

THE LEPTOGRAPHIUM COMPLEX TWO NEW SPECIES OF PHIALOCEPHALA¹

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Abstract

Two new species are described in the lignicolous Hyphomycete genus *Phialocephala* Kendrick, and a key is given to the members of the genus.

All members of the *Leptographium* complex produce a darkly pigmented, mononematous conidiophore which bears at its apex a compound sporogenous apparatus made up of one to several multiplicative series of metulae, the ultimate series of which bears a variable, but often large, number of sporogenous cells. The conidia are slimy amerospores, and accumulate in a mucilaginous droplet around the sporogenous apparatus. Wood is the characteristic substrate of the group, some members being associated with disease in living trees, and many producing blue stain.

Closer examination of this seemingly homogeneous group has shown that three distinct methods of spore ontogeny are exhibited by different species (Hughes 1953, Kendrick 1962). Because methods of spore production are now recognized to be of great importance in the delimitation of major groups in the Hyphomycetes, the erection of segregate genera within the *Leptographium* complex became necessary. The type species of *Leptographium* Lagerberg & Melin (1927), *L. lundbergii*, has sporogenous cells of the type known as annelophores (Hughes 1953). *Verticicladiella* Hughes (1953) was erected for species producing sympodulae, and more recently *Phialocephala* Kendrick (1961) has been proposed for species with phialides. *Penicillium repens* C & E has recently been transferred to *Phialocephala* (Kendrick 1963). In this communication two further new species are described in *Phialocephala* and a brief key is given to members of this genus.

Phialocephala fusca Kendrick sp. nov.

Fungi Imperfecti, Hyphomycetes dematiaceae.

Ex culturis in agaro descripta.

Coloniae in agaro 'malt' dicto radiantes, diam. aetate 15 dierum 7–8 cm, olivaceo-brunneae; margine regularis vel irregularis, diffusa. Zonae concentricae angustae pallidae versus marginem adsunt. Mycelium immersum fuscum. Hyphae septatae, ad 4 μ lat., irregulares, frequenter ramosae et densiter intertextae, interdum excrescentia lateralibus digitata vel inflata gerentes. Mycelium aerium perpaucum vel deest. **Conidiophora** numerosa, mononematea, densissima versus centrum coloniae, sed in margine desunt. **Stipites** ad 300 μ long., ad 10-septati, ad 7.0 μ lat. versus basas, ad 4.4 μ lat. versus summas; ad basas brunnei, saepius apicem versus pallidiores, pariete ad 1.0 μ crass. versus basas, apicem versus attenuato; hyphae rhizoidales paucae vel desunt.

¹Contribution No. 285.

Apparatus sporogenus dilute coloratus, ad 45 μ long., ex 1–3 seriebus metularum distaliter phialides ferentes. Metulae primariae 2–3, ad 16 μ long., ad 4.5 μ lat., saepius angustae ad basas. *Phialides* densae, subparallelae, graciles, laeves, ad 15 μ long., ad 3.2 μ lat. versus basas, gradatim attenuatae versus summas ad 0.8–1.4 μ , insuper abrupte dilatatae in variabile, sed saepius conspicuum, strophium cupulatum vel laxe expansum et laceratum. Interdum phialides externae proliferant. *Phialoconidia* glutinosa, continua, plusminusve ellipsoidea vel ovoidea, interdum paullum curvata, 2.0–4.8 \times 1.0–2.6 μ , saepius hilum basalem exhibentia; a subhyalino dilute fusca, aggregantia et capitulum mucosum nigrum formantia.

In agaro 'potato dextrose' dicto, coloniae radiantes, diam. aetate 15 dierum 7–8 cm. Conidiophora proprio perpauca. Centrum coloniae interdum convolutum; tectum cum cespite humili ex mycelio aereo, a cinereo pallide griseo-olivacea, gerens phialides singulatim vel paullum fasciculata. Zonae concentricae adsunt.

Status perfectus, si occurrit, mihi ignotus.

Typus: siccus ex vitro, DAOM 75852.

The *colonies* on malt agar approximate to 'olive brown'² in color and are spreading, with a diameter of 7–8 cm after 15 days; toward the margin narrow concentric diurnal zones of alternately lightly and heavily pigmented immersed hyphae are present; the margin is very finely fibrillose, slightly diffuse, and usually, but not invariably, regular. The immersed hyphae are brown, up to 4 μ wide, irregular, frequently branched and densely interwoven, sometimes exhibiting swollen or digitate lateral outgrowths. Aerial hyphae are few or absent.

