hyphae fuscae, irregulariter sinuosae, a glabris perrugosae, saepius 3.5-5.0  $\mu$  crass. Conidiophorae majores ad 88  $\mu$  long., inconspicuae, ex medio extus natae, minores ex hyphis aeriis natae. Conidiophorae si in vitro minus differentiatas quam in solo, sed phialides phialosporaeque, parum si diversae, phialides 9.4-18.0  $\times$  2.4-4.0  $\mu$ . Phialospora primaria 4.6-6.4  $\times$  1.9-2.7  $\mu$ , alterae 2.0-2.7  $\times$  2.0-2.6  $\mu$ . Apparatus sporogenus in primis subhyalinus, deinde stipite colorato, in aetate omnino coloratus.

<sup>2</sup>Colors cited from R. Ridgway, "Color standards and color nomenclature" (Washington, 1912).

*Typus*: On rotten deciduous wood, one mile south of Manotick, Ontario, November 1960. S. J. Hughes. DAOM 71465(c).

*Other collection examined*: On rotten deciduous wood, Gatineau Park, Province of Quebec. November 1960. S. J. Hughes. DAOM 71381(b).

On the natural substrate the very inconspicuous conidiophores are erect, scattered or rarely in small clusters. The mononematous stipe is thick-walled

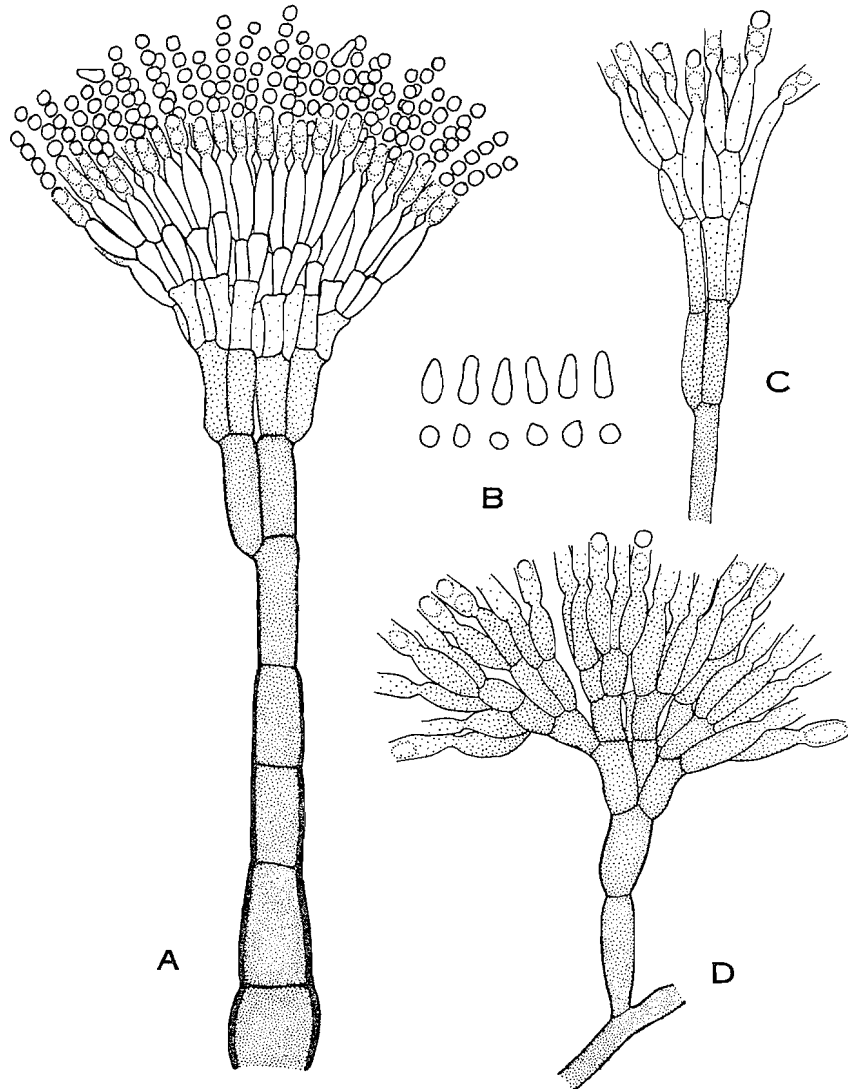


FIG. 1. *Phialocephala dimorphospora*: A, conidiophore and phialospores from host; B, first (ovoid) and subsequent (spherical) phialospores from culture; C, D, conidiophores from culture. All  $\times 1000$ .

