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(FUNDADO EM 1880 PELO DR. JÚLIO HENRIQUES)

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ESTUDIOS CARIOLOGICOS SOBRE LA FLORA ESPAÑOLA

por

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MATERIAL Y MÉTODOS

PARA estudiar las meiosis se fijaron yemas florales * directamente en el campo, con una mezcla de alcohol etílico absoluto y ácido acético glacial (3:1). El material así fijado se conservó 2-4 días a 4° C, tras lo que se cambió a alcohol de 70°, guardándolo así hasta el momento de su estudio. Doce horas antes de teñir, cambiamos las yemas a una solución mordiente formada por tres partes de alcohol absoluto y una de disolución saturada de acetato férrico en ácido acético glacial. Las preparaciones se consiguieron por aplastamiento de anteras, la tinción se hizo con carmín acético *1%.* Antes de cerrar las preparaciones, se calentaron a la llama de un mechero de alcohol hasta el desprendimiento de vapores pero evitando la ebullición. En resumen, seguimos aproximadamente la técnica que describe SAÑUDO (1971).

Para estudiar las mitosis se utilizaron meristemos apicales jóvenes, que se obtuvieron para el caso de *Arum* y *Biarum* de bulbos conseguidos en sus poblaciones naturales y para el caso de *Hyosciamus* por germinación de semillas en cápsulas de Petri. Una vez conseguidos los meristemos apicales realizamos el pretratamiento con 8-hidroxiquinoleina 0,002 M., durante dos a seis horas. Posteriormente los fijamos con alcohol-acético (3:1) durante dos horas y los sometimos a hidrólisis en ácido clorhídrico 1 N., a 60° C, durante cuatro minutos y medio. Tras media hora

de tinción en orceina acética 1% realizamos las preparaciones por aplastamiento.

Todo el material utilizado es silvestre y en cada caso se indicará los datos de recolección. Los ejemplares testigo y las microfotografías correspondientes se conservan en el herbario del Departamento de Genética y en el herbario particular de J. FERNÁNDEZ *CASAS* a quién agradecemos la ayuda prestada.

RESULTADOS

Arum arisarum L. $\eta = 28$, $2n = 56$

Material. Granada, Sierra Elvira, VG 32, 700 m. s. m., *Fdez. Casas & M. Ruiz Rejón* 1-1975.

El número encontrado para esta espécie (Lám. I, fig. 3) coincide con el que reseñan GARBARI & MONTI (1970) para plantas de Italia.

El cariotipo que nosotros hemos encontrado (Lám. I, fig. 4) está constituido por:

26 parejas heterobraquiales
2 parejas hiperheterobraquiales.

De las parejas heterobraquiales 4 son satelitíferas. (Lám. I, fig. 3 y 4).

El estudio de la meiosis revela la presencia de 3-4 configuraciones tetravalentes por célula, ademas de bivalentes y algunos univalentes (Lám. II, fig. 5).

Biarum carratracensis (Haenseler) Font Quer $2n = 98$

Material. Granada, Sierra Elvira, VG 32, 800 m. s. m., *Fdez. Casas & M. Ruiz Rejón* III-1975.

Consultados los índices cromosómicos que se citan en la bibliografía del presente trabajo, pensamos que es la primera vez que se efectúa una investigación cariológica en este taxon. El número que nosotros encontramos, $2n = 98$, corresponde a un cariotipo constituido por:

8 parejas isobraquiales
25 parejas heterobraquiales
16 parejas hiperheterobraquiales (Lám. I, fig. 1 y fig. 2).

Hyosciamus albus L. η — 34, 2n — 68

Material. Granada, Padul, VF 49, 800 m. s. m., *M. Ruiz Rejón* III-1975.

En esta especie existe un recuento cromosomico efectuado por BHATTACHARYA, S. S., M. M. KHALIFA & I. I. CHAUDHRI (1971) en el que aparece un número somático 2n = 34.

Si embargo, en el Vol. 3 de Flora Europaea el número somático que figura para esta especie es 2n = 68.

Nuestro estudio coincide tanto en meiosis (n = 34) como en mitosis (2n = 68) con este último dato. El análisis de la meiosis, que se hace aquí por primera vez, revela que esta especie tiene un comportamiento meiótico regular.

Scrophularia crithmifolia Boiss. n = 12

Material. Málaga, Carratraca, VF 38, 600 m. s. m., *Fdez. Gasas* 2-IV-1974.

Consultados los índices cromosómicos que aparecen en la bibliografia del presente trabajo, no hemos encontrado recuento cromosomico alguno para esta especie. Sin embargo, Flora Europaea presenta como número somático de esta espécie, 2n = 24, sin indicar la procedencia del dato. Nuestro análisis de la meiosis nos indica la presencia de 12 bivalentes en todos los casos y un comportamiento meiótico regular (Lám. Π, fig. 6).

Ulex argenteus Welw. ex Webb subsp. erinaceus (Welw. ex Webb), η = 16

Material. Almería, Cabo de 'Gata, *Fdez. Piqueras & M. Ruiz Rejón* XII-1975.

El número que nosotros hemos encontrado es la primera vez que se reseña para esta especie. Con anterioridad DE CASTRO (1943) había reseñado para este taxon un número somático 2n = 96, sin indicar la subespécie. Flora Europea, por su parte, presenta como número somático de esta especie 2n = 64 y pone en duda el número 2n — 96. El análisis de la meiosis revela un comportamiento meiótico regular (Lám. Π, fig. 7).

Ulex parviflorus Pourret n = 16

Material. Véase Mapa adjunto. *Fdez. Piqueras & M. Ruiz Rejón* XII-1975; 1-1976.

Ante la gran variabilidad morfológica que presenta este taxon, hemos realizado un análisis detallado de las poblaciones de esta especie en el S. E. español (véase Mapa adjunto).

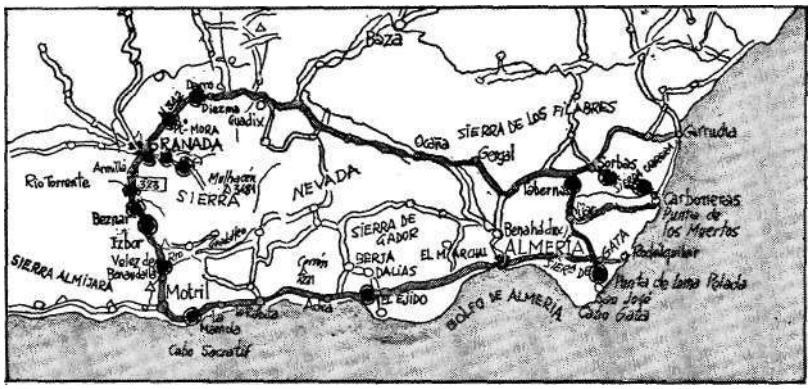
Concretamente, se han estudiado 15 localidades de las provincias de Almería y Granada. En todos los casos aparecen 16 bivalentes y el comportamiento meiótico es regular (Lám. II, fig. 8).

En esta especie se han dado los siguientes números somáticos, 2n = 32, 64 y 96 (Flora Europea).

Nuestro recuento coincide con el realizado por LOVE & KJELLQUIST (1974) en material español, 2n = 32.

SUMMARY

The chromosome number of six wild phanerogamic species from Spain is studied: the number η = 28 of *Arum arisarum* L., is the first analysis for meiotic behaviour of this species with quadrivalents; the number 2n = 98 of *Biarum carratracensis* (Haenseler) Font Quer is reported presumably for the first time; the chromosome level η = 16 is also reported for the first time in *Ulex argenteus* Welw. ex Webb subsp. *erinaceus* (Welw. ex Webb).



Mapa con indicación de las localidades de las poblaciones de *Ulex parviflorus* Pourret que se estudian en este trabajo.

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NOTA —Los trabajos señalados con asterisco no han sido consultados directamente, sino a través de los índices de números cromosomico».

LAMINAS

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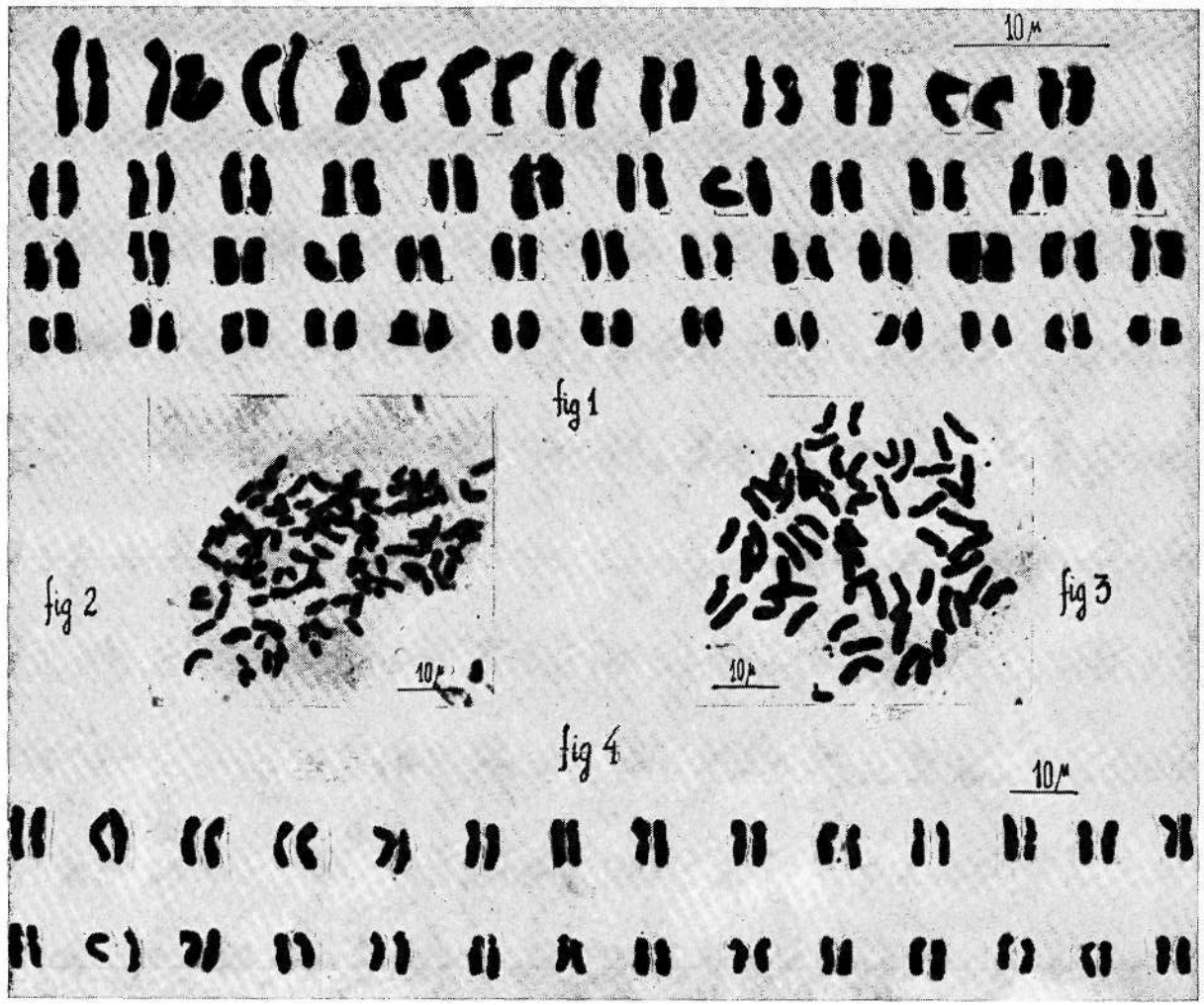
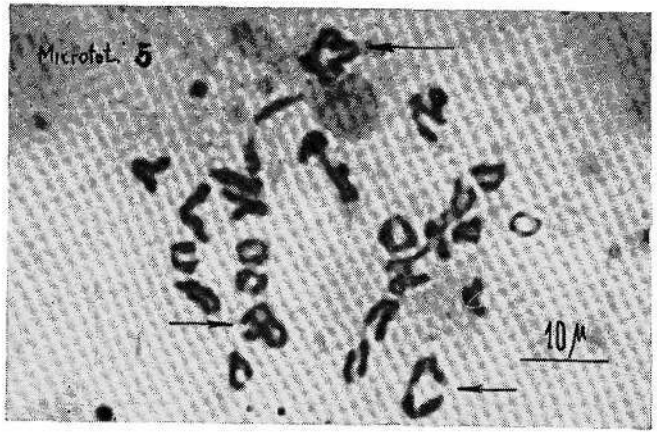
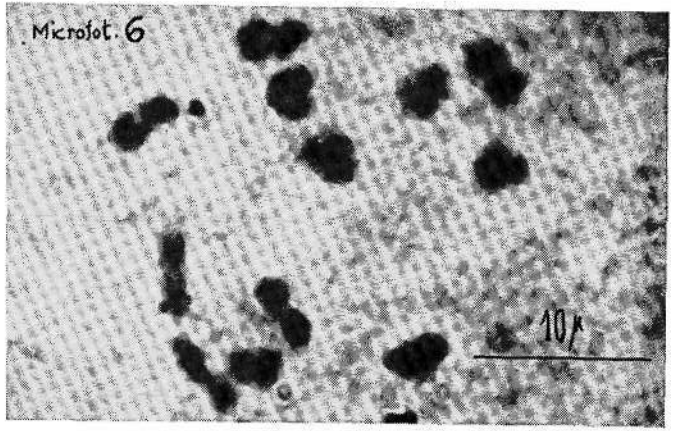
LAMINA I

Fig. 1. — Cariotipo de *Biarum carratracensis.*
Fig. 2, — Metafase somática de *Biarum carratracensis.*
Fig. 3. — Metafase somática de *Arum arisarum.*
Fig. 4. --- Cariotipo de *Arum arisarum.*




Microfot. 5
10 µ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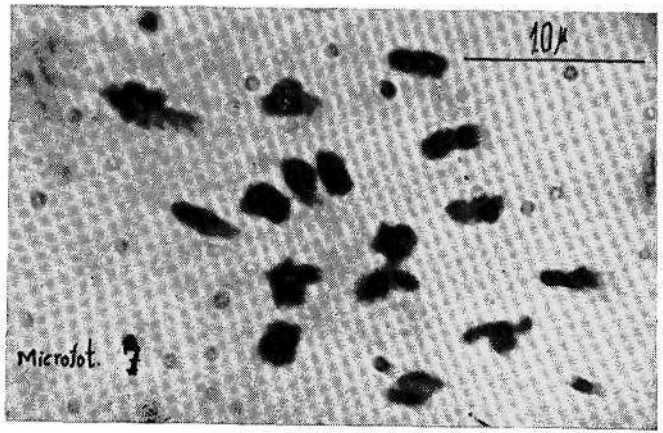
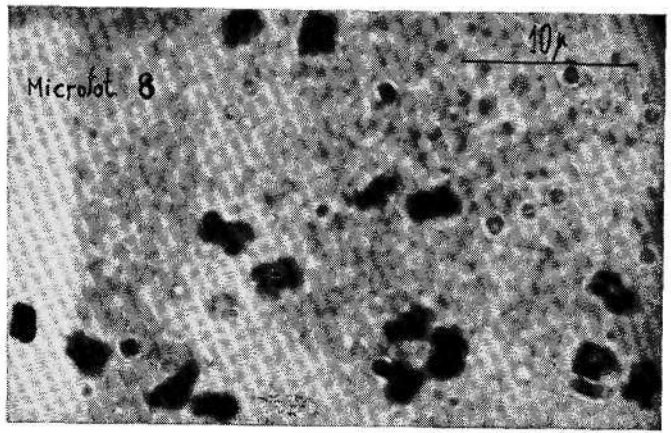

10 µ
Microfot. 7


10 µ
Microfot. 8

LAMINA Π

Fig. 5. — Diacinesis de *Arum arisarum* (obsérvense las formaciones cuadrivalentes).
Fig. 6. — Diacinesis de *Scrophularia crithmifölia.*
Fig. 7. — Diacinesis de *Ulex argenteus* subsp. *erinaceus.*
Fig. 8. — Diacinesis de *Ulex parviflorus.*

Γ

ON THE DETERMINISM AND BIOLOGICAL SIGNIFICANCE OF STERILE FRUIT-BODIES, IN BIFACTORIAL SPECIES OF *COPRINUS*

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I. INTRODUCTION

IN bifactorial [1] — tetrapolar heterothallic — species of *Hymenonvycetes,* in order that *normal* hymenophores [2] develop in a mycelium, it is necessary that this mycelium be dikarvotic with its two haploid nuclei completely compatible, in relation to the incompatibility *factors,* i. e. „*A*= B= according to the symbolism of RAPER (1966; cfr; PINTO--LOPES, 1952)3 The dikaron is, therefore, «the mycelial

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[1] On the meaning of the expressions «bifactorial» and «uni factorial», see KÓLTIN, STAMBEBG & LEMKE (1972).

[2] Among the designations used for the more or less differentiated structure which bears the hymenium, we prefer the term *hymenophore.* The hymenium is, in fact, its characteristic feature, since it only arises in that structure. The term *hymenophore* is given the same meaning as sporophore, carpophore, basidioearp, fructification and fruit-body. The expression «sterile fruit-body» is used "here in the sense of a hymenophore without spores or with a reduced or irregular number of spores. Those hymenophores in which every basidium is fertile (having four basidiospores) are *normal* fruit-bodies;

3 According to present knowledge, each incompatibility factor is a genetic regulatory region (cfr. RAPER, 1966; RAFER & RAFER, 1964; KOLTIN, STAMBERG & LEMKE, 1972; RAFEE & RAPEE, 1973)- This expression «incompatibility factors» was introduced into genetic terminology by QUINTANILHA (1937), and since then it has been universally adopted. Earlier, this Author had, used the expression «incompatibility between haplonts» (QUINTANILHA, 1934). Later on, the-same Author (QUINTANILHA, 1941, 1942) used the expressions «sexuality»,

phase crucial to fruiting» (RAPER, 1966: 34), or in other words, the fruit-body «is typically the prerogative of the dikaryon» (PERKINS & RAPER, 1970).

However, examples of tetrapolar species of the genus *Coprinus* have been reported, in which other types of mycelium can also develop fruit-bodies under laboratory conditions. These are heterokaryotic mycelia, whose nuclei have in common one of the incompatilibity factors (A= $B^{=}$) [1] or heterokaryons resulting from mating between a diploid and a haploid mycelium (2n X n) [2], or between two diploid mycelia (2n X 2n) [3], or even between an aneuploid mycelium (n + 1) and a haploid mycelium (n).

Cases are also known of hymenophores developing in wild homokaryons[4], or in mutant homokaryons arising in heterokaryons A= B= or A= $B^{=}$. Other cases are known in

«sterility», and «incompatibility» factors, indiscriminately (e. g.: «une paire de facteurs de stérilité *Aa* provoque une incompatibilité absolue entre haplontes possédant un facteur commun»). DICKSON (1934) had previously used the expression «incompatibility factors» with a different meaning. This Author observed in the uni-factorial species *Coprinus sphaerosporus,* an occasional production of more or less sterile sporophores, having assumed that this variability of the fertility was due to the presence of «sterility (or incompatibility) factors» (DICKSON, op. cit.: 544). These factors would exist apart from the «sex factor», and would not only affect the degree of fertility of the dikaryon when this has been produced, but should also condition the fusion of the haplonts when there is no dikaryon production. However, that Author did not find enough evidence for the existence of these «incompatibility factors» (DICKSON, 1934: 546).

[1] No cases are known of fruit-bodies with a heterokaryotic structure occurring in heterokaryons A= B= or A= B=.

[2] The diploid nucleus may result from: i) a dikaryon A= B=; ii) a hetehokaryon A= B=; a heterokaryon A= B=; iv) a heterokaryon A= B=.

3 When the two nuclei remain diploid, fruit-bodies do not develop.

[4] BULLER (1965: 265) stated: «One of the most remarkable discoveries in connection with Hymenomycetes is that, in certain species of Agaricineae, there are haploid fruit-bodies as well as diploid». (This Author used the term *«diploid»* to mean dikaryotic).

In the next chapter we will give other quotations referring to species which under laboratory conditions develop sterile fruit-bodies In the haploid mycelium.

which chimeric hymenophores have been developed in heteroyarkons. Therefore, BULLER'S (1941: 367) assertion according to which «the presence of conjugate nuclei is not necessary for fruit-body development», is justified.

In the cases already mentioned, the hymenophores are either completely fertile or completely sterile, or sub-sterile, or may have a variable sterility. On the other hand, in dikaryons, i. e., heterokaryons which are hetero-allelic for the two pairs of the incompatibility factors, there may be a simultaneous development of both sterile and fertile fruit-bodies.

Sterility is detected by the absence of spores, or by a reduction in their number, and a consequent whitish, instead of dark 1 colouring of the hymenophores [2]. This is why sterile hymenophores have been called «pale fruit-bodies» (e. g. DAY, 1959).

For BULLER (1909), «one of the most curious phenomena»... «in studying the Hymenomycetes, is the occasional sterility of *Coprinus* fruit-bodies». According to the same Author *(BULLER*, 1922: 69), «occasional sterility of fruit-bodies seems to be a phenomenon by no means limited to the Coprini but to be widely spread throughout the Hymenomycetes» [3]. However, it is in the genus *Coprinus,* that

[1] According to DAY'S (1959) estimates, the dikaryotic (double heterozygotic) fruit-bodies have 3000 tetrads of dark purple spores per mm^2 of hymenial surface, giving them a dark appearance. In pale hymenophores, the tetrad density is from 0 to 600 per mm^2.

[2] The term *«fertility»* has been used in relation to different stages of development, giving it different meanings: i) in homothallic species we say that homokaryons are self-fertile, since they have no incompatibility factors; ii) in heterothallics, inter-/ertiZe homokaryons are those which show different incompatibility factors; ili) a mycelium is said to be *fertile* if it gives origin to fruit-bodies; iv) a fruit-body or a basidium is *fertile* if it gives rise to spores. In the same way, the term «sterility» has various applications for mycelia, fruit-bodies and basidia,

[3] Buller (1922) referred to the following cases of fruit-body sterility found in the literature: *Lactarius vellereus* (LEVEILLÉ, 1837), *Stropharia obturata* (STEVENSON, 1886), *Clitocybe sadleri* (OOOKE, 1883), *Russula integra* (E. FRIES, seg. STEVENSON), *Stropharia semiglobata* (GROVE, 1891), *Paneolus campanulatus* (GROVE, 1910). DICKSON

we know the greatest number of cases of sterile fruit-bodies developing in different types of mycelia under laboratory conditions.

*

The knowledge of these cases of fruit-body sterility roused the curiosity of the present Authors as to the cause or the several causes determining it.

In the literature, various factors, namely environmental, physiological, cytological, genetic (chromosomic and non-chromosomic) factors have been considered as responsible for the different cases of fruit-body sterility.

On the other hand, from a *stock* of *Coprinus radiatus*, we isolated (unpublished) a few pairs of compatible homokaryons whose mating regularly gave rise to clamped secondary mycelia from which pale fruit-bodies developed. Mating of other compatible homokaryons of the same *stock* gave rise to mycelia with clamps, where black fruit-bodies developed regularly. Heterokaryons produced through the pairing of homokaryons from both of these two groups gave also origin to normal fruit-bodies. These observations led us to collect the bibliographical data on this subject, while carrying out laboratory tests to try and explain the causes of sterility.

The present paper is in the first place a critical review of the literature relative to the different cases of incomplete fertility or total sterility of hymenophores and respective determinism. On the other hand, it seems worth while to discuss the biological significance of those hymenophores which show incomplete fertility. These two points have been considered in relation exclusively to bifactorial species having clamped secondary mycelium, of *Coprinus.*

(1934: 533) observed, in *Coprinus sphaerosparus*, the occasional production of fruit-bodies which were sterile, or only «vey sparingly» produced. KOMATSU & KIMURA (1964, 1968) found that the production of white sterile fruit-bodies of the tetrapolar species *Lentinus élodés* is a heritable character which is controlled by a single recessive gene.

II. REVIEW OF LITERATURE

The present chapter deals with the analysis of the eases of the different types of pale hymenophores which have been described in the literature.

In the first place we may make the distinction between homokaryotic and heterokaryotic pale fruit-bodies. Among the homokaryotic fruit-bodies it is possible to distinguish the monokaryotic (haploid) and the monordikaryotic (haplo--diploid) hymenophores. Among the heterokaryotic fruit--bodies, we may consider the following sub-types: i) mixed monokaryotic (haploid) ; ii) mixed mono-dikaryotie (haplo--diploid) ; iii) dikaryotic. In the latter, we can still distinguish various cases according to the genome of the basidia (diploid, triploid, or aneuploid).

1. — Homokaryotic fruit-bodies

a. —· Monokaryotic (haploid) fruit-bodies

A first example of hymenophore sterility is given by homokaryotic, haploid, fruit-bodies, of which only a few cases were studied in detail[1].

[1] BRUNSWICK (1924) reported the development, in the unifactorial species *Coprinus ephemerus* Fr. ex Bull., of haploid fruit--bodies with a reduced production of basidiospores. The same Author as well as VANDENDRIES (1929) reported the development of haploid fruit-bodies in a bifactorial species, *Coprinus micaceus.* MORTEN LANGE (1952) observed the production of sterile and of sub-sterile haploid fruit-bodies in monosporic mycelia isolated from *Coprinus ephemerus* Fr ex Bull, and from *C. congregatus* Fr. ex Bull. These cases referring to bipolar heterothallic species are not discussed here. The known cases of bipolar and tetrapolar species of *Hymenomycetes* which do not belong to the genus *Coprinus* and whose homokaryons produce sterile fruit-bodies (cfr. SMITH, 1934; KLUSHNIKOVA, 1939; BULLER, 1941; RAPER, 1953; SARAZIN, 1955; RAPER & KRONGELB, 1958; RAPER, 1959, 1966) are not also dealt with here. Also KOLTIN, STAMBERG & LEMKE (1972) mentioned four species whose monokaryotic mycelia gave rise to fruit-bodies (see also QUINTANILHA & PINTO-LOPES, 1950). On the other hand LEONARD & RAPER (1969) proved the existence, in the bifactorial species *Schizophyllum commune,* Of a gene which acts in the induction, by exogenous chemical agents, of the production of haploid fruit-bodies.

The hymenophores developed in homokaryotic mycelia, have the following characteristics (cfr. also BULLER, 1931: 265; QUINTANILHA, 1935: 14; BULLER, 1941: 366):

a — they emerge later than the normal dikaryotic f ruit--bodies which arise from a secondary mycelium, and show a slower «rythm» in development;
b — the cap is much lighter than that of the normal dikaryotic fruit-bodies, being almost colourless or greyish, due to the variable sterility of the hymenium which may even be completely devoid of spores;
c — their basidia sometimes have a different number of spores than normal: diads, triads, pentads, besides tetrads;
d — sometimes the cap remains closed and does not deliquesce; when autolisis occurs, the deliquescent liquid is hyaline or brownish;
e — the spores usually remain attached to the sterigmata of the basidia;
f — all the spores produced in a haploid sporophore have the same incompatibility factors as the spore originating the mycelium which produced that sporophore, so that the tetrads are monocratic (or monotypic); the spores, have, therefore, an apomictic development 1.

