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Cladosporium carrionii n. sp. and the problem of Cladosporia isolated from chromoblastomycosis

by

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HISTORICAL REVIEW

No discussion will be made here of the generic position of *Fonsecaea pedrosoi, F. compacta,* and *F. dermatitidis,* which some authors include in the genus *Hormodendrum* (at present considered synonymous with the genus *Cladosporium***).

I believe these species should be maintained in the genus *Fonsecaea;* and in consequence, only those fungi will be studied here which show sporulation exclusively on *Cladosporium*-type conidiophores and which, like the above species and *Phialophora verrucosa,* have been isolated from cases of chromoblastomycosis.

The problem of *"Hormodendra"* isolated from chromoblastomycosis first came to light with the studies of SIMSON *et al.* (25) and of O'DALY (18). In 1947 CARRIÓN & SILVA (6) already referred to these strains as different from all other known causative agents of chromoblastomycosis, and called them *"Hormodendrum species".* O'DALY (18) did not discuss the taxonomic position of his isolate, merely stating that it sporulated like a *Hormodendrum;* CARRIÓN & SILVA (6) agreed with him on that point.

The same year SIMSON, HARINGTON, and BARNETSON (25) described the first six cases reported from South Africa, and obtained cultures from two of

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** The opinion, that *Hormodendrum* or *Hormodendron* should be considered a synonym of *Cladosporium,* is accepted here, as expressed by VUILLEMIN (31), THOM (29), SKINNER *et al.* (26), LANGERON (14), EMMONS (1), DE VRIES (10), and others.

them. One of the cultures showed the characteristics of *Fonsecaea pedrosoi* (=*Hormodendrum pedrosoi*), as the photomicrographs illustrating their description clearly show. The other strain isolated, of much slower growth than the first, showed the peculiarity of having only *Cladosporium* (=*Hormodendrum*) type of sporulation in six different culture media. The authors also included very good photomicrographs of the conidiophores, and stated this strain had not been classified as yet.

In 1946 SIMSON (24) published six other cases of chromoblastomycosis. He mentioned that, out of a total of 12 cases seen by him, he had obtained cultures from 6, and that out of these 6, three had been determined as *Fonsecaea pedrosoi var. typica**. With regard to the other 3, he referred them first to a species of *Hormodendrum* similar to O'DALY's, but further on he called them *"Fonsecaea pedrosoi var. cladosporium"*. At the beginning of his paper he mentioned the varieties described by CARRIÓN (3-4), including *Fonsecaea pedrosoi cladosporioides,* but later on he wrote *"Cladosporium"* instead of *cladosporoides.* Since there appeared no description of a *Fonsecaea pedrosoi Cladosporium* as a new variety, we must assume SIMSON meant to refer to *F. pedrosoi cladosporioides* and that a typographical error had been made.

In any case, the lack of conidiophores with "radula spores" in the sense used by Mason (pseudo-*Acrotheca* or *Trichosporium* type) and the lack of phialides (even though the presence of the latter is less significant because of their occurrence in some cultures and their absence in others) preclude the inclusion of these fungi in the genus *Fonsecaea* (Negroni 1936), *emend.* Carrión 1940, 1942 *et* 1950. The very *Cladosporium*-type conidiophores illustrated by SIMSON exhibit certain differences, to be discussed later from the *Cladosporium* type conidiophores of the species *F. pedrosoi* and *F. compacta,* and still greater differences from the blastospores in short branched chains reported by CARRIÓN (5), recently in *F. dermatitidis.*

SIMSON, in the same paper (24), reported sending to CARRIÓN the first isolated strain of this species, and receiving from him in a personal communication the opinion that the organism was a species of *Hormodendrum* quite similar to that isolated by O'DALY from a case in Venezuela.

At this point, POWELL (19) published in October 1952 an analysis of 31 Australian cases of chromoblastomycosis, which, added to the 5 cases previously known (13-27) raised the total for the country to 36. From these cases there have been isolated 19 strains, two of which have been classified as *Fonsecaea pedrosoi cladosporioides,* while the other 17, according to POWELL, resemble closely those described by SIMSON in South Africa in that they exhibit only *Cladosporium* (*Hormodendrum*) type conidiophores, of which he published very convincing illustrations.

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* SIMSON, like CARRIÓN, wrote *F. pedrosoi typicus* but, *"varietas"* being feminine, the correct term is as set down above.

POWELL assumed SIMSON to have described *F. pedrosoi var. cladosporium* as a new variety, different from *F. pedrosoi cladosporioides.* As already pointed out, if the former name is not held to be a typographical or ortographical error, at least it must be assumed to be a *nomen ambiguum et dubium.* POWELL, though, raised this ambiguous name to specific rank, making it *Fonsecaea cladosporium.* Apart from the fact that neither SIMSON (24) nor POWELL (19) gave the Latin diagnosis of either the new variety or the new species, the same reasons could be adduced here to conclude that these strains can not be held to belong to the genus *Fonsecaea.* POWELL considered the habitat as the principal argument for the inclusion in that genus; but this criterion has been shown in numerous instances to be misleading, since the same species may give diverse clinical pictures. I recently pointed this out with regard to *Phialophora jeanselmei* (30). Also diverse species may produce a given clinical picture.

Relying, then, as much as possible on botanical criteria, the morphologic uniformity of O'DALY'S strain, SIMSON'S three and POWELL'S 17 leads to the grouping of all of them under one single specific designation, and the only genus which may harbor them without complicating mycological taxonomy is the genus *Cladosporium.*

In a recent publication, CAMPINS & SCHARYJ (2) described 34 cases of chromoblastomycosis, from 24 of which it was possible to isolate a *Cladosporium* (*Hormodendrum*) with the characteristics already noted for the other Venezuelan isolate, and for those from South Africa and Australia. The Venezuelan authors considered those 24 strains as undetermined. Four other strains studied by them were determined as *Fonsecaea pedrosoi cladosporioides,* and two as *F. compacta.*

Holding the ability of these dematiaceous fungi to produce in man the cutaneous granulomatous reaction known as chromoblastomycosis as a biological characteristic with taxonomic value equal to any other's, the possibility was considered of referring these cultures to *Cladosporium langeronii*[*] before proceeding to describe a new species. *Cladosporium langeronii* (Fonseca, Leao *et* Penido, 1927 (12)) Vuillemin 1931 *n. comb.* (31) was isolated from ulcerating lesions along the course of the lymphatics of the arm and forearm, suggesting sporotrichosis; but subsequently has been referred to in the literature by ROTTER & PEÑA-CHAVARRÍA (23), ROTTER (22), CONANT & MARTIN (8) and REDAELLI & CIFERRI (21) as causative agent of chromoblastomycosis.

The investigation of this possibility proved quite difficult. When ROTTER & PEÑA-CHAVARRÍA isolated their strain, they sent it to DODGE, who determined it as *Hormodendrum langeronii.* No culture having been kept in Costa Rica, I requested it from DODGE, who answered (11) that it had been lost. As CONANT & MARTIN (8) mentioned working with it when discussing "*H. langeronii* 282 C.B.S. (Costa Rica)", I wrote to CONANT, who answered (7) that no culture

[*] The species *C. langeronii* was described, and appears in the literature, as *H. langeroni;* but, according to recommendation XL of the Rules of Botanical Nomenclature, it should be spelled - - ii.

remained in his laboratory, and that he had obtained it from the Centraalbureau voor Schimmelcultures. I wrote then to Dr. WESTERDIJK, who answered (32) that in the C.B.S. there was no culture of *H. langeronii* isolated from Costa Rica, nor did she remember there having been any; she offered the Brazilian strain, which I had secured already from the collection of the Instituto Oswaldo Cruz.

In my opinion, the Costa Rican material labelled by DODGE as *H. langeronii* is a strain of *Fonsecaea pedrosoi* similar to the other 43 I have been able to observe in autochthonous cases, while the material CONANT & MARTIN worked with is the Brazilian strain kept at the C.B.S. Even though I was unable to see the Costa Rican strain for the reasons given above, I find enough support for my opinion in the fact that the photomicrographs published by ROTTER & PEÑA-CHAVARRÍA, here reproduced by courtesy of Dr. PEÑA-CHAVARRÍA (figure 1), resemble the *Cladosporium*-type conidiophores of *F. pedrosoi* rather than those of *C. langeronii* (cf. CONANT & MARTIN (8), plate 3, figures 11-15). In addition *C. langeronii,* as we have been able to observe recently, is proteolytic, like the saprophytic species of the same genus, as MONTEMAYOR (17) pointed out, and as DE VRIES (10) recently remarked. The latter author, furthermore, proved that the strain of *C. langeronii* isolated in Brazil by FONSECA, LEÃO & PENIDO (12) is merely a strain of *Cladosporium sphærospermum* Penzig, 1882. Other strains of *C. sphærospermum* have been isolated from nail tissue, from the air, and from diverse plant materials.

In a photograph shown by ROTTER & PEÑA-CHAVARRÍA (*op. cit.*) of a culture in Löffler coagulated serum (figure 2) it is clearly apparent that the Costa Rican strain had no digestive action whatever on this medium. Lastly, the rate of growth of the culture studied by CONANT & MARTIN and its macro and micromorphologic characteristics correspond more closely to the Brazilian strain of *C. langeronii* than to the Costa Rican strain which bore the same name and which, as gathered from the publications of ROTTER and ROTTER & PEÑA-CHAVARRÍA (22-23), grew at a slower rate.

There being, then no similarity between the original strain of *C. langeronii* and those now under consideration, it seems justifiable to establish a new species. The characteristics separating *C. carrionii n. sp.* from *C. trichoides* Emmons, 1952 will be discussed below.

MATERIAL

We have been able to study 4 Australian strains, 2 received through the kindness of Dr. Chester W. Emmons of Bethesda, Md., U.S.A., and 2 obtained through the kindness of Dr. R. E. Powell of Brisbane, Queensland, Australia. They are:

Strain Nº 27. Received from Emmons in 1951 with the label, "*Hormodendrum* from Australia, Nº 8619".

Nº 28. Received from Emmons with the same date and label, with the number 8620.

Nº 35. Received from Powell in December 1952 with the indication, "*Fonsecaea cladosporium,* case A.M., isolated August 1952".

Nº 36. Received from Powell in December 1952 with the indication, *"Fonsecaea cladosporium,* case J.T., isolated in November 1952".

Also, 3 strains from Venezuela, kindly forwarded by Dr. Humberto Campins, of Barquisimeto, as follows:

Strain Nº 40. Received from Campins April 2, 1954, with the annotation, "Cr. 2."

Nº 41. Received from Campins on the same date, *with the annotation,* "Cr. 3".

Nº 42. Received from Campins on the same date, with the annotation, "Cr. 4".

The following species of *Cladosporia* have also been studied for purpose of comparison:

Strain Nº 37. *Cladosporium trichoides.* Received from Emmons in March 1954 with the annotation, "Baltimore 8579. ATCC Nº 10858".

Nº 38. *C. trichoides.* Received from Emmons in *March 1954 with the annotation,* "Pennsylvania, 8580".

Nº 23. *Cladosporium sphœrospermum.* Received from Instituto Oswaldo Cruz in September 1952 with the annotation, *"Hormodendrum langeronii* Nº 1127. *isoado de lesoes nodulares e ulcerosas, semelhando esporotricose. Obs.* Dr. Penido, 1927". This is the original strain of Fonseca, Leao & Penido.