The complex *conidiophores* (Fig. 1) are usually numerous toward the center of the colony, but absent from the margin. The conidiophore stipe is mononematous, and rhizoidal hyphae are sometimes present. The *stipe* is variable in length with a maximum of about 300 μ ; it is up to 10-septate, up to 7.0 μ wide at the base and up to 4.4 μ just below the sporogenous apparatus; it is warm brown in color below, usually becoming somewhat paler toward the apex; the wall is up to 1.0 μ thick near the base, thinner above.

The *sporogenous apparatus* is generally dilute brown throughout; it is up to 45 μ long, excluding conidia, and is composed of one to three series of metulae, the distal series bearing phialides. The primary metulae number two or three, and are up to 16 μ long and 4.5 μ wide, often constricted at their point of origin. *Phialides* are crowded, subparallel, often rather slender, smooth-walled, up to 15 μ long and 3.2 μ wide near the base, tapering gradually above to 0.8–1.4 μ , then abruptly expanding into a very variable, but usually conspicuous collarette, which is sometimes cupulate with margin somewhat involute, but more usually loosely flaring and irregularly split. The extremely expanded and membranous nature of many collarettes (Fig. 1A) is explained by the fact that a number of conidia often accumulate within the apex of the phialide, which becomes correspondingly thin-walled and inflated before rupturing to form the collarette. Unbroken apical vesicles containing numerous conidia have occasionally been seen in slide preparations of this species. Sometimes a few of the peripheral phialides develop lateral outgrowths

²Colors cited from Ridgway, R. Color standards and color nomenclature. Washington 1912.