and fuscous at the base, gradually becoming paler toward the apex. It is 2-5 septate, 19-81  $\mu$  long, 6.7-10.1  $\mu$  thick at the base, and 4.9-6.7  $\mu$  thick near the apex. The complex obconical sporogenous head produced at the apex of the stipe (Figs. 1A, 5, 6, 8) consists of 2-5 series of metulae ultimately bearing a cluster of phialides. The total length of the sporogenous head ranges from 30 to 70  $\mu$ . The primary metulae usually number 2 and measure 8.0-16.2  $\times$  3.5-5.4  $\mu$ ; they are thick-walled and almost concolorous with the stipe. Secondary metulae, commonly 3-7 in number, are thinner-walled and paler, measuring 8.0-13.5  $\times$  2.7-3.8  $\mu$ . Tertiary metulae, commonly 15-20 in number, are subhyaline, and measure 6.7-13.5  $\times$  2.4-3.2  $\mu$ . The numerous quaternary metulae are hyaline, and are 5.4-9.4  $\times$  2.2-3.0  $\mu$ . The phialides all arise at approximately the same level and are consequently packed together in a tight fascicle at the summit of the conidiophore. They are hyaline, subcylindrical, slightly wider in the middle than at either end, and above a distal construction develop a long cylindrical collarette which measures 3.8-5.4  $\times$  2.3-2.8  $\mu$  (Fig. 5). The entire phialide, including collarette, measures 10.8-17.5  $\times$  2.5-3.1  $\mu$ . The first phialospore, which is formed within the unbroken extension of the phialide, is ovoid, measuring 3.6-5.4  $\times$  2.3-2.8  $\mu$ . After the rupture of the apex of the outer wall, the first phialospore is freed, and subsequent phialospores are almost spherical, measuring 1.7-2.4  $\times$  1.7-2.4  $\mu$ . This accounts for the two types of phialospore to be found in slide preparations of this species (Fig. 1B). The spherical type is naturally much more numerous than the ovoid type (Fig. 7). Up to three of the spherical spores may be found within the collarette of an active mature phialide, and spore chains observed emerging from phialides commonly comprise up to nine conidia. The chains of phialospores are slimy and accumulate at the apex of the conidiophore in a slimy head.

Both collections of *P. dimorphospora* were made on rotten deciduous wood, and both were successfully isolated on laboratory media.

In pure culture at room temperature, colonies spread slowly, attaining a diameter of 4 cm after 5 days on malt agar and 3.5 cm on potato dextrose agar. After 2 months, however, the plate is completely covered. General appearance of colonies on malt and potato dextrose agars is very similar. There is a moderately thick turf of 'deep mouse gray' to 'dark mouse gray' aerial mycelium, while the reverse of the colony appears 'fuscous black'. The margin is slightly diffuse and finely striate. Hyphae are brown, irregularly sinuous, smooth to quite rough-walled, and usually 3.5-5.0  $\mu$  wide. Conidiophores (Figs. 1 C and D, 3, 4) are very inconspicuous in the floccose mycelium. Those forming on aerial hyphae are rather reduced, and those arising directly from the substrate, while more complex and up to 88  $\mu$  long, do not attain the degree of organization characteristic of conidiophores found in nature. The phialides and phialospores, however, retain their characteristic appearance, and their dimensions are little changed, phialides measuring 9.4-18.0  $\times$  2.4-4.0  $\mu$ , primary phialospores 4.6-6.4  $\times$  1.9-2.7  $\mu$ , and other phialospores 2.0-2.7  $\times$  2.0-2.6  $\mu$ . The sporogenous apparatus ranges with advancing age from subhyaline, through a condition in which only the stipe is pigmented, to a final generalized brown coloration of all but the tips of the collarettes.