Nº 30. *Cladosporium fulvum.* Received from I.O.C. in September 1952 with the annotation, *"Cladosporium fulvum* Nº 2204 - proveniencia Lab. Dr. Negroni, *Buenos Aires, com a nota* Nº 680 *C. fulvum,* Washington, Dr. Ch. Thom. Date 1/IV/47".

Cladosporium carrionii n. sp.

TAXONOMY

Class: Fungi imperfecti (=Deuteromycetes).

Order: Moniliales (=Hyphales = Hyphomycetes).

Family: *Dematiaceæ.*

SYNONYMY

Hormodendrum species Carrión *et* Silva, 1947 (6).

Fonsecaea pedrosoi var. cladosporium Simson, 1946 (24), *nomen dubium et confusum.*

Fonsecaea cladosporium Powell, 1952 (19), *nomen nudum.*

Hormodendrum species Conant *et al.,* 1954 (9).

GEOGRAPHIC DISTRIBUTION

Of the 46 known isolates of this species, 25 are from Venezuela, 18 from Australia, and 3 from South Africa. Of the Australian strains, 17 correspond to cases already published (19), the 18th. having been communicated subsequently by POWELL (20).

Fig. 1: Conidiophores of the Costa Rican strain isolated by Rotter & Peña-Chavarría, which in our opinion is a strain of *Fonsecaea pedrosoi* but which was classified by Dodge as *Hormodendrum langeronii* (now *Cladosporium sphærospermum*). After Rotter & Peña-Chavarría.

Fig. 2: Culture in Löffler coagulated serum of the Costa Rican strain isolated by Rotter & Peña-Chavarría from a case of chromoblastomycosis. Note no digestion of coagulated serum. After Rotter & Peña-Chavarría.

Fig. 3: *Cladosporium carrionii n. sp.*, Australian strains grown 1½ months on Sabouraud's maltose agar.

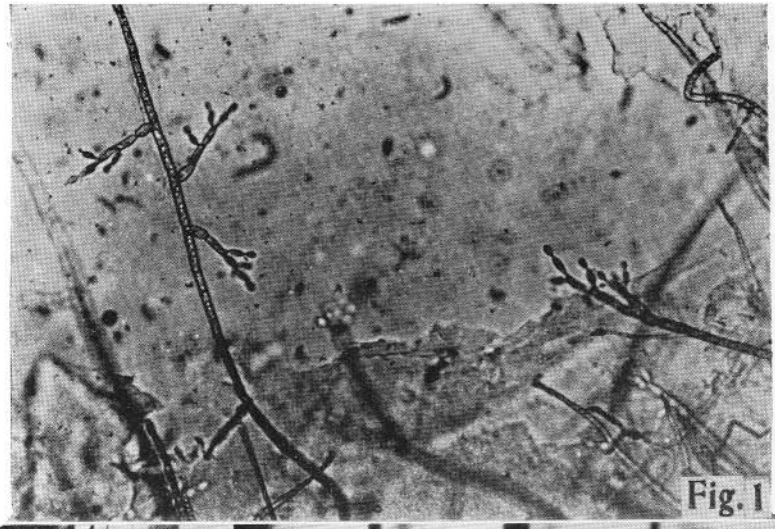


Fig. 1

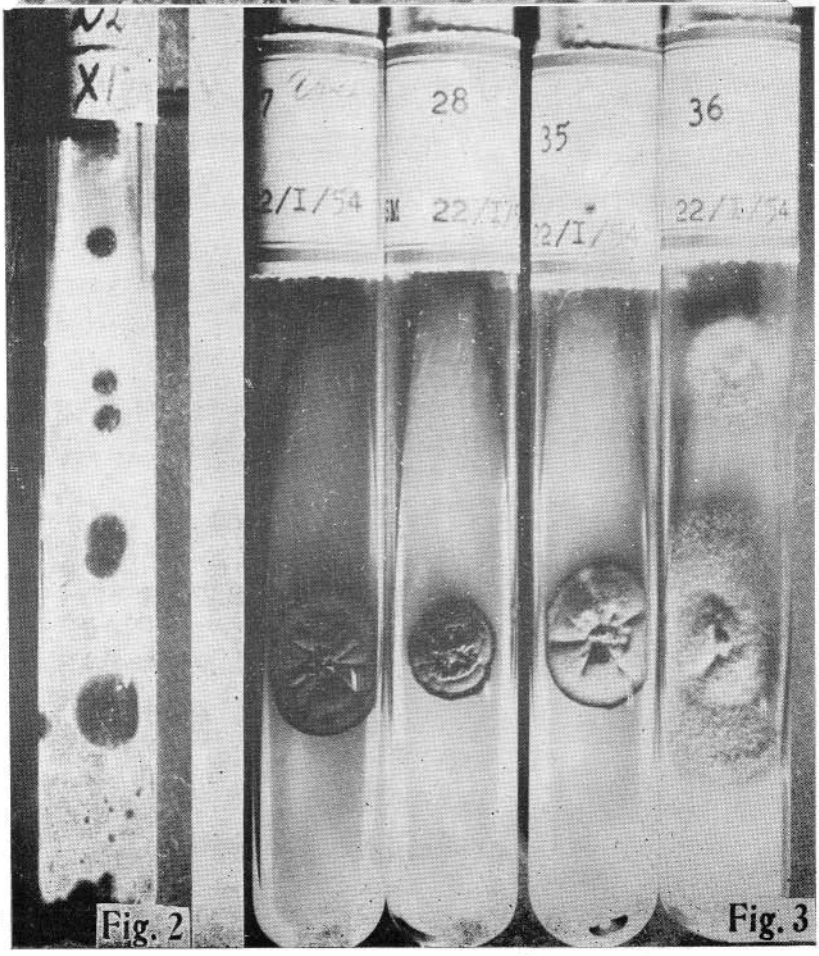


Fig. 2

Fig. 3

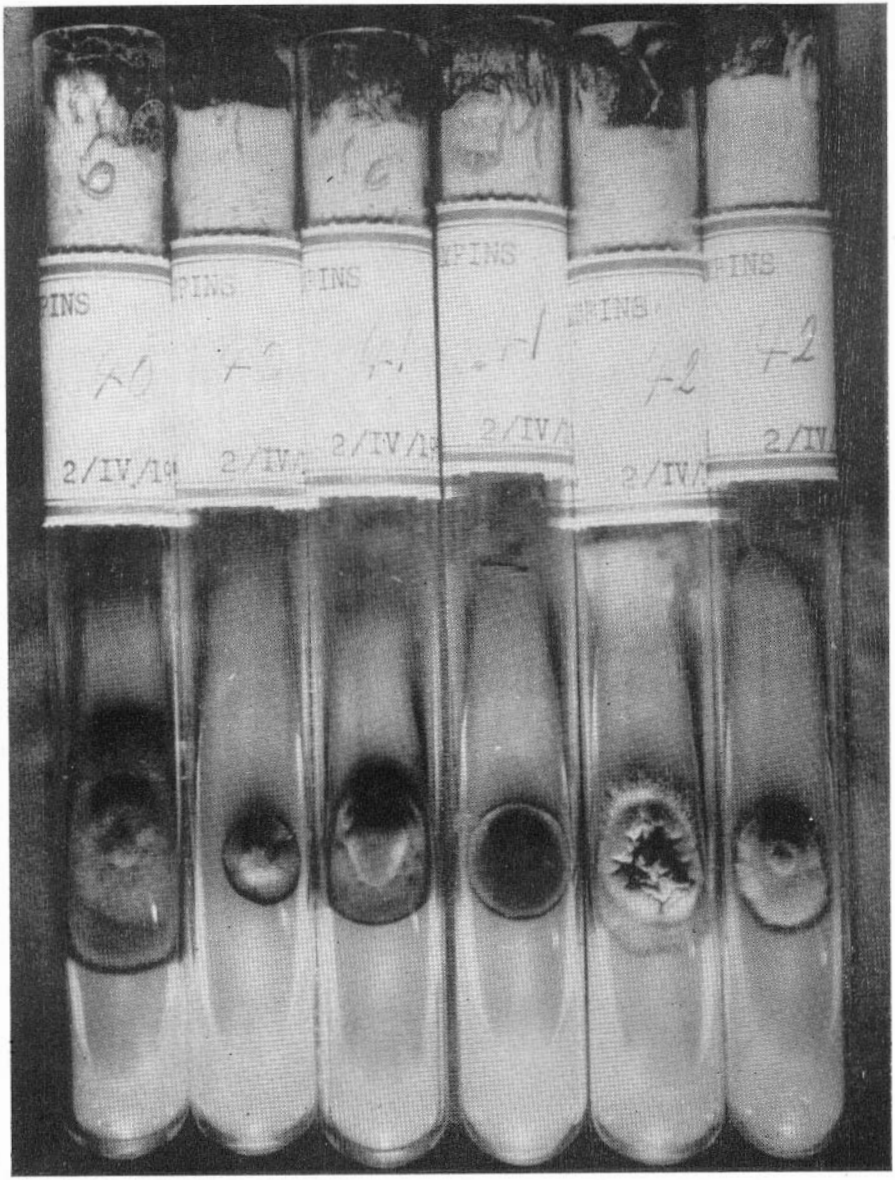


Fig. 4: *Cladosporium carrionii n. sp.* Venezuelan strains. Cultures 7 weeks old. Each of the three strains grown in Sabouraud's glucose agar on the left and on Sabouraud's maltose agar on the right.

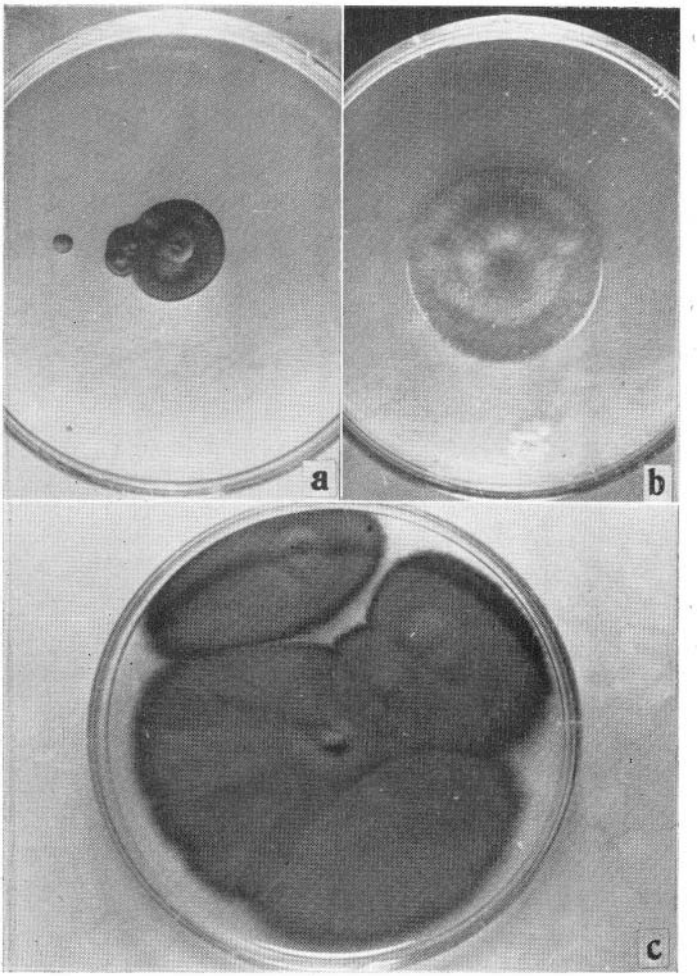


Fig. 5: Thirty-five-day cultures in Sabouraud's glucose agar of a, *Cladosporium carrionii n. sp.* Australian strain; b, *Fonsecaea pedrosoi,* strain received from Instituto Oswaldo Cruz; c, *Cladosporium sphærospermum* (=*Hormodendrum langeronii*), original strain of Fonseca, Leao & Penido, isolated from a case of "mycosis resembling sporotrichosis".