The homokaryotic mycelium developing fruit-bodies shows the characteristics of the primary mycelium. All the monosporic mycelia isolated from a haploid sporophore, have the same incompatibility factors of the haploid mycelium in which it developed. Therefore, in species with clamped secondary mycelium, the polysporic cultures from haploid fruit-bodies give rise to mycelia without clamp

[1] This fact was demonstrated experimentally in bifactorial species, by several early Authors such as KNIEP (1920), in *Schizophyllum commune;* HANNA (1925), and QUINTANILHA (1935), in *Coprinus radiatus;* VANDENDRIES (1929), in *Coprinus micaceus,* and later by other Authors in other species.

connections. The monosporic cultures from a haploid sporophore developed in a haploid mycelium also possess the ability to fruit. From the mating of two compatible homokaryons, both forming fruit-bodies, a dikaryotic mycelium results where normal fruit-bodies develop, such as in the case of dikaryotic mycelia arising from anastomosis of haplonts devoid of fruiting ability[1].

*
* *

QUINTANILHA (1935; 1941) found in the progeny of a normal sporophore (A= B=), of known genetic constitution (A'b + aB), of *Coprinus radiatus* ss. Lange (under *C. fimetarius)2,* a homokaryon which he called B_4. It was characterized not only by the ability to fruit which was considered to be a consequence of a mutation, but also by «une légère modification génotypique» of one of the incompatibility factors (mutation from *a* to a1). The results of crossings with the testers led that Author to attribute the symbols a_ab to the B_4 homokaryon. The cytological analysis of the pale fruit-bodies developed in this primary mycelium revealed that all the basidia were monokaryotie[3], and that two successive mitosis had occurred later. The nuclei of the

[1] ZATLER (1924) observed that, in *Collybia velutipes*— a tetrapolar heterothallic species (VANDENDRIES, 1927; ZATLER, op. cit.) --, the ability to develop haploid fruit-bodies is hereditary, segregating as if it were controlled by a Mendelian factor. More recently, TAKEMARU (1961) thought that the results of his investigations confirmed ZATLER'S opinion (op. cit.) that the ability of haploid mycelia to fruit is hereditary.

[2] On the nomenclature of the species which have been used in cytological and genetical researches, see PINTO-LOPES & ALMEIDA (1972).

[8] BULLER (1931: 266, in a footnote) had already stated that «presumably, in *Coprinus lagopus,* as in other *Agaricineae,* in a haploid fruit-body, the cells contain isolated non-conjugate nuclei and each young basidium a single nucleus», but the Author himself revealed he «has not verified these suppositions by means of cytological observations». (It must be pointed out that his *Coprinus lagopus* corresponds to *C. radiatus* ss. Lange; see PINTO-LOPES & ALMEIDA, 1972).

basidia were the size of haploid nuclei, being less than half the size of diploid nuclei of normal dikaryotic fruit-bodies; this fact supported the idea of the non-existence of karyogamy. Some basidia aborted before or after the first division, others after the second; this fact was believed to be the cause of the production of the small number of spores. No explanation was given on the aborting of the basidia, but the mutation is apparently unrelated to the sterility.

The genetical analysis showed that all the tetrads developed in these pale monokaryotic (haploid) fruit-bodies were monocratic. The respective homokaryons possessed the same incompatibility factors as the mycelium where the fruit-bodies developed, as HANNA (1928) had already observed (see below). In QUINTANILHA's (op. cit.) opinion, this fact confirms the absence of meiosis in the basidia.

The spore to spore cycle would be totally haploid, and so the reproduction would be apomictic. Homokaryons isolated from haploid fruit-bodies were also able to fruit, which demonstrates the hereditary transmission of this character, also shown previously by HANNA (op. cit.).

According to QUINTANILHA (op. cit.), the ability to fruit in the haploid stage, is not, in this species, common to all.:. primary mycelia. He observed the development of fruit-bodies in a single monosporic culture among the thousands he studied, isolated from various Sources. On the other hand, it was not a consequence of favourable environmental conditions, but was actually a mutation which persisted through successive, generations, while other haplonts under the same conditions did not fruit \.

1 Some Authors Who, before QUINTANILHA, had done research on the same species, also reported the development of haploid fruit-bodies in some or all monosporic cultures. BENSAÚDE (1918) reported in her investigations that haploid mycelia of *Coprinus radiatus* ss. Lange (under -*C*- fimetarius), did not develop fruit-bodies. Later, however, she informed BULLER (cfr. BULLER, 1931) that, after the publication of her papers dated 1917 and 1918, she had observed the development of fruit-bodies in one of her haploid cultures. MOUNCE (1922, *in* BULLER, vol. 2: 73, under C. lagopus; 1924, *in* BULLER, vol. 3: 312; 1931 *in* BULLER, vol. 5: 215) observed, in the same species, that in some haploid mycelia, sterile fruit-bodies developed. HANNA (1924,

QUINTANILHA (1935: 41) stated very explicitly, that «des fructifications haploïdes ne surviennent que sur des mycéliums frappés de mutation». One may assume that there is no relation between this mutation for fruiting and the simultaneous mutation of the incompatibility factor.

According to QUINTANILHA (op. cit.), sterility of haploid sporophores is due to the aborting of the basidia, but BULLEE (1924:314) stated that this sterility is due to lack of «vigour», which in turn is a consequence of its «abnormal assexuai condition». We are allowed to assume that both statements will not explain the primary cause of the sterility.

HANNA (1928), also disagreeing with QUINTANILHA (1935), stated that, in sterile haploid fruit-bodies which she observed in the same species, the basidia were at first binucleate, karyogamy occurring later. This was the first example of self-fertility of homokaryons in a heterothallic species of *Coprinus.* All the homokaryons behaved as if the incompatibility between their genetically similar nuclei had been disrupted, allowing a nuclear fusion and therefore leading to a complete eu-sexual cycle, identical to the homothallic type.

CHOW (1934) also reported the development in *Coprinus radiatus* ss. Lange (under «C. lagopus sec. BULLER»), of partially or completely sterile fruit-bodies in all primary myeelia. According to this Author, in haploid fruit-bodies, haploid and diploid basidia occur, both producing four basidiospores with the same «sex» as the mycelium in which they developed, as shown experimentally by HANNA (op. cit.). However, it is worth while to point out that the

in BULLER, vol. 3: 313, under C. lagopus) isolated 70 monosporic mycelia, having ascertained that all of them produced fruit-bodies which were sterile «in a greater or lesser degree». The same Author (HANNA, 1928; see also BULLER, 1931: 235) obtained, in ten successive haploid generations, fruit-bodies in the haploid stage, from monosporic cultures, of the same species. BREFELD (1877) also observed the development of fruit-bodies in monosporic mycelia of *Coprinus lagopus.*

occurrence of karyogamy in the diploid basidia was not confirmed. CHOW (op. cit.) stated that he did not know the cause of sterility of the fruit-bodies, but believed that the primary stage of the mycelium was not responsible, since also in the secondary mycelia, there was development of sterile fruit-bodies (see below).

DAY (1960, 1963) [1] reported the development of monokaryotic, haploid, pale fruit-bodies, on a homokaryotie mycelium from a common-A heterokaryon, A_5B_5 + A_5B6, of *Coprinus cinereus* ss. Konr. (under C. lagopus). All the homokaryons from the basidiospores of these haploid fruit--bodies were A_5B_5. These monokaryons, as well as the haplonts isolated from fruit-bodies of the next generation had false clamp connections and the faculty of fruiting. Their monokaryotie fruit-bodies had pale gills «bearing varying proportions of abnormal tetrads with less than four spores». It is not a mutation of the factor *A,* as the homokaryons are incompatible with the wild tester A_5B6.

From the crossing between the mutant $A5B_5$, a homokaryon characterized by false clamp connections and producing fruit-bodies, and the wild strain A_eB_6, a dikaryon was produced where normal sporophores developed. From these, 21 tetrads (84 spores) were isolated for analysing mutant segregation; of the respective homokaryons, 42 had false clamp connections, and of these, 18 produced pale fruit-bodies, but from the mycelia devoid of clamps, none fruited. The presence of the mutation is not sufficient for fruiting. As to the compatibility factors in this progeny, it was concluded that they segregated independently of the factors responsible for the faculty of producing false clamps and for fruiting.

According to DAY (op. cit.), the production of false clamps is due to a gene which suppresses *(suppressor gene)* the normal function of the *A locus,* and which he called *su-A* [2]. On the other hand, according to the same Author,

[1] See also FINCHAM & DAY (1965).

[2] FINCHAM & DAY (1965) stated: «Evidently the mutant phenotype was due to a recessive gene, called *su-A,* which was not

the production of fruit-bodies by a monokaryon is not dependent only on the presence of the factor which suppresses the action of the *A* factor, but it must be accompanied by one or more other factors not present in the progenitor of wild type $A6B_6$. In fact, as referred above, only some mutants produced haploid, pale, fruit-bodies.

We give the following salient facts from DAY'S (op. cit.) report, apart from the development, due to mutation, of sterile haploid fruit-bodies, in a homokaryon with false clamp connections (this character being the result of another mutation) :

a — occurrence of mutation, in a heterokaryon having the A factor in common ($A^{=}$ B=) ;
b — occurrence of mutation, not of an incompatibility factor, but in another unlinked *locus;*
c — existence of a suppressor gene;
d — detection of the function of an incompatibility factor when its normal function is repressed.

It must also be pointed out that DAY (op. cit.) did not carry out any cytological study of the homokaryotic mycelia having false clamp connections. Nevertheless, it would be interesting to know the distribution of nuclei in the terminal cell of the hyphae and in the anastomotic cells.

SWIEZYNSKI & DAY (1960) and DAY (1963) have also reported the segregation of non-parental A-factors in progenies of normal, heterokaryotic fruit-bodies developed in mycelia originating from common-A heterokaryons (A= B=). The mutant homokaryons have pseudo-clamps. Pale homokaryotic fruit-bodies developed in some of these homokaryons. Different A-mutants were compatible among them as well as with each of the four parental wild testers. This is an example of a kind of mutation Of an incompatibility factor, when there is A-community of the hetero-

linked with *A* but which suppressed its normal function allowing the development of false clamps». 'About *suppressor genes,* see, for example, DAY (1963), and CASSELTON (1971).

karyon. The mutation resulted in the loss of the discriminatory function of the factor, which converts the homokaryon into an A= B= heterokaryon-mimic (cfr. also RAPER, I960: 133).

b. — Mono-dikaryotic (haplo-diploid) fruit-bodies

A second example of sterility in homokaryotic fruit--bodies is seen in haplo-diploid fruit-bodies, i. e. those showing simultaneously haploid monokaryotic basidia, and dikaryotic basidia, karyogamy occurring in these with the production of diploid nuclei.

Here we must recall two cases, both described by QUINTANILHA (1935), referring to fruit-bodies developed in mycelia which initially were heterokaryotie with nuclei carrying in common one incompatibility factor (A= B= or A= B=). In both cases, according to that Author, this was not due to the arresting of heterothallism and the regression to a homothallic condition. Nevertheless, the monokaryons in both cases behaved as if it were a case of homothallism, since in their fruit-bodies there were dikaryotic basidia with genetically identical nuclei which fused, this karyogamy being followed by meiosis. These would be examples of disruption of the incompatibility, whose consequence is the intra-fertility of homokaryons of a heterothallic species — *Coprinus radiatus* ss. Lange (under C. fimetarius) —, with production of meiospores.

One of these cases reported by QUINTANILHA (op. cit.) concerns the development of a pale fruit-body from one of the parental homokaryons of a eommon-2? heterokaryotie mycelium (Ab + ab). According to that Author, the ability to fruit in the haploid stage is due to mutation. The mutant, Ab, called *K,* had false clamp connections whose development was, in rare cases, simultaneous with a conjugate mitosis. In these cases, the homokaryon is similar to a common-B heterokaryon (A= $B^{=}$). The homokaryons from the first fruit-body did also show the faculty of fruiting, their fruit-bodies being pale and similar to common-B fruit-bodies.

These fruit-bodies are homokaryotic but they are also mono-dikaryotic (haplo-diploids). In fact, although almost all the basidia are monokaryotic haploid, a few are dikaryotic; in the latter, nuclear fusion occurs, followed by meiosis. However, all the tetrads are monocratic, all the haplonts having the characteristics of the *K* mutant, which means that the two fusing nuclei are genetically the same.

According to QUINTANILHA (op. cit.), in this homokaryon there would be no mutation of an incompatibility factor[1], as shown by the results of matings with homokaryons of the four *testers*. Nevertheless, that Author did not determine the nature of this mutation. In DAY'S (1963) opinion, «we cannot tell whether *K* was an *A* factor mutant or, as seems more likely, a recessive suppressor like *su-A*» (see above)[2]. On the other hand, according to RATER (1966: 131), the disruption of the control of the incompatibility was not in this case due to a mutation of an incompatibility factor but to a mutation of another *locus,* «to a mutation of the type now termed modifier mutations»[3 4 5].

QUINTANILHA (op. cit.) described another mutation which he called *A"*[*]. Homokaryotic mutants showing many

[1] QUINTANILHA, at this period, called *sexual factors* what he later termed *incompatibility factors.*

[2] However, had we been dealing with the mutation of an incompatibility factor, the results of mating· with testers would be different from those reported by QUINTANILHA.

[3] According· to QUINTANILHA'S interpretation, there was, in this case, no mutation of an incompatibility factor. It appears that this interpretation was not emphasized by RAPER who stated that QUINTANILHA «attributed the anomalous behaviour in each case» *(A', K, A")* «to a mutation of the *A* factor» (RAFER, l. c.; see also RAFER, op. cit.: 144).

[4] On «modifier mutations», see RAPER & RAPER (1964).

[5] This case, reported by QUINTANILHA (1935), of a homokaryon having false clamp connections together with the ability to develop pale fruit-bodies, was published prior to DAY'S (1963) observations described above. The criterium of systematization that we adopted here is not a chronological one; this is the reason why this and thé following case are given after DAY'S.

* This mutation, originally called *A"* (QUINTANILHA, 1935), was later called A_3 (QUINTANILHA, 1967).

pseudo-clamps and a few clamps were isolated from a normal fruit-body developed in a common-A heterokaryon [1]. All the homokaryons carried the same non-parental factor and the faculty of fruiting, giving origin to pale fruit-bodies. The normal fruit-body might have been produced after the mutation had occurred in one of the parental homokaryons of the heterokaryon.

The homokaryotic fruit-bodies had monokaryotic and dikaryotic basidia, the latter being rarer than those on the «illegitimate» (A= B=) fruit-bodies. In the dikaryotic basidia, caryogamy occurred followed by meiosis. Thus, the pale fruit-bodies were haplo-diploid. All the tetrads of basidiospores from analysed fruit-bodies were monotypic, so that the fused nuclei were genetically the same, differently from what happens in the «illegitimate» fruit-bodies.

This *A″* mutation is similar to B_4 mutation (see above), since both have a genotypical alteration of one of the incompatibility factors, but they differ in the kind of the mutation. DAY (1963) and RAPER (1960) have also agreed that this *A″* mutation is a mutation of an incompatibility factor. On the other hand, *A″* mutant differ from the B_4 mutant by possessing both true and false clamps, and also because the fruit-bodies of the former are not totally haploid. No explanation was given for the existence of both false and true clamps, in these homokaryons.

We are inclined to assume that the A"-mutation of the homokaryons is not a change to a new functional specificity but it resulted in the loss of its discriminatory function, converting the homokaryons into A^ B= heterokaryon-mimics (cfr. RAPER, 1966: 133).

2. — Chimeric fruit-bodies

Among chimeric, mixed, fruit-bodies developed in heterokaryotic mycelia, we may consider: a) those which are

[1] The possibility of occurrence of mutation of incompatibility factors was irrefutably demonstrated for the first time by QUINTANILHA (1935), even though other investigators (e. g. KNIEP, 1923) had already attributed to mutations the abnormal behaviour of the expression of incompatibility (cfr. RAPER, 1966).

chimeric haploide, i. e. with some basidia having one parental nucleus, and other basidia having the nucleus of the other progenitor; b) those which are chimeric, but show simultaneously haploid and diploid basidia, the haploid basidia having one or the other nucleus.

a. — Mixed monokaryotic (haploid) fruit-bodies

In *Coprinus radiatus* ss. Lange (under C. fimetarius), OOET (1929, 1930) [1] observed occasional development of fruit-bodies in a mycelium devoid of clamp connections, which grew from the pairing of two homokaryons having one *B* factor in common; however, these fruit-bodies are not true A= B= , but are rather mixed haploids, i. e. with the separate participation of both homokaryotic parents. In the basidia, all the tetrads are monocratic, but of two types, each one corresponding to a homokaryon. Therefore we are dealing in these cases with chimeric fruit-bodies, which have not developed from a true heterokaryon.

b. — Mixed mono-dikaryotic (haplo-diploid) fruit-bodies

QUINTANILHA (1935) [2] reported the development of pale fruit-bodies with a small number of spores, in A= B= heterokaryons having false clamp connections, of *Coprinus radiatus* (under C. fimetarius).

These pale fruit-bodies had both monokaryotic haploid basidia, and dikaryotic basidia with two haploid nuclei which fuse into a diploid nucleus. The diploid basidia gave rise to dicratic tetrads, through meiosis. In turn, haploid basidia where no meiosis occurs, give rise to monocratic tetrads or they abort before dividing, or they divide only once, and then the two resulting nuclei either degenerate or will belong to two spores. However, most of the uninucleate basidia abort, due to irregularities in chromosome

[1] BRUNSWIK (1924) had already shown development of fruit-bodies in common-i heterokaryotic mycelia, in the same species.

* See also QUINTANILHA (1934, 1941, 1967).

distribution. The monocratic tetrads are of two types, each one corresponding to one of the homokaryon progenitors.

These are, therefore, chimeric fruit-bodies with haploid basidia. A greater or lesser degree of sterility of the haploid basidia is the determining cause of a greater or lesser degree of paleness in the fruit-bodies.

3. — Dikaryotic fruit-bodies

We can here distinguish: a) dikaryotic fruit-bodies with diploid basidia; b) dikaryotic fruit-bodies with triploid basidia; c) dikaryotic fruit-bodies in which the basidia are aneuploid.

a. — Fruit-bodies with diploid basidia

Sterile sporophores, developed in dikaryotic A= B= mycelia, differ from normal fruit-bodies by being yellowish--white, apart from the disc of the pileus, which is brown.

BULLER (1909) reported the development of sterile fruit-bodies next to normal ones in dikaryotic mycelia of *C, cinereus* (under C. fimetarius var. c*inereus)* and of C. *curtios* (under C. plicatiloides) [1]. To this Author, the cause of sterility «seems to be somewhat obscure», as it also does not seem to be conditioned by temperature, light, humidity or atmospheric gases. «Perhaps the phenomenon is due to some accident happening to the mycelium at the time when its contents are being poured into the young fruit-body» (BULLER, 1909: 16). «The diminution in the supply of food materials might lead to the non-development of the basidia» (BULLER, 1909: 17).

Later, the same Author (BULLER, 1922) again reported on the occasional development in *Coprinus radiatus*[2], of

[1] According to BULLER (1931, vol. 4: 11), «the name *O. plicatiloides* must therefore now be regarded as a mere synonym for C. *curtus*».

2 HANNA (1925, under C. lagopus; see also BULLER, 1924: 313) demonstrated experimentally that these sterile fruit-bodies (imperfect fruit-bodies looking similar to those produced by a primary mycelium), arise from the secondary, and not from the primary mycelium.

some sterile fruit-bodies, showing variable sterility, among normal fruit-bodies. He considered that cause of the arresting of hymenial development is still a mystery. «It cannot be the action of a parasite, because sterile fruit--bodies make their appearance in pure cultures; and it cannot be a deficiency of food material in the substratum, for sterile fruit-bodies come up in pure cultures on fresh sterilised horse dung» (BULLER, op. cit.: 73). «Perhaps the sterility is due to a too great luxuriance in the production of fruit-bodies». «Some of the fruit-bodies do not obtain the quantity of growth materials requisite for their full development and suffer partial starvation when just about to expand. Possibly, therefore, the imperfect development of the hymenium in some of the fruit-bodies is simply due to lack of vigour brought about by imperfect food supply from the mycelium» (BULLER, op. cit.: 74).

BULLER (1924: 314) reaffirmed that the sterility of dikaryotic fruit-bodies is due to «lack of vigour» and that this, in turn, «may be due to partial starvation brought about by the development of more fruit-bodies than the mycelium can properly support with nutriment».

BULLER (1931, vol. 4: 9) attributed fruit-body sterility, in *Coprinus curtus*[1], to the effect of «fumes given off by the fresh horse dung»[2].

[1] Having admitted the possibility of *Coprinus curtus* being- a tetrapolar heterothallic species (NEWTON, cfr. BULLER, 1931: 6) which however, according· to BULLER (1. a), is not proved, we were led to include this species in the present paper which concerns only the tetrapolar heterothallic species of the genus *Coprinus.* The fact that *G. curtus* has no clamp connections (cfr. for instance MOUNCE, 1921), may explain why the bipolar or tetrapolar type of heterothallism has not yet been determined.

[2] We shall quote Buller's (loc. cit.) original text: *«Fruit--bodies rendered Sterile by Fumes from Fresh Manure:* In a large glass case (3 feet long, 1.5 feet wide, and 2 feet high), one-half of the floor was covered by horse-dung balls which were two or three weeks old and which had produced and were producing many normal fruit-bodies of *Coprinus curtus.* Into this chamber there was introduced a mass of new horse dung sufficient to cover the other half of the floor, and then the door of the case was shut tightly. Two

SASS (1932) observed the development of an almost completely sterile fruit-body, among normal ones, from a mycelium of a strain of a *Coprinus*[1]. From a fragment of the stipe of this fruit-body, a mycelium bearing clamp connections was obtained, showing many other sterile fruit--bodies identical to the previous one. The secondary mycelium was maintained by successive subcultures for five years, always giving rise exclusively to sterile fruit-bodies.

Having carried out the cytologieal study of the sterile fruit-bodies, SASS (op. cit.) concluded that possibly most of the basidia did not develop beyond the stage of nuclear fusion. He thought that sterility was the result of the non-occurrence of meiosis in the basidia, disagreeing thus with BULLER'S (see above) suggestion that sterility was provoked by nutritional deficiences. However, SASS (op. cit.) did not explain what had originated the arresting of basidia development at the nuclear fusion stage.

Later, the same Author (SASS, 1935), attributed the sterility of fruit-bodies in the case described above, to the genetic inhibition of meiosis in the basidia; it is therefore completely independent of nutritional or other environmental conditions. SASS (op. cit.) considered that in the *Hymenorrvycetes,* it is necessary to consider the existence of another type of sterility in which «inadequate nutritional conditions may merely prevent the full development of hymenial elements, or may produce aberrations in the nuclear mechanism of the basidium». The same Author considered that those cases of variable sterility studied in Professor BULLER'S

days later a considerable number of *O. curtus* fruit-bodies came up on the old dung and expanded; but, instead of becoming· grey with ripened spores, they all remained pale and were partially or wholly sterile. There can be but little doubt that this sterility was due to the effect of fumes given off by the fresh horse dung».

Tests were carried out recently on the same species, do not corroborate BULLER'S opinion (op. cit.). In fact, in polisporic cultures on sterilized horse dung, three pale sporophores developed among 50 normal fruit-bodies. One of the pale sporophores was completely sterile.

[1] Probably C. *boudieri* or a very closely related species (SASS, op. cit.).

laboratory in *Coprinus radiatus* would belong to this type 1. There would be, therefore, two types of sterility, one of genetic nature, the other physiological.

CHOW (1934) reported the development in the secondary mycelium of *Coprinus radiatus* ss. Lange (under «C. lagopus sec. Buller»), of partially or completely sterile fruit-bodies, and «de temps à autre», of sterile fruit-bodies «côte à côte avec des normaux».

According to the same Author, the basidia of these sterile fruit-bodies are binucleate and their cytoplasm is altered. However, they regularly show normal nuclear fusion in a diploid nucleus. In some basidia, CHOW (op. cit.) observed two or four nuclei in the altered cytoplasm. This Author stated that he was not «en mesure d'expliquer cette stérilité» [2].

[1] According to the same Author, the case reported in the same paper, concerning the development of sterile fruit-bodies from dikaryotic mycelium with clamp connections, of the homothallic species *Coprinus sterquilinus* Fr., also belongs to this type. The fruit-bodies of this species were sterile when the culture was «overcrowded with several simultaneously expanding fruit-bodies, or after a culture has produced several crops». «In basidia of sterile fruit-bodies, usually only one division of the fusion nucleus occurs» (SASS, 1936).

[2] In the homothallic species *O. tomentosus* and *C. hendersonii*, the same Author (CHOW, op. cit.) also observed the development of partially or completely sterile fruit-bodies, in which most or all the basidia did not produce spores. In *O. tomentosus*, according to the same Author, all the basidia suspended their development in the diploid stage; therefore meiosis did not occur. In *C. hendersonii*, sterile basidia are those in which development is suspended either in the nuclear fusion stage, or in the two or the four meiotic nuclei stages.

DICKSON (1934) admitted that the variations in the degree of fertility of sporophores in the unifactorial species *Coprinus sphaerosporus* are due to the presence of «sterility (or incompatibility) factors», which must be numerous, «too numerous to be capable of analysis from the available data and must affect not only the degree of fertility of the diploid when formed, but must also, in those cases in which the diploid was not produced, condition the fusion of the haploids». According to this Author,, «the evidence is not sufficient to show whether the various degrees of sterility exhibited are due to the presence of incompatibility factors, and if so, how many such

QUINTANILHA (1935) reported having isolated from a tetrad of an «illegitimate» pale fruit-body, Ab + ab (A= B=), of *Coprinus fimetarius,* a mutant homokaryon which he called A'b. This would be an alteration of the valency of a «sexual» factor as a result of which the mutant homokaryon gave positive reactions with two testers, aB and AB, and in both these crosses, fruit-bodies developed.

According to that Author, this mycelium originated because «dans les noyaux du mycélium qui proviennent de celui de la spore (ab), une mutation doit être survenue quelque part» — the a factor would have mutated to *A'*. This mycelium would not be a heterokaryon, but a mixture of hyphae, wild and mutant. The demonstration of the occurrence of this mutation was also obtained through the analysis of random spores (not of tetrads) of the first generation, spores produced by one fruit-body developed from the cross between the mutant A'b and the tester aB. As a matter of fact, on analysing 27 spores, the matings with the testers gave: 6 ab, 12 *A'b,* 3 aB and 6 *A'B.* There was thus mendelization of the four factors *A', a, B, b.*

The first fruit-bodies developed in the mycelium obtained from the mating of the mutant *A'b* and the tester aB «avaient l'air de fructifications illégitimes — croissance lente, pieds courts, petite quantité de spores, des chapeaux qui souvent ne s'épanouissent pas, couleur pâle, etc». However, fruit-bodies which developed later in the same culture showed phenotypes becoming successively more and more normal until completely normal ones, which led that Author to conclude that the abnormalities in the development of the fruit-bodies came from the cytoplasm. This would be another case besides CHOW'S (1934), in which the cytoplasm was considered responsible for the development of pale fruit-bodies. However, QUINTANILHA (op. cit.) did not mention the possibility of abnormalities of

factors are concerned». (We recall that the significance attributed to this Author, of the expression «incompatibility factors» is different from that given by QUINTANILHA, in 1937).

the cytoplasm being determined by non-chromosomie genes.