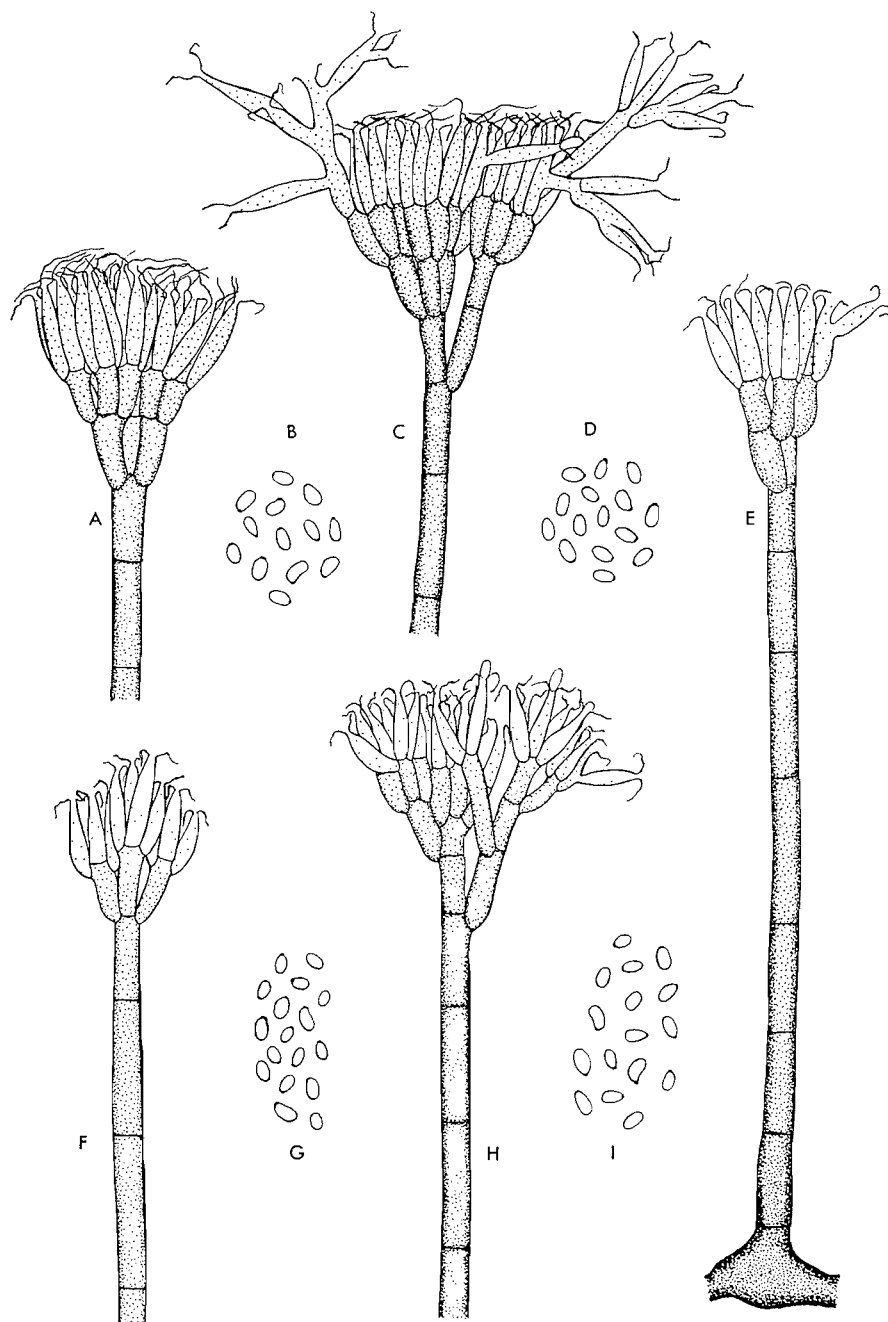


FIG. 1. *Phialocephala fusca*, conidiophores and phialoconidia from culture on malt agar. A-E, from the type, DAOM 75852. Note the very expanded collarettes in A, and the proliferated phialides in C. F-I, DAOM 33774. (All $\times 800$)

which may produce a single secondary phialide, or, by repeated elongation and branching, produce several such phialides (Fig. 1C). The *phialoconidia* are slimy, ellipsoid to ovoid, and sometimes slightly curved; they are continuous, smooth-walled, and measure $2.0\text{--}4.8 \times 1.0\text{--}2.6 \mu$, often exhibiting an abstriction scar on the long axis (Fig. 1B); they are subhyaline to dilute fuscous under the microscope, accumulating around the sporogenous apparatus and forming a spherical, almost black, mucilaginous head. Toward the center of the colony adjacent heads of conidia often coalesce. Isolates vary somewhat in their ability to produce well-developed conidiophores, but their phialides, colarettes, and phialoconidia are always true to type.

On potato dextrose agar, colonies are spreading, with a growth rate comparable to that on malt agar. However, very few conidiophores are produced, and the central portion of the colony is covered by a 'smoke gray' to 'light grayish olive' turf of low growing aerial hyphae bearing phialides singly or in small clusters. This area occasionally becomes irregularly ridged. The concentric zones of pigment variation noted on malt agar are also present on potato dextrose agar. A reddish brown coloration is sometimes observed to be evenly diffused throughout the culture medium.

Phialocephala fusca has been isolated four times in eastern Canada from wood of *Pinus*, *Picea*, and *Acer*, and once in western Canada from wood of *Tsuga*, and an almost certainly conspecific fungus has been isolated from *Bougainvillea* in Sierra Leone. This species has not been found fruiting on the natural substrate, and no associated perfect state is known. Attempts to produce a perfect state by mating the various strains of *P. fusca* in culture have so far been unsuccessful, but are being continued on an extended range of substrates.

Collections Examined

(1) Type: isolated from *Picea glauca* post, Petawawa Forest Experiment Station, Chalk River, Ontario, October 1959, K. Shields, dried cultures DAOM 75852.

(2) Isolated from *Pinus* sp. picket, Barrie, Ontario, June 1956, K. Shields, dried cultures DAOM 75851.

(3) Isolated from *Pinus strobus* roof rafter, Ottawa, Ontario, 19 June 1959, K. Shields, dried cultures DAOM 67995.

(4) Isolated from *Tsuga heterophylla* top rot (77 ft), Victoria, British Columbia, received November 1951, DAOM 33774.

(5) Isolated from rotten wood of *Acer*, Gatineau Park, Quebec, November 1960, DAOM 74598.

(6) Isolated from dead twig of *Bougainvillea spectabilis* var., Njala (Kori) Sierra Leone, 8 February 1954, F. C. Deighton (Herb. Mycol. Dept. Agr. Sierra Leone No. M5615(B), Herb. IMI 56093(b)) dried culture DAOM 63904.

Phialocephala canadensis Kendrick sp. nov.

Fungi Imperfecti, Hyphomycetes dematiaceae.