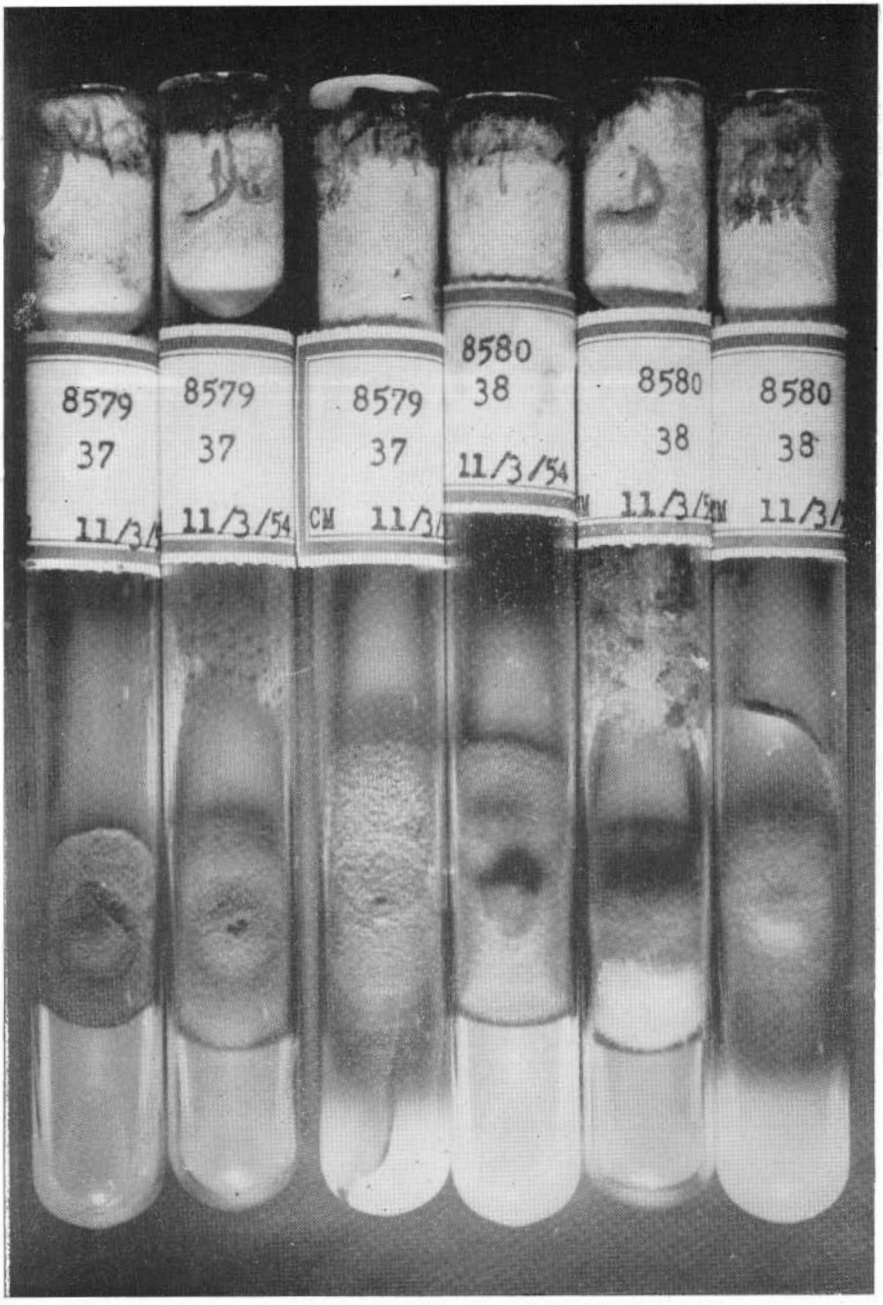


Fig. 6: *Cladosporium trichoides.* Strains received from Emmons. Cultures 1 month old. Aspect of each of the two strains in Sabouraud's glucose agar, Sabouraud's maltose agar, and corn-meal agar.

HABITAT

All the cultures have been isolated from cases of chromoblastomycosis in man. The lesions show no clinical or histopathological differences from those caused by species of *Fonsecaea* or *Phialophora.* As in other known cases of the disease, lesions caused by *C. carrionii n. sp.* are cutaneous granulomatoses. The localization on patients' bodies is variable. The 3 cases reported by SIMSON (24) from South Africa were located on the legs. In Australia, on the other hand, location is preferently on the arms, as is the case in Venezuela according to the studies of CAMPINS & SCHARYJ (2). With regard to sex, the first South African case caused by the species in question and reported by SIMSON, HARINGTON & BARNETSON (25) was on a male. The Australian cases were all on males (19); but the Venezuelan cases were on approximately equal numbers of males and females.

As in the case of other dematiaceous fungi, causing chromoblastomycosis, this species probably exists in nature as a saprophyte on higher plants; but its life cycle is unknown so far.

MACROSCOPIC CHARACTERS

The aspect of colonies varies within certain limits; in some cases it is somewhat smoother in Sabouraud's glucose agar than in Sabouraud's maltose agar. In corn-meal agar the colony is almost wholly submerged.

Some cultures show radial furrows and are umbonate; other have folds and furrows giving them a somewhat cerebriform aspect. There may or may not be a central group of longer hyphae, but in general the aerial hyphae are quite short, giving the colony a furry aspect. The aerial hyphae are scarcely long enough for the surface to be mat and not glossy (figures 3 and 4).

The outline of the colony is usually well defined, with a darker halo of submerged mycelium around it (figures 3 and 4). However, in one of the Australian strains studied, that most recently isolated by POWELL (in December 1952), (figure 3, tube 36) there was no well defined outline, the rate of growth was slightly greater, and superficial hyphae were slightly longer.

The underside of the colony is black and the mycelium penetrates more or less deeply into the culture medium.

Cladosporium carrionii n. sp. grows more slowly than most typical strains of *Fonsecaea pedrosoi* studied, and much more slowly than *C. sphærospermum,* as shown in figure 5, illustrating cultures 35 days old. It also grows more slowly than *C. trichoides,* as may be seen comparing figures 3 and 4 showing, respectively, six- and seven-week cultures of *C. carrionii,* with figure 6, showing four-week cultures of *C. trichoides.*

Two-month-old cultures, depending on the culture medium (Sabouraud's glucose agar, Sabouraud's maltose agar) range from 14 to 40 mm. in their greatest diameter when grown in 18 x 150 mm. tubes at room temperature (25 ± 2°C).

Well-developed cultures show a grayish-brown color ranging in different cultures from "taupe" to "rose taupe", 16A4 and 16A6 of the color atlas of MAERZ & PAUL (16).

MICROSCOPIC CHARACTERS

The morphological characteristics of the fungus in human tissues do not permit distinguishing it from other causative agents of chromoblastomycosis, as may be gathered from the publications of those who observed the clinical cases.

The following characteristics are based on observations of slide cultures on Sabouraud's glucose agar, Sabouraud's maltose agar, and corn-meal agar, incubated for periods ranging from 1 week to 1 month at room temperature (25 ± 2°C). The microscopic aspect of slide cultures varies from one strain to another within bounds which can not be considered of specific rank. The morphologic characters of the strains studied are described collectively, with reference to peculiarities shown by a particular strain where pertinent.

The mycelium is formed by hyphae which may be of several types in a single strain. In general, there are aerial hyphae more or less tinged with a greenish color, or sometimes similar to the fumagoid forms observed in tissues during the parasitic phase. There are also decumbent hyphae, in various proportions with regard to the aerial hyphae and usually lighter in color than the latter. Both types are usually cylindric, uniformly pigmented, smooth-walled, and measure from 1 to 3 μ in diameter, generally 1.5 to 2.5 μ. Hyphae are septate, with septa at variable intervals. In a corn-meal agar slide culture of one of the Venezuelan strains (Nº 41) pigment deposits were observed irregularly scattered at various points on the walls, similar to those described by TAKAHASHI (28) in the fungus he named *Torula poikilospora.* The latter designation I consider a *nomen nudum*, since his description was based on elements in an incomplete stage of growth, which we have observed in the first transfers of diverse strains of chromoblastomycosis fungi whose classification is possible only after normal cultures have been obtained. TAKAHASHI's cultures, in my opinion, should be considered "Ankulturen" and not "Normkulturen", to follow the concepts of APPEL & WOLLENWEBER cited by LANGERON (14).

Besides the more frequent types of hyphae described above, it is possible to find decumbent hyphae with very thick, pigmented walls, formed by cubic or rounded cells (figure 7a). Sometimes such hyphae bear at the tips elements which may be considered analogous to chains of atypical spores (figure 7b). In other instances there may be found elements resembling chlamydospores (figure 7c), which may or may not have one or more septa.

In two of the Venezuelan strains (Nº 40 and 41) there appear with relative frequency some hyphae similar to those described by DE VRIES (10) in *Cladosporium macrocarpum* and which I have also observed in *Fonsecaea compacta* (figures 7d-e). The name of "coralloid hyphae" given by DE VRIES to such elements is most descriptive, but has the disadvantage of not suiting exactly the form of these hyphae, since it connotes growth in all planes and the hyphae

in question are extremely flattened, showing only lateral excrescenses on the plane parallel to the surface of the culture medium. Besides, the name "coralloid hyphae" has been used previously to designate somewhat different structures, such as the rhizoids of *Venturia circinans*. The hyphae termed "coralloid" by DE VRIES seem to have a structure similar to what LANGERON (14) designated generically "mycélium en palmettes", and their function is that of rhizoids or appresoria. In particular circumstances, an aerial hypha will descend until it comes in contact with the surface of the culture medium, from which point it will continue to grow more irregularly, developing lateral prolongations.

After a while there may appear at the tip of the "coralloid" hypha prolongations which again become aerial hyphae (figure 7e).

In Figure 7d septa are visible in "coralloid" hyphae, contrary to DE VRIES' (10) observations in those of *Cladosporium macrocarpum*. Figures 7f-g illustrate the aspect of such hyphae in strain Nº 40 of *C. carrionii n. sp.* from Venezuela.

Besides the chlamydospores observed in the somewhat toruloid decumbent hyphae (figures 7a-c), similar elements were found in aerial hyphae of two Venezuelan strains (Nº 40 - 41) (figures 8a-b). Such vesiculous elements, more or less pigmented and with more or less thickened walls, may be terminal, intercalary, or lateral in the aerial hyphae. Similar structures were also found in strain Nº 40 at the end of a chain of spores (figure 8c) or intercalary on sporophores and on atypical and irregular spore chains (figure 8d). On one occasion I observed what may be considered a chlamydospore linked laterally by a short peduncle to a hypha, and giving rise to a branched chain of spores (figure 8e).