Later, RAPER (1966: 131) did not confirm QUINTANILHA'S (op. cit.) interpretation, according to which this was a case of mutation of factor *a* into *A',* i. e., a change to a new functional specificity. According to that Author, after a «careful examination»..., «in the light of more recent findings»..., the anomalous, behaviour of the *A-f*actor — which gives rise to fruit-bodies in the mycelium resulting from the mating A'b X aB — was due to disomy[1]. As a matter of fact, the analysis of the progeny (see above) of one normal fruit-body, supports that opinion.

That Author also considered the abnormal cytoplasm to be responsible for the dwarfing of numerous mycelia arising from the initial «illegitimate» fruit-body, Ab + ab. This opinion is based on the results of crossings between dwarf and normal haplonts. The mutation *A'* would not be responsible for this dwarfing, which should be phenotypically determined (see, however, PAPAZIAN, 1958). «Un trouble

[1] In RAPER'S opinion (1966: 131), out of the three cases of abnormal strains arising from «illegitimate» fruit-bodies, developed in heterokaryons with a common factor, interpreted by QUINTANILHA (1935) as due to mutations of the factor *A,* only one *(A"),* a strain derived from a A= B= heterokaryon, should be attributed to this cause. Prom the other two cases *(A'* and *K)* of anomalous behaviour of factor *A,* both derived from A= B= heterokaryons, one *XA') A=* more likely to be due to disomy and the other *(K)* to a «modifier mutation». However, QUINTANILHA'S own interpretation is different from that attributed to him by RAFEE (1. α), since for the former, although the strains *A'* and *A"* were, in fact, mutants of *A,* strain *K* had not arisen from mutation of an incompatibility factor. On this subject, it seems worth while to quote QINTANILHA'S text (op. cit.: 41-42): «Les mutations étudiées peuvent ne frapper que la valence des facteurs dits de sexualité» *(A',* p. ex.). «D'autres fois, sans rien changer à cette valence, elles déterminent la formation de pseudo--anses dans des cultures monospermes *(K,* p. ex.). Ces mycéliums donnent origine à des carpophores haplo-diploïdes, comme ceux des copulations illégitimes mais ceux-là ne produisent que des tétrades monocrates. D'autre foie encore la mutation frappe, simultanément la valence d'un facteur de «sexualité» et la faculté de produire des mycéliums monospermes, fèrtils, à pseudo-anses (*A",* p. ex.). Les carpophores produits sur ces mycéliums sont aussi haplo-diploïdes, mais ne produisent que des tétrades monocrates».

quelconque, et qui n'intéresse pas le génotype, s'est produit simultanément avec la mutation» (QUINTANILHA, 1935: 25). And even as QUINTANILHA stated, «les noyaux sont sains et ne transmettent pas la *maladie;* c'est, peut-être, le plasma qui est *malade*, plus ou moins incapable de se nourrir. Déversés dans un plasma bien portant, les noyaux se conduisent d'une façon normale et les anomalies disparaissent». These abnormalities are of interest, not only in relation to dwarfing in haplonts, but also to the «illegitimate» phenotype of fruit-bodies. This interpretation, applied to abnormal fruit-bodies, implies the acceptance that the disturbed cytoplasm and having nutritional difficulties does not influence fruit-body development, but only that of the basidia.

In any case, the fact to emphasize is the development of sterile fruit-bodies in a normal dikaryon, sterility being attributed to a cytoplasmic disturbance which has arisen simultaneously with an alteration (structural or numerical mutation) of the genome. QUINTANILHA stated that this character of the cytoplasm is not genotypically determined, but this was not proved.

DAY (1957, 1959) described in *Coprinus cinereus* ss. Konr. (under C. lagopus), a mutation interpreted as being of an extra-chromosomic nature, which would be responsible for the production of sporophores whose gills have fewer spores than normal. The sterility of many basidia gives these sporophores the «pale» phenotype. Besides completely sterile basidia, the pale fruit-bodies have basidia (12.9%) with spore numbers inferior (2, 3) or superior (5, 6, 8) to four, and others (87,1 %) with the normal number of four basidiospores.

Obtaining two different phenotypes, normal and abnormal, in reciprocal crosses of two haplonts arising from the same hymenophore was the main criterium which allowed the conclusion that the abnormal character is cytoplasmically controlled. But according to DAY (1959), the repetition of the same reciprocal crosses with sub-cultures of the same haplonts, carried out three months later on a larger scale, did not duplicate the previous results. In the same way, the mycelium obtained by sub-culturing the dikaryon which

had given rise to the development of normal sporophores, gave normal and abnormal sporophores. The same Author accepted that the cytoplasmic mutation had been a result of ageing. The occurrence in a common inoculum of both the phenotypes and the production of sectorial sporophores, inferred the existence of segregation of cytoplasmic elements.

The reciprocal crosses of monokaryons (neo-haplonts) obtained from the hyphal tips arising from dikaryotic chlamydospores (formed in mycelia in which both normal and abnormal pale sporophores developed), gave rise to mycelia, which in some cases developed sporophores having the phenotype of the parental dikaryon; in other cases, however, this did not happen. On the other hand, of the four dikaryotic chlamydospores produced in the mycelium resulting from the cross where normal sporophores developed, three gave rise to pale sporophores, and one to a normal, black sporophore. DAY (op. cit.) considered it likely that these cases were due to variation of the relative amounts of cytoplasm in the different chlamydospores of a given mycelium. All the crosses of compatible homokaryons showing normal growth, derived from spores produced by abnormal (white) sporophores, gave rise to normal sporophores (black). According to DAY (op. cit.), «the abnormality is determined by the cytoplasm and is a result of ageing». The abnormal cytoplasm provoked irregularities in meiosis, such as the non-occurrence of meiosis II, formation of bridges between chromosomes, of chromosomic fragments and of micro-nuclei. The aborted meiosis had, therefore, as consequences, the production of less viable spores and of stunted mycelia. Another effect of the abnormal cytoplasm was the appearance of an early mitosis in the basidium, leading to the formation of more than four spores in each basidium. The abnormal cytoplasm had the effect of determining the production of an abnormal basidiospore number, as there is no correlation between the number of nuclei and the number of sterigma. According to ESSER & KUENEN (1965), .the extra-chromosomic cha-

racter of the basidium abnormality «is unequivocally established».

In this example, the only criterium which supports the hypothesis of the existence of a cytoplasmic determinism resides in the differences revealed by the reciprocal crosses. However, JINKS (1963) called attention to the fact that the results of the reciprocal crosses in DAY'S experiments cannot be sufficient proof of cytoplasmic heredity, since the difference was not persistent 1. Referring to DAY'S experiments (op. cit.), ESSER & KUENEN (1965) held the opinion that «it is unfortunate that the first attempt to gain an insight into the function of the plasmone in fungi has so far failed to yield significant results».

We feel DAY'S experiments (op. cit.) do not show conclusively the existence of non-chromosomic factors (genes or states), but, on the other hand, the results do not allow a different interpretation.

Kimura (1963a, 1963b) [2] found evidence o fthe control of the production of «undeveloped fruit-bodies» by a recessive factor *f,* unlinked to the incompatibility factors, in ff heterokaryons (dikaryons and common-!? heterokaryons) of *Coprinus cinereus* (under C. macrorhizus f. microsporus; cfr. KEMP, 1975).

b. — Fruit-bodies with triploid basidia

CASSELTON (1965), in *Coprinus cinereus,* and PRUD'HOMME (1965), in *Coprinus radiatus,* obtained fruit-bodies with triploid basidia, or mixed, chimeric, fruit-bodies with basidia having a triploid segregation. These fruit-bodies were pale and developed in heterokaryons arising from

[1] In H*ymenomycetes,* there are only a few other recorded cases of differences attributed to non-chromosomic determinism, all of them concerning bifactorial species of *Coprinus:* DICKSON (1936: *O. macrorhigus);* QUINTANILHA & BALLE (1938, 1940: C. *radiatus,* under C. fimetarius); KIMURA (1954: *O. cinereus,* under C. macrorhizus for. microsporus; cfr. KEMP, 1975); CASSELTON & CONDIT (1972: C. *cinereus,* under C. lagopus); CASSELTON & KIRKHAM (1975: C. *cinereus,* under C. lagopus).

[2] According to English abstracts.

matings between a diploid and a haploid mycelia or between two diploids.

CASSELTON (op. cit.) obtained a dikaryon with clamp connections, by crossing a common-1 diploid, which resulted from a heterokaryon having in common the factor *A,* with a haploid homokaryon. The fruit-bodies which developed in the resulting dikaryotic heterokaryon, had triploid basidia which became aneuploid (hyperdiploids).

The same Author also obtained a dikaryon with clamp connections, from a mating of a common-AB diploid mycelium with a haploid. In the cases in which the diploid nucleus remained diploid, pale fruit-bodies developed. She also obtained a dikaryotic mycelium with clamp connections, from a mating of two common-A diploid mycelia. In one of the diploid nuclei of this heterokaryon, haploidization occurred. The pale fruit-bodies that developed had triploid or aneuploid basidia. Fruit-bodies did not develop when the two nuclei remained diploid.

PRUD'HOMME (op. cit.) obtained pale fruit-bodies in heterokaryons produced by mixed culture of a diploid and a haploid, or of an aneuploid and a haploid. The diploids were produced either from a dikaryon with clamp connections, or from a common-B heterokaryon, having false clamp connections, or from tri-heterokaryons obtained from mixing three inocula. In the dikaryon (2n + n) with clamp connections resulting from the mating of a diplont (A= B=) and a haplont, pale mixed chimeric fruit-bodies developed, with three types of basidia: 2n + n, or (n + 1) + + n, or η + n. In the dikaryotic mycelium (2n + n), produced from the mating of a haplont and a diplont (originating in a common-B heterokaryon having false clamp connections), pale mixed chimeric fruit-bodies also developed; in these, there were two types of basidia: 2n, with normal segregation, and 2n + n, with a triploid segregation. When there were triploid basidia, the fruit--bodies were abnormal besides **being pale.**

α— Fruit-bodies with aneuploid basidia

PRUD'HOMME (1965) obtained pale fruit-bodies which developed in heterokaryotic mycelia resulting from crossing an aneuploid, disomic II, with a haploid. This aneuploid had arisen by haploidization from a diplont originating in a common-*B* heterokaryon. According to that Author however, not all disomies give, by crossing with a haploid, pale fruit-bodies. The chromosome Π determines the production of fruit-bodies which are abnormal besides having pale gills.

4. — Trikaryotic fruit-bodies

KIMURA & KAYODA (1962) [1] obtained two trikaryotic fruit-bodies having spores somewhat scanty and generally larger than the normal spores, some of them being angular, in C*oprinus cinereus* (under C. macrorhizus f. microsporus; cfr. KEMP, 1975). These fruit-bodies might have been developed from trikaryotic cells resulting from the migration of nuclei of four different mating types from four inocula, through the hyphae of a previously established monokaryon compatible with all of them. Among the monosporous mycelia, isolated from one of the fruit-bodies, there were monokaryons and dikaryons, some of the former being disomies for *B*=*f*actor. Among the monosporous myeelia isolated from the other fruit-body, there were monokaryons, some of which being disomies for *A*- or B=factors, and dikaryons.

*
* *

The cases of fruit-body sterility we have compiled from the literature, and have reported on the previous pages, may be presented according to the following classification concerning types of sterile fruit-bodies and conditions

[1] According to the English abstract.

determinig them, as well as the factors influencing their respective development:

1 —homokaryotic fruit-bodies

a. — monokariotic (haploid) fruit-bodies

i) — ability to fruit simultaneous with the mutation of an incompatibility factor *(A)*, in a homokaryon isolated from the progeny of a fruit-body developed in an A=B= heterokaryon (dikaryon) *(Coprinus radiatus:* QUINTANILHA, 1935; «mutante B4»)[1];

ii) — ability to fruit simultaneous with a mutation which supresses *(suppressor gene)* the action of an incompatibility factor (presence of pseudo--clamps), of a homokaryotic mycelium from a common-A heterokaryon (A= B=) *(Coprinus cinereus:* DAY, 1963, 1965; «mutant *su-A»)* ;

iii) — ability to fruit of A-mutant homokaryons having pseudo-clamps, isolated from normal fruit-bodies developed in A B = heterokaryons *(Coprinus cinereus:* SWIEZYNSKI & DAY, **1960; DAY, 1963;** *A-mut)*[2] ;

b. — mono-dikariotic (haplo-diploid) fruit-bodies

i) r— ability to fruit, unrelated to a mutation, of all the homokaryons isolated from normal fruit--bodies (A= B=) ; pale haploid fruit-bodies having haploid and diploid basidia *(Coprinus radiatus:* CHOW, 1934) ;

ii) — ability to fruit simultaneous with a suppressor mutation (presence of false clamp connections), in a parental homokaryon of a common-B (A= B=)

[1] This mutation is a change to a new functional activity of an A-faetor.

[2] This mutation results from the loss of the discriminatory function of **an A-factor.**

heterokaryon *(Coprinus radiatus:* QUINTANILHA, 1935; «mutante *K»)* [1]*;*

iii) — ability to fruit simultaneous with the mutation of an incompatibility factor, of homokaryons having true and false clamps, coming from a normal fruit-body after the occurrence of the mutation in an A= B= heterokaryon *(Coprinus radiatus:* QUINTANILHA, 1935, «mutante A») ;

2. — chimeric fruit-bodies

a. — monokaryotic (haploid) mixed fruit-bodies, developed in a pairing of homokaryons having community of a B-factor (A= B—) *(Coprinus radiatus:* OORT, 1929, 1930);

b. — mono-dikaryotic (haplo-diploid) mixed fruit-bodies developed in an A=B$^{=}$ heterokaryon having false clamp connections *(Coprinus radiatus:* QUINTANILHA, 1935);

3. — dikaryotic fruit-bodies

a. — fruit-bodies with diploid basidia [2] :

i)—phenotypic determinism of sterility: *Coprinus cinereus* (BULLER, 1909) ; *C. radiatus* (BULLER, 1909, 1922, 1924; HANNA, 1925, 1928; QUINTANILHA, 1935: sterile fruit-bodies in which one homokaryon has a mutation *A'* — see also below) ; *C. curtus* (BULLER, 1931) ; *C. sterquilinus* (SASS, 1935) ;

ii)—genotypic, chromosomic, determinism of sterility: *Coprinus boudieri* (SASS, 1932) ; *C. radiatus* (QUINTANILHA, 1935: according to RARER, 1966, *A'-ho*mokaryon would be a disomic — see also above)[8];

[1] This is RAPER'S (1966) interpretation of a «modifier» mutation.

[2] Types of determinism of sterility are here given in agreement with the interpretations of the Authors cited.

[8] KIMURA (1963a, 1963b) found evidence of the control of the production of «undeveloped fruit-bodies» by a recessive factor *f,* unlinked to the incompatibility factors, in *ff* heterokaryons (dikaryons and common-B heterokaryons) of *Coprinus cinereus* (under C. macrorhizus f. microsporus; cfr. KEMP, 1975). (From **English abstracts).**

iii) —genotypie, non-chromosomie, determinism of sterility: *Coprinus cinereus* (DAY, 1957, 1959) ; *C. radiatus* (CHOW, 1934) ;

b. — fruit-bodies with triploid basidia:

i)—diploid X haploid matings: *Coprinus cinereus* (CASSELTON, 1965: common-A diploid X n; com-*mon-AB* diploid X n) ; *Coprinus radiatus* (PRud' HOMME, 1965: 2n X n; common-5 diploid X n) ;

ii)—diploid X diploid matings: *Coprinus cinereus* (CASSELTON, 1965: common-A diploid X common-A diploid: tri-haploid fruit-bodies) ;

c. — fruit-bodies with aneuploid basidia: aneuploid X X haploid mating: *Coprinus radiatus* [PRUD'HOMME, 1965: (n + 1) X n] ;

4. — trikaryotic fruit-bodies: *Coprinus cinereus* (KIMURA & KADOYA, 1962).

III. ON THE DETERMINISM OF HYMENOPHORE STERILITY

The compiling from the literature of interpretations and data on the development of sterile fruit-bodies in bifactorial species of *Coprinus,* dealt with in the previous chapter, help towards a better understanding of the determinism of sterility. This is the aim of the present chapter.

In these heterothallic tetrapolar species, whose life--cycle includes various types of mycelium[1], fruit-bodies may develop either in the different homokaryons, these being dikaryotic (A= B=) or not (A= B=). From this, we may infer that *hymenophore development is independent of the momokaryotic or heterokaryotic condition of the vegetative mycélium.* Thus, the existence of a dikaryon, i. e. a heterokaryon which is di-hetero-allelic for the factors of incom-

[1] The different types of mycelium which may originate either from spores or from the various types of hyphal anastomoses were described and redefined by PINTO-LOPES (1952).

patibility, is not essential for the production of fruit-bodies (cfr. BULLER, 1941).

In rare cases, the development of fruit-bodies occurs in all the numerous haplonts of the four genetical types, isolated from the same genetic stock. This points to the conclusion that *the development of homokaryotic fruit--bodies is independent of the respective incompatibility factors.*

In certain types of heterokaryon (A= B= and A= $B^{=}$), fruit-bodies with a heterokaryotic structure do not develop. From this fact we may conclude that *the development of heterokaryotic fruit-bodies depends, to a certain extent, on the incompatibility factors present in the heterokaryon.*

In the other types of heterokaryon (A=$B^{=}$ and A= B=), hymenophores may develop. Comparing this with the previous statement, leads us to conclude that *the community of the factor A with or without the community of the factor B, prevents the development of fruit-bodies in heterokaryotic mycelia,* this non-development being a consequence of A-factor function, related, in the heterozygotic condition, to the production of clamps (different *Bs)* or of false clamps (common-B).

A homokaryon can give rise to fruit-bodies which are also homokaryotic and haploid, karyogamy and meiosis not occurring in their basidia. In a variable number of basidia of a homokaryotic fruit-body, and from the respective haploid nucleus, four haploid non-recombining nuclei may arise through two somatic mitosis, followed by the development of a monocratic (or monotypic) tetrad of basidiospores, genetically equal to the initial homokaryon. Cases are known, however, in which the basidia of homokaryotic fruit-bodies, as well as some basidia from chimeric fruit-bodies, have two nuclei genetically identical, which fuse into one diploid nucleus, the fusion being followed by the two mitosis of meiosis, with the production of four haploid spores which are genetically identical to the initial homokaryon.

From these facts, we conclude that *the basidium is a specialized hymenophore cell — the only one in the life--cycle -—, where two successive mitosis may take place from a haploid or a diploid nucleus. In the basidia in which these mitosis do not take place, there is no spore production. Thus the greater or lesser degree of sterility of a hymenophore is a consequence of the higher or lower frequency of basidia where those mitosis occur. On the other hand, the basidium, being the structure where either karyogamy or meiosis may or may not occur, is nevertheless, the only structure of the whole life-cycle where meiosis may occur*[1]. On the other hand, *the occurrence of karyogamy means that the two nuclei are sexually different, and that sexual differentiation taking place in the basidia is, in fact, phenotypic:* besides this, the fact that the nuclei which fuse, are genetically the same, favours the idea that *incompatibility factors are not actually sex factors.*

The ability of homokaryons of a given genetic stock to fruit is, in some experimentally proved cases, a consequence of mutation which is not related to incompatibility factors, regular mendelization of the difference having been demonstrated [2].

Under experimental conditions, the ability to fruit was also observed in homokaryons coming from fruit-bodies developed in different types of non-dikaryotic heterokaryons; such homokaryons showed, simultaneously with that ability, a mutation of one of the incompatibility factors, or a mutation which suppresses the activity of incompatibility factors, or even disomy concerning one of these factors 3.

[1] Although we may infer that the development leading to the production of a basidial cell determines the type of process which ultimately occurs inside it, the reasons are not yet known why sometimes a basidiocarp shows simultaneously basidia with somatic or meiotic mitosis, and others without them.

2 However, in identical cases, no factual evidence was obtained of the genetic determinism of the ability of homokaryons to fruit.

» Cfr.: PRUD'HOMME & GANS (1958), in *Coprinus radiatus.*

Fruit-bodies developing in haploid mutants may have haploid basidia (in monokaryotic fruit-bodies) or, side by side, haploid and diploid (in mono-dikaryotic fruit-bodies) basidia; these fruit-bodies show a greater or lesser degree of sterility.

On the other hand, as a consequence of segregation occurring in non-dikaryotic heterokaryons, different homokaryons may participate in the production of chimeric fruit-bodies which will be sterile to a greater or lesser degree.

Prom the above mentioned facts, we may draw the following conclusions:

a — *the greater or lesser degree of sterility of monokaryotic fruit-bodies results from their haploid condition, i. e. from the absence of meiosis, as well as from the greater or lesser irregularity in the two mitosis which may occur in the basidia*[1]*;*

b — *the sterility of chimeric, homokaryotic and homo--heterokaryotic, fruit-bodies, results from the haploid state of all or some of the basidia, and from the absence of meiosis in diploid, ones*[2] ;

c — *the occurence of karyogamy and meiosis is not dependent on the incompatibility factors present in the haploid nuclei of the basidia.*

Laboratory tests leave no doubt as to the conditions necessary for the development of fruit-bodies having all their basidia fertile. These fruit-bodies develop in dikaryotic mycelia (A= B=) having true clamp connections, while

[1] The fact that dikaryotic fruit-bodies may also be sterile, should not be used as an argument against the interpretation that the haploid condition determines the sterility of homokaryotic fruit--bodies.

[2] In the cases where meiosis takes place, the basidia will be fertile, even when the haploid nuclei that take part in the karyogamy are genetically identical.

they maintain the genetic blocking for somatic nuclear fusion[1].

The dikaryon is formed as a result of hyphal anastomosis between two compatible homokaryons or of dikaryotization (« A = phenomenon»)[2] of a homokaryon by a heterokaryon under genetically controlled conditions, or even from anastomosis of two compatible dikaryons.

The conclusion to be drawn from this is that *complete fertility of fruit-bodies depends on their complete dikaryotic condition 3* which cannot be substituted by a somatic stage of diploid development, even though both cases are

The complete fertility of fruit-bodies in dikaryons (A= B=) results from the presence in all the basidia, of

[1] As a consequence of breaking this blocking·, a diploid mycelium is produced, sometimes stable (in *Coprinus cinereus:* CASSELTON, 1964; CASSELTON & LEWIS, 1966; *in Coprinus radiatus: PRUD'HOMME*, 1958, 1965, 1966, 1970; in *Schisophyllum commune:* PAEAG & NACHMAN, 1966; KOLTIN & RAFEE, 1968; MILLS & ELLINGBOE, 1969). Diploid mycelia do not have the ability to fruit. This fact deserves attention in order to interpret the biological significance of the dikaryon. Although this behaves as if it is a diploid stage, and although it is assumed (cfr. MATHER, 1965) that the usual lack of nuclear fusion in this vegetative mycelium is·due to these fungi not having developed a mechanism for maintaining diploidy — meiosis occurring immediately after karyogamy in the basidium, — the fact is that the mycelia where the nuclear fusion takes place do not possess the ability to fruit. Indeed, in order to fruit *normally,* a dikaryon is necessary. The somatic diploidization which therefore precedes the development of fruit-bodies prevents the subsequent progress to this stage, 1. e. the karyogamy, which normally takes place in the basidia, is not replaceable by an occasional occurrence of karyogamy in the vegetative mycelium. However, it should be recalled that from the point of view of the essential consequence — which is the genetic recombination —• of this process, and of those that follow — meiosis or haploidization — it can be stated that somatic karyogamy substitutes or adds to the basidial karyogamy.

* The expression « A = phenomenon» was introduced by QUINTANILHA (1937); PAPAZIAN (1950) adopted the more explicit expression «di-mon mating». RAPER (1966) presumes that this phenomenon is frequent in Nature.

[s] The «complete dikaryotic condition» of the fruit-body means that all their basidia are developed from dikaryotic hyphae.

two pairs of allelic factors of incompatibility, distributed in two distinct haploid nuclei, and from their immediate fusion. On the other hand, the fertility, represented by the production of a tetrad of viable basidiospores in every basidia, implies a previous regular functioning of the meiosis of the diploid nuclei resulting from the above mentioned basidial karyogamy.

Under laboratory conditions, it is possible to produce heterokaryons whose nuclei have one (A= B=, A= B$^{=}$) or two (A= B=) incompatibility factors in common, and from them fruit-bodies develop which are completely fertile in some cases, and partly sterile in others. The fruit-bodies are fertile when there is in the heterokaryon one of the genetic causes of disruption of the incompatibility control (cfr. RAPER, 1966). Conversely, if the heterokaryon maintains the initial genetic constitution, fruit-bodies do not develop — in cases of A= B= and A= B= —, or they are partly sterile — in cases of A=B= The sterility of some basidia of these latter fruit-bodies is due to their being haploids derived from homokaryotic hyphae of one or other of the parents, although in some haploid basidia there are somatic mitosis with the subsequent production of tetrads of basidiospores.

From these facts we may conclude:

a — in a fruit-body, all the basidia are fertile when two nuclear genetic characteristics of the eu-sexual process take place regularly — karyogamy and meiosis;

b — the necessary condition for the occurrence of karyogamy followed by meiosis is the development into basidia of cells having two nuclei; these may be: i) two nuclei from a dikaryon (A= B=); ii) two nuclei from a heterokaryon A= B= ;

c — the sterility of some basidia, in fruit-bodies developed in heterokaryons A= B=, is a result of the absence of karyogamy and meiosis;

d— variable sterility of heterokaryotic fruit-bodies, results from a greater or lesser participation of haploid hyphae in their structure.

e — complete fertility of the fruit-bodies developed in my celia originating from A= B= and A= B⁼ matings is a result of a previous genetic disruption of incompatibility control.

In all types of heterokaryons, A= B=, A= B=, A= B=, A= B⁼ , somatic karyogamy has already been obtained experimentally, which allows us to conclude that:

a — *karyogamy is independent of the incompatibility factors of the partner nuclei;*

b — the community of A-f actor does not prevent karyogamy;

c — in common-A heterokaryons, which do not fruit, para-sexuality is an alternative mechanism, and not an additional process, to the sexual mechanism.

Under experimental conditions, it is possible to obtain dikaryons in which one or both nuclei are diploid. These dikaryons may give rise to fruit-bodies, but here the karyogamy as well as the subsequent meiosis do not take place normally, and, as a consequence, many basidia are not fertile, in spite of some being able to produce spores.

Also under experimental conditions, it is possible to produce dikaryons in which one of the nuclei is diploid and had its origin in a heterokaryon with one or two incompatibility factors in common (common-A diploid + n; common-B diploid + n; common-AB diploid + n).

It is equally possible to provoke the formation of dikaryons with diploid nuclei, in which each of these came from a heterokaryon having one factor in common (common-A diploid + common-A diploid), and even the formation of dikaryons coming from the mating of an aneuploid and a haploid mycelium.

In all these cases, fruit-bodies may be produced with a greater or lesser degree of sterility. We must remember,

however, that in these cases the basidia are not diploids but are triploids, or tri-haploids, or aneuploids, and therefore meiosis does not follow the normal pattern.

From the above mentioned facts, we may conclude that *the irregularities in meiosis arising from alterations in the number of chromosomes, results in the absence of sporulation and therefore in the sterility of both basidia and hymenophores.*

In Nature or under laboratory conditions simulating natural ones, the development of sterile fruit-bodies, sometimes together with fertile ones, may be interpreted as the result of the occasional production of semi-compatible and of incompatible heterokaryons.