The second species of *Phialocephala* has not been found fruiting on wood, but has been obtained in pure culture twice, in isolations derived from stained *Tilia* veneers in England, and from *Populus trichocarpa* in Canada.

***Phialocephala bactrospora*** Kendrick sp. nov.

Ex culturis in agaro descripta.

**Coloniae** radiantes, diam. aetate 15 dierum 4.8 cm in agaro 'malt' dicto, 5.6 cm in agaro 'potato dextrose' dicto. In agaro 'malt' dicto mycelium aerium 'chaetura drab', laxe floccosum, densior ad medias, laxissimum ad marginem diffusissimam. **Conidiophorae** plurimae ex medio extus et ex hyphis aeriis natae. **Stipites** mononematei a fuscis, pallide fuscis, pariete tenue, multiseptati, 460–1060  $\mu$  long., 4.0–7.0  $\mu$  crass. **Apparatus sporogenus** in apice stipitis natus, 38–90  $\mu$  long., ex 1–2 seriebus metularum, cum stipite concolores, interdum irregulariter dispositae, atque distaliter phialides ferentes. **Phialides** pallidiores, strophium elongatum

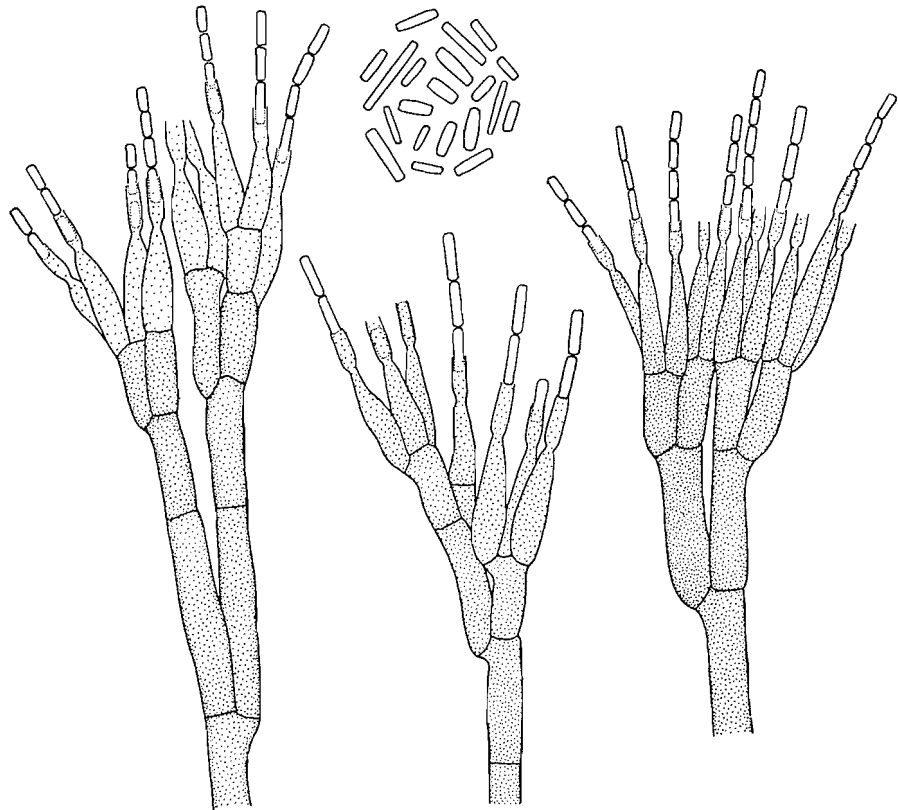


FIG. 2. *Phialocephala bactrospora*: sporogenous heads and phialospores from culture,  $\times 1000$ .