DE VRIES (10) pointed out that true anastomoses are exceptional in the various species of *Cladosporia* studied by him. In Venezuelan strains of *C. carrionii n. sp.* I could not find true anastomoses either, but they do appear in variable numbers in Australian strains, in some slide cultures of which anastomoses were quite frequent, as shown in figure 8f. Besides true anastomoses, I observed, as did DE VRIES, hyphae which come in contact with each other, the walls remaining unaltered, and continue to grow parallel and close to each other without fusing.

A fact which I have not found mentioned in the literature, and which seems to me to have great trascendency with regard to the nature of spores in *Cladosporium,* is the occurrence of anastomoses between hyphae and spore chains (figure 9a) and between two neighboring spore chains (figures 9b-c). Other aspects of such anastomoses, which occur with some frequency in corn-meal agar slide cultures of strain Nº 36, are shown in figures 9d-f.

Sporulation in *C. carrionii n. sp.* occurs only in basifugal, branched spore chains. None of the investigators who have studied strains of this species have been able to find in any culture medium any other type of sporulation besides that typical of the genus. The conidial structures are what DE VRIES (10) called "*Hormodendrum*" type, symmetrical or asymmetrical, and more or less branched.

Sometimes chains of spores are seen to form on tips of hyphae without

Fig. 7: a. *Cladosporium carrionii n. sp.*, strain N° 28. The margin of a month-old microcolony growing on Sabouraud's glucose agar. Note decumbent, thick-walled, pigmented hyphae made up of cubic or rounded cells. Most of the mycelium in this slide consisted of hyphae of this type. Lactophenol, × 450.

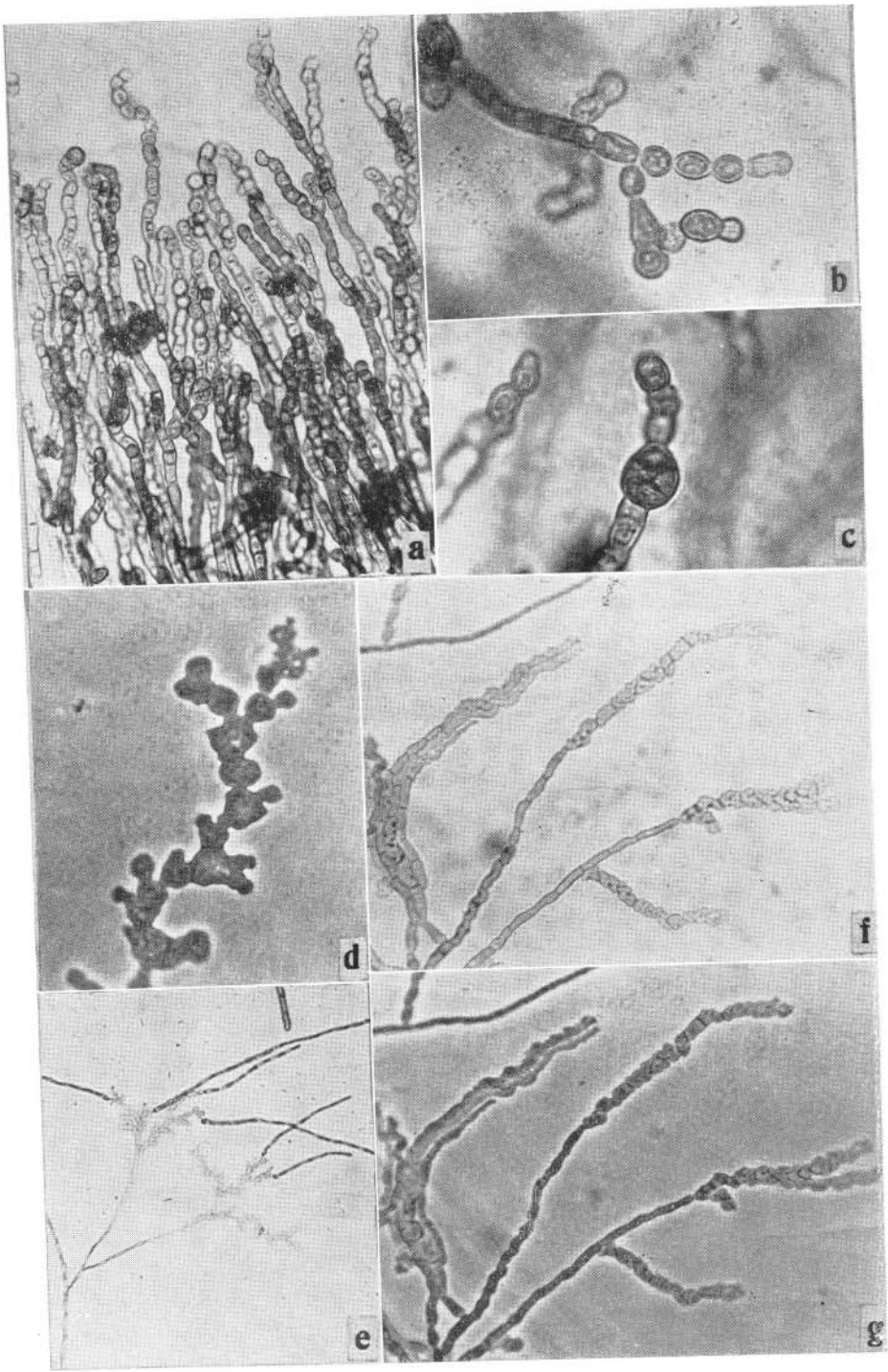
b. *Cladosporium carrionii n. sp.* strain N° 28. Terminal chain of elements which may be considered atypical spores, in a month-old slide culture on Sabouraud's glucose agar. Lactophenol, × 450.

c. *Cladosporium carrionii n. sp.,* strain N° 28. Chlamydospore-like elements observed in the hyhae of figure 7 a. Lactophenol, × 1000.

d. "Coralloid" hypha of *Fonsecaea compacta,* strain 1982 I.O.C. Corn-meal agar slide culture 1 month old. Lactophenol. Phase-contrast, × 1000.

e. Another view of the same preparation shown in d. Note coralloid hyphae formed from an aerial hypha and giving rise in turn to other aerial hyphae. Lactophenol, × 200.

f-g. Ordinary and phase-contrast photomicrographs of "coralloid" hyphae in strain N° 40 of *C. carrionii n. sp.* Sabouraud's glucose agar slide culture one month ol.d. Lactophenol, × 450.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d
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Fig. 8: a-e. *Cladosporium carrionii n. sp.,* strain N° 40, from Venezuela. Sabouraud's glucose agar slide culture one month old. Lactophenol, × 450.

a-b. Terminal and lateral enlargements in aerial hyphae. c. Deeply pigmented, vesicular element, terminal on a spore chain.

d. The same type of elements, intercalary and terminal on irregular sporophores.

e. Lateral, well-pigmented, vesicular element which gave rise to a branched spore chain.

f. *Cladosporium carrionii n. sp.,* strain N° 36, from Australia. Corn-meal agar slide culture, 20 days old. Note numerous anastomoses. Lactophenol. Phase-contrast, × 200.

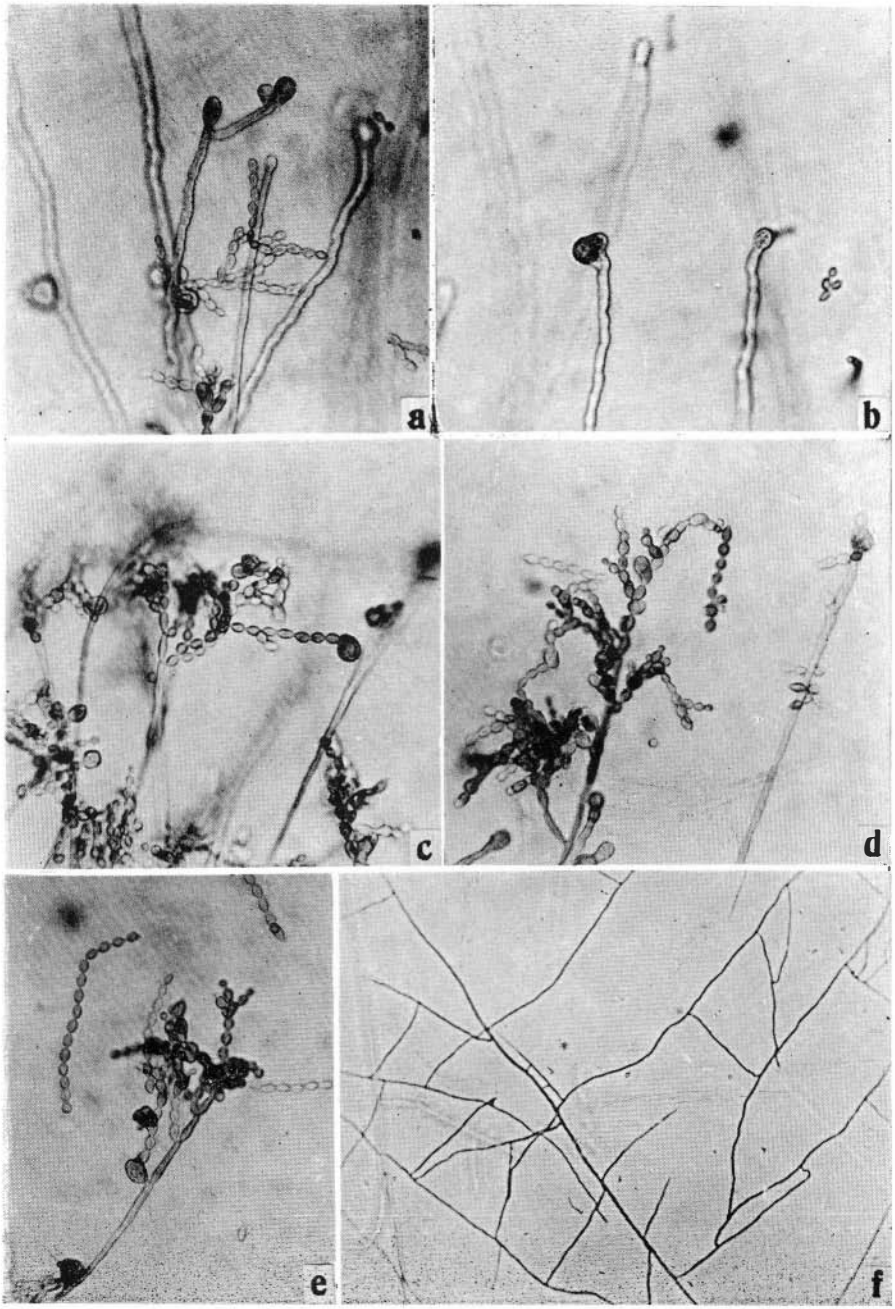
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Fig. 9: *Cladosporium carrionii n. sp.*, strain N° 36, from Australia. Corn-meal agar slide cultures of various ages.

a. Anastomosis between the terminal spore of a chain and a neighboring hypha. Culture 20 days old. Lactophenol. Phase-contrast, × 1000.

b-c. Anastomosis between spores of two neighbor chains arising from the same sporophore. Culture 10 days old. Lactophenol, × 1000. b, ordinary optic; c, phase-contrast.

d. Anastomoses between spore chains, and between spore chains and hyphae. Cultures 30 days old. Lactophenol, × 450.

e-f. Details of photomicrograph d. Lactophenol. × 1000.