The conclusion which we may draw here is that *hymenophore sterility is always genetically determined.*

The production of sterile fruit-bodies, *in vitro,* in cases of factual evidence for the existence of normal segregation of known factors and absence of mendelization of sterility, and which may be attributed to the influence of non--chromosomic factors must also be considered as genetically controlled. These factors would be responsible for the irregularities of meiosis which originate sterility in the basidia 1.

The conclusion is that the *genetic determinants of sterility can be chromosomic or non-chromosomic.*

In fruit-bodies, the production of lateral or superimposed sectors, some with fertile and others with sterile basidia, can be interpreted as due to the joint participation in the structure of the fruit-body, either of homokaryons segregated from heterokaryons or of heterokaryons. It may also be the result of somatic segregation of non-chromoso-

[1] There is still no conclusive experimental data about non--chromosomic genetical determinism of sterillity. The hypothesis, however, of responsibilising· extra-chromosomic elements for hymenophore sterility allied with the occurrence of anomalies in the meiotic process (cfr. DAY, 1959), would point to those elements as partly influencing these anomalies. According- to ESSER & KUENEN (1967: 460), the plasmone in the Basidiomycete *Coprinus cinereus* is partially responsible for the normal course of meiosis.

mic genes, resulting in a differentiation of different types of hyphae in the development of the hymenophore, and in the development of the hymenium.

Considering all these facts, we come to the conclusion that *the determinism of sterility in fruit-bodies is related to either disturbance or absence of the meiotic process.*

The hypothesis that hymenophores owe their occasional sterility to physiological alterations directly influenced by environmental factors cannot be considered as experimentally proved, but taking into consideration the above mentioned conclusions, we may state that *determinism of sterility is not phenotypic.*

In conclusion, *basidium sterility is due to the absence of meiosis or to irregularities in the meiotic process, both motivated by genetic determinants, either chromosomic or possibly, non-chromosomic*[1].

IV. ON THE BIOLOGICAL SIGNIFICANCE OF INCOMPLETELY FERTILE HYMENOPHORES

The observation of development, under laboratory conditions, of sterile or more or less sterile hymenophores, and the knowledge of sterility determinism, lead us to a discussion concerning the biological significance of these structures.

The importance of this subject is evident when we assume that also in Nature, basidiocarp development may occur showing irregularities in basidiospore production. On the other hand, the subject involves problems of General Biology related either to genetic recombination, and therefore with genetic diversity, or to absence of recombination, and therefore with genetic continuity. In fact, these two opposite genetic events are always present, together and balanced, in all living organisms possessing sexual reproduction (cfr. MATHER, 1942; KOLTIN, STAMBERG & LEMKE,

[1] It is not proved that there is no meiosis in haploid hymenophores. But it is still not possible to explain the variable sterility which is generally shown by fruit-bodies developed in homokaryons.

1972). In the present case, we are dealing with organisms whose eu-sexual cycle includes a long dikaryophase, with a possible production of various types of dikaryons 1. and whose sexual pattern includes a tetrapolar, and even multipolar heterothallism, which are genetically determined and, concomitantly, with a phenotypical sexual determinism. These conditions increase the probabilities of cross fertilization, and therefore of recombination, and restrict those of self-fertility. We are also dealing with organisms which besides their eu-sexual cycle, present various types of assexuai cycles, and through these, various kinds of recombination and which may even present a para-sexual process as a result of the existence of heterokaryosis. In fact, the vegetative mycelium, having a potentially unlimited growth in the substratum, may, as a result of multiple vegetative fusions with other mycelia, become a mosaic of combinations of nuclei in each population of individuals of a genetic *stock* — a social organization of nuclear level — which, for this motive, also allows various types of genetic recombination. «The common concept of the dikaryon as a homogeneous, stable and genetically balanced heterokaryon having a nuclear ratio of unity is no longer wholly adequate» (RAPER, 1961).

This versatility gives rise to various types of recombination — somatic [2], nuclear, intrachromosomic — which are obtainable experimentally, but may also arise effectively and efficiently in Nature. The knowledge of the existence of this variety of possible recombinations, leads to the idea

[1] Various types of dikaryons can be distinguished in the *Hymenomycetes,* according to their origin. PINTO-LOPES (1952: 29) suggested the terms *homodikaryon* and *heterodikaryon* to distinguish a mycelium in which all its dikaryons were equal, and one in which there are different dikaryons. The same Author (PINTO-DOPES, l. c.) also suggested the designations d*ihomokaryon* and *diheterokaryon* for dikaryons which had the two nuclei equal or different respectively. Later, OLIVE (1953) suggested the terms *homodikaryon* and *heterodikaryon,* but gave them the meaning which the first Author had attributed to the terms dihomokaryon and diheterokaryon.

[2] With regard to the meanings of the expression «somatic recombination», see also SHALEV, STAMBERG & SIMCHEN **(1972).**

that «higher fungi became and in general remained the sexiest of haploid organisms» (KOLTIN, STAMBERG & LEMKE, 1972).

The production of basidiospores on surfaces (where genetic recombination takes place) supported and held above the substrata by the hymenophore, favours the dispersion of those immediate products of meiosis, being especially important when dispersion reaches distant areas and substrata different from the initial one; from this results, in its genetic significance, simultaneously, either the possibility of genetic diversity which involves recombination processes, and therefore the genetic development of each species, making speciation difficult, or alternatively, the genetic discontinuity through the isolation which favours speciation (cfr. INGOLD, 1965).

Bearing the above facts in mind, we may state that hymenophores are naturally specialized organs of recombination as well as of dispersion, being particularly important this dispersion of the recombined products. One must remember, however, that the hymenophore is not the only structure where karyogamy and the reduction, to the haploid number, of the diploid number of chromosomes occur, so that it is not the only structure where inter--chromosomic and intra-chromosomic recombinations take place. In fact, under experimental conditions these types of recombination may also occur in the vegetative mycelium through somatic *crossing over*, followed by haploidization in the para-sexual process. One may easily assume that this process also occurs in Nature. According to BURNETT (1965), «if somatic recombination is indeed widespread»... «sporophores could be more significant as agents of dispersal than as localized organs for recombination» 1.

However, the two processes cannot be dissociated, but we should rather consider the simultaneous advantages

[1] According- to RAPER (1961), «the frequent occurrence of novel nuclear types in legitimate combinations»... «indicated somatic recombination to be a regular — and possibly a very common — feature of the dikaryon».

gained by the enormous probabilities of both meiotic recombination and dispersion of meiospores, as consequences of the extraordinary high number of spores produced in each totally fertile hymenophore. Indeed, both for recombination and dispersion, the hymenophores and respective basidiospores are more important than the vegetative mycelium and the spores developed in it.

The, problems under consideration is comparing the fruit-body, as an organ where recombination occurs regularly, with the vegetative mycelium in which this may occur through a para-sexual process.

Since karyogamy and meiosis are the two nuclear genetic events characteristic of the sexual process, and since meiosis does not take place except in basidia, then we can state that the hymenophore is an indispensible structure for completing the eu-sexual process, this cycle having greater advantages regarding the probabilities of recombination, than the para-sexual process.

The rarity of cases where there is a direct production of basidia in the mycelia, without the development of hymenophores (ROGERS, 1936; BIGGS, 1937; DODGE, 1938; LEMKE, 1969), shows that these are not exclusive organs of recombination and sporulation. However, in these cases, basidiospore dispersal is not so great as in the case of spore production above the substrate, in hymenophores. These cases are relevant for understanding the hymenophore as a favourable agent for basidiospore dispersion.

Similarly, when discussing the biological significance of more or less sterile hymenophores, we must take into account these inter-related processes — recombination and dispersion — as well as their genetic consequences.

In extreme, rare cases of completely sterile sporophores, their production is of no consequence, differing thus from what happens when they are partially fertile.

Dispersal of spores originating in partially fertile hymenophores is genetically important because of the posibility of its intervention in later processes of genetic recombination. Thus, the results are the same as in fully

fertile hymenophores but in these there is the advantage of the production of a greater number of spores.

The exclusive production of non-recombined spores — mitospores — by some types of more or less sterile hymenophores, is also interesting because of the consequences of dispersion. In haploid fruit-bodies, developed in homokaryons, the sporulation guarantees the dispersion of spores with genetic constitution which is identical to that of the initial spore. In chimeric haploid fruit-bodies — mixed monokaryotic — sporulation maintains the genetic constitution of the two parents. Finally, in mixed mono-dikaryotic, haplo-diploid, fruit-bodies, the two initial genomes aré preserved without recombination, and are dispersed by the spores, at the same time as the recombination between the two genomes occurs through meiosis, in spite of the incompatibility factor which they have in common[1].

All these facts and their respective interpretations, show that fruit-bodies are fundamentally dispersion agents, especially of recombined diaspores.

Experimental results furnish us with a variety of «abnormal» situations which do not actually prevent the development of fruit-bodies, but cause them to be sterile; this sterility is usually incomplete which results in spore production. So a greater or lesser degree of sterility of basidia is a way of restricting the dispersion of non--recombined genotypes.

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[1] In this last case, there are two simultaneous processes in the same sporophore, one conservative, maintaining the genotype, and the other revolutionary, of genetic recombination.

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ETUDES CYTOLOGIQUES SUR LES ALGUES JAUNES *(CHRYSOPHYCEAE)*

I. ULTRA STRUCTURE DE *CHRYSOCAPSA EPIPHYTICA* LUND. *

par

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RÉSUMÉ

Dans cet article on fait l'étude de *Chrysocapsa epiphytica* Lund (Chrysophycée).

C'est la première référence de cette espèce au Portugal et aussi la première fois qu'on envisage la cytologie du genre *Chrysocapsa* au point de vue ultrastructural.

Cette étude a compris alors, fondamentalement, les deux aspects suivants: 1. Identification, isolement et mantien de l'espèce en culture; 2. Etude de sa cytologie, aux microscopes photonique et électronique, incluant des aspects morphologiques et cytochimiques.

INTRODUCTION

Depuis l'application de la microscopie électronique aux études cytologiques des Algues, la connaissance de la structure de ces plantes a subi un très grand progrès grâce aux contributions de nombreux chercheurs.

Avec ces résultats on essaye maintenant d'élaborer de nouvelles classifications phycologiques et parallèlement, d'appendre la signification phylogénétique de quelques détails ultrastructuraux (GIBBS, 1970; STEWART & MATTOX, 1975).

Cependant, il y a encore beaucoup de difficultés, celles-ci ne pourrant être surmontées qu'après la connaissance de l'infrastructure de beaucoup plus de représentants des grands

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groupes algologiques, particulièrement de ceux moins étudiés, c'est-à-dire des algues non-vertes. C'est avec ce but que nous décrivons ici, pour la première fois, l'ultrastructure de *Chrysocapsa epiphytica* Lund (Chrysophycée), espèce qui n'avait pas encore été citée au Portugal.

MATÉRIEL ET MÉTHODES

Â partir d'une récolte d'algues réalisée dans la région de Cantanhede (au banlieu de Coimbra), on a isolé quelques espèces (selon la technique de l'épuisement sur des plaques à Petri) qui, actuellement, croissent dans une chambre de culture à température, humidité et illumination réglables. L'espèce que nous étudions ici *(Chrysocapsa epiphytica* Lund) a été cultivée dans un milieu essentiellement minéral, en phase liquide ou solidifié par l'addition d'agar-agar (gélose) à 1,2%.

Ces cultures se sont développées à la température de 16-18° C, dans une atmosphère à 75% d'humidité à peu près, et soumises à une photopériode de 10-12 h lumière sur 24 h.

Le renouvellement du milieu a été assuré par des répiquages efectués périodiquement; on a alors constaté que les cultures (particulièrement en phase solide) se mantiennent en bonnes conditions pendant 4-6 semaines.

Pour les études cytologiques (morphologiques ou cytochimiques) nous avons utilisé les méthodes suivantes:

1. Microscopie optique

   a) Observation des cellules «in vivo», utilisant comme milieux de montagne de l'eau ou de l'encre de la Chine (fond noir artificiel).
   b) Coloration des cellules «in toto» par le noir-Sudan B (solution saturée dans l'alcool à 70%).
   c) Même coloration sur des coupes semi-minces du matériel inclu pour microscopie électronique (MCGEE-RUSSEL & SMALE, 1963).

d) Coloration des cellules par le Sudan III (sol. alcoolique).
e) Coloration par le bleu de crésyle (sol. aqueuse à 0,01%) et le rouge neutre (sol. aqueuse à 0,01%).

2. Microscopie électronique

a) Fixation dans le glutaraldéhyde (2,5 %, tampon phosphate 0,025M, pH 6,8) à la température du laboratoire, pendant 1,5-2 heures. Quelques gouttes d'une solution de $CaCl_2$ 0,1M sont ajoutées au fixateur au moment de l'emploi.
b) Même fixateur en tampon eacodylate 0,1M pH 7, pendant 1-2 heures, à la température de 0-4° C.
c) Fixation dans le tétroxyde d'osmium (1%, tampon phosphate 0,025M, pH 6,8) à la température de la pièce, pendant 1,5-2 heures. Dans les processus a) et b), les cellules, après plusieurs rinçages dans le tampon, ont été post-fixées par le tétroxyde d'osmium à 1% en conditions identiques à c).

Toutes ces opérations ont été réalisées dans des tubes d'une centrifugeuse. Â la fin de chaque étape (fixation, rinçages, etc.), on fait une légère centrifugation (5 min. à 1500-2000 r/min.) pour concentrer le matériel; alors, on décante le surnageant et on dilue le culot obtenu avec le réagent suivant. Après l'osmiation, le matériel a été rincé encore une fois dans le tampon et inclu dans l'agar-agar.

Alors, ce bloc de gélose a été découpé en des petits fragments qui ont subi le traitement habituel pour une étude au M. E. L'inclusion a été faite dans une résine selon la méthode préconisée par SPURR (1969). Les coupes, faites au microtome Ultratome III (LKB) et ramassées sur des grilles de 200-400 «meshes», ont été contrastées ou non par l'acétate d'uranyle et/ou du citrate de plomb (REYNOLDS, 1963; VENABLE & GOGGESHALL, 1965) et observées dans un microscope Siemens Elmiskop 101.

d) En çytochimie ultrastructurale nous avons essayé les techniques suivantes:

— Extraction par l'acétone: les cellules ont été traitées par l'acétone, pendant 24 h à la température de 0-4° C, entre la fixation au glutaraldéhyde et Fosmiation.
— Oxidation des coupes par le peroxide d'hydrogène: des coupes dorées, obtenues à partir du matériel fixé selon les conditions *2c,* ont été recueillies sur des grilles et ensuite oxidées par le peroxyde d'hydrogène à 3% pendant 20 minutes. Après un lavage soigneux quelques coupes ont été traitées par le tetroxyde d'osmium à 1% (3-4 h) ou par l'acétate d'uranyle en solution aqueuse saturée (30 min.) (MESQUITA-GUIMARÃES, 1974).

Bien que sans obtenir des résultats positifs, nous avons essayé d'induire la formation de cellules mobiles. Pour cela et à partir de cultures développées dans des conditions déjà décrites, nous avons fait plusieurs repiquages pour un milieu liquide frais, préalablement enrichi en $C0_2$, et mantenu à l'obscurité.

RÉSULTATS

A. Microscopie photonique

1. Les colonies

Ã l'habitat naturel les cellules forment des colonies mucilagineuses très consistantes, brunâtres, epiphytes et aux formes et dimensions très variables. En effet, les jeunes colonies sont normalement sphériques et vides; néanmoins, au fur et à mesure qu'elles se développent, deviennent progressivement sacculiformes ou parfois laminaires, les lames pouvant atteindre quelques centimètres en longueur (PI. I, fig. 7).

Apparemment les cellules se placent d'une façon plus ou moins régulière à la surface du mucilage formant une seule assise (Pl. I, fig. 6). Cependant, lorsque le montage est fait dans l'encre de la Chine et les colonies sont légèrement écrasées, l'organisation du thalle devient évidente. On peut alors constater, d'acord avec BELCHER (1973), que ce thalle est constitué par des tubes mucilagineux qui, par suite de l'écrasement, se séparent. Ces tubes, souvent bifurqués à l'extrémité externe, portent normalement une cellule dans chaque rameau de la fourche.

Au laboratoire, la morphologie des colonies varie selon les conditions de culture. C'est ainsi que dans le milieu solide les cellules forment des amas facilement dissociables, où il n'est pas très évidente une matrix (PI. I, fig. 5). Par contre, dans le milieu liquide, les jeunes colonies (âgées d'un mois) montrent une morphologie assez irrégulière et pas très facile à étudier. Néanmoins, en fond noir artificiel, on peut constater que l'aspect le plus fréquent est celui de rassemblements de tubes, parfois ramifiés ou bifurqués simulant des oursins; en règle, chaque tube montre 1 ou 2 cellules à l'extrémité (PI. I, figs. 3 et 4). Il se peut que ces colonies hérissées évoluent vers des formes sphériques au «paroi» plus consistente, c'est-à-dire, semblables à celles qu'on trouve dans la nature. De toute manière, dans les cultures âgées de six mois, la plupart des colonies mantiennent leur forme originale en «oursin» mucilagineux.

Puisque nous n'avons pas utilisé des techniques convenables pour faire ressortir la structure du mucilage (BELCHER, 1966), il se présente à l'aspect homogène, c'est-à-dire sans structure apparente. Cependant, enfoncées dans ce mucilage, on peut voir nettement quelques parois cellulaires isolées, disons des cellules sans contenu (PI. Π, fig. 7).

2. Cellules

Les cellules jeunes, au début ovoïdes, deviennent sphériques avec un diamètre de 9 à 11 /um. Le protoplaste, parfois apparemment nu, est réèlement enveloppé par une paroi très mince qu'on voit bien lorsque le contenu cellulaire sort

de la cellule totalement ou partiellement (PL II, fig. 7). Dans ce contenu on peut observer nettement 2-4 plastes jaunes-brûnatres en position périphérique (PL I, figs. *2a, b).*

Dans les cultures plus âgées apparaissent souvent des cellules plus grandes (17 *µm* en moyenne) et avec un nombre de plastes plus élevé (PL I, fig. 2c).

Ces cellules montrent normalement des vacuoles assez développés, où on voit des granules animés de mouvements du type brownien.

En èe qui concerne les substances de réserve on sait que la leucosine est contenue dans quelques vacuoles et se révèle très difficile à conserver par les fixateurs. En dehors de cet hydrate de carbone, les cellules présentent encore, comme matériel de. réserve, des inclusions plus ou moins sphériques et colorables par les colorants généraux des lipides (lysochromes). En effet, après un traitement des cellules par le noir Sudan B ou le Sudan III, on y voit de nombreuses gouttes aux dimensions très variables (0,4-3,5 *µm)* et colorées, respectivement, en bleu noir foncé ou en orangé (PL IV, fig. 3). Cette diversité de dimensions provient, sans doute, du fait des gouttelettes pouvoir se fusionner très facilement, en donnant des inclusions assez volumineuses. On obtient le même résultat sur des coupes semi-minces de matériel inclu pour microscopie électronique et colorées au noir Sudan B selon la technique de MCGEE-RUSSEL & SMALE (1963). Toujours au M. O., on remarque en plus, à la périphérie des cellules, la présence de nombreuses et petites granulations (0,1-0,4 µm) fortement colorables en bleu par le bleu de erésyle (PL IV, fig. 5) et en rouge par le rouge neutre (PL IV, fig. 6), mais sans affinité pour le noir Sudan B.

Nous avons vu aussi des cellules au paroi beaucoup plus épaisse et très riches en globules sudanophiles (PL II, fig. 6) qui, d'après LUND (1949), doivent représenter des âkinètes.

Par contre, on a jamais observé des zoospores, malgré les essais qu'on été faits pour induire leur formation.

2. Microscopie électronique

Nous n'avons pas constaté des différences significatives entre l'ultrastructure des cellules développées dans le milieu liquide par rapport à celles cultivées dans le milieu agaré.

1. Paroi

L'étude au M. E. montre que toutes les cellules, indépendemment de croître dans le milieu liquide ou solide possèdent une paroi complète. Néanmoins, celle-ci, par le fait d'être extrêmement mince dans quelques cellules jeunes, est difficille à voir au M. O. (LUND, 1949). De toute façon, le décollement de la pellicule ectoplasmique sur des régions assez étendues, que parfois on observe dans la cellule (PI. II, fig. 2), ne laisse aucun doute sur l'existence d'une enveloppe rigide. Cette paroi squélettique, lorsqu'elle est bien développée, montre une structure fibrillaire dont l'aspect rappelle celui de la paroi cellulaire des plantes supérieures (PI. II, fig. 4).

En effet, sur des microphotographies favorables, on voit nettement des microfibrilles (peut-être de nature cellulosique) de 25-30 nm d'épaisseur. Ces fibrilles se disposent parallèlement à la surface de la cellule formant très souvent des couches bien nettes, séparées par d'autres couches beaucoup plus minces et très denses aux électrons. On peut admettre que ces dernières couches ont une nature chimique essentiellement différente puis qu'elles disparaissent très souvent laissant à leur place des espaces claires apparement vides (PI. II, figs. 4 et 5). Alors, ces couches pourront représenter une sorte de «zones de séparation» tout au long desquelles la paroi s'effeuille très facilement. Cela pourrait expliquer la variabilité de l'épaisseur présentée par la paroi, parfois dans une seule cellule (PL Π, fig. 1). Lorsque deux ou plus que deux cellules se trouvent associées, nous n'avons jamais trouvé aucune structure du type plasmodesmique qui laisse prévoir continuité entre cellules adjacentes (PI. I, fig. 1). La membrane cytoplasmique ou plasmalemme, au trajet plus ou moins sinueux, est bien évidente et, **parfois,**

forme des corps paramuraux (plasmalemmasomes) (PI. II, fig. 3) homologues de ceux décrits dans les cellules des plantes supérieures et dans quelques Algues (pour la bibliographie voir MESQUITA, 1970).

2. Contenu cellulaire (protoplaste)

a) ***Hyaloplasme, vacuoles e d'autres inclusions***

Une vue générale de la structure de *Chrysocapsa epiphytica* montre un hyaloplasme riche en ribosomes, la plupart étant «libres», tandis que d'autres, moins nombreux, se trouvent fixés sur les membranes du reticulum endoplasmique (PI. I, fig. 1).

La morphologie. du système vacuolaire varie selon l'âge et, probablement, l'état physiologique des cellules. Alors, les cellules jeunes (provenant d'une division cellulaire récente) montrent normalement quelques petites vacuoles au contour irrégulier, placées entre un noyau, plus au moins central, et les chloroplastes pariétaux (PI. I, fig. 1). Sur quelques photos on voit nettement que ces vacuoles représentent des dilatations locales du R. E. (PL III, fig. 2) et sont parfois en continuité avec l'espace périnucléaire (PI. IП, fig. 3). Dans d'autres cellules plus âgées, lorsque l'orientation de la coupe est favorable, on voit un noyau entouré par une mince couche cytoplasmique, continue avec le cytoplasme périphérique au moyen de trabecules extrêmement fines et très difficiles à préserver pendant la fixation (PI. II, fig. 1).

Le contenu vacuolaire montre un aspect différent selon le tampon utilisé dans la préparation du fixateur. C'est ainsi que, avec le tampon phosphate, les vacuoles semblent être complètement vides ou bien elles montrent une mince couche de matériel très attaché au tonoplaste (PI. m, fig. 1). Par contre, lorsque le fixateur est préparé avec le tampon cacodylate, les vacuoles se présentent remplies d'un précipité à structure réticulo-granuleuse (PL ПI, fig. 6). Cela est dû, probablement, à une réaction chimique quelconque entre le tampon et un ou plusieurs constituants du suc vacuolaire. Outre ces vacuoles, on voit fréquement d'autres du type lysosomique contenant des «structures myéliniques»

et des débris cellulaires en état plus ou moins avancé de dégénérescense (PL III, figs. 4, 5 et 7). Bien que nous n'avons pas fait une étude cytochimique de ces structures pour nous assurer si elles contiennent ou non de la phosphatase acide, leur morphologie montre nettement qu'elles doivent représenter des cytolisomes (vacuoles autophagiques).

En ce qui concerne les autres inclusions du cytoplasme, l'étude ultrastructurale, s'acorde assez bien, sous plusieurs aspects, avec les observations au microscope photonique. En effet, parmi ces inclusions, quelques-unes (type I) plus grandes et localisées de préférence à l'intérieur de la cellule, n'ont pas de membrane, sont peu osmiophiles et insolubles dans l'acétone (PI. IV, fig. 1) ; elles correspondent sans doute aux gouttes sudanophiles observées au M.O. D'autres inclusions (type II), par contre, sont plus fréquentes près de la paroi cellulaire, beaucoup plus petites, montrent souvent une membrane et réduisent très fortement le tétroxyde d'osmium (PL IV, fig. 1) ; par leur dimension et leur localisation dans la cellule, elles doivent représenter les granulations qui, en microscopie optique, se colorent par le bleu de crésyle et le rouge neutre. L'osmiophilie de ces inclusions disparaît lorsque les coupes sont soumises a une oxidation par le peroxide d'hydrogène (PL IV, fig. 8) et ne se «récolorent» pas ultérieurement avec un traitement, soit par l'acétate d'uranyle soit par le tétroxyde d'osmium (PL IV, fig. 9). Parfois la membrane est plus ou moins éloignée du matériel osmiophile et alors on a l'impression que celui-ci s'est accumulé dans une vésicule (PL *TV,* fig. 4) ; néanmoins, l'accotement de ce matériel osmiophile avec la membrane est souvent si intime que l'inclusion semble plutôt n'avoir aucune enveloppe (PL II, fig. 3; PL IV, figs. 1 et 4).

Dans le matériel fixé seulement par l'acide osmique ces inclusions se présentent parfois plus ou moins elliptiques ou bien sphériques et avec une «ligne limitative» plus dense que la «matrix»; celle-ci est alors moins homogène qu'habituellement (PL rv, fig. 10α, b). Cette variabilité doit être en rapport avec la méthode de fixation, car d'autres composants cellulaires, comme par exemple le chloroplaste (PL IV, fig. 10b), montrent une structure assez altérée par rapport

au matériel fixé au glut/$Os0_4$. Apparemment, ces globules très osmiophiles sont plus nombreux dans les cellules assez vacuolisées. De toute façon, les deux types d'inclusions dont nous venons de parler ne sont pas obligatoirement présents à la fois dans une même cellule.

b) ***Structures tubulaires***

Bien que nous n'avons pas observé des flagelles, nous avons trouvé, dans plusieurs cellules, des structures tubulaires à organisation typique de corpuscule basal ou de centriole.

En effet ces structures, avec un diamètre de 0,2 jam en moyenne, sont constituiées par 9 triplets (groupes de trois microtubules) disposés en cercle (PL VII, figs. 1-3) et parfois attachés à un tubule axial par des filaments très délicats (Pl VII, fig. 1). Comme d'habitude, les triplets sont orientés obliquement par rapport au rayon de la circonférence, formant avec celui-ci un angle supérieur à 90° (PL VII, fig. 2). Les microtubules qui constituent ces triplets ont un diamètre moyen de 17 nm.