cyndricumque gaudentes. Metulae primariae  $16-48 \times 3.6-5.6 \mu$ , metulae secundariae  $9.8-16.8 \times 3.2-4.6 \mu$ . Phialides  $16.2-27.6 \times 3.5-4.3 \mu$ , strophium  $4.7-7.5 \times 1.8-2.2 \mu$ . **Phialosporae** bacilliformae, hyalinae,  $3.2-10.6 \times 0.7-1.7 \mu$ , in catenis mucosis pertinacibus extrusae. Phialosporae omnes, et primaria et alterae, consimiles. Coloniae si in agaro 'potato dextrose' dicto gaudent mycelium aerium sterile copiosus et conidiophorae perpaucae quam si in agaro 'malt' dicto.

*Typus*: Isolated from *Populus trichocarpa*, Cinema, British Columbia, J. Foster, 1949. Dried cultures DAOM 28736, also Herb. IMI 47572.

*Other collection examined*: Isolated from stained *Tilia* veneers, Wisbech, England, J. G. Savory, August 1952. Herb. IMI 52633, DAOM 63900.

At room temperature colonies are spreading, reaching a diameter of 4.8 cm after 15 days on malt agar and 5.6 cm on potato dextrose agar. On malt agar the 'chaetura drab' aerial mycelium is loosely floccose, being most dense in the center and gradually becoming scantier toward the margin, which is very diffuse. Some fine sterile hyaline aerial mycelium is often found overgrowing the aerial mat of darker hyphae. Numerous conidiophores arise both from the surface of the medium and from the aerial mycelium. The mononematous stipe is medium to light fuscous, thin-walled, polyseptate, from 460 to 1060  $\mu$  long, not tapered and 4.0-7.0  $\mu$  thick. The sporogenous head (Figs. 2, 11) borne at the apex of the stipe consists of 1-2 series of metulae sometimes rather irregularly arranged and usually concolorous with the stipe, and a distal cluster of characteristic phialides which are usually paler in color and possess a long cylindrical collarette (Figs. 9, 10, 11). The total length of the sporogenous head ranges from 38 to 90  $\mu$ . The primary metulae measure  $16-48 \times 3.6-5.6 \mu$ , and the secondary metulae measure  $9.8-16.8 \times 3.2-4.6 \mu$ . The entire phialides, including collarettes, measure  $16.2-27.6 \times 3.5-4.3 \mu$ . The collarettes measure  $4.7-7.5 \times 1.8-2.2 \mu$ . The phialospores (Figs. 2, 12) are bacilliform and hyaline, measuring  $3.2-10.6 \times 0.7-1.7 \mu$ , and are produced in long persistent chains (Fig. 12) which are held together in a minute drop of slime. In this species no differentiation was evident between the first phialospore and those produced subsequently. Colonies on potato dextrose agar differ from those on malt agar in the greater preponderance of sterile aerial mycelium, which imparts a 'smoke gray' color to the mycelial mat, and in the reduced number of conidiophores produced.