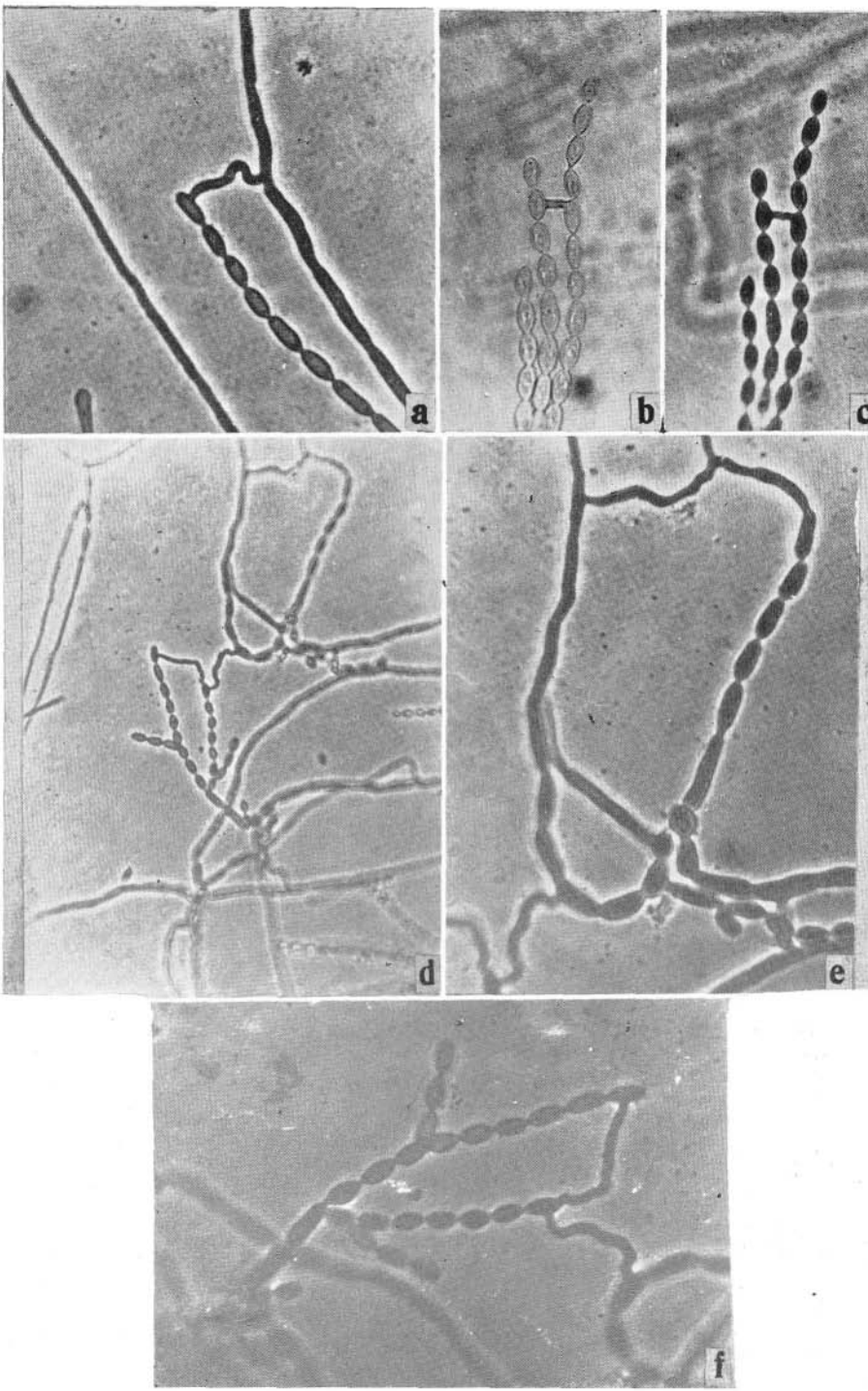
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Fig. 10: *Cladosporium carrionii n. sp.* Strain N° 27, from Australia. Sabouraud's glucose agar slide cultures of various ages. a, b, c, e, g, phase-contrast; d, f, ordinary optic.

a. Acrogenous spore chain. Culture 15 days old. Lactophenol, × 1000.

b. Branched, acrogenous spore chain. Culture 15 days old. Lactophenol, × 1000.

c. Pleurogenous spore chain. Culture 15 days old. Lactophenol, × 1000.

d. A group of sporophores of various lengths, with branched, rather short spore chains. Culture 1 month old. Lactophenol, × 200.

e. A typical sporophore. Lactophenol, × 1000.

f-g. Groups of sporophores. Lactophenol, × 450.

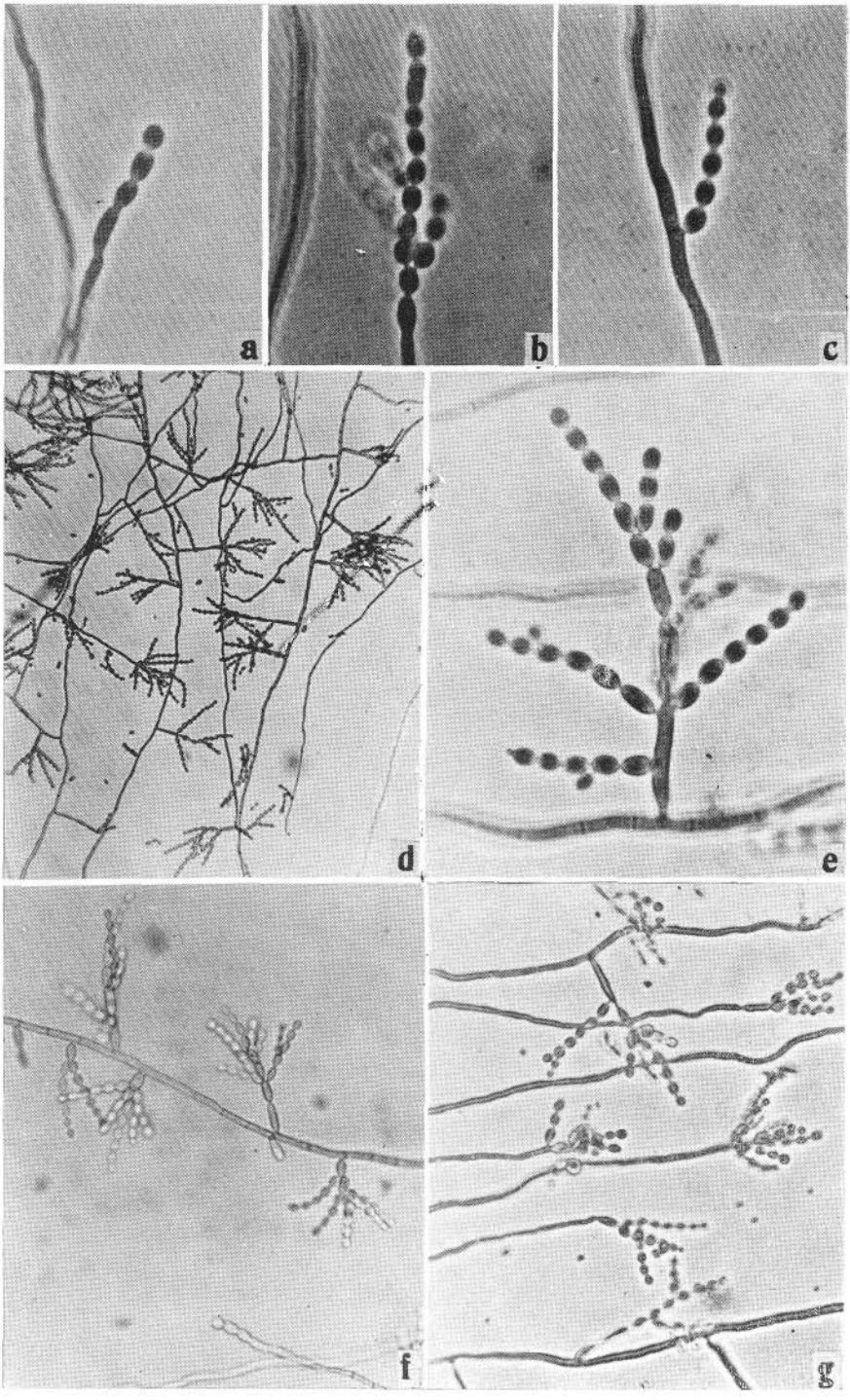
a
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Fig. 11: a, b, c, d. Various sporophores of *C. carrionii n. sp.* strain N° 28, from Australia. Sabouraud's glucose agar slide culture, 15 days old. a, with phase-contrast. Lactophenol, × 450.

e-f. *Cladosporium carrionii n. sp.* Strain N° 36, from Australia. Corn-meal agar slide culture, 10 days old. Phase-contrast. Lactophenol, × 1000.

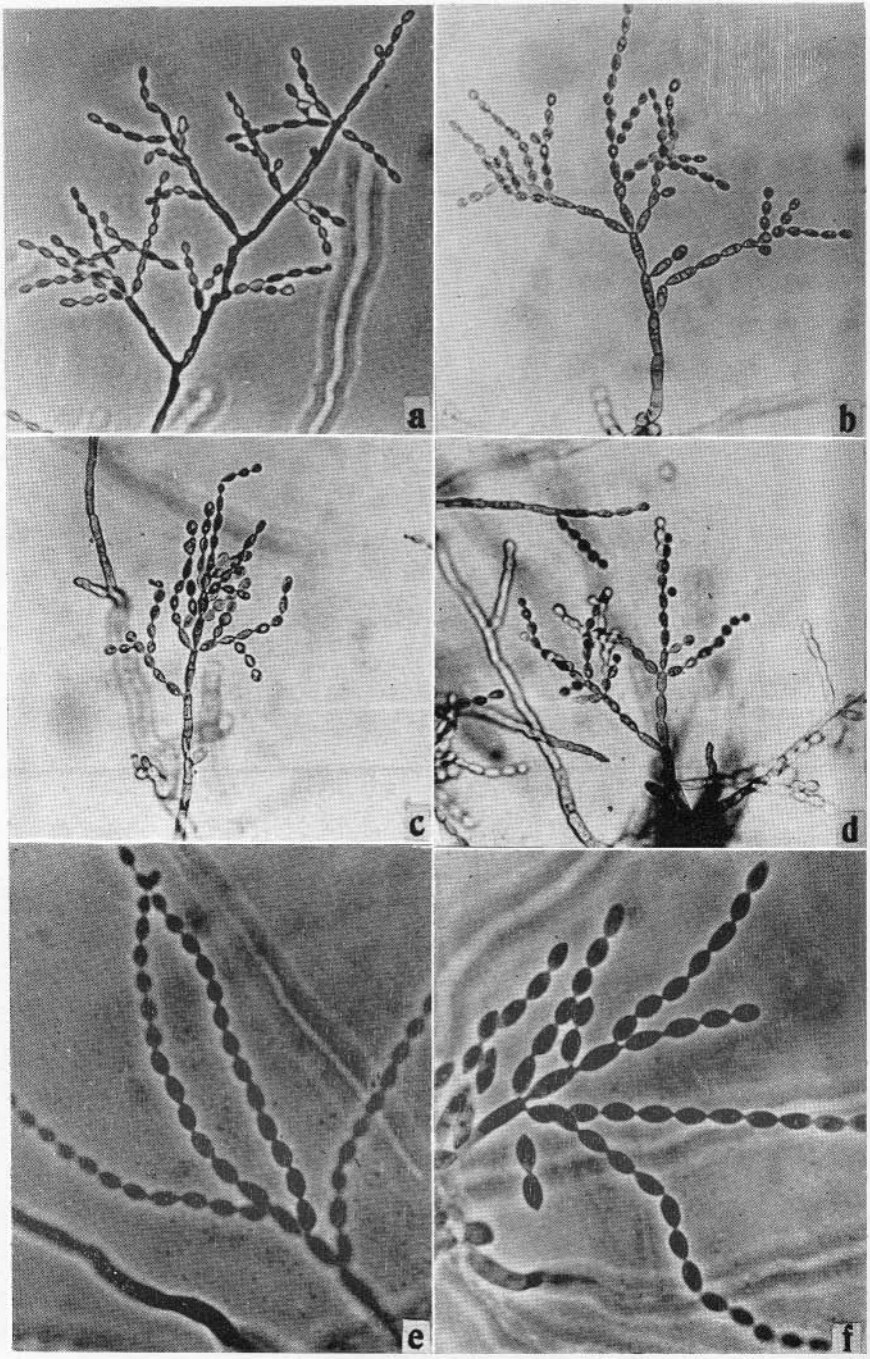
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Fig. 12: *Cladosporium carrionii n. sp.*

a. Strain Nº 36, from Australia. Corn-meal agar slide culture, 20 days old. Terminal and lateral sporophores. Lactophenol, × 450.