Fréquement, on voit les structures tubulaires réunies en paires (PL VII, figs. 1, 3, 4). Le plus souvent, les deux structures de chaque paire apparaissent sectionées de la même façon, c'est-à-dire transversal (PL VII, fig. 3) ou longitudinalement (PL VII, fig. 4). Cependant, quelques images plus rares montrent une structure en coupe transversale, tandis que l'autre apparaît sectionée plus ou moins obliquement (PL VII, fig. 1).

c) ***Noyau***

Le noyau interphasique montre un aspect typiquement eucaryotique : l'enveloppe nucléaire est formée par deux «unit-membranes» délimitant un espace périnucléaire qui, en plusieurs endroits, est continu avec le reticulum endoplasmique; le nucléole est bien différencié en zone fibrillaire, plus ou moins centrale et zone granulaire normalement localisée à la périphérie (PL VII, fig. 6; PL VIH, fig. 1);,

la chromatine se trouve fondamentalement à l'état disperse (PI. VIII, fig. 1) bien qu'on voit ça et là des petits aggrégats plus denses correspondants sans doute, à l'hétérochromatine (PI. VII, fig. 6). La membrane nucléaire mantient des intéressants rapports avec l'appareil de Golgi ainsi qu'on le verra ci-après.

d) ***Chloroplaste***

L'appareil photosynthétique des chloroplastes, sans différenciation en zones granaires et intergranaires, est constitué par des thylacoïdes associés en faisceaux ou bandes qui, normalement, s'étendent d'un extrême à l'autre du chloroplaste (PL V, fig. 3; PL VI, fig. 1). Dans quelques de ces organites il n'est pas possible de compter exactement le nombre de thylacoïdes par bande car, du fait d'être fortement serrés les uns contre les autres, leurs locules subissent une extrême réduction, ou bien ils disparaissent complètement. Alors, les faisceaux prennent l'aspect de longues zones denses à structure très mal définie (PL V, fig. 1). Néanmoins, dans d'autres chloroplastes, les lamelles photosynthétiques apparaissent bien individualisées (PL V, fig. 4). On peut alors constater que les bandes sont en règle formées par 3 thylacoïdes bien que ce nombre puisse s'élever à 4 ou 7 (Pl. V, figs. 2 et 4). Parfois un ou plusieurs thylacoïdes peuvent appartenir à deux bandes voisines, ce qui donne origine à des images ae bifurcation plus ou moins nettes (PL V, fig. 2). Exceptionellement, on a compté 16-25 ou même plus thylacoïdes superposés, en conséquence du rapprochement de bandes adjacentes (PL V, figs. 5 et 6). Une caractéristique commune à tous les chloroplastes est l'existence d'un faisceau périphérique («girdle band») qui s'étend tout au long de la région bordante du stroma, parallèlement à la membrane plastidale (PL V, fig. 4; PL VI, fig. 1).

Le stroma se présente uniformément dense et, en dehors de quelques plastoglobuli montrant parfois des différents dégrés d'osmiophilie (PL VIII, fig. 1), on y voit de nombreux ribosomes plus petits que ceux du cytoplasme. Les régions à l'aspect de «nucleoide», lorsqu'elles sont évidentes, se loca-

lisent systématiquement aux extrêmes de la section du chloroplaste, à l'intérieur de la bande périphérique (PI. V, fig. 3; PI. VI, fig. 1). Ce fait provient sans doute de l'existence d'un seul nucleoide annulaire, semblablement à ce qu'il arrive chez d'autres Chromophyta.

Nous n'avons trouvé aucune différenciation du stroma qui puisse être interprétée indubitablement comme un pyrénoïde. Les cellules ne doivent posséder non plus un stigma puisqu'une telle structure n'a jamais été observée à l'intérieur ou en dehors du plaste.

Les chloroplastes sont entourés d'un saccule de R. E. qui parfois s'observe en continuité avec la membrane externe de l'enveloppe nucléaire (PI. VI, figs. 1 et 2). Ce saccule, nommée R. E. chloroplastidale (BOUCK, 1965), bien que très proche de la membrane du chloroplaste sur une grande extension (PI. V, fig. 2; PI. VI, fig. 1), s'en éloigne dans quelques endroits. Alors, l'espace compris entre les deux «enveloppes» apparaît parfois occupé par des vésicules plus ou moins applaties (PL VI, figs. 3 et 4) qui réprésentent, apparemment, des sections d'un reticulum tubulaire. Lorsque les cellules sont fixées tout simplement dans le tétroxyde d'osmium, les bandes des chloroplastes se présentent fortement ondulées, au même temps que la matrix devient beaucoup moins dense (PL IV, fig. 10). Celles-ci et d'autres altérations par rapport au matériel fixé au glut/$Os0_4$, représentent sans doute des artefacts.

e) ***Mitochondries***

En section, les mitochondries montrent des profils variés, c'est-à-dire, circulaires, elliptiques, allongés, annulaires ou bifurqués (PL IV, fig. 1; PL VII, figs. 6-11). Souvent on voit des images qui suggèrent la division de ces organites par étranglement à peu près dans la région moyenne (PL VII, figs. 7, 8, 11) ou par bourgeonnement (PL V, fig. 1; PL VII, fig. 9). La membrane interne de l'enveloppe mitochondriale forme des crêtes ou bien des invaginations en doigts de gants très nombreuses, et, pour la plupart, étranglées à la base (PL VII, fig. 11). Le stroma

est uniformément dense et les régions claires aux fibrilles (nucleoides) ne sont pas évidentes (PL VII, fig. 6-10).

f) ***Appareil de Golgi***

L'appareil de Golgi semble être constitué par un seul dictyosome qui se localise, sistématiquement, près du noyau (PL I, fig. 1; PL VIII, figs. 1 et 2), car nous n'avons jamais trouvé plus qu'un de ces organites dans chaque cellule.

Un dictyosome est composé par un empilement de 5 à 10 saccules golgiens et montre une polarité bien nette. En effet, tandis que sur un pôle (face de maturation) les saccules se tuméfient particulièrement à la périphérie produisant des vésicules de sécrétion aux dimensions variables, sur l'autre pôle (face de formation), orienté vers le noyau, on voit presque toujours de petites vésicules en différents états de coalescence assurant le renouvellement des saccules (PL III, fig. 3; PL VIII, figs. 1 et 2). Ces petites vésicules de transition montrent un contenu plus ou moins dense et sont produites par évagination de la membrane externe de l'enveloppe nucléaire (PL VIII, figs. 1 et 2).

g) *« Microb odies » (peroxisomes)*

Au cours de cette étude nous avons pu constater l'existence, dans cette Chrysophyeée, d'organites qui, par leurs morphologie, dimensions et caractères ultrastructuraux, sont comparables au «microbodies» décrits dans des matériaux les plus divers, tant d'origine animale que végétale (PL V, fig. 3; PL VI, figs. 2 et 3).

L'étude détaillée de ces organites au point de vue morphologique et cytoehimique, constitue matière d'un autre article à publier bientôt.

DISCUSSION

D'après l'étude au M. O., les caractéristiques fondamentales de l'algue qui a fait l'objet de cette étude s'accordent assez bien avec l'espèce *Chrysocapsa epiphytica,* décrite par LUND (1949).

Ces caractéristiques sont essentiellement les suivantes: morphologie et dimensions des colonies et des cellules isolées à l'habitat naturel ; paroi cellulaire peu évidente en conditions normales mais devenant bien nette après le videment total ou partiel du contenu cellulaire; absence de vacuoles contractiles et de stigma ; existence d'inclusions lipidiques comme substance de réserve plus importante; difficultés en trouver des zoospores, que nous n'avons pas non plus observé, malgré les efforts qu'on été faits pour provoquer leur formation.

Cependant, LUND (1949) reconnaît qu'il est difficile à délimiter, d'une manière précise, le genre *Chrysocapsa.* D'après ses observations et les données d'autres auteurs (PASCHER, 1925; FRITSCH, 1935), il considère la possibilité de *Chrysocapsa epiphytica* pouvoir être inclue dans le genre *Phaeogloea.* Malgré cela, LUND (1949) décide retenir l'espèce dans le genre *Chrysocapsa,* étant donné la morphologie des colonies et la ténuité de la paroi sauf dans les ákinètes.

L'étude au M. E. nous a permis de confirmer et préciser quelques points plus au moins douteux. C'est ainsi que: 1) Toutes les cellules possèdent effectivement une paroi qui, parfois, est extrêmement fine, étant alors difficile à observer au M. O. 2) En conditions non encore déterminées, cette espèce est sans doute capable de produire des cellules mobiles, puisqu'on a observé des structures à organisation typique de corpuscule basal ou de centriole.

PASCHER (1925) considère que dans les espèces du genre *Chrysocapsa,* les zoospores, bien qu'observés très rarement, seront du type chromulinoïde (à un seul flagelle). Néanmoins, chez les Chromulinales (typiquement à un seul fouet) ont été décrites des formes apparement n'ayant qu'un seul flagelle mais qui, effectivement, en possèdent deux, le deuxième étant rudimentaire et difficile à observer BOURREUY (1968).

C'est le cas de *Chromulina psammobia* (FAURÉ-FREMIET & ROUILLER, 1957) et *Chromulina placentula* (BELCHER & SWALE, 1967) qui, en dehors du long flagelle externe caratéristique du genre, montrent un second très court et intracytoplasmique, à peine décelable au M. E.

Il se peut que ça arrive aussi chez *Chrysocapsa epiphytica,* étant donné qu'on voit des cellules à deux complexes

tubulaires très proches l'un de l'autre. Bien sûr que ces images pourront représenter tout simplement la duplication du corpuscule basal ou du centriole précédant la division cellulaire, ou bien correspondre à une seule structure coupée à deux niveaux différents par suite de son trajet sinueux.

Nous n'avons pas fait des coupes sériées pour en tirer l'épreuve. N'importe comment, le plus important c'est que, effectivement, l'espèce peut former des zoospores, puisque les cellules possèdent des structures génératrices de flagelles.

En ce qui concerne le système vacuolaire, des images comme celles de la PL ΠI, fig. 2 confirment encore, cette fois-ci dans les organismes inférieurs, que les vacuoles typiques réprésentent une spécialisation du reticulum endopîasmique (voir MESQUITA, 1969).

Quant aux autres inclusions paraplasmiques, il faudrait expérimenter beaucoup plus de réactions (LISON, 1960; GANTER & JOLLÈS, 1969; MESQUTTA-GUIMARÃES, 1974) pour connaître à la rigueur leur composition chimique, ce qui, évidemment, n'est pas dans le cadre de ce travail. Quoi qu'il en soit, les essais cytochimiques que nous avons fait nous suggèrent, pour le moment, les considérations suivantes : les inclusions plus grandes (type I), par suite d'être sudanophiles, représentent sans doute des lipides. Cependant, leur insolubilité dans l'acétone et leur faible osmiophilie indiquent qu'il peut s'agir, au moins partiellement, de phospholipides très peu insaturés (LISON, 1960). Par contre, la forte osmiophilie des autres inclusions plus petites et périphériques (type II) nous a fait penser qu'elles seraient aussi de nature lipidique, mais à caractère insaturé, d'autant plus que cette affinité pour l'osmium disparaît avec l'oxidation par le peroxyde d'hydrogène. Néanmoins, il faut remarquer que ces inclusions ont une très faible ou même nulle affinité pour le noir Sudan B. Alors, ceci et la possession d'une vrai «unit-membrane», montrent qu'elles ne doivent pas être considérées de simples globules gras, au moins d'un type courant. Étant donné leur intense coloration par les colorants vitaux (bleu de crésyle et rouge neutre) il se peut qu'elles représentent plutôt un des plusieurs types de corps mucifères ou d'autres structures semblables (corps physoïdes)

décrites depuis longtemps chez les *Chrysophycées* et d'autres Chromophytes (CHADEFAUD, 1936; BOURRELLY, 1957; 1988). Bien que nous n'ayons pas essayé la réaction de la phloroglucine, leurs fortes propriétés osmioréductrices pourront être dues, au moins partiellement, à la présence de quelques substances tannoïdes (CHADEFAUD, 1936). D'ailleurs, BOUCK (1965), chez quelques algues brunes, interprète des inclusions tout à fait comparables à celles-ci comme de vrais «physodes». Néanmoins, plus tard, dans les zoospores de deux Xantophycées, elles sont considérées tout simplement des lipides (MASSALSKI & LEEDALE, 1969).

Le problème n'étant pas définitivement éclairci, nous pensons, en étudiant d'autres Chromophytes, y revenir pour en faire une étude plus approfondie.

La corrélation enveloppe nucléaire-Golgi, que nous avons observée systématiquement dans cette espèce, est parfaitement d'accord avec le processus bien connu de «turnover» des saccules golgiens, souvent démontré, tant dans des cellules animales que végétales (voir MORRÈ & col., 1971).

Relativement aux Algues, bien que ce mécanisme semble être aussi assez fréquent, il n'a pas été décrit souvent chez les Chrysophycées (MASSAUSKI & LEEDALE, 1969).

L'ultrastructure du chloroplaste des Algues est une des plus importantes caractéristiques qui, dernièrement, ont été utilisées pour des objectifs taxonòmiques (délimitation des groupes) et comme base d'élaboration de systèmes phylogénétiques nouveaux (établissement du degré de parenté parmi les différents groupes).

Les caractères ultrastructuraux du chloroplaste de *Chrysocapsa epiphytica* sont d'accord avec ceux qu'ont été décrits chez d'autres Chrysophycées et, d'une manière générale, dans la plupart des Chromophytes[1] (GIBBS, 1970). Ces caractéristiques sont essentiellement les suivantes: appareil photosynthétique agranaire, bandes les plus fréquentes à trois

[1] Dans le sens de CHRYSTENSEN (1962) ce groupe comprend les classiques Pyrrophyta, Chrysophyta et Phaeophyta ou, plus spécifiquement, les 9 classes suivantes: Chryptophyceae, Raphidophyceae, Dinophyeeae, Xantophyceae, Chrysophyceae, Bacillariophyceae, Craspedophyceae, Haptophyceae et Phaeophyceae.

thylacoïdes, présence constante d'une bande à la périphérie («girdle band»), conformation annulaire du nucleoide et enveloppement du chloroplaste par une travée de reticulum endoplasmique.

Chez les Chromophytes, le degré d'association des thylacoïdes dans chaque bande est une caractéristique extrêmement variable, tant d'espèce pour espèce (LEPORT, 1962; DESCOMPS, 1963; FALK & KLEINING, 1968) que chez la même espèce (MASSALSKI and LEEDALE, 1969). Le fixateur et l'état physiologique des cellules doivent jouer un rôle décisif à cet aspect structural (GIBBS, 1962a, 1970).

Chez *Chrysocapsa epiphytica* cette variabilité est aussi bien évidente, même lorsque les conditions de fixation se maintiennent. Alors, il paraît que, au moins chez la plupart des Chromophytes, le degré de cohérence des thylacoïdes n'est pas significatif (MASSALSKI & LEEDALE, 1969) et, par conséquence, il ne doit pas être considéré une caractéristique à grande valeur taxonomique ou phylogénétique.

La continuité entre l'enveloppe externe du chloroplaste (GIBBS, 1962b), c'est-à-dire, le R. E. chloroplastidale (BOUCK, 1965) et l'enveloppe nucléaire, déjà signalée chez plusieurs Chromophytes (GIBBS, 1962a, 1962b; BOUCK, 1965; COLE, 1969; GIBBS, 1970; SLANKIS & GIBBS, 1972), a été aussi constatée par nous dans les cellules de *Chrysocapsa epiphytica.*

Cette corrélation n'est pas seulement évidente lorsque le chloroplaste et le noyau sont très proches l'un de l'autre, ce qui détermine, parfois, la superposition des enveloppes respectives. En effet, même si les deux organites se trouvent relativement éloignés, on établit entre eux une sorte de «pont» qui assure la continuité de l'espace périnucléaire avec le lumen du R. E. chloroplastidale (PL VI, figs. 1 et 2).

Étant donné la petite épaisseur de ce pont-là, la relative rareté de ces images doit résulter uniquement de l'impossibilité de la visualiser, sauf si l'on a la chance d'avoir fait les coupes selon une orientation favorable. Alors, nous ne pensons pas que ce détail ultrastructural soit fortuit, c'est-à-dire, uniquement determiné par les rapports de voisinage occasionels entre le chloroplaste et le noyau (OLIVEIRA &

BISALPUTRA, 1973). Bien au contraire, il doit avoir effectivement une signification phylogénétique (MASSALSKI & LEEDALE, 1969), ou, tout au moins, métabolique, c'est-à-dire, intervenant à l'échange de metabolites entre les deux organelles (GIBBS, 1962b; MANTÓN, 1966).

Nous n'avons jamais observé des pyrénoïdes, ce qui n'est pas étonnant, puisque la plupart des genres de Chrysophycées ne les possède pas (DODGE, 1973). Cependant, la conclusion n'est pas définitive, étant donné qu'on n'a pas fait l'étude des zoospores et que, il paraît, quelques organismes peuvent ne pas maintenir les pyrénoïdes pendant tout leur cycle de vie (DODGE, 1973).

REMERCIEMENTS: NOUS tenons à remercier au Dr. JORGE RINO ses précieuses suggestions concernant la determination de l'espèce.

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EXPLICATION DBS PLANCHES

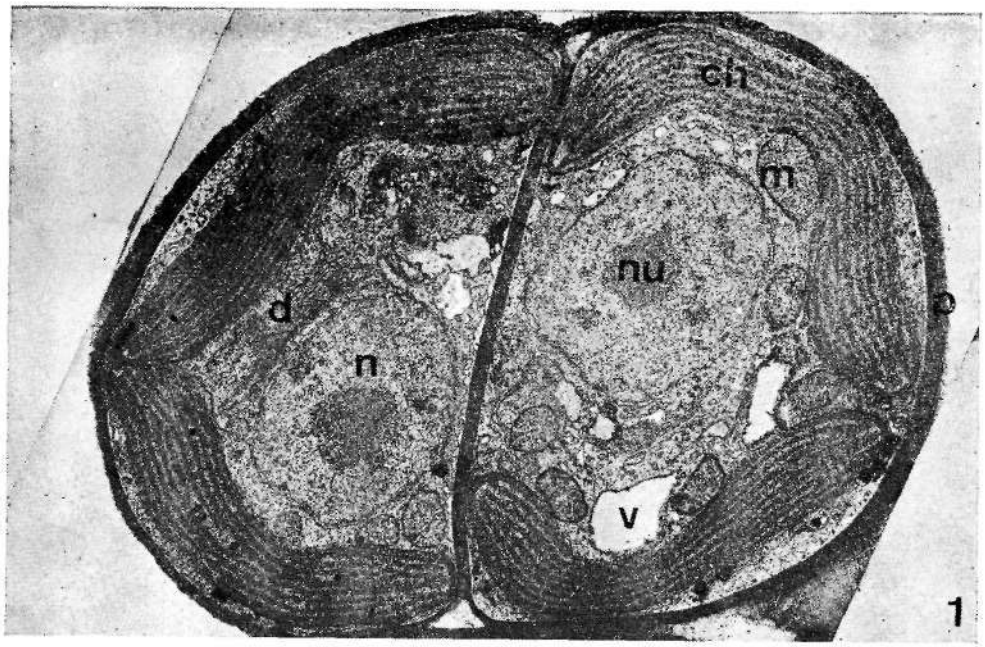
1. Toutes les figures concernent l'espèce C*hysocapsa epiphytica* Lund (Chrysophycêe) et montrent des aspects microscopiques ou ultrastructurales.
2. Microphotographies au M. O.: PI. I, figs. 2-7
   PI. II, figs. 6 et 7
   PI. IV, figs. 3, 5 et 6

   Microphotographies au M. E.: toutes les autres.
3. Méthode de préparation du matériel: toutes les microphotographies électroniques, pour lesquelles on ne fait aucune référence particulière dans les légendes, concernent des cellules fixées au glut/$Os0_4$ selon les conditions *Za* (voir Matériel et méthodes), et contrastées par l'acétate d'uranyle et le citrate de plomb.
4. *Abréviations:* eh, chloroplaste; cy, cytolisome (vacuole autophagique); d, dyctiosome; I, inclusion paraplasmique (lipide); II, inclusion paraplasmique (corps mucifère); m, mitochondrie; mi, microbody; n, noyau; nu, nucléole; p, paroi cellulaire; re, reticulum endoplasmique; v, vacuole.
5. Le titre de chaque Planche représente la (les) structures cellulaires qu'y sont essentiellement documentées.

PLANCHE I

Morphologie générale

1. — Aspect général de l'ultrastructure de deux cellules jeunes où on peut voir tous les organites. Remarquer la présence de deux plastes et d'un seul dietyosome dans chaque cellule et le faible développement du système vacuolaire. x 12 000.
   Comparer avec la fig. 2b.
2. — Trois vues des cellules «in vivo» au microscope Optique. En c la cellule est plus grande et montre un nombre plus élevé de plastes, *a* et *b* χ 1800; c X 1600.
3-4. — Morphologie d'une colonie jeune en milieu liquide (montage dans l'encre de la Chine). La fig. 3 (détail de la fig. 4) montre l'extrémité bifurquée d'un tube mucilagineux (voir le texte). X 1400; X 100.
5. — Aspect amorphe des colonies en milieu solide, x 280.
6. — Vue microscopique de la superficie du thalle. X 80.
7. — Morphologie des colonies à l'habitat naturel (voir le texte).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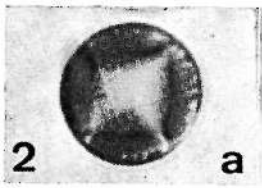
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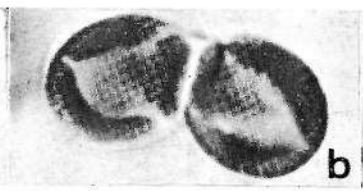
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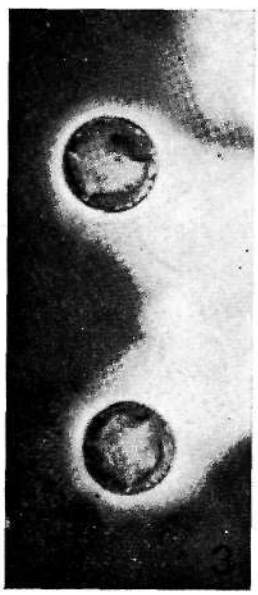
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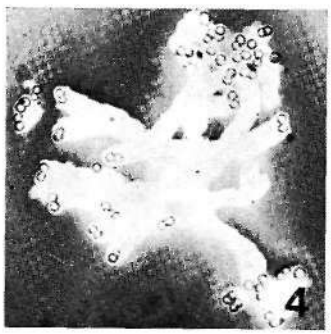
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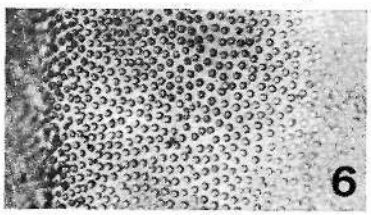
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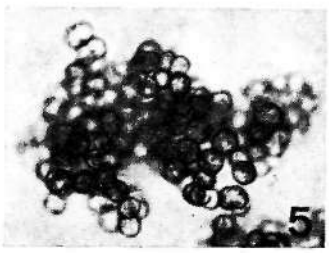
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PLANCHE Π

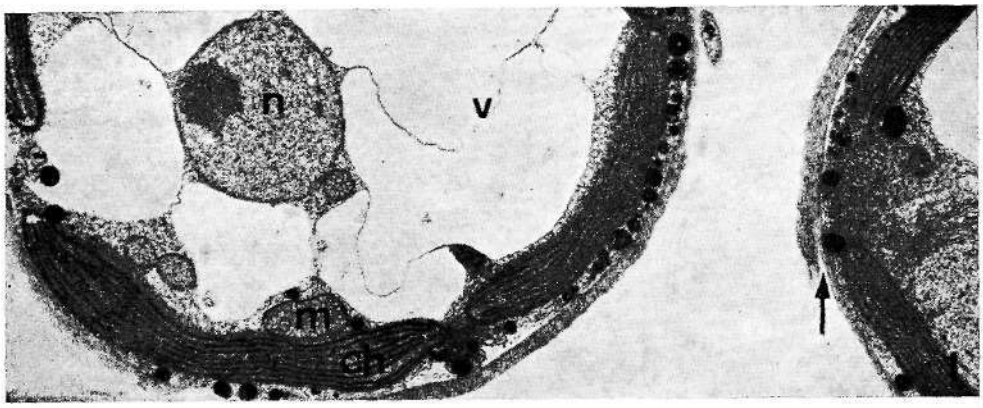
Paroi cellulaire et ses anexes

Fig-, 1. — On voit partiellement deux cellules. En comparaison avec celles de la fig. 1 on remarque essentiellement le suivant: appareil vacuolaire beaucoup plus développé, nombre de plastes plus élevé (quatre) et inclusions osmiophiles périphériques (corps mucifères) plus abondantes.
Dans la. cellule à droite remarquer le «clivage» de la paroi (flèche) (voir le texte), x 10 000.

Fig. 2. — Le détachement du plasmalemme (flèche) confirme l'existence d'une paroi squélletique (p), bien qu'ici elle soit très ténue. X 28 000.

Fig. S. — Corps paramural d'origine plasmalémmique (plasmalemmasome). X 32 000.

Fig. 4-7. — Différents aspects de la paroi cellulaire au M. E. (figs. 4, 5) et au M. O. (figs. 6, 7).
Sur les figures 4 et 5 ressort la stratification de la paroi avec les «zones de séparation» (flèches) (voir le texte). Les figs. 6 et 7 montrent, respectivement, un akinète bourré de lipides colorés au noir Sudan B, et des parois cellulaires sans contenu.
Fig. 4 X 64 000; Fig. 5 X 42000; Fig. 6 X1600; Fig. **7 X 1400.**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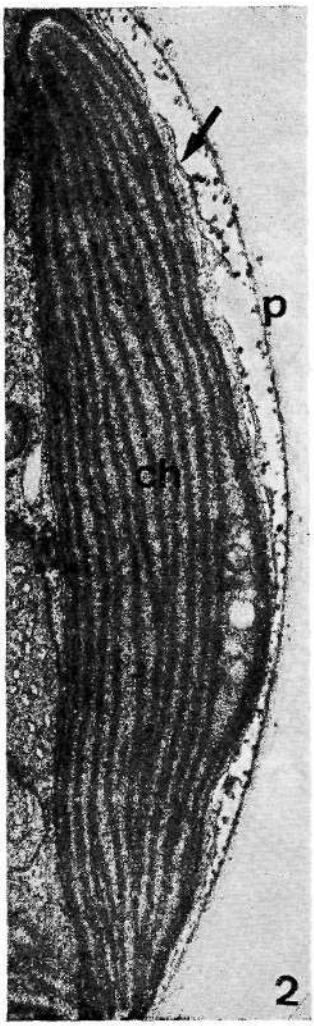
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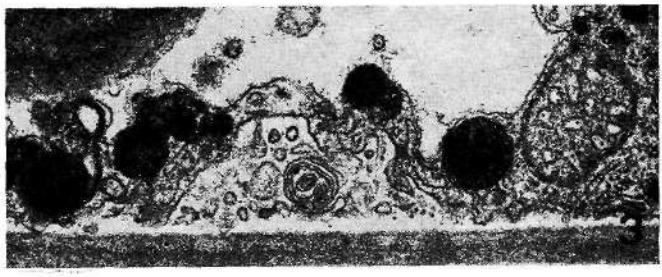
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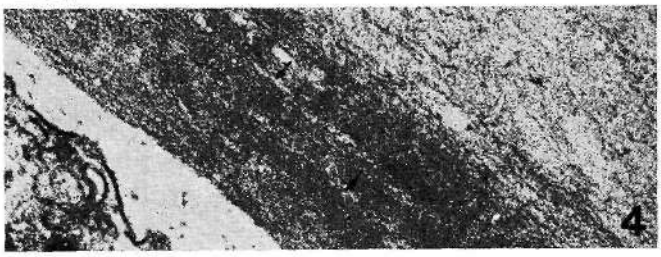
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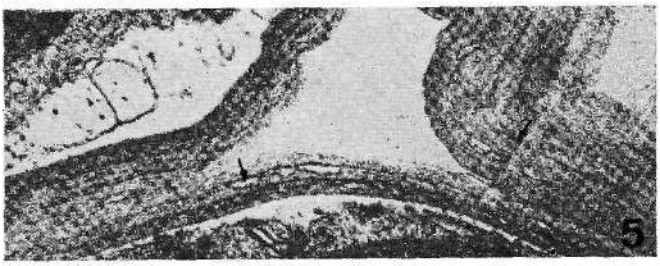
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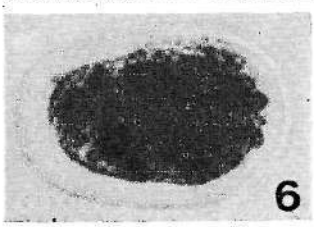
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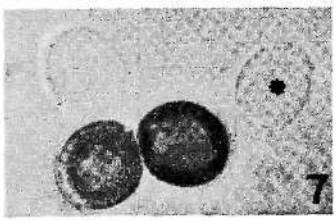
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PLANCHE III

Appareil vacuolaire

Fig. 1. — Les vacuoles (v) montrent une mince couche de matériel très dense attachée au tonoplaste. x 12 000.