In vitro, *coloniae* in agar 'malt' dicto lente crescentes, diam. aetate 30 dierum 2 cm, aetate 60 dierum 3–4 cm, fusco-nigrae; mycelium immersum ex hyphis septatis fasciculatis, ad marginem dendriticis. Zonae concentricae desunt. Mycelium aerium saepius deest. *Conidiophora* numerosa, mononematea,

saepius densissima versus centrum coloniae, in margine nata solummodo ex fasciculis immersis. *Stipites* ad 210 μ long., ad 8-septati, ad 4.4 μ lat. ad basas, versus summas ad 3.8 μ lat.; ad basas brunnei, paullulum pallidiores apicem versus, pariete ad 0.6 μ crass. ad basas, apicem versus attenuato. Stipes gerens excrescentia lateralialia sterilia, saepius opposita vel interdum singulatim, nata sub septo et saepius versus apicem stipitis. Excrescentia simplicia, recta, nonseptata, divergentia 30–45°; ad 26 μ long., ad 3.8 μ lat., cum stipite concolora ad basas, rapide pallidiora supra, et hyalina cum pariete attenuato versus apicem inflatum.

Apparatus sporogenus cum stipite concolor, ad 40 μ long., raro ad 55 μ long., ex 1–3 seriebus metularum distaliter phialides ferentes. Metulae primariae 2–4, ad 12 μ long., ad 4.2 μ lat. *Phialides* numerosae, subparallelae, laeves, ad 16 μ long., ad 2.8 μ lat. versus basas, attenuatae versus summas ad 0.8–1.4 μ lat., insuper abrupte dilatatae in variabile, sed saepius conspicuum, strophium cupulatum vel saepius laxum et laceratum. Interdum phialides externae proliferant. *Phialoconidia* glutinosa, transverse ellipsoidea, cum parvo hilo laterali; interdum paullum curvata cum hilo in convexitate; 1.4–2.2 \times 2.2–3.6 μ , dilute fusca, aggregantia et capitulum mucosum nigrum formantia.

In agar 'potato dextrose' dicto, coloniae restrictae, diam. aetate 90 dierum 2 cm. Centrum coloniae tumescens et convolutum, tectum cum cespite humili ex mycelio aereo sterili pallide olivaceo-griseo, sed mycelium haec deest versus marginem. Mycelium immersum fusco-nigrum. Margo coloniae et irregularis et dendriticus. Conidiophora proprio desunt.

In ligno, *coloniae* inconspicuae, olivaceo-nigrae, irregulariter radiantes et tenues, ex *conidiophoris* numerosis quae singulatim vel fasciculatim ex stromatibus minutissimis vel ex hyphis immersis nata. *Stipites* ad 180 μ long., ad 5-septati, ad 5.4 μ lat. versus basas, ad 4.0 μ lat. versus summas; fusci ad basas, paullum pallidiores versus apicem; pariete ad 0.6 μ crass. versus basas, attenuato supra. Excrescentia lateralialia quae ex vitro descripta adsunt in ligno quoque. *Apparatus sporogenus* ad 32 μ long., saepius cum stipite concolor, pallidior supra; ex 2–3 seriebus metularum, distaliter phialides ferentes. Metulae primariae 2–4, ad 12 μ long. et ad 3.8 μ lat. *Phialides* densae et plusminusve parallelae, saepius simplices et laeves, ad 15 μ long., et ad 2.2 μ lat. versus basas, gradualiter attenuatae supra, abrupte dilatatae in variabile sed saepius conspicuum strophium cupulatum vel interdum laxum expansum. Interdum phialides externae proliferant.

Phialoconidia ut in vitro.

Status perfectus, si occurrit, mihi ignotus.

Typus: siccus ex vitro DAOM 71971.

Colonies on malt agar are fuscous black and slow-growing, reaching a diameter of about 2 cm after 30 days and 3–4 cm after 60 days; the immersed mycelium is composed of dark, septate hyphae aggregated in radiating fibrils which become extremely dendritic toward the margin. No concentric zones of pigment variation are present. Aerial mycelium is usually absent. *Conidiophores* (Fig. 2) are numerous, especially toward the center of the colony; toward the margin they are produced only from the immersed fibrils. The *stipe* is mononematous, usually lacking rhizoidal hyphae; its length is up to