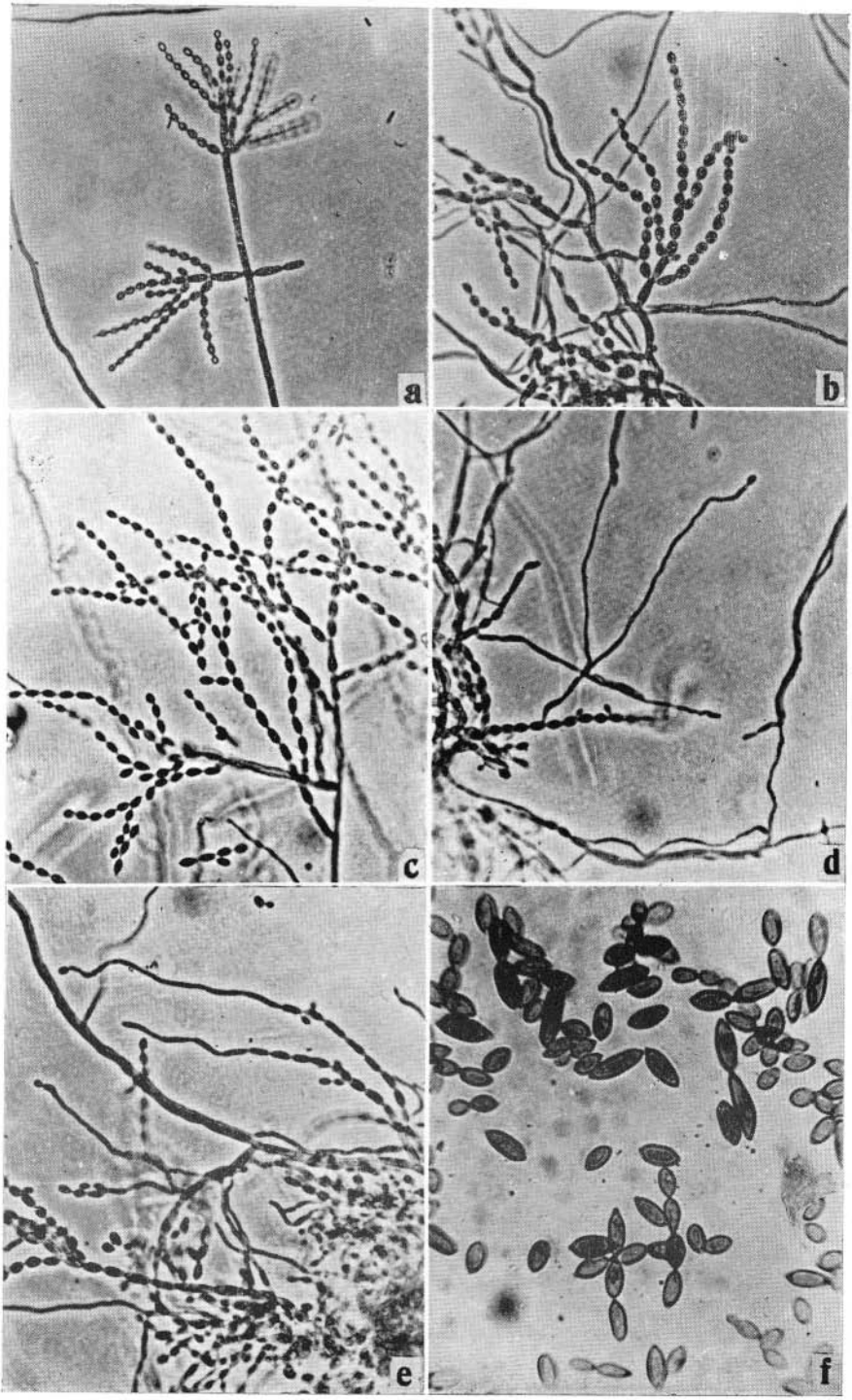
b. Strain Nº 42, from Venezuela. Sabouraud's glucose agar slide culture, one month old. Lactophenol. Phase-contrast, × 450.

c. Strain Nº 40, from Venezuela. Sabouraud's glucose agar slide culture, one month old. Group of sporophores seen with phase contrast. Lactophenol. × 450.

d-e. Strain Nº 42, from Venezuela. Sabouraud's glucose agar slide culture, one month old. Phase-contrast. Lactophenol, × 450.

Note in d a mycelial thread arising from an intercalary spore in an unbranched chain, and bearing a spore terminally. In e, note spore chains whose distal members give rise to mycelial threads some of which bear spores terminally.

f. Strain Nº 27, from Australia. Mature, detached spores from a month-old Sabouraud's glucose agar slide culture, × 1000.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the differentiation of a sporophore. Such spore chains are simple at first (figure 10a) and may branch later on (figure 10b) without any intermediate structures.

There are also more or less elongated and more or less branched sporophores, pigmented like the hyphae from which they originate.

The degree of sporulations is variable in the diverses strains and culture media; in general it is greater in corn-meal agar. Photomicrograph 10d shows a frequent aspect of sporulation in this species. In general, the longer spore chains are, the less branching they exhibit. Unbranched chains have been found with as many as 50 elements.

Small disjunctors are to be seen between spores in almost all cultures, showing like dark dots when the latter are dispersed (figure 11f).

Figures 10-12 illustrate diverse aspects of sporulation in Australian and Venezuelan isolates, which offer no differences worth considering with those of South Africa as shown in SIMSON's (24) excellent photomicrographs.

In figures 12d-e some abnormalities may be seen which I refrain from interpreting. In Sabouraud's glucose agar slide cultures of strain Nº 42 from Venezuela, I have found with some frequency very fine mycelial threads, about 1 μ thick, originating from a spore and often bearing a new spore at the distal end. Such filaments rarely form from intercalary spores (figure 12d); they usually form from the terminal spores of the various chains, those preceding that from which the filaments arise already appearing more elongated than the spores of the proximal part of the same chain.

The shape of the spores varies within rather narrow limits according to the strain and the culture medium employed. In general, they are elliptic or oval (figure 12f); sub-cylindric or irregular shapes are rare, and in some cases sub-sphaeric elements are found. The walls are smooth and more or less pigmented. No typical septate spores have been observed; in the few instances of spores with one septum, the doubt always remained as to their being or not being fragments of a sporophore.

Thus far I have deliberately avoided referring to spores and sporophores as conidia and conidiophores. The nature of the spores in *Cladosporium* (=*Hormodendrum*) is a matter which repeatedly has provoked controversy. DE VRIES did not express a categorical opinion, remarking that the term "blastospore" is incorrect according to LANGERON and MASON. DE VRIES called them "conidia", or simply "spores". LANGERON (14) states that they must be considered thallospores, and, since they break off at the least mechanical disturbance, they must be considered to be dry arthrospores or xerospores. However, it seems difficult to harmonize this concept of arthrospores, forming in basifugal chains by budding of the last element, with the same author's definition of them as "being always formed by disarticulation of the thallus". It does seem, though, that the chains of spores in *Cladosporium* function as part of the thallus, since there must be necessarily a cytoplasmic connection between them. Such a concept is supported by the occurrence of true anastomoses between the chains of spores, and between spores and hyphae, as described above. In any case, it seems best to consider *Cladosporium* as having a particular type of spo-

rulation, and to designate its spores with the term, "thalloconidiospores". LANGERON (14) had already mentioned the "particular case of the Hormodendra" discussing conidiospores.

The dimensions of thalloconidiospores vary in the different strains of *C. carrionii n. sp.* studied. The smaller diameter is fairly constant, ranging from 2 to 3 μ, the most frequent size being 2.5 μ. The greater diameter varies as indicated in figures 13 and 14, which show frequency curves of longitude in μ of, respectively, the four Australian and three Venezuelan strains. The dimensions are seen to be from (3) 4 to 5 (9.5) μ; the means and other statistical constants are shown in Table 1, in which those obtained from two strains of *C. trichoides* are included for purposes of comparison.

TABLE 1

Statistical analysis of the length in micra of spores of seven strains of CLADOSPORIUM CARRIONII *and two strains of* C. TRICHOIDES.

Strain Nº	Species	Mean ± the standard error	Standard deviation	Coefficient of variation	Mode	Median
	C. carrionii					
27	" "	5.10 ± 0.17	1.65	32.35 %	4.47	4.84
28	" "	4.48 ± 0.11	1.08	22.31	4.87	4.89
35	" "	5.16 ± 0.06	0.62	12.02	5.51	5.48
36	" "	4.99 ± 0.09	0.85	17.03	5.32	5.21
40	" "	4.73 ± 0.08	0.81	17.12	4.65	4.76
41	" "	4.97 ± 0.10	1.01	20.32	4.89	5.15
42	" "	4.89 ± 0.08	0.76	15.54	5.33	5.23
37	*C. trichoides*	6.93 ± 0.18	1.78	25.68	7.28	7.27
38	" "	7.16 ± 0.22	2.18	30.44	6.75	7.61

DE VRIES' (10) methods have been followed in the statistical analysis of spore dimensions, as I think all future studies of new species of the genus *Cladosporium* must have as a point of reference his magnificent work which has come to fill an urgent need already recognized by EMMONS (1) when he described *C. trichoides.* One hundred spores were measured of each of the seven strains of *C. carrionii n. sp.* and of each of the two strains of *C. trichoides.* Measurements were made with a 45x objective and a 10x ocular with an eyepiece micrometer calibrated with a stage micrometer.

Statistical significance of the differences between means was evaluated with basis on the 99% probability interval, observing whether the difference

between means falls within that interval or not— i. e., observing, as DE VRIES did, whether the difference between two means is or is not at least 3 times greater than the standard error of that difference. In table I are shown, in addition to the mean spore length ± the standard error, the standard deviation and the coefficient of variation; and to complete the data, the median and the mode. DE VRIES did not include in his tables the median and the coefficient of variation, but I place them on record for the usefulness they may have in the future for purposes of comparison.