Fig. 2.— Une travée de R. E. (flèche) se dilate pour originer une vacuole, x 50 000.

Fig. 3. — Une petite vacuole (= vésicule de R. E.) est en continuité avec l'espace perinucléaire (flèche). On voit aussi un dictyosome tout près du noyau. X 32 000.

Figs. 4 et 5. — Deux aspects différents de vacuoles du type autophagique ou cytolisomes (cy). χ 32 000; X 48 000.

Fig. 6. — La fixation a été faite dans le glutaraldéhyde preparé avec le tampon cacodylate selon les conditions *2b* (voir Matériel et méthodes). Remarquer que les vacuoles sont pleines d'un precipité. Comparer avec la fig. 1. X 14 000.

Fig. 7. — A gauche du noyau on voit une vacuole (cy) contenant une structure multilamellaire (une phase évolutive des cytolisomes). Remarquer encore le noyau typiquement eucariotique et très pauvre en chromatine. X 28 000.

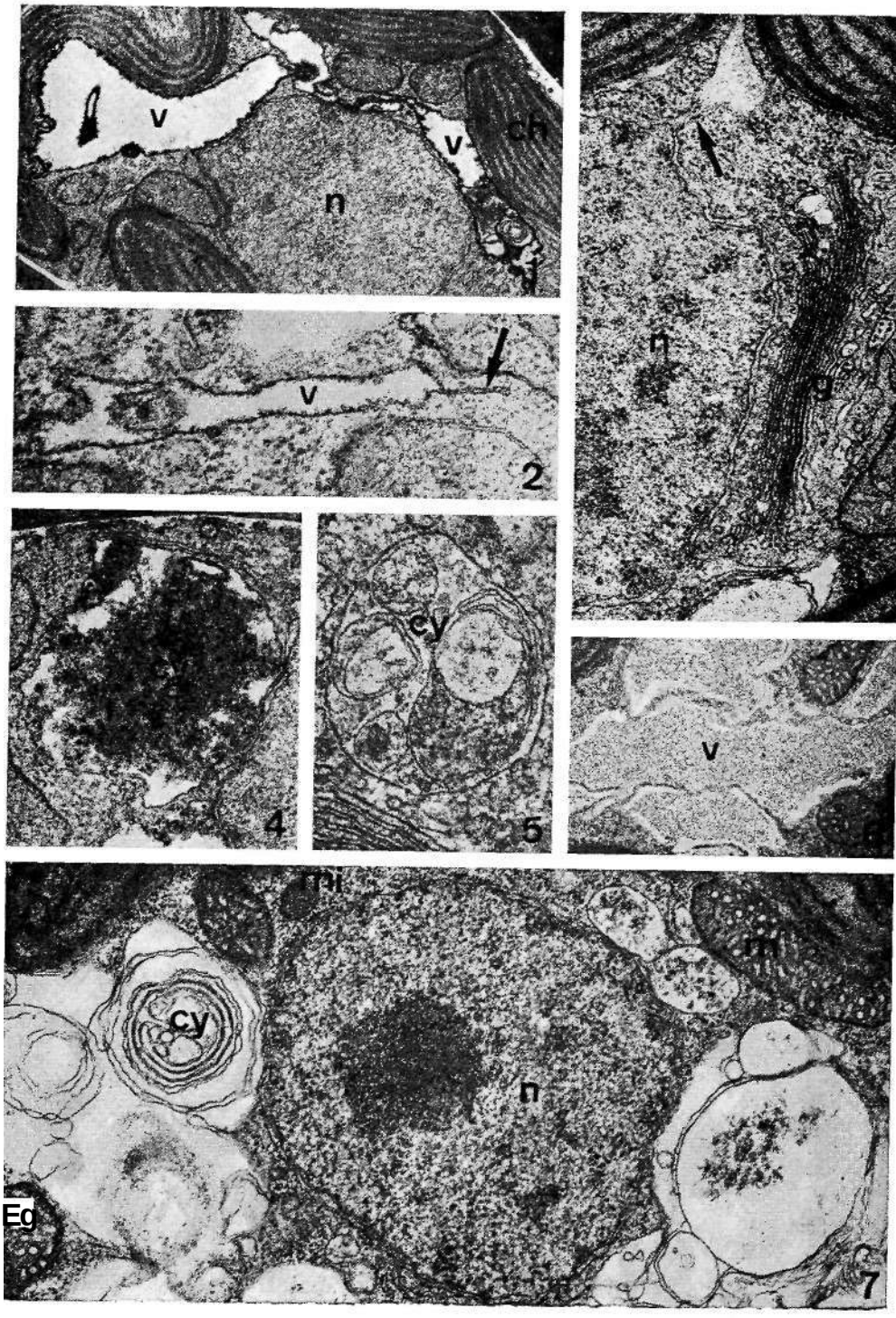
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PLANCHE IV

Inclusions «paraplasmiques»

Fig. 1. — Cette cellule montre, à la fois, les deux types d'inclusions «paraplasmiques»: I (lipides); II (probablement des corps mucifères ou des corps physoides) (Voir explication dans le texte). X 32 000.
On voit encore une mitochondrie bifurquée (m) et un microbody (mi). X 32 000.

Figs. 2 et 3. — Les inclusions I résistent au traitement par l'acétone (fig. 2) et se colorent très nettement par le noir Sudan B (fig. 3 — en haut) et le Sudan III (fig. 3 — en bas), x 16 000; X 1600.

Figs. 4-10. — Différents aspects des inclusions du type II (étude ultrastructurale et cytochimique). Ces inclusions se montrent fortement osmiophiles, soit dans des cellules fixées au glut/$Os0_4$ et contrastées (fig. 4) ou non (fig. 7), soit dans le matériel fixé uniquement par le tétroxyde d'osmium (fig. 10α et *b).* L'osmiophilie disparaît par oxidation au peroxyde d'hydrogène (fig. 8-flèehe) et ne se manifeste plus, après un traitement par le létroxyde d'osmium (fig. 9 — flèche). En plus, au M. O., ces inclusions se colorent ortochromatiquement par le bleu de crésyle (fig. 5 — flèche) et par le rouge neutre (fig. 6 — flèche).
La fig. 4 est un montage de deux photos où on peut suivre, du haut vers le bas, le «masquage» progressive de la membrane de l'inclusion, par suite de l'accroissement du matériel osmiophile. (Pour plus de détails voir le texte). En plus, dans le chloroplaste, on voit nettement des ribosomes (flèches).
Fig. 4 X 51 000; Figs. 5 et 6 X 1600; Fig. 7 X 12 000; Figs. 8 et 9 X 38 000; Figs. 10α et *b* χ 16 000; X 32 000.

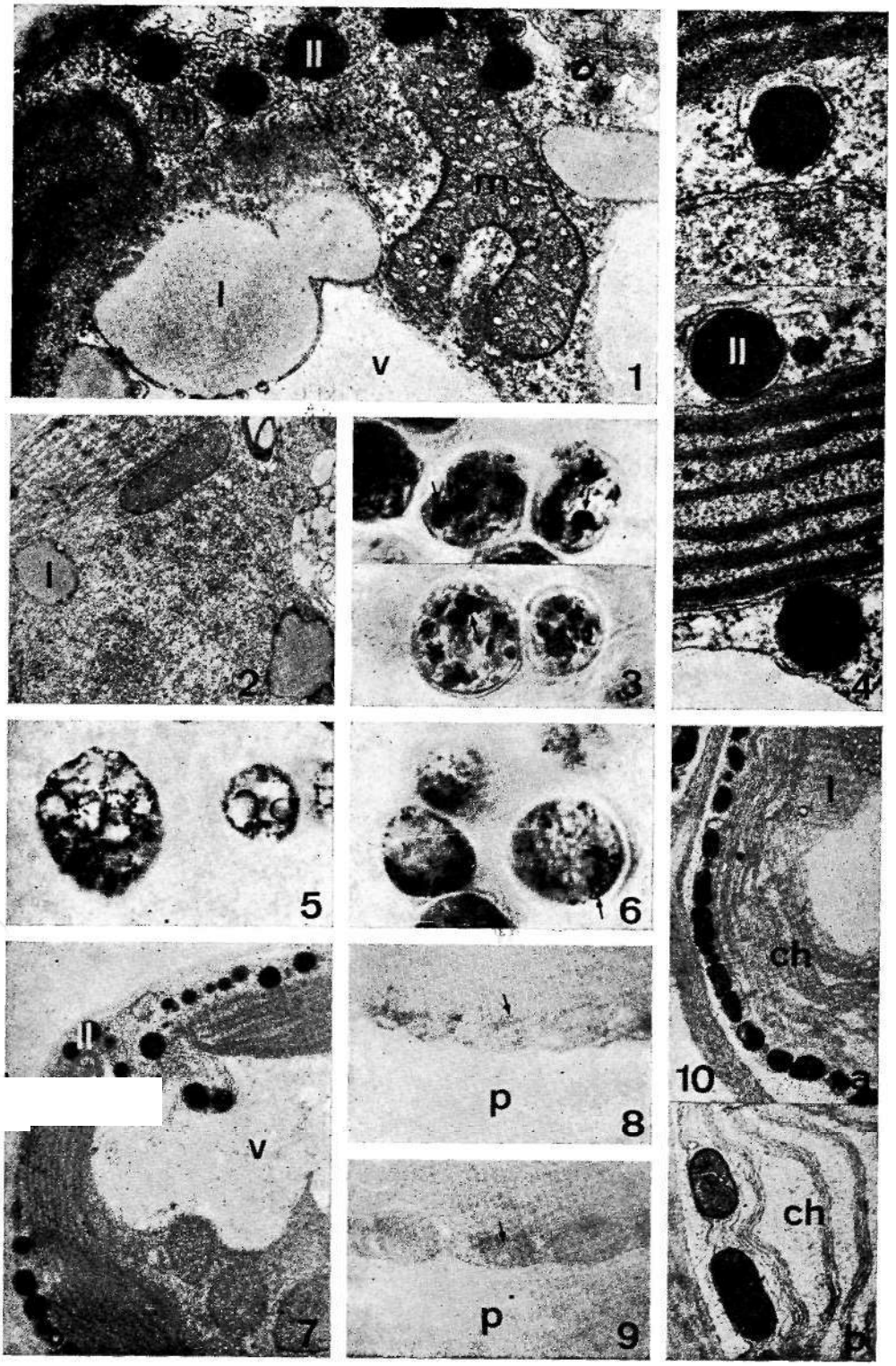
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PLANCHE V

Plastes

Fig. 1. — Les thylacoïdes ne sont pas individualisés dans les bandes du chloroplaste. On remarque encore une mitochondrie en bourgeonement. x 48 000.

Fig. 2. — Détail de la fig. 3 : les deux «enveloppes» du chloroplaste sont accolées et l'externe montre des ribosomes liés à sa surface (flèche simple). Remarquer un thylacoïde qui appartient, à la fois, à deux bandes adjacentes (flèche double). X 64 000.

Fig. 3. — Aux deux extrémités de la section du chloroplaste est bien évident le nucleoide. Au-dessus de l'organite on voit aussi un microbody (mi). X 32 000.

Fig. 4. — Les 3-4 thylacoïdes qui composent chaque bande typique des chloroplastes sont ici bien évidents. En bas, on voit très bien la bande périphérique («girdle band») (flèche). X 54 000.

Fig. 5. — Plaste à structure anormale: dans cet organite, les bandes montrent un nombre de thylacoïdes beaucoup plus élevé que l'habituel. X 28 000.

Fig. 6. — Détail de la fig. 5. Malgré la «fusion» des membranes des thylacoïdes superposée leurs locules sont encore visibles. X 70 000.

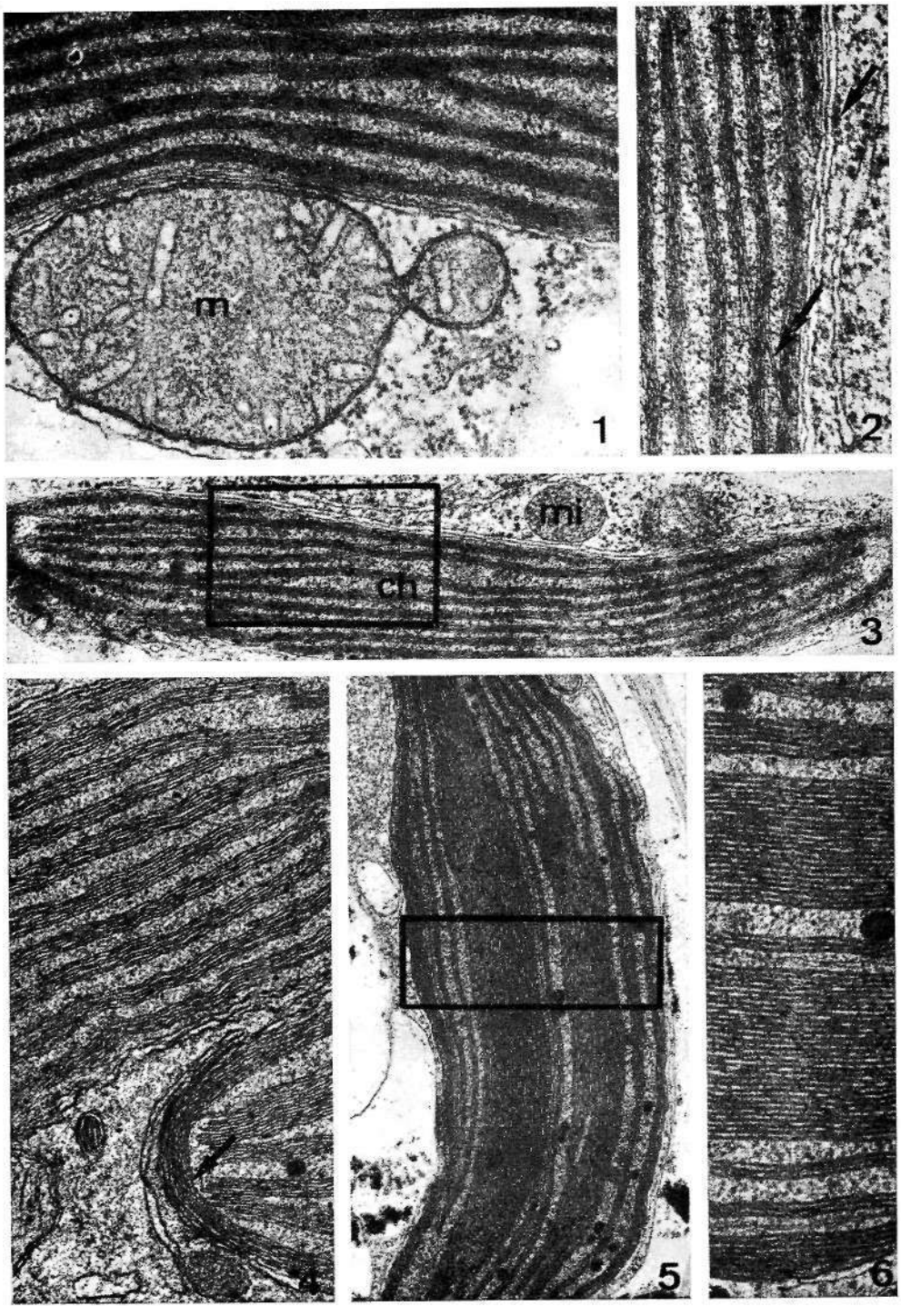
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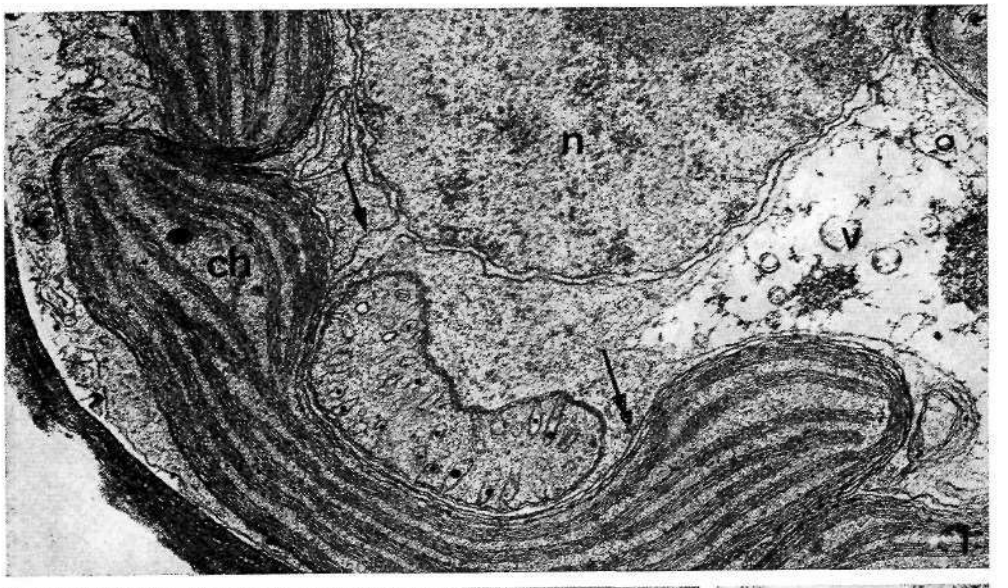
PLANCHE VI

Plastes (continuation)

Bigs. 1 et 2.— Remarquer les «ponts» (flèches simples) qui établissent une parfaite continuité entre le R. E. chloroplastidale (flèche double) et l'espace périnucléaire. X 35000; X 37 000.

Figs. 3 et 4.·—Ces images montrent le R. E. chloroplastidale relativement éloigné de la membrane du plaste. Entre les deux «enveloppes» on voit de nombreuses vésicules plus au moins aplaties (flèches). X 35 000; X 38 000.



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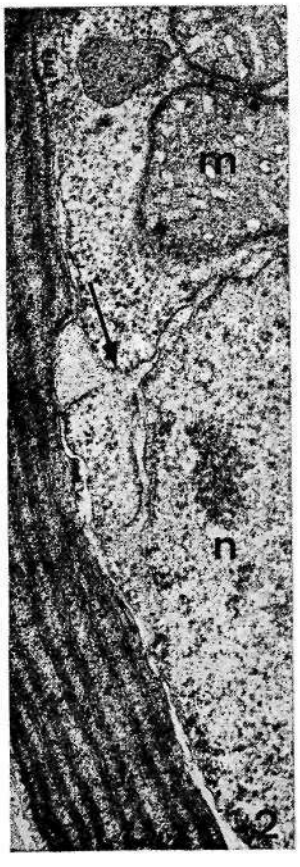

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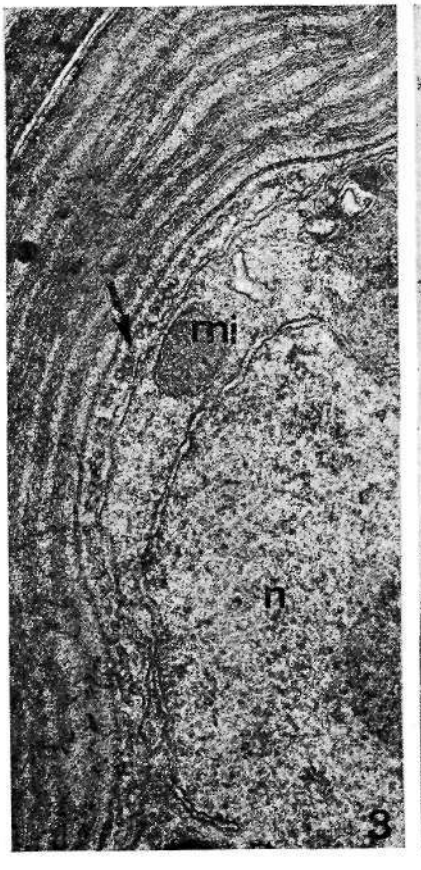

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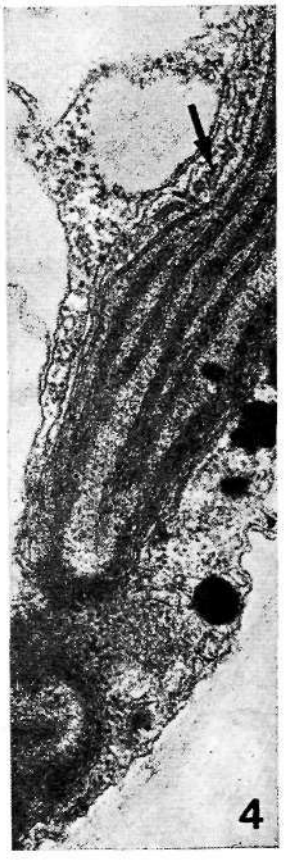

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PLANCHE VII

Structures tubulaires, noyau, mitochondries

Figs. 1-5. — Différents aspects des structures tubulaires observées dans quelques cellules (voir explication dans le texte). Fig. 1 X 48000; Fig. 2 X 80 000; Fig. 3 X 54 000; Fig. 4 X 40 000; Fig. 5 X 35 000.

Fig. 6. — Dans le noyau ressortent la pauvreté en chromatine condensée et la ségrégation nette des zones granuleuse et fibrillaire du nucléole. A droite, on voit des mitochondries à section plus au moins circulaire et très riches en crêtes tubulaires. X 32 000.

Figs. 7-11. — Profils variés des mitochondries. En dehors de la fig. 10 (probablement une section annulaire d'une mitochondrie en cloche), toutes les autres images suggèrent la division des organites.
Fig. 7 X 24 000; Fig. 8 X 20 000; Fig. 9 X 28 000; Figs. 10 et 11 X 32 000.

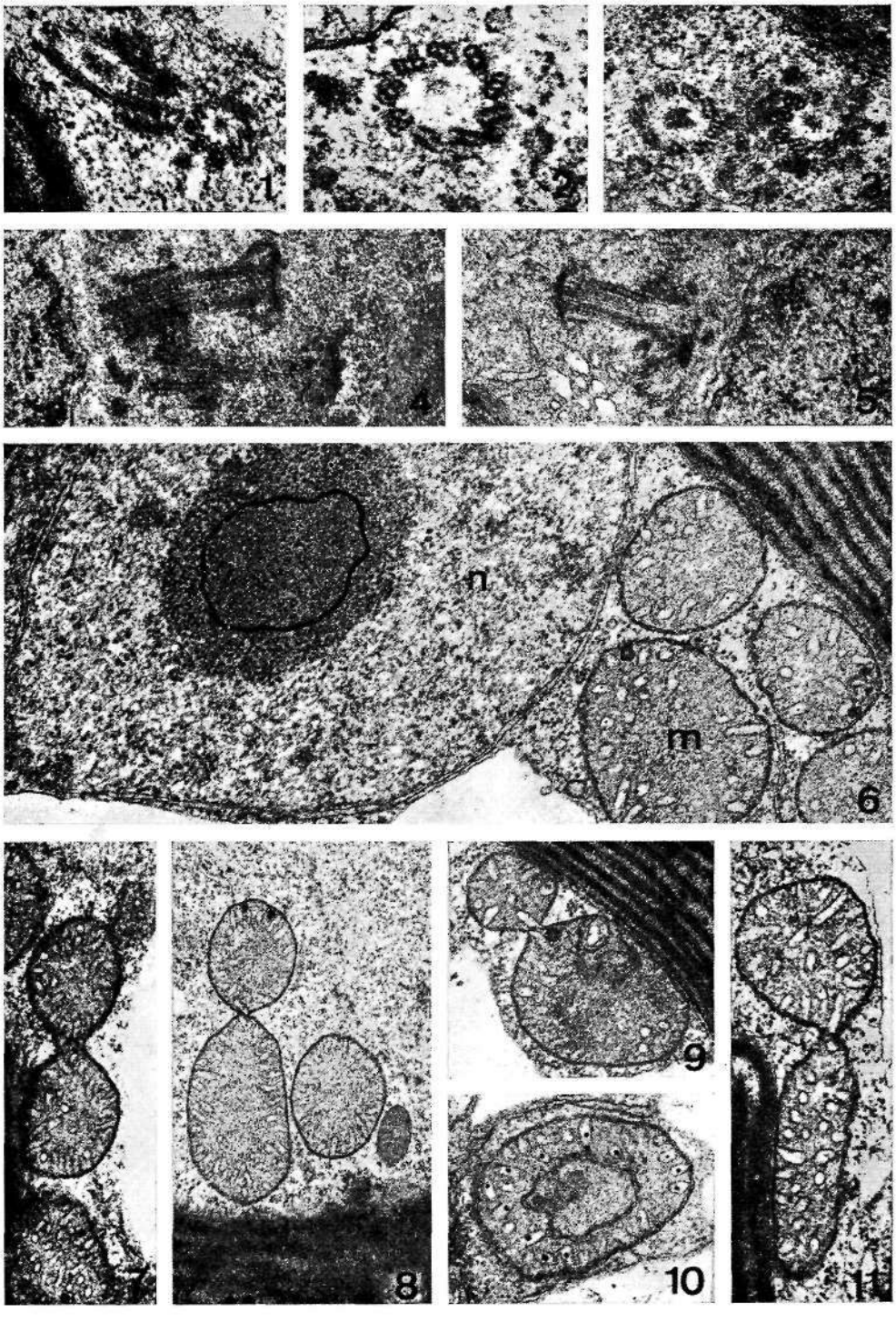
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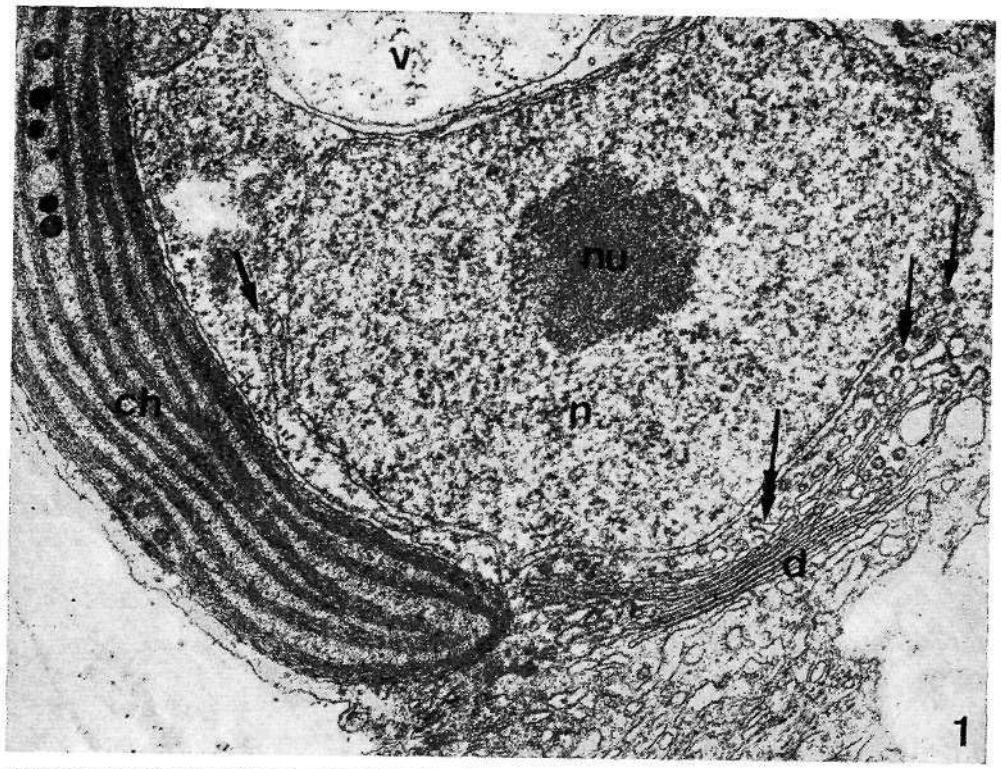
PLANCHE VIII