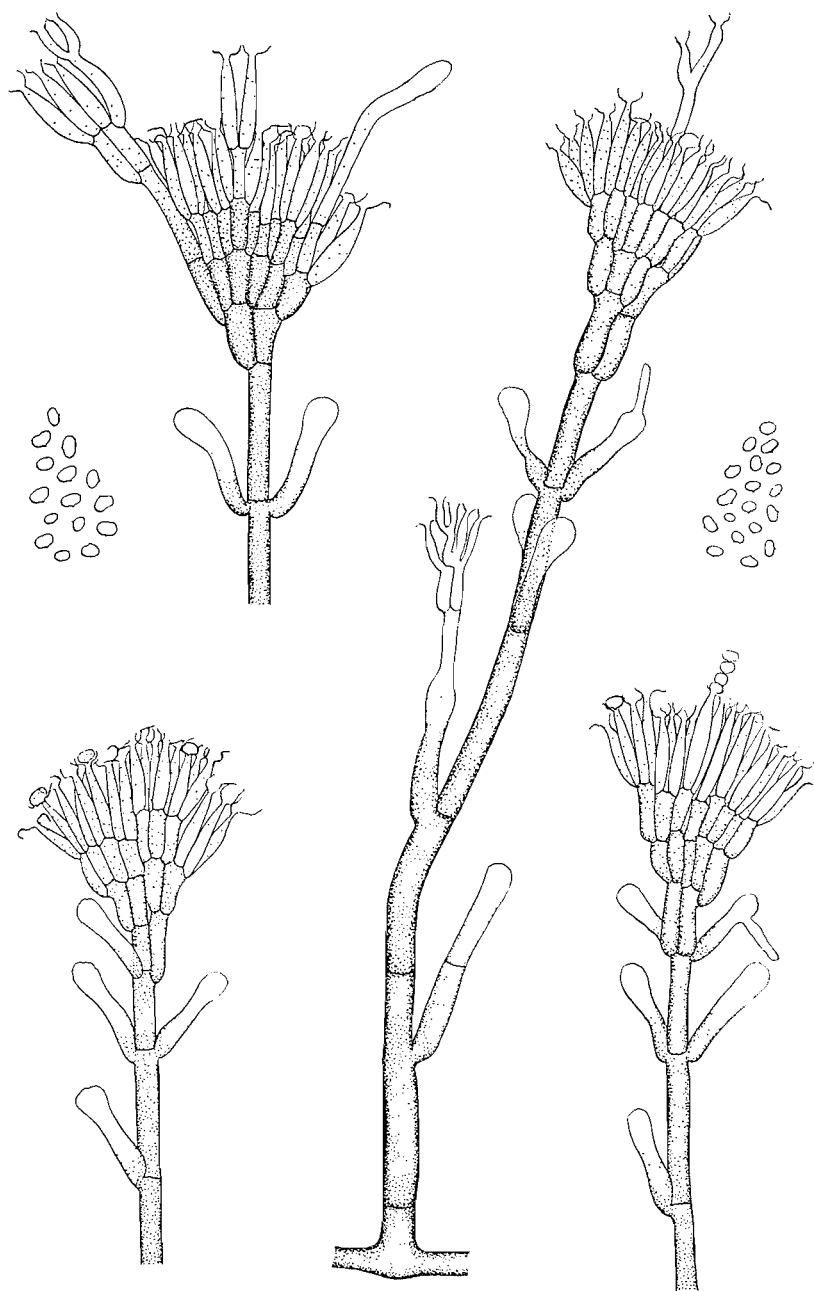


FIG. 2. *Phialocephala canadensis*, conidiophores and phialoconidia from the type culture on malt agar, DAOM 71971. Note the characteristic lateral stipe outgrowths, occasional proliferated phialides, and inconspicuous apiculate abstriction scar on the short axis of the conidia. (All $\times 800$)

210 μ , and it is up to 8-septate; its width is up to 4.4 μ near the base, up to 3.8 μ just below the sporogenous apparatus; it is deep brown in color at the base, very little lighter toward the apex; the stipe wall is up to 0.6 μ thick near the base, usually thinner above. Characteristically, two sterile outgrowths are produced from opposite sides of the stipe immediately below its penultimate septum; frequently one or two further outgrowths develop adjacent to the septum below, and other such outgrowths may often be seen lower on the stipe, or developing alongside the primary metulae. These outgrowths are simple, straight, non-septate, and develop at an angle of 30–45° to the stipe; they are up to 26 μ long, and up to 3.8 μ wide; they are concolorous with the stipe at their point of origin, but become rapidly paler above, and are hyaline and thin-walled at their somewhat swollen, rounded apex.

The *sporogenous apparatus* is usually concolorous with the stipe; it is commonly up to 40 μ , rarely up to 55 μ long, excluding conidia, and is composed of one to three series of closely aggregated metulae, the distal series of which bears phialides. The primary metulae number two to four, and are up to 12 μ long and 4.2 μ wide. *Phialides* are numerous, subparallel, smooth-walled; they are up to 16 μ long, and 2.8 μ wide near the base, tapering above to 0.8–1.4 μ , then abruptly expanding into a very variable but usually conspicuous collarette, which is sometimes cupulate but more usually loosely flaring and irregularly torn, sometimes extremely expanded and membranous. Occasionally a peripheral phialide may develop a lateral outgrowth and produce one or more secondary phialides.