Analysis of the data obtained leads to the following conclusions:

1. Comparing the two most divergent mean values for strains of *C. carrionii n. sp.*, 4.73 ± 0.08 μ for Nº 40 and 5.16 ± 0.06 μ, of strain Nº 35, the difference obtained is $5.16 - 4.73 \pm \sqrt{0.06^2 + 0.08^2}\ \mu = 0.43 \pm 0.10\ \mu$. The difference between the two means is 4.3 times greater than the standard error of that difference. In other words, an interval with 99% probability gives 0.10 x 3 = 0.30 μ, and the difference of 0.43 falls outside such an interval. Therefore, the difference between the two means is statistically significant.

2. The comparison of each of the two extreme mean values with that of each of the other strains, and of the other strains with each other, (strains Nº 27, 28, 36, 41, 42) brings out no statistically significant differences.

3. The difference between the mean of 400 spores measured from the 4 Australian strains (5.02 ± 0.056 μ) and that of the 300 spores measured from the 3 Venezuelan strains (4.86 ± 0.041 μ) is not statistically significant.

Strains Nº 35 and 40 of *C. carrionii n. sp.* show no biological or morphological characteristics to justify specific separation. Neither do such differences exist between either strain and the other five studied (27, 28, 36, 41, 42). Yet there is a statistically significant difference between the mean spore lengths of strains 35 and 40. It appears, then, that small statistically significant differences between spore dimensions of two different strains should be given no taxonomic value when other morphological or biological differences are lacking. Likewise, it is possible to find spore dimensions with statistically equal means in two strains of species otherwise easily distinguishable, as in the case of the mean spore length obtained by me in strain Nº 35 of *C. carrionii* (5.16 ± 0.06 μ) and that found by DE VRIES in strain Nº 7 of *Cladosporium sphærospermum* (5.17 ± 0.08 μ).

On the other hand, the statistical study of spore dimensions contributes one more difference between *C. carrionii n. sp.* and *C. trichoides*. The mean length of the 700 spores measured of *C. carrionii* (4.95 ± 0.038 μ) shows a highly significant difference with that of 200 spores measured of *C. trichoides* (7.04 ± 0.140 μ).

Comparing the greatest mean length obtained from a strain of *C. carrionii* (strain Nº 35, 5.16 ± 0.06 μ) with the smallest from a strain of *C. trichoides* (strain Nº 37, 6.93 ± 0.18 μ), the difference obtained is slightly greater than 9 times the standard error of that difference (1.77 ± 0.19 μ), also a highly significant result.

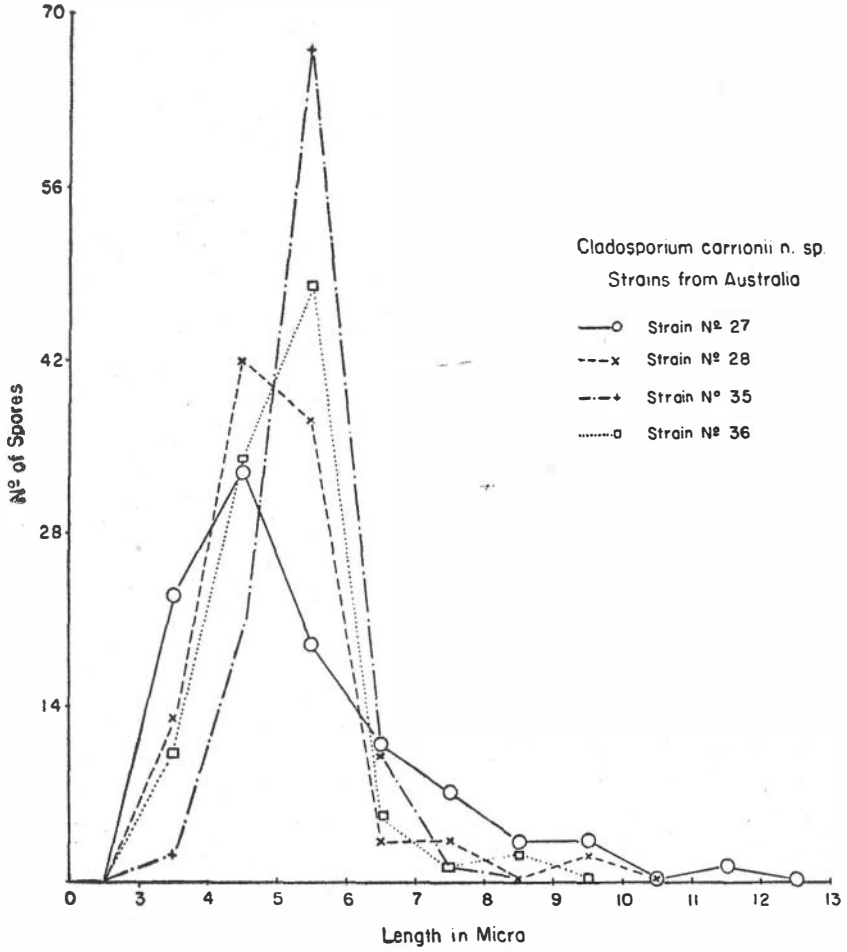

Fig. 13: Frequency curves of the length in μ of spores from four Australian strains of *C. carrionii n. sp.*

The frequency curves of spore lengths of the Australian and Venezuelan strains of *C. carrionii n. sp.* (figures 13 and 14) compared with those of the two strains of *C. trichoides* (figure 15) give also an objective idea of the differences existing between the two species.

Biochemical characters

Neither carbon source nor nitrogen source utilization data are discussed in this study, as they offer no important specific characteristics in the Dematiaceae. No pigment was observed to difuse in any culture medium.

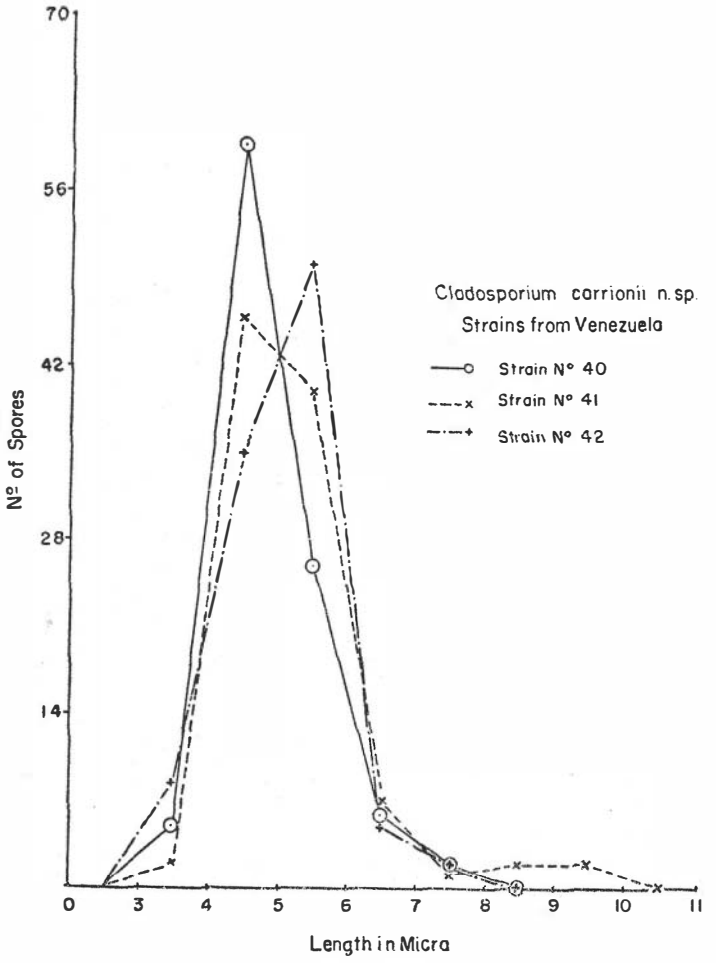

Fig. 14: Frequency curves of the length in μ of spores of three Venezuelan strains of *C. carrionii n. sp.*

The fact that *C. carrionii n. sp.* is devoid of proteolytic power for Löffler's coagulated serum seems to me of great importance, for reasons to be discussed further on.

Pathogenicity in laboratory animals

Simson *et al.* (25) made sub-cutaneous inoculations in *Cavia cobaya* and *Macacus rhesus* from the first South African isolate. The inoculum consisted of a thick suspension of spores and hyphal fragments. The animals were examined repeatedly during the next six months, but no lesions were observed to appear.

At present I am carrying out comparative studies on the pathogenic power of the species *C. carrionii n. sp., C. sphærospermum, C. trichoides,* and *Fonsecaea pedrosoi* on mice, inoculating a million spores intravenously. Spore

suspensions are filtered so that no hyphal fragments are included, to prevent embolisms. The results are to be presented in full in a separate paper, but the data regarding *C. carrionii* may be given here, as obtained so far.

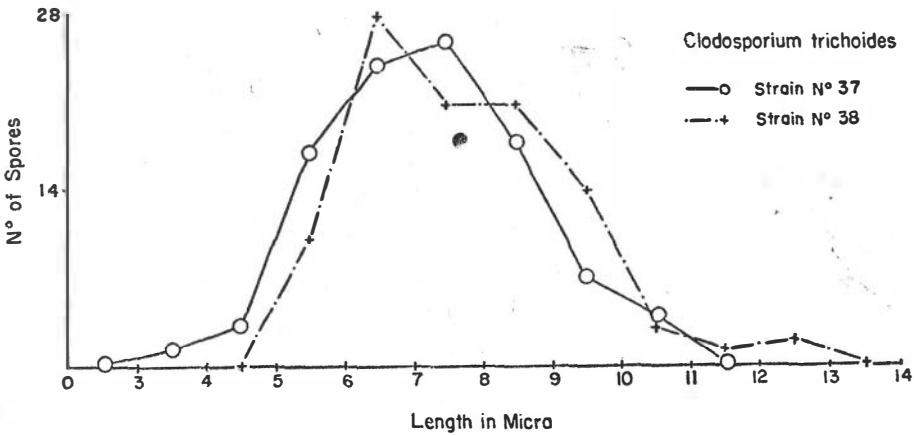

Fig. 15: Frequency curves of the length in μ of spores of two strains of *C. trichoides* Emmons, 1952.

A million spores suspended in 0.25 cc. of physiologic saline solution were injected into one of the tail veins of each of five adult male mice. In the morning of the 24th. day after inoculation, one of the mice was found dead; decomposition prevented carrying out an autopsy. The other mice were apparently normal.

On the 41st. day after inoculation, one of the four remaining mice was killed. Autopsy showed no macroscopic alterations in the organs of the thoracic and abdominal cavities or in the brain. Fragments of the organs were fixed in 10 % formaldehyde, and 4 corn-meal agar tubes were inoculated with small portions of brain tissue. Two months after inoculation the tubes were still sterile and were discarded. The three remaining mice retain a normal appearance three months after inoculation, at the date of writing, and are to be killed subsequently.

The microscopic alterations found in the sections of the diverse organs examined are the following.

Myocardium and spleen, without any appreciable inflammatory alterations. Hyperemia in the spleen. In the lung, very small foci of infiltration and condensation were found, similar to those observable in bronchopneumonia, but neither large enough nor abundant enough to warrant such a diagnosis. In the liver there were several small foci of lymphatic infiltration, scattered throughout the parenchyma near the vessels. The kidneys showed small foci of infiltration similar to those found in the liver, as well as a few somewhat larger, and some perivascular foci.