Appareil de Golgi

Fig's. 1 et 2.— Ces images documentent la corrélation systématique, dans cette espèce, entre le dictyosome et le noyau. La membrane externe de l'enveloppe nucléaire forme des vésicules (flèches simples), qui migrent vers le pôle proximal du dictyosome (flèche double) et s'y fusionnent (flèche triple) pour former un saccule golgien.
Remarquer encore, sur la fig. 1, la continuité entre le R. E. chloroplastidale et la membrane nucléaire (flèche grosse) et l'existence dans le même plaste (en haut et à gauche) de deux types de plastoglobuli à différents degrés d'osmiophilie.
X 32 000; X 48 000.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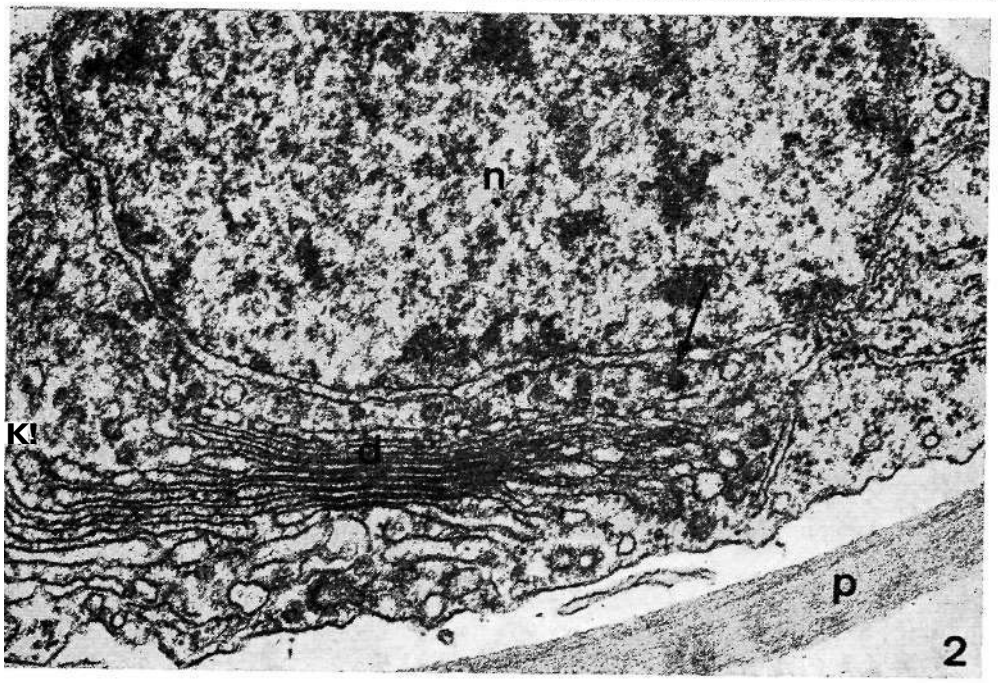
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DEUX NOUVEAUTÉS POUR LA FLORE BRYOLOGIQUE DES AÇORES:

KIAERIA BLYTTII (B. S. G.) BROTH. ET *ORTHODICRANUM FLAGELLARE* (HEDW.) LOESKE

par

CECILIA SÉRGIO *

Résumé

Parmi les Dicranacées récoltées aux Açores par R. T. PALHINHA et L. SOBRINHO en 1937 et L. SOBRINHO et G. DA CUNHA en 1938, deux espèces ont été reconnues qui sont des nouveautés pour la flore açorienne: *Kiaeria blyttii* (espèce nouvelle pour les Iles Atlantiques) et *Orthodicranum flagellare* (espèce qui n'avait pas été retrouvée depuis longtemps soit à Madère soit aux Canaries). La découverte de ces deux mousses aux Açores présente de l'intérêt biogéographique et écologique.

A l'occasion de l'examen des échantillons non identifiés des Açores de l'herbier de l'Institut Botanique de Lisbonne (LISU), il nous a été donné de trouver entre les Dicranacées deux espèces nouvelles pour ces Iles Atlantiques.

Ces échantillons appartiennent à deux collections récoltées une au cours de l'année 1937 par R. T. PALHINHA et L. SOBRINHO, et l'autre, en 1938, par L. SOBRINHO et G. DA CUNHA.

1. **Kiaeria blyttii** (B. S. G.) Broth.

Mousse récolté dans l'île du Pico, à une altitude d'environ 2000 m, parmi *Rhacomitrium lanuginosum, Isopaches bicrenatus* et *Bryum* sp.

L'examen de cette récolte nous montre qu'elle correspond presque exactement aux exemplaires européens de la

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collection de SCHIMPER de notre herbier, puisqu'elle s'en écarte seulement par la coloration un peu moins brune et plus jaune du touffe et aussi par le pédicelle moins rougeâtre et plus jaunâtre.

À notre avis, comme la coloration est un caractère très variable chez les mousses, ces divergences sont parfaitement explicables par les différences écologiques et climatiques entre cette localité et les stations sptentrionales et même boréales où cette espèce existe.

Dans bien des cas, cette Dicranacée se distingue aisément des deux espèces plus proches, *K. starkei* et *K. falcata*, par les feuilles crispées à l'état sec et jamais falciformes-secondes. Cependant, parfois il est difficile d'identifier certains échantillons seulement par ce caractère. La distinction la plus sûre entre *K. blyttii* et les deux espèces est essentiellement basée sur la localisation des gamétangescences et encore sur la papillosité du tissu foliaire supérieur.

Spécimen: Açores, Ilha do Pico, Caldeira, ± 2000 m, parmi *Rhacomitrium lanuginosum*, *Isopaches bicrenatus* et *Bryum* sp., 7.8.1938, *G. da Cunha & L. Sobrinho* 12 (Lisu).

Nous donnons ici une description de ce spécimen:

Plante en touffes denses, vert jaunâtre à l'extérieur, brun décoloré à l'intérieur. Tiges de 1,5-2,5 cm de haut, dressées, rameuses, peu tomenteuses. Feuilles de 3-4 mm de long, dressées-étalées ou étalées en tous sens, crispées à l'état sec, de base concave, longement linéaires-acuminées au sommet, papilleuses sur le dos, finement denticulées aux bords supérieurs; nervure percurrente un peu rugueuse sur le dos, dépourvue de stéréides. Cellules foliaires supérieures bi-ou pluristratifiées, de 10-28 μ sur 6-10 μ, carrées ou subrectangulaires, les inférieures de 20-60 μ sur 8-12 μ, à parois peu épaisses et un peu poreuses près de la base, les angulaires formant des oreillettes peu distinctes à parois plus au moins minces et brunâtres. Gamétangescences situées à la base de la tige ou des innovations, ordinairement terminales sur un court rameau spécial. Gamétangescences à feuilles périchétiales internes longuement engainantes, subulées. Pédicelle de 8-12 mm, jaune-rougeâtre. Capsule presque dressée, à

urne de 1,5 mm, ovoïde plus ou moins arquée, lisse à l'état sec ou sillonnée irrégulièrement après la sporaison, à col faiblement goitreux. Peristome rougeâtre, à 16 dents de 0,6 mm, divisée jusqu'au milieu en 2 branches, et papilleuse au sommet. Anneau à 3 rangées de cellules, caduc. Spores de 16-20 μ de diamètre, jaunâtres, finement papilleuses (PI. I, fig. 1-13).

Distribution géographique: Kiaeria blyttii possède une aire disjointe dont les éléments se répartissent sur la zone holarctique surtout dans les hautes montagnes: Europe septentrionale, occidentale et centrale, dès l'Islande aux Monts Oural; Amérique septentrionale (au nord du 40° N environ), Groenland; Asie au Japon (PI. II).

De l'examen de cette aire actuellement connue, nous pouvons déduire que *K. blyttii* a une distribution disjointe qui présente de grandes similitudes avec celle de *Bryoxiphium,* aussi bien que d'autres Bryophytes *(Amphidium mougeotii, Mylia taylori,* etc.). Il s'agit d'espèces holarctiques, arcto-alpines ou alpines s. 1. et qui existent aussi dans la Macaronésie (PI. II).

On a beaucoup écrit sur l'aire de *Bryoxiphium norvegicum* et les taxa parents *(B. madeirense* Love & Love; *B. norvegicum,* ssp. *japonicum* Love & Love et *B. norvegicum* var. *mexicanum* Scharp.), groupe réparti depuis si longtemps en populations isolées qui ont subi des modifications importantes à créer des unités taxonòmiques bien individualisées (LOVE & LOVE, 1953 et 1955).

Nous pouvons aussi penser que l'aire de *Kiaeria blyttii* et aussi des autres *Kiaeria* révèlent l'ancienneté de ce groupe, mais dans ce cas il n'existe pas une évolution, au moins au niveau morphologique, dans les éléments isolés, mais bien une stabilité plus au moins parfaite.

Kiaeria blyttii est une espèce qui aurait une large distribution au Quaternaire. Elle a pu se mantenir dans les endroits propices (hautes montagnes, même les plus méridionales) et plus tard, chassée par le période xérothermique qui s'ensuivit, a disparu des stations intermédiaires.

H faut cependant rappeler que nous ne donnons ici qu'une des possibles interprétations, car divers hypothèses

ont été émises (voir SCHOFIELD, 1972) quant à l'explication des disjonctions, mais jusqu'à présent elles ne sont pas bien expliquées.

Des disjonctions semblables ont été traitées avec détail soit pour les Bryophytes (TH. HERZOG, 1926; W. B. SCHOFIELD, 1969, 1972; W. C. STEERE, 1969, etc.), soit pour les Lichens (R. G. WERNER, 1973, 1975) et aussi pour les Phanérogames (E. HOLTEN, 1958; G. E. WOOD, JR., 1972, etc.).

Outre l'importance que l'établissement de l'existence de cette espèce dans les Iles Atlantiques possède en ce qui concerne la connaissance de la végétation bryologique de ces Iles, le fait est encore important pour les études biogéographiques, écologiques et paleogéographiques. *K. blyttii* nous apparaît comme une espèce très ancienne, parfaitement stabilisée, arctico-alpine, vivant dans la région holarctique, plus aux moins disjointe, ce qui serait un indice de régression de l'espèce après le Quaternaire.

2. Orthodicranum flagellare (Hedw.) Loeske

Cette espèce a été récoltée pour la première fois aux Açores en 1937, par R. T. PALHINHA et L. SOBRINHO dans l'île du Faial sur la base de *Juniperus* sp.

Spécimen: Açores: Ilha do Faial, Baldio da Caldeira, 800 m, associé a *Hypnum ericetorum, Frullania germana* et *Lophozia* sp., 4.5.1937, *R. T. Palhinha & L. Sobrinho* 123a (LISU).

Cette récolte se présente stérile avec une couleur vert pâle à jaunâtre, très compacte, portant des rameux flagelliformes à petites feuilles énervées et imbriquées caractéristiques (PI. I, fig. 14),

Distribution géographique: Orthodicranum flagellare est une Dicranacée répandue dans les régions montagnardes ou submontagnardes de toute l'Europe centrale et septentrionale, Sibérie, Madère et Canaries, d'Asie jusqu'en Chine, Corée et Japon, et dans l'Amérique septentrionale jusqu'à la Guatemala.

La découverte dans l'archipel des Açores de cette espèce élarge sa distribution géographique et confirme son existence dans la Macaronésie.

Remarquons toutefois que ce Dicranacée sera très rare dans les Des Atlantiques, puisqu'elle n'y a été jamais rétrouvée soit à Madère soit aux Canaries après 1876 d'après SHIMPER. Cependant, cet auteur avait considéré que cette espèce ne serait pas si rare aux Canaries «Haud rarum in America septentrionali et in Insulis Canariensibus».

Par contre, nous avons constaté qu'aucun bryologiste plus récent (A. LUISIER, P. & V. ALORGE, H. PERSSON, P. SUNDING, P. STORMER, A. VON HÜBSCHMANN et E. SJÖGREN) ont rapporté ce mousse dans les inventaires floristiques des Iles Atlantiques.

O. flagellare est un espèce holarctique qui a une aire géographique très étendue, mais peu abondante. Est-ce .qu'elle sera une espèce aussi en régression?

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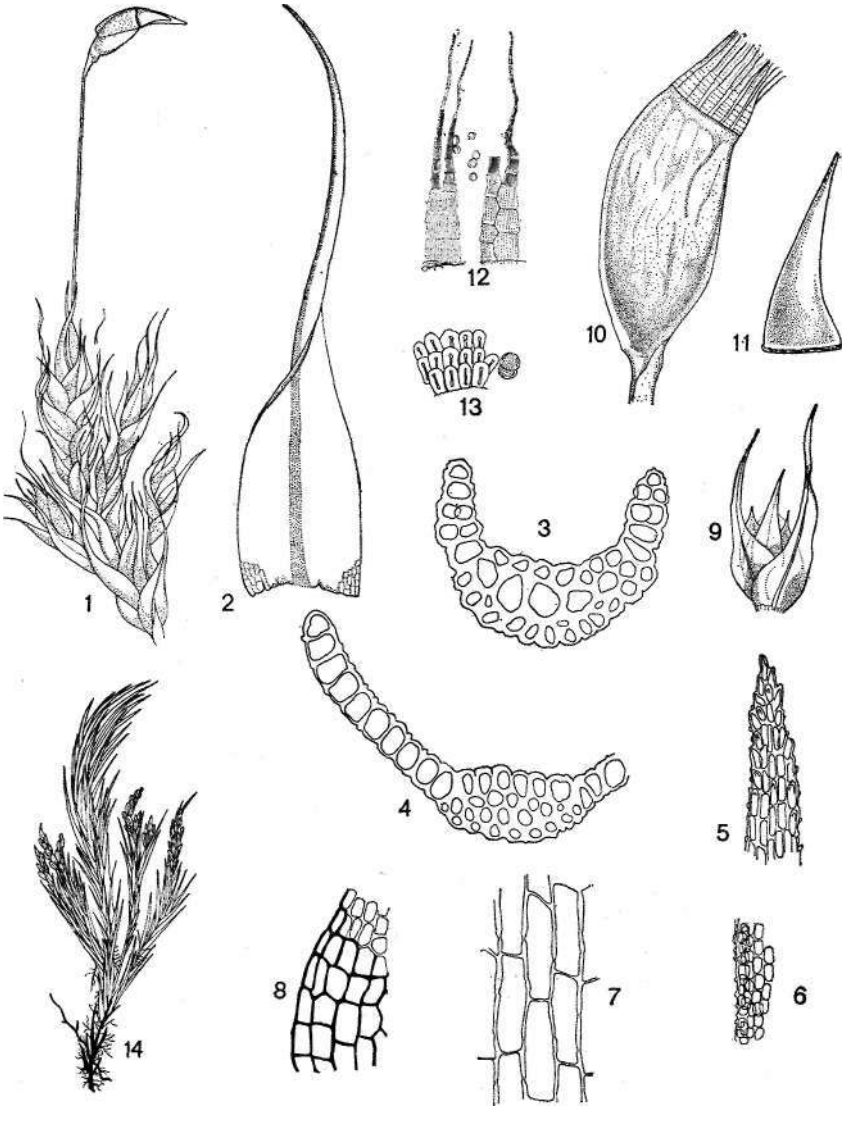
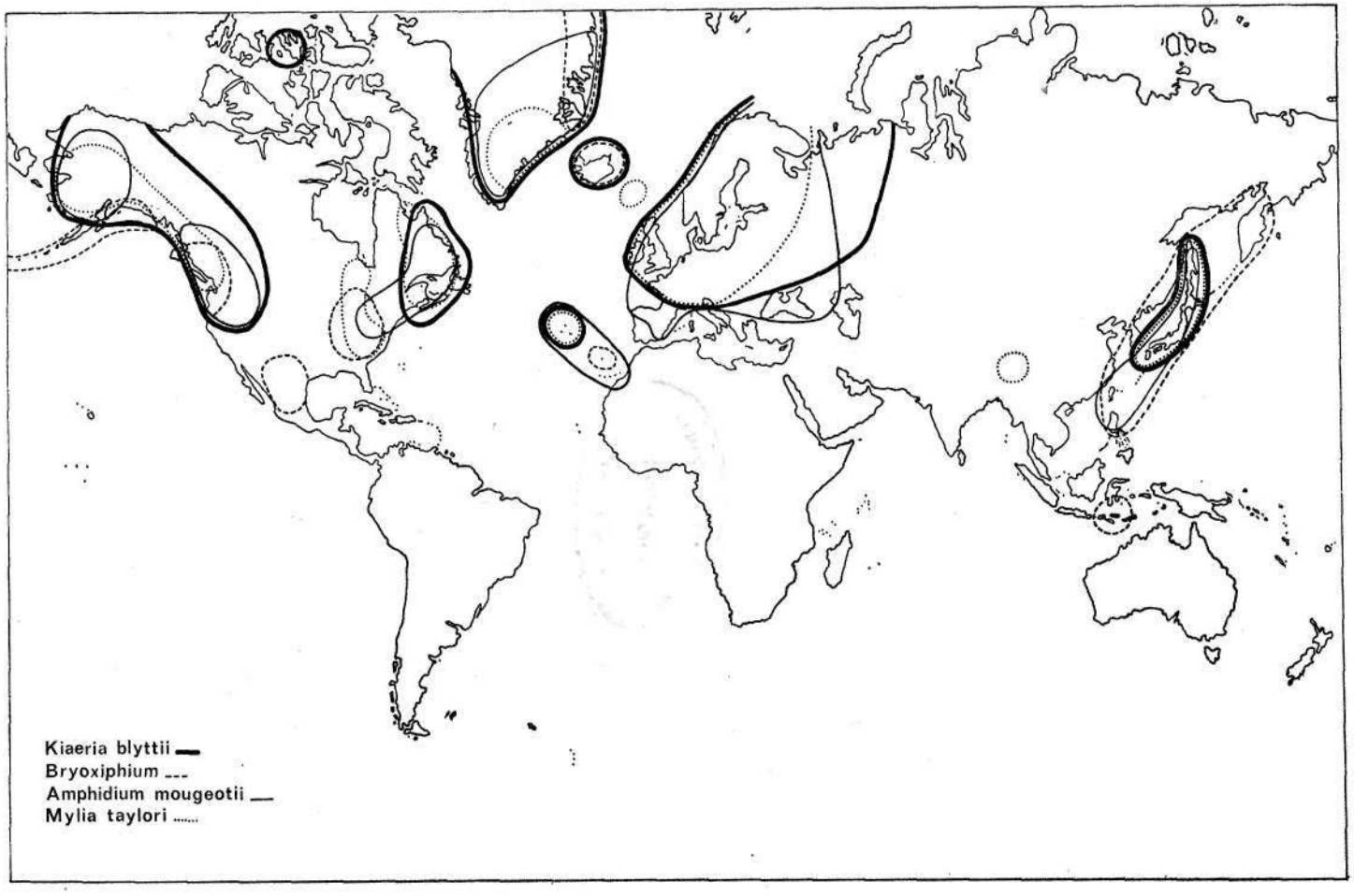


Fig. 1-13: *Kiaeria blyttii* (B. S. G.) Broth. — 1: habitus de la plante (X5). — 2: Feuille caulinaire (X20). — 3-4: Coupes transversales de la feuille à différentes hauteurs (X 250). — 5: Apex foliaire (X100). —6: Cellules de la partie centrale de la feuille (X100).— 7: Cellules basilaires intermédiaires (χ 300). — 8: Cellules angulaires (X100).— 9: Gamétangescence (X 8). —10: Capsule (X20).— 11: Opercule (X20). —12: Dents du peristome sur les deux côtés (X50). —13: Cellules de l'anneau et spores (X 100). Fig. 14: *Orthodicranum flagelare* (Hedw) Loeske, plante stérile avec les rameux flagelliformes (X 2,5).



Aire générale de: *Kiaeria blyttii* (B. S. G.) Broth., *Bryoxiphium norvegicum* (Brid.) Mitt, (sensu amplo), *Amphidium mougeotii* (B. S. G.) Schimp. (incl. va r. *formosicum* Card.) et *Mylia taylori.*

CONTRIBUIÇÃO PARA O CONHECIMENTO CITOTAXONÓMICO DAS *SPERMATOPHYTA* DE PORTUGAL

XII. ONAGRACEAE

por

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PROSSEGUINDO a série de trabalhos que empreendemos (FERNANDES & QUEIRÓS, 1969, 1971 e QUEIRÓS, 1972, 1973a, *b*, *c*, 1974a, *b*, 1975a, *b)*, apresentamos aqui os estudos que efectuámos sobre algumas *Onagraceae* da flora de Portugal.

Nesta exposição, adoptámos a classificação de MELCHIOR, publicada no «Syllabus der Pflanzenfamilien», ed. 12, vol. *2*, 1964. A ordenação das espécies fez-se segundo a «Flora Europaea», vol. *2*, 1968.

Aos *índices* mencionados nos primeiro e segundo trabalhos (FERNANDES & QUEIRÓS, 1969, 1971) acrescentamos os seguintes:

MOORE, R. J. — Index to plant chromosome numbers 1967-1971. Utrecht, 1973. *Regnum Vegetabile*, 90.

MÁJOVSKY, J. & al. — Index of chromosome numbers of Slovakian flora (Part. 3). *Acta F. R. N. Univ. Comen. Bot.*, *22*, 1974.

Mem — Idem (Part. 4). *Ibid. Bot.*, 23, 1974.

LOVE. A. & LOVE, D. — Citotaxonomical Atlas of the Slovenian flora. Lehre, 1974.

FEDOROV, A. A.—Chromosome numbers of flowering plants. Koenigstein, 1974 (Otto Koeltz, Science Publishers).

Empregámos as mesmas técnicas de fixação e coloração dos vértices vegetativos das raízes que foram descritas pormenorizadamente em FERNANDES & QUEIRÓS (1969).

Apesar desta família se encontrar representada em Portugal Continental somente por 15 espécies distribuídas por quatro géneros, não nos foi possível estudar todos os taxa. No entanto, tentaremos apresentar, em futuros suplementos, os resultados que obtivermos pelo estudo dos não incluídos neste trabalho.

Todas as figuras de metafases somáticas que publicamos, são reproduzidas com uma ampliação de ca. 3000.

Agradecemos ao Prof. Dr. ABÍLIO FERNANDES a revisão crítica que se dignou fazer do nosso manuscrito.

Trib. JUSSIEUEAE

Ludwigia palustris (L.) Elliott (= *Isnardia palustris* L.) — Santana Ferreira, Matas de Foja (n.^{os} 1720, 6038).

Esta espécie americana, introduzida na Europa, foi estudada por GREGORY & KLEIN (1960). O material americano citado por estes autores apresentava $\eta = 8$. SCHOTSMAN (1970a), estudando material europeu, contou, de harmonia com o número gamético $\eta = 8$ encontrado pelos primeiros autores, $2n = 16$. No nosso material, contámos igualmente 16 cromossomas somáticos (fig. Iα). Estes são de pequenas

Fig. 1. — α, *Ludwigia palustris,* n.° 6038 ($2n = 16$). *b, Epilobium hirsutum,* n.° 2925 ($2n = 36$). *c, E. parviflorum,* n.° 1718 ($2n = 36$).

dimensões, apresentando constrições medianas ou submedianas. Talvez devido à pequenez dos cromossomas, não pudemos identificar com precisão o par de cromossomas de constrição subterminal citado por SCHOTSMAN.

Trib. EPILOBIEAE

Epliobium hirsutum L. — Miranda do Douro, São Martinho de Angueira (n.° 2822) ; Praia do Guincho (n.os 7686, 2925); Vale de Lobos (n.° 7862).

Esta espécie foi estudada por diversos autores, entre os quais HAKANSSON (1924), SCHWEMMIJE (1924), HARA (1952), RAVEN & MOOKE (1964), GADELLA & KLIPHUIS (1966, 1968) e PODLECH & DIETERLE (1969), que citam 2n = 36.

As nossas contagens confirmam as destes autores (fig. 16).

KISCH (1941) refere o número 2n—18 e MICHAELIS (1928, 1942) cita 2n = 54. Estes resultados sugerem que existe nesta espécie uma série poliplóide em que são conhecidos os termos 2x, 4x e 6x. As formas tetraplóides seriam, porém, as mais frequentes.

Epilobium parviflorum Schreber — Coimbra, Coselhas (n.° 1718).

Vários autores, tais como SCHWEMMUE (1924), RAVEN & MOOKE (1964) e, mais recentemente, MURÍN (in MÁJOVSKY & ai., 1973) referem para esta espécie 2n = 36. Confirmamos este resultado (fig. 1e).

Epilobium lanceolatum Seb. & Mauri — Penacova (n.° 6035).

Tal como RAVEN & MOOKE (1964), determinamos para esta espécie 2n = 36. Os cromossomas são de dimensões muito pequenas (fig. 2α).

Epilobium tetragonum L. subsp. tetragonum (= *E. adnatum* Griseb.)—Valongo, Carvoeira (n.os 2823, 4009); Porto, Monte Aventino (n.° 5364) ; Bodiosa, a 8 km de Viseu (n.° 4555) ; Serra da Estrela, na estrada para a Torre (n.° 5665) ; Lousã, Candal (n.° 6037) ; Figueira da Foz, Gala (n.° 1719) ; Serra de Monsanto (n.° 2042) ;, Manique (n.° 3801) ; Sintra, Algueirão (n.os 6596, 7863) ; Lisboa, Rio de Mouro (n.° 7687). ,

SCHWEMMLE (1924) e RAVEN & MOORE (1964) determinaram para este taxon 2n = 36; nós confirmamos este resultado (fig. 2b).