The *phialoconidia* are slimy, transversely ellipsoid, with a small abstriction scar on the short axis; sometimes slightly curved, with the abstriction scar on the convex side (Fig. 2); they measure 1.4–2.2 \times 2.2–3.6 μ ,³ and are individually very dilute brown, accumulating around the sporogenous apparatus and forming a black spherical mucilaginous head which may sometimes coalesce with adjacent heads.

On potato dextrose agar colonies are restricted, with a diameter of only 2 cm after 90 days; the central portion of the colony is raised and convoluted, and is covered with a low turf of 'light grayish olive' sterile mycelium which is absent toward the margin. The immersed mycelium is 'fuscous black' and the margin of the colony is dendritic and irregular. No characteristic conidiophores are produced.

On the host, the inconspicuous 'olivaceous black' *colonies* are irregularly spreading and thin, the visible portion being composed of numerous fairly short, dark *conidiophores*, arising singly or in small groups from minute stromata, or directly from the mycelium, which is largely immersed in the substrate. The *stipe* is up to 180 μ long and up to 5-septate; it is up to 5.4 μ wide near the often somewhat expanded base, tapering slightly and up to 4.0 μ wide just below the sporogenous apparatus; it is deep brown at the base; slightly lighter in color toward the apex; the wall is up to 0.6 μ thick at the

³In order to draw attention to the mode of production of the phialoconidia in *Phialocephala canadensis*, the length of these conidia is being regarded, not as their maximum dimension, but as the distance from base to apex, which in this case is less than their width. Few conidia are usually seen attached to the parent phialide, and the apiculate dehiscence scar of free conidia is often extremely inconspicuous.

base, and up to $0.3\ \mu$ at the apex. The very characteristic lateral outgrowths described in detail from culture are typically present on host material also. The *sporogenous apparatus* is up to $32\ \mu$ long, exclusive of conidia, and may be concolorous with the apical portion of the stipe, or more lightly pigmented, becoming paler above; there are usually 2, rarely 3, series of subparallel metulae, the ultimate series bearing phialides. The primary metulae number two to four and are up to $12\ \mu$ long and $3.8\ \mu$ wide. The *phialides* are crowded and more or less parallel, usually unbranched, and smooth-walled; they are up to $15\ \mu$ long, and $2.2\ \mu$ wide near the base, tapering gradually above, then expanding into a cupulate or flaring collarette which is, however, much less conspicuous and more regular than that produced in culture; secondary branching and extension growth of phialides is occasionally seen. The *phialoconidia* are identical with those produced in culture.

Phialocephala canadensis is in many ways very similar to *P. fusca* described above. However, it can be easily differentiated from that species by the characteristic lateral outgrowths produced on the stipe; by the position of the conidium abstriction scar on the short axis of the spore; and perhaps by its dendritic and very restricted growth in culture. *P. canadensis* has been collected once in fruiting condition on wood in eastern Canada and was successfully obtained in pure culture from that collection. No associated perfect state is known.

Collection Examined

Type: on *Acer* wood, Gatineau Park, Quebec, 25 November, 1960, S. J. Hughes, host material and dried cultures, DAOM 71971.

It is believed that further species will be added to this genus as our knowledge and understanding of the *Leptographium* complex grows. For the present, a key to the described species of *Phialocephala* is set out below. This should facilitate not only the identification of known species, but the recognition of new ones.

Key to the Species of *Phialocephala*

1. Phialides possessing a long cylindrical collarette.....2
1. Collarette not cylindrical.....3
 2. Phialoconidia bacilliform, in long chains.....*P. bactrospora*
 2. Phialoconidia of two types (ovoid and spherical), not bacilliform nor in long chains.....*P. dimorphospora*
3. Collarettes inconspicuous, phialoconidia more or less spherical.....*P. repens*
3. Collarettes conspicuous, often irregular and flaring, phialoconidia not spherical....4
 4. Conidiophore stipe producing sterile, often opposite, lateral outgrowths below the sporogenous apparatus, phialoconidia with abstriction scar on short axis.....*P. canadensis*
 4. Conidiophore stipe lacking lateral outgrowths, phialoconidia with abstriction scar on long axis.....*P. fusca*

Acknowledgments

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