In the brain only 2 minute perivascular infiltration foci were found. Direct microscopic examination of a fragment of brain tissue squashed between a slide and a cover glass showed no hyphae or other structures of the fungus inoculated.

LATIN DIAGNOSIS

*Cladosporium carrionii n. sp.**: *Communibus in mediis, glucoso ac maltoso Sabouraudii medio maydisque infusione agaro, cultus hic fungus, colonias leviter e substrati superficie eminentes, explicatas, sæpe radiatim sulcatas, centroque tuberanti, interdum umbilicato, bene limitato vulgo ambitu, format. Incrementum eius tardum, quod laboratorii temperatura (25 ± 2° C) culturarum magnitudo in tubulis 18 x 150 mm. duos post menses inter quattuordecim et quadraginta millimetra variare potest. Opaca coloniæ superficies brunneo-grisea (inter "taupe" et "rose-taupe", 16-A-4 et 16-A-6 secundum* MAERZ *et* PAUL*), tergum autem subatrum. In medium pigmentum non diffundit. Lœffleri serum coagulatum non liquefacit.*

Hyphæ aeriæ breves, septatæ, plus minusve pigmentæ, olivaceo-brunneæ, (1) 1,5-2,5 (3) μ crassæ, ex quibus laterales ac terminales sporophori, magnitudine varii, erecti procumbentesque, hyphis æque pigmentati, oriuntur. Sporulatio tantummodo secundum typum Cladosporium. Sporæ seu thalloconidiosporæ in catenis plus minusve longis ramosisque dispositæ, subhyalinæ vel olivaceo-brunneæ, continuæ, ellipsoideæ vel ovoideæ interdum subsphæricæ (3)4-5(9,5)x(2)2,5(3)μ metientes. Hyphæ decumbentes communiter membranis claris ac levigatis, in quibus et sporophori, interdum atypici, formari queunt. Coralloideæ structuræ atque toruloideum mycelium brevibus cellulis crassimembranatis ac pigmentatis compositum, cellulæ etiamque ampulliformes adesse possunt. Constantes non sunt anastomoses, hæ vero non tantum inter hyphas duas, sed etiam inter hypham et sporam vel inter sporas duas inveniri possunt.. Patentem statu parasitico reductionem morphologicam præbet thallusque subsphæricis elementis fumagoidibus, circiter 10 μ diametro, cassimembranatis, flavobrunneis, per septa producentibus, constitutus est.

Hominis cutis in læsionibus substratisque artificialibus habitat. E variis chromoblastomycosis casibus in Venetiola, in Africa Australi, in Australia collectus est.

DISCUSSION

Cladosporium carrionii n. sp. is easily distinguishable from the other species in the same genus studied by DE VRIES (10) by the absence of proteolytic activity.

It is interesting to note that, according to MONTEMAYOR (17) and MACKINNON *et al.* (15), this lack of proteolytic activity is common to the strains of *Fonsecaea* and *Phialophora* which also cause chromoblastomycosis in man and to *Phialophora jeanselmei,* the agent of black-grain maduromycosis and of chromoblastomycosis. In the course of this study I also found *Cladosporium*

* The specific name is given in honor of Professor Arturo L. Carrión, of San Juan, Puerto Rico, whose many studies on the etiologic agents of chromoblastomycosis constitute an outstanding contribution to the knowledge of this interesting group within the Dematiaceæ.

trichoides Emmons, 1952 to lack a digestive action on Löffler's coagulated serum. Since *C. trichoides* and *C. carrionii n. sp.* are two species whose pathogenicity in man has been thoroughly established, further investigation is needed to determine whether the proteolytic activity of species of *Cladosporium* is, as it seems to be, a valid differential character between the saprophytic (proteolytic) and pathogenic in man (non-proteolytic) groups.

Cladosporium carrionii n. sp. is distinguished from *C. trichoides* by the slower rate of growth in all culture media employed, by the mean spore length, which is much greater in the latter species (figures 12-14) and, lastly, as accessory differential characters, by the neurotropism of *C. trichoides* as contrasted with the dermotropism of *C. carrionii* during their parasitic life in man.

As to the lesions caused by the two species in laboratory animals experimentally inoculated intravenously with similar quantities of spores, no definitive data may be given at this time. Observations so far seem to indicate that *C. trichoides* is easily recovered from brain tissue of inoculated animals, while *C. carrionii* does not grow on cultures made from similar material. Results of inoculations in laboratory animals must be interpreted with reservations, as the mere intravenous injection of inert particles produces inflammatory alterations in diverse organs. Yet, according to the observations of BINFORD *et al.* (1) on experimental lesions caused by *C. trichoides* inoculated intravenously in rats and rabbits, and to my own observations on two mice and 1 rat killed so far from the group inoculated intravenously with 1 million spores of the same species, it would seem that *C. trichoides* shows a marked neurotropism, although it may also form lesions in other organs. Cerebral lesions are quite evident, consisting of proliferative inflammation of the meninges and focal proliferative inflammation, with very scanty exudative component, in the brain parenchyma. In the two cultures of brain material numerous colonies of *C. trichoides* were obtained, and in three cases I have found hyphae in fresh fragments of brain tissue squashed between a slide and a cover glass.

In the case of the mouse killed 41 days after inoculation with *C. carrionii,* no important cerebral lesions were found; direct examination of brain tissue showed no hyphae or other structures of the fungus, and cultures were negative. Therefore, the results obtained so far seem to indicate that, in animals inoculated intravenously, *C. trichoides* exhibits a marked neurotropism not observed in *C. carrionii.* The experiments now in progress should yield further information on the comparative pathogenic action of *C. sphærospermum, C. carrionii, C. trichoides,* and *Fonsecaea pedrosoi.*

ACKNOWLEDGEMENTS

I wish to thank my colleagues Lic. Hernán Badilla and Prof. Armando Ruiz for their collaboration in the present study. The Latin diagnosis of the new species was prepared by Prof. Ruiz, and revised by Dr. Domenico Vitola. Doctors Marcial Fallas and Rodolfo Céspedes have given their invaluable

cooperation in the interpretation of histological lesions observed in experimental animals. Sr. Leonardo Mata prepared most of the histological sections. Sr. Mario Romero, of the Dirección General de Estadística y Censos, carried out the statistical analysis of the data from spore measurements. I am indebted to Dr. R. L. Rodríguez for the English translation of the manuscript.

The present study would not have been possible without the generous collaboration of Doctors Chester W. Emmons, of Bethesda, Maryland, U.S.A.; R. E. Powell, of Brisbane, Queensland, Australia; and Humberto Campins, of Barquisimeto, Venezuela, who sent strains of *C. carrionii* and reprints of their publications. Dr. Libero Ajello, of Chamblee, Georgia, U.S.A., gave me valuable bibliographic references and posed questions which are answered in the present article.

To these, and to all other persons who in one way or another have contributed to the preparation of this work, I acknowledge my profound gratitude.

SUMMARY

An historical revision is made of the problem of *Cladosporia* isolated from cases of Chromoblastomycosis in various parts of the world, leading to the conclusion that the diverse strains isolated correspond to a single new species, whose description is given after a detailed study of seven strains which the author was able to study personally. The characters are also given which separate the new species from the other saprophytic members of the genus, and from *C. trichoides,* isolated from brain abscesses in the U.S.A.

The new species of chromoblastomycosis-causing fungus is named *Cladosporium carrionii* in honor of the illustrious Porto Rican mycologist Prof. Arturo L. Carrión.

RESUMEN Y CONCLUSIONES

Se realiza una revisión histórica del problema de los *Cladosporia* aislados de casos humanos de cromoblastomicosis. Estas cepas se admiten como diferentes de las especies de los géneros *Fonsecaea* y *Phialophora,* pues únicamente presentan conidióforos tipo *Cladosporium* (=*Hormodendrum*).

Se dan las razones por las cuales se considera que la cepa aislada de un caso costarricense por ROTTER & PEÑA-CHAVARRÍA y que aparece en la literatura como perteneciente a la especie *Hormodendrum langeronii* (actualmente *Cladosporium sphærospermum*), en realidad no era un representante de esta especie.

Se llega a la conclusión de que la uniformidad de las cepas que presentan únicamente conidióforos tipo *Cladosporium,* y que fueron aisladas de cromoblastomicosis en Venezuela, Sur Africa y Australia, justifica el que sean todas incluídas en una nueva especie cuya descripción se da, proponiendo para ella el nombre de *Cladosporium carrionii* en homenaje al ilustre micólogo puertorriqueño Prof. Dr. Arturo L. Carrión.

La descripción de la especie es la siguiente:

Cladosporium carrionii n. sp.

Cultivado en los medios comunes (Sabouraud glucosado y maltosado y "Corn-meal agar") este hongo forma colonias ligeramente elevadas de la superficie del substrato, extendidas, a menudo con surcos radiales, con el centro abultado o algunas veces umbilicado y generalmente con el contorno bien delimitado. Su crecimiento es lento ya que el tamaño de las colonias puede variar en distintas cepas entre 14 y 40 mm después de dos meses a la temperatura del laboratorio (25 ± 2°C), cuando cultivado en tubos de 18 x 150 mm. La superficie de la colonia es opaca, de color pardo grisáceo, que varía entre "taupe" y "rose taupe", 16 A 4 y 16 A 6 según MAERZ & PAUL. El reverso de la colonia es negro. El pigmento no difunde en el medio de cultivo. No tiene acción proteolítica sobre el suero coagulado de Löffler.

Hifas aéreas cortas, septadas, más o menos pigmentadas, pardo olivaceas, de (1) 1,5-2,5(3) μ de diámetro, de las cuales se originan esporóforos laterales y terminales de diversos tamaños, erectos y decumbentes e igualmente pigmentados que las hifas. Esporulación únicamente del tipo *Cladosporium.* Esporas o taloconidiosporas dispuestas en cadenas más o menos largas y más o menos ramificadas, subhialinas o pardo oliváceas, sin septos, elipsoides u ovoides, algunas veces subesféricas, que miden de (3)4-5(9,5)x(2)2,5(3) μ. Hifas decumbentes por lo general con membranas claras y lisas, en las cuales también se pueden formar esporóforos, algunas veces atípicos. Pueden observarse estructuras coraloides y micelo toruloide, compuesto de células cortas, de membranas gruesas y pigmentadas, así como también células ampuliformes. Las anastomosis no son constantes, pero éstas pueden encontrarse no sólo entre dos hifas, sino también entre una hifa y una espora, o entre dos esporas. Durante el estado parasitario muestra una acentuada reducción morfológica y el talo está constituído por elementos fumagoides subesféricos, alrededor de 10 μ de diámetro, con gruesas membranas pardo amarillentas y que se reproducen por septos.

Habita en lesiones de la piel del hombre y en los medios artificiales de cultivo. Ha sido aislado de varios casos de cromoblastomicosis en Venezuela, Africa del Sur y Australia.

Se sugiere que el poder proteolítico de las especies del género *Cladosporium* permite, por el momento, separar las especies saprófitas (proteolíticas) de las patógenas (no proteolíticas). Entre estas últimas figuran las especies *C. trichoides* y *C. carrionii.* La diferenciación entre estas dos se hace tomando en cuenta las dimensiones de las esporas, el aspecto macroscópico y velocidad de crecimiento de las colonias en los medios artificiales de cultivo, el neurotropismo de la primera y el dermotropismo de la segunda cuando parasitan al hombre y la patogenicidad para los animales de laboratorio.

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