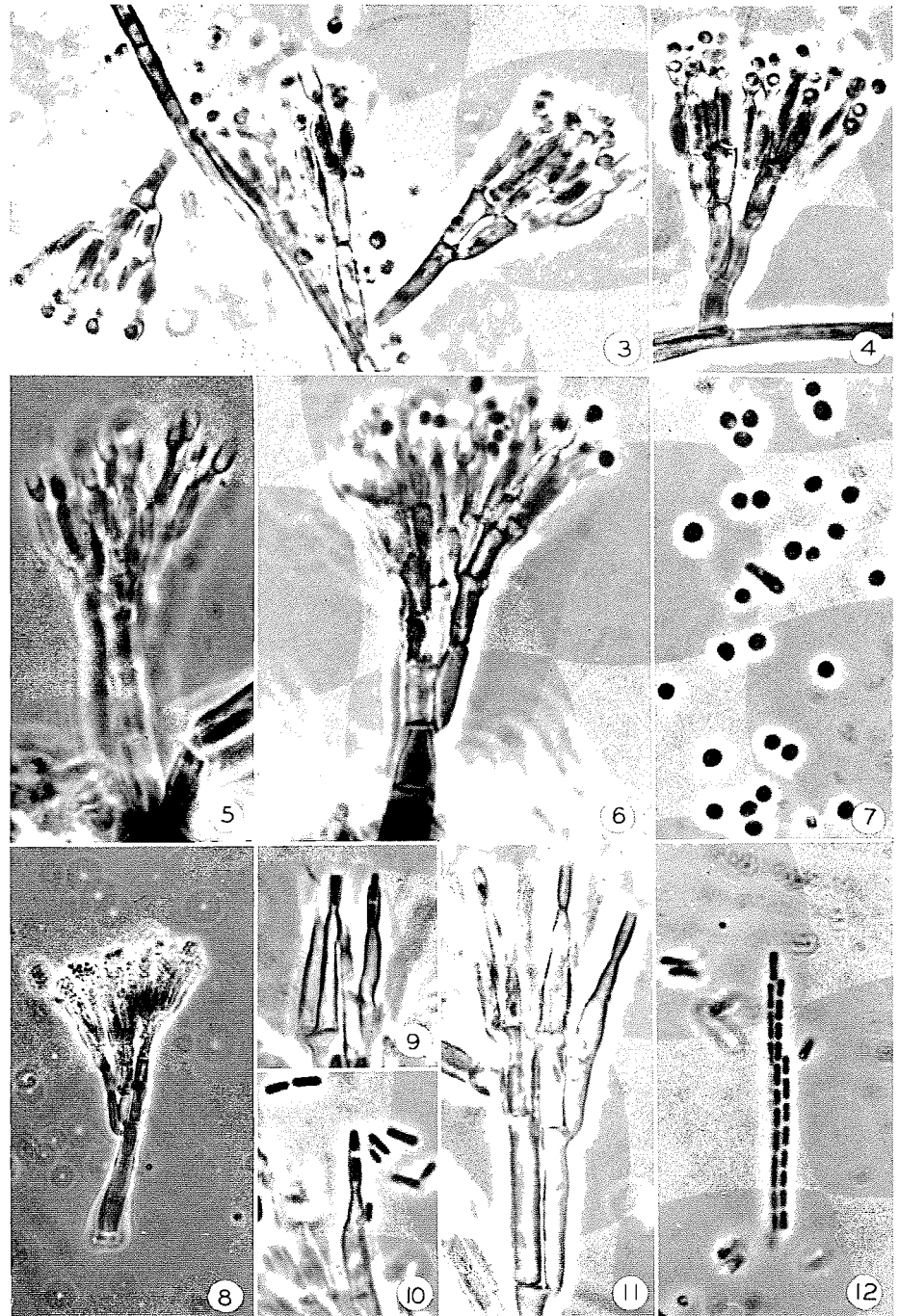
In this paper only two species are placed in the genus *Phialocephala*, but other collections and isolates in Herb. DAOM represent at least eight further undescribed species of *Phialocephala*. However, these are at present represented by single collections only, and it is hoped to publish descriptions of them when additional material becomes available.

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FIGS. 3-8. *Phialocephala dimorphospora*. Figs. 3, 4. Conidiophores in culture,  $\times 1000$ . Figs. 5, 6. Conidiophores from host,  $\times 1000$ . Fig. 7. Phialospores,  $\times 1000$ . Fig. 8. Conidiophore from wood,  $\times 400$ .

FIGS. 9-12. *Phialocephala bactrospora*. Figs. 9, 10. Phialides and phialospores,  $\times 1000$ . Fig. 11. Sporogenous head,  $\times 1000$ . Fig. 12. Phialospore chains,  $\times 1000$ .

All photomicrographs taken under phase contrast.



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LAGERBERG, T., LUNDBERG, G., and MELIN, E. 1928. Biological and practical researches into blueing in pine and spruce. *Svensk Skogsvards. Tidskr.* **25**, 145-272.