Fig. 2. — *a, Epilobium lanceolatum,* n.° 6035 (2n = 36). *b, E. tetragonum* subsp. *tetragonum,* n.° 7863 (2n = 36).

Trib. OENOTHEREAE (ONAGREAE)

Oenothera biennis L. — Porto, Aguas Férreas (n.° 5366).

A maior parte dos autores que estudaram este taxon (ver *Indices)* atribuem-lhe 2n = 14. Todavia, formas tetraplóides são citadas por LEVIVELD (1931) e SEITZ (1935).

Os indivíduos que observámos apresentam 2n = 14 (fig. 3α).

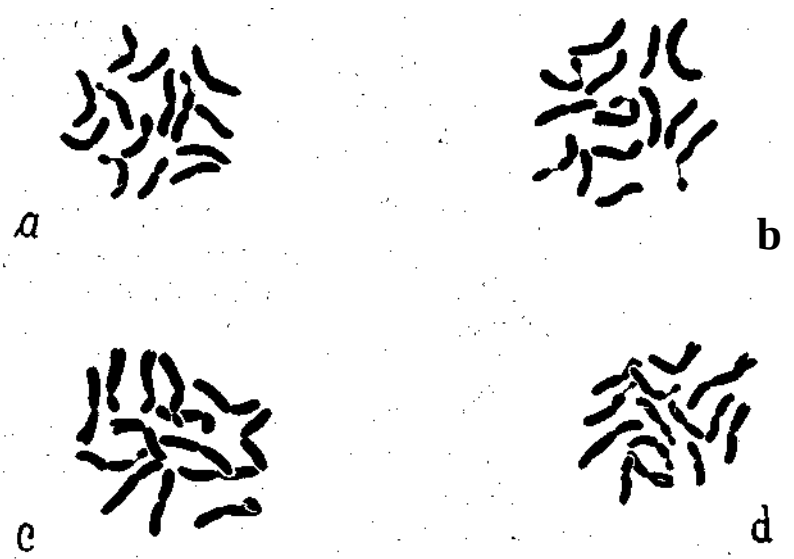

Fig. 3. — *a , Oenothera biennis, nº* 5366 (2n = 14). *b, O. erytrosepala* n.° 2824 (2n = 14). *c, O. grandiflora,* n.° 1721 (2a = 14). *d, ídem,* n.» 6162 (2n = 14).

Oenothera erythrosepaïa Borbás—-Porto, Arrábida (n.os 2824, 2825) ; Barracão, na estrada de Cantanhede a Mira (n.° 6039); Marinha Grande (n.° 4556).

Determinamos 2n = 14 (fig. 3b), de acordo com os resultados de GADELLA & KLIPHUIS (1966).

Oenothera grandiflora Aitón — Coimbra, Choupal (n.os 1721, 6162, 6040).

Os *índices* referem para esta espécie 2n = 14. BOEBIJN, 1924 (trabalho que não tivemos possibilidade de consultar) cita, segundo FEDOKOV (1969), 2n = 28. Os nossos resultados estão de acordo com o primeiro daqueles números (fig. 3c, 3d).

Oenothera affinis Camb. — Praia das Maçãs (n.° 7864).

HECHT (1950) e TANDON & HECHT (1953) enumeram para esta espécie 2n=14. Confirmamos este número (fig. 4α).

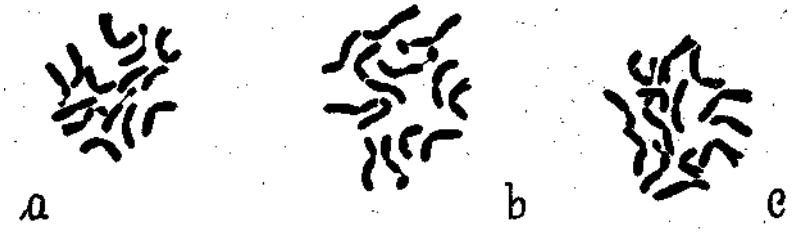

Fig. 4.— *a, Oenothera affinis, n.°* 7864 (2n = 14). *b,* O. *strida,* n.° 2826 (2n = 14). c, *Idem,* n.« 7281 (2n = 14).

Oenothera stricta Ledeb. ex Link — Matosinhos, Guifões, Ponte do Carro (n.° 7281); Vila Nova de Gaia, Miramar (n.° 2826); Figueira da Foz, Gala (n.os 1722, 6042); Praia das Maçãs (n.° 7865); Próx. de Azeitão (n.º 2043).

HAKANSSON (1926, 1928), HECHT (1950) e SCHWEMMIE & SIMON (1956) contaram nesta espécie 2n==14 e nós confirmamos este número (fig. 4b, 4c).

Oenothera indecora Camb. — Corroios (n.° 7688); próx. de Corroios (n.° 2926).

Planta originária do Brasil, Uruguai e Norte e Centro da Argentina foi encontrada em Corroios e arredores \ Contámos 2n = 14 (fig. 5α), de acordo com os estudos de HECHT (1950).

Fig. 5.—*a, Oenothera indecora,* n.° 7688 (2n = 14). *b, Circaea lutetiana,* n.° 1717 (2n = 22).

Trib. CIRCAEAE

Circaea lutetiana L. — Lousa, Alfucheira (n.os 1717, 6034).

UDDLING (1929), DELAY (1947, 1948), RAVEN (1983), JUNGBLUT (1967), *GABELLA* & KLIPHUIS (1968), JANKUN (in SKALINSKA & al., 1968), SCHOTSMAN (1970&) e JANKUN (in SKALINSKA & al., 1971) são unânimes em atribuir a esta espécie 2n = 22. Os nossos resultados estão de acordo com os destes,autores (fig. 5b).

[1] Para a identificação deste material, consultou-se a obra de A. L. CABRERA — *Manual de la Flora de los Alrededores de Buenos Aires,* 1953.

Números cromossómicos determinados

Nome do taxon	2n	2n determinado por outros autores
Ludwigia palustris (L.) Elliott , . .	16	16
Epilobium hlrsutum L. . . . . ' .	36	18, 36, 54
Epilobium parviflorum Sehréber . .	36	36
Epilobium lanceolatum Seb. & Mauri	36	36
Epilobium tetragonum L. subsp. tetra-	36	36
	14	14, 28
Oenothera erythrosepala Borbás . .	14	14
Oenothera grandiflora Aitón	14	14, 28
Oenothera affinis Camb.	14	14
Oenothera strieta Ledeb. ex Link . .	14	14
Oenothera indecora Camb.	14	14
Circaea lutetiana L.	22	22

RESUMO

O autor apresenta os números cromossómicos de 12 taxa de *Onagraceae* existentes em Portugal e provenientes de várias localidades do País.

Os resultados obtidos estão de acordo com os dos nossos antecessores.

RÉSUMÉ

L'auteur a déterminé les nombres chromosomiques de 12 taxa *d'Onagraceae* récoltés dans plusieurs localités du Portugal. Les résultats obtenus confirment les numérations de nos prédécesseurs.

SUMMARY

Somatic chromosome numbers for 12 taxa of *Onagraceae* from Portugal have been determined. The results are in accordance with previous reports.

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FRESHWATER ALGAL VEGETATION IN THE AZORES

by

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ABSTRACT

The investigations of the algal material collected in the Azores in the summer of 1975 showed a wide variety of algal communities (27) from 8 types of biotopes.

THE Azorean archipelago is situated in the middle of * the Atlantic Ocean. It is made up of three groups of islands (Fig. 13), the western: Flores, Corvo; the central: Faial, Pico, S. Jorge, Graciosa, Terceira; the eastern: S. Miguel, Santa Maria. The topography of the islands is considerably dissected by a large number of periodically dry ravines and by river valleys. The nine islands are all of volcanic origin and the geological conditions are rather uniform. Basaltic rock predominates. The climate of the Azores Islands is oceanic, with small variations in temperature, large amounts of precipitation, and high air humidity. The mean annual temperature is about 17.5° C.

The present notes are based on the algal material collected during a short excursion to the islands of Terceira (T), S. Jorge (J), Pico (P) and Faial (F) in the summer of 1975. The purpose of the investigation was to study different types of algal biotopes.

A review of sparse algological literature concerning the Azorean Islands, is to be found in CEDERCKEÜTZ (1941) and Bourrelly & MANGUIN (1946). It is evident from these two publications that most investigations have been carried out on S. Miguel, the main island. The papers of BOHLIN (1901) and CEDERCREUTZ (1941) are of greater interest since

they also deal with algae from Terceira, S. Jorge, Pico and Faial. Moreover, there is some information about these islands in BOURRELLY & MANGUIN (1946) and MANGUIN (1942). When studying algal lists from the Azores one finds that the species listed are all, with few exceptions, to be found in Europe and most of them have a wide distribution there.

The algal flora of isolated islands and its origin has always been much discussed by phycologists. BOHLIN (1901) has outlined the following ways for dispersal:

transportation by sea-birds	ornithochorous	dispersal
transportation by man	anthropochorous	dispersal
transportation by ocean currents	anemochorous	dispersal
transportation by winds	hydrochorous	dispersal

One of the most important means of dispersal is probably the ornithochorous one. Often sea-birds make leeway with storms from original routes to the Azorean archipelago. Algal fragments, spores, cysts, diatoms and desmids are attached to the toes of the birds. Man, as a dispersal agent between the main land and the archipelago and between the islands themselves has become more and more important during the last decades due to the growing plane- and shipconnections. Anemochorous dispersal demands that the algae are able to tolerate desiccation. This ability is known to exist among many blue-green algae, diatoms and desmids, which can survive several months of dryness, and when moistured grow again. Aerial algae form the most important group of freshwater algae in the islands. PETERSEN (1928) has characterized them as following: «As aerial algae I will then define all algae that do not grow in water, or which, at any rate, in periods are able to grow without being immersed in water, even if they pass into a resting stage during protracted periods of desiccation.

THE MATERIAL·

The algae, which have been collected on Terceira, S. Jorge, Pico and Faial, originate mostly from temporarily flooded areas in craters, caves and ravines. The following

biotopes and sampling stations (Fig. 14, 15) for algal vegetation have been studied:

1. Rock surfaces in craters: Santa Barbara (T), Pico das Caldeirinhas (J), Caldeira (F)
2. Streams: Ribeira da Ponta Furada (J), Ribeira do Cedro (J), Ribeira do Cavalete (J), Caldeira (F)
3. Fumaroles: Furnas do Enxofre (T)
4. Cave walls: Serra do Topo area (J), Furna do Frei Matias (P)
5. Ravine sides and bottoms: Pico das Caldeirinhas area (J), Pico do Areeiro area (J), Serra do Topo area (J)
6. Epiphytic algae on vascular plants, and bryophytes: Ribeira da Fonte (J), Pico do Areeiro area (J), Pico Volcano (P), Caldeira (F)
7. Lakes: Lagoa do Capitão (P), Caldeira (F)
8. Steep road sides: Pico das Caldeirinhas area (J)

1. **Rock surfaces in craters**

The largest caldeira, Santa Bárbara, located on Terceira, has a rather different micro-habitat structure compared to the Caldeiras on Faial and S. Jorge. The algal floras are consequently of different composition. The habitats are:

a. Rock surfaces with no vegetation but algae (samples from T, J and P)
b. Rock surfaces with Sphagnum spp. (T)
c. Rock surfaces with other mosses than *Sphagnum* spp. (F).

Stigonema-Chroococcus community. On the first type (a) of substrate there is a vegetation of brown algal cushions (T, J), 2-3 cm in diameter, mostly of *Stigonema minutum, 8. informe, Chroococcus turgidus* (Fig. 2), *Schizothrix* species and a few diatoms (the algae are arranged according to decreasing frequency in the sample, above and below).

Frustulia-Gloeocapsa community. Another association (T) contains a great deal of *Frustulia rhomboïdes* v. *viridula*

and *Lyngbya allorgei* along with some *Stigonema-* and *Gloeocapsa* species.

Synedra-Cymbella community. This type of substrate (a) also occurs on Faial, but has a completely different species structure. Most samples consist almost exclusively of diatoms, with a pronounced dominance of *Synedra ulna, Cymbella turgida, AchnantJies exigua, Epithemia turgida, Rhopalodia gibberula* v. *producta and AchnantJies lanceolata.*

Chroococcus-Tetmemorus community. The second type (b) of rock habitat is a very rich vegetation of *Sphagnum* species on the rock surfaces. It is characteristic for the Santa Bárbara crater on Terceira, but is completely lacking in the Caldeira on Faial. The samples contain mainly C*hroococcus minutus, Tetmemorus laevis* v. *minutus, Frustulia rhomboïdes* v. *saxónica* and the forma *capitata, Frustulia vulgaris* v. *parva,* and *Navícula subtilissima.*

Chroococcus-Eunotia community. *Sphagnum* covered crater walls exist both on S. Jorge and in the big Caldeira on Terceira. *Tetmemorus laevis* v. *minutus* is abundant on Terceira but has not yet been found by the author on S. Jorge. On S. Jorge there is instead *Eunotia pectinalis* together with *Chroococcus turgidus* among *Schizothrix pallida, Gloeocapsa grevillei,* and *Anomoeoneis exilis.*

Rhopalodia-Schizothrix community. From the material collected in the third (c) type of rock habitat, epiphytic algae on mosses have been studied. Dominating algae on *Marchantía* sp. are *Rhopalodia gibberula* (Fig. 6), *Schizothrix pallida, Nitzschia amphibia, Melosira roseana,* and *Phormidium pachydermaticum.*

Cylindrospermum maius community. The *Cylindrospermum maius* occurs as the only species on *Rhynchostegium* sp.

Nostoc-Chroococcus community. In the samples on *Fissidens* sp. were found *Nostoc* spp., *Chroococcus turgidus,* and *Schizothrix telephorides.*

2. Streams

The island of S. Jorge has, compared with the other islands, many places with running water. These are located especially in ravines at high altitudes. Small streams also

occur in the cultivated landscape. The following communities have been observed.

Zygnema-Oscillatoria community. The main algal association in a rather large stream at 680 meters a.s.l. in the Ribeira do Cedro in the Serra do Topo area in the SE part of S. Jorge. The dominating species in this community are *Zygnema* sp., *Oscillatoria irrigua, Phormidium retzii* (Fig. 7), *Microspora wittrockii, Mougeotia* sp., and *Frustuìia rhomboïdes* v. *saxónica* fo. *capitata.*

Oedogonium-Achnanthes community. Studied in a stream running in a ravine in the NW part of S. Jorge called Ribeira da Ponta Furada. The samples were taken at 600 meters a.s.l. in an exposed biotope and showed a very striking vegetation of *Oedogonium* sp., *Achnanthes minutissima* and *Gomphonema constrictum* (Fig. 8).

Oedogonium-Nostoc community. In a partly dried habitat in the same ravine occurs *Oedogonium* sp., *Nostoc sphaericum* and many diatoms, e. g. *Achnanthes minutissima, Navícula gracilis* and *Nitzschia palea.*

Achnanthes minutissima community. Some parts of the stream were completely covered with moss, *Rhyncostegium rusciforme* completely covered with *Achnanthes minutissima.*

Phormidium-Navicula community. Below a small water fall in a shaded place was a growth of *Phormidium pachydermaticum* and *Navícula radiosa.*

Rhopalodiu gïbberula community. Observed under an overhanging lava block is made up of almost pure growths of that diatom.

Zygnema-Eunotia community. A very dense vegetation of *Zygnema* sp. and *Eunotia pectinalis* v. *minor* forms this community in Ribeira do Cavalete, a ravine surrounded by pasture land.

Nostoc-Radiophilium community. In the Caldeira of Faial was found a small stream containing *Radiophilium flavescens* (Fig. 1), *Phormidium retzii* and some *Nostoc* species. Otherwise there was very little running water available there because of the unsually dry summer.

3. Fumaroles

Microspora-Tetmemorus community. The presence of fumaroles on Terceira and S. Miguel indicates that volcanic activity is not extinct. The temperature in and close to the fumaroles is rather high. MOSELEY (1875) measured a maximum temperature of 93° C. Luxuriant algal vegetation^ generally blue-green species, occurs at about 40° C (BOHLIN, 1901). At Furnas do Enxofre (T), the fumaroles are surrounded by a grey-green-red substance, which consist predominantly of *Microspora stagnorum, M. quadrata, Tetmemorus laevis* v. *minutus.* Reddish grains intermingle with colonies of *Gloeocapsa gelatinosa.*

4. Cave walls

Most of the sampling was carried out in the cave Furna de Frei Matías (675 m a.s.l.) on Pico. The vascular plant flora close to the mouth consists of *Bellis azorica, Culcita macrocarpa, Diplazium caudatum, Carex vulcani* and other plants.

Melosira-Chroococcus community. The macroscopic algal substance on the cave walls can be described as an amorphous, loose layer of about 5 cm thickness. The algal vegetation contains mainly blue-greens and diatoms. Close to the cave opening, *Aphanothece stagnina* dominates, together with *Chroococcus turgidus* and *Navícula cincta* (Fig. 3). In addition, there are small numbers of *Navícula* cfr. *dispersa* (Fig. 4), *Eunotia valida, Navícula pupula* v. *genuina* (Fig. 5) and *Melosira roseana.* The abundance of the last taxon increases further inside the cave, likewise that of *Nostoc sphaeroides.* The community also consists of a great deal of *Rhopalodia gibberúla, Eunotia pectinális, Navícula pupula* v. *elliptica,* and a large number of *Cymbella* species.

Another type of cave is rather open with walls covered by the hepatic *Conocephalum conicum.* On S. Jorge, in the Serra Topo area, one of the studied caves has an algal vegetation of *Chrococcus turgidus* like the cave on Pico,

but, among the diatoms, *Cymbella ventricosa* and *Rhopalodia gibberula* dominate.

5. Ravines

This type of habitat was mainly studied on S. Jorge. The following communities have been observed.

Vaucheria community. Completely covering the lava material on a ravine bottom in the Pico das Caldeirinhas area. Besides the steril *Vaucheria,* the diatoms *Achnanthes lanceolata, Nitzschia dissipata* and *Rhopalodia gibberúla* occur sparsely.

Phormidium-Navicula community. Moss-covered ravine walls in the Pico do Areeiro area are dominated in this community, made up of *Phormidiumretzii, Navícula gracilis, Microspora lauterbornii* (Fig. 9), *Phormidium durum* and many diatoms.

Stigonema-Eunotia community. On ravine walls in the area of Serra do Topo at an altitude of 800 meters in the Ribeira do Cavalete dominating algae in this community are *Stigonema multipartitum* and *Eunotia exigua.*

Gloeocapsa-Eunotia community. In the same ravine, there is a rich algal vegetation on branches of *Juniperus brevifolia.* Their presence is favoured by the high air humidity generally over 90%. This community is very conspicious. It is mainly composed by *Gloeocapsa rupestris, Eunotia exigua,* and *Clamydomonas* sp. as is also the *Scytonema-Zygnema* community, with *Scytonema hofmannii* (Fig. 10), *Zygnema* sp., *Phormidium rotheanum, Microspora loefgrenii, Tabellaria flocculosa* and *Eunotia pectinalis.*

6. Epiphytic algae on vascular plants and bryophytes

Vaucheria-Nitzschia community. In the highest sections of Ribeira da Fonte (J) grows a small planted forest of *Cryptomeria japonica.* Samples from the bark contain *Vaucheria* sp., and small amounts of diatoms, such as *Nitzschia dissipata, Achnanthes lanceolata,* and *Synedra ulna.* A bark sample of *Cryptomeria* from another area (Pico dos Frades)

showed a dense cover of *Glamydomonas* sp. and *Radiophilium flavescens* (Fig. 1).

Zygnema-Rhopalodia community. On *Erica azotica* in the Pico do Areeiro area (J) was recorded a sparse growth oí *Zygnema* sp., *Phormidium retzii,* and *Rhopalodia gibberula.*

Schizothrix-Gloeocapsa community. Epiphytic on *HymenophyUum tunbrigense* in the same area grow *Schizothrix vaginata* together with *Gloeocapsa rupestris.*

Homoeothrix africana community. A *Blechnum spicant* specimen on the island of Pico from the slope of the Pico vulcan at about 900 meters showed a growth of *Homoeothrix africana.* This species is a primary colonizer and supports the further colonization of certain preferentially epiphytic hepatics,

Schizothrix-Anomoeoneis community. In the Caldeira (F) specimens *Marchantía polymorpha* were found to be covered by *Schizothrix pallida* and a lot of diatoms, such as *Anomoeoneis serians* v. *brachysira, Navícula gracilis,* and *Rhopalodia gibberula* v. *producta.*

7. Lakes

Only a few samples were collected from lakes and may therefore give only a vague idea about algal vegetation.

Bulbochaete community. Among *Litorella uniflora* leaves in Lagoa do Capitão (P), there is a dense growth of *Bulbochaete* sp., *Scenedesmus quadricauda, Tabellaría flocculosa, Zygnema-,* and *Mougeotia* species.

Oedogonium-Tolypella community. In the bottom of the Caldeira (F) there are two crater lakes which usually dry out at the end of summer. In one of them, among *Eleocharis palustris* and *Litorella uniflora* grow long threads of *Oedogonium* sp. and *Tolypella prolifera.* The second lake contains mainly *Vaucheria* sp.

8, Steap road sides

Aphanocapsa-Epithemia community. Along the road, in in the Pico das Caldeirinhas area, this type of community covers the cut out earth walls and contains mainly *Apha-*

nocapsa grevillei, Eunotia arcus (Fig. 11), *Rhopalodia gïbberüla* and *Cymbella cesatii.*

Cocconeis-Achnanthes community. In the same area, another patch consisted of different diatom species, e.g. *Cocconeis hustedtii, Achnanthes* cfr. *marginulata, Achnanthes linearis* v. *pusilla,* and *Navícula dicephala* v. *neglecta.*

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TABLE I

Checklist of fresh water algae from the Azores (material collected during the excursion 1975)

Islands		T	J	P	F	O
CYANOPHYTA						
Cyanophyceae						
Aphanocapsa	grevillei (Hass.) Rab.	.	●	.	.	.
Aphanothece	stagnina (Spreng.) A. Br.	.	.	●	.	.
	spp.	.	.	.	.	X
Chrooeoccus	turgidus (Kütz.) Näg.	●C	●	●	●	X
	minutus (Kütz.) Näg.	●	.	.	.	X
Gloeocapsa	gelatinosa Kütz.	●	●	.	.	.
	rupestris Kütz.	●	●	.	.	X
	thermalis Lemm.	●	.	.	.	.
	quaternaria (Brèb.) Kütz.	●	.	.	.	.
	caldariorum Rab.	●	.	.	.	.
	montana Kütz.	●	.	.	.	X
	sp.	.	.	.	.	X
Stigonema	hormoides (Kütz.) Bornet et Flan.	●C	●	.	.	X
	tomentosum (Kütz.) Hieron.	.	●	.	.	X
	minutum (Ag.) Hass.	●	●	.	.	X
	ocellatum Thur.	●	.	.	.	X
	mamiHosum (Lyngb.) Ag.	.	●	.	.	X
	informe Kütz.	●	●	.	.	.
	multipartitum Gardner	.	●	.	.	.
	robustum Gardner	.	●	.	.	.
Hapalosiphon	intricatus W. et G. S. West	●	.	.	.	.
Scytonema	hofmannii Ag.	.	●	.	.	X
	dilatatum Bharandwaja	●	.	.	.	.
Homoeothrix	africana G. S. "West	.	●	.	.	.
Nostoc	sphaeroides Kütz.	.	.	●	●	.
	commune Vauch.	.	.	●	.	.
	sphaericum Vauch.	.	●	.	●	X
"	carneum Ag.	.	.	.	●	.
-	sp.	.	.	.	.	X

T = Terceira
J = S. Jorge
P = Pico
F = Faial
O = other islands within the Azores

● = C. Johansson
C = C. Cedercreutz
H = J. Holmboe
X = other records

TABLE I *(Continuation)*

Islands		T	J	P	F	O
Cylindrospermum	maius Kütz.	.	●	.	●	X
	sp.	.	.	C	.	.
Oscillatoria	tenuis Ag.	.	.	.	.	X
	irrigua Kütz.	.	●	.	.	X
Phormidium	rotheanum Itzigs.	.	●	.	.	.
	retzii (Ag.) Gom.	.	●	.	●	.
	pachydermaticum Frémy	●	●	.	.	.
	durum Gardner	.	●	.	.	.
Lyngbya	martensiana Menegh.	●	.	.	.	X
	allorgei Frémy	●	.	.	.	.
Schizotrix	fuscescens Kütz.	●	.	.	.	.
	symplocoides (Gardner) Geitl.	●	.	.	.	.
	lacustris A. Br.	●	.	.	.	.
	telephorides (Mont.) Gom.	.	.	.	●	.
	pallida (Näg.) Geitl.	.	●	.	.	.
	vaginata (Näg.) Gom.	.	●	.	.	.
CHROMOPHYTA						
Heterokontae						
Tribonema	sp.	●	.	.	.	X
Vaucheria	sp.	C	●	.	●	X
Bacillariophycae						
Achnanthes	affinis Grun.	.	●	.	.	X
	cfr. calcar Cleve	.	●	.	.	.
	conspicua v. brevistriata Hust.	.	.	●	●	.
	exigua Grun.	.	.	.	●	X
	flexella (Kütz.) Brun	.	●	.	.	.
	lanceolata (Brèb.) Grun.	.	●	.	●H	X
	lanceolata v. elliptica Cleve	.	●	.	●	X
	lanceolata v. rostrata Hust.	.	.	●	.	X
	linearis W. Smith	●	.	.	.	X
	linearis v. pusilla Grun.	.	●	.	.	.
	ef. marginulata Grun.	.	●	.	.	.
	minutissima Kütz.	.	●	.	.	X
	minutissima v. cryptocephala Grun.	.	.	.	●	X
Anomoeoneis	exilis (Kütz.) Cleve	●	●	.	●	X
	exilis v. azotica Mang.	.	.	.	●	X
	serians v. brachysira (Brèb.) Hust.	.	.	.	●	X
Anomoeoneie	styriaca (Grun.) Hust.	●	.	.	●	.
Caloñéis	elevei v. undulata cfr. fo. inaequalis Mang.	.	.	.	●	X
Cocconeis	hustedtii Krasske	.	●	.	.	.

TABLE I *(Continuation)*

Islands		T	J	P	F	O
Cocconeis	placentula Ehr.	·	●	·	·	·
	placentula v. euglypta (Ehr.) Cleve	·	●	·	·	·
Cymbella	cesatii (Rab.) Grun.	·	●	·	·	X
	delicatula Kütz.	·	●	·	·	·
	gracilis (Rab.) Cleve	●	·	·	·	X
	microeephala Grun.	·	●	·	·	X
	perpusilla A. Cleve	·	●	·	·	X
	pusilla Grun.	·	·	●	·	·
	turgida (Gregory) Cleve	·	·	·	●	X
	ventricosa Kütz.	●	●	·	●	X
Diploneis	ovalis (Hilse) Cleve	·	●	·	·	X
Epithemia	argus Kütz.	·	●	·	·	·
	turgida (Ehr.) Kütz.	·	·	·	●	X
Eunotia	arcus Ehr.	●	●	·	·	X
	arcus v. fallax Hust.	·	●	·	·	·
	exigua (Brèb.) Grun.	●	●	·	·	X
	exigua v. compacta (Brèb.) Grun.	·	●	·	·	·
	paludosa Grun.	·	●	·	·	·
	pectinalis (Kütz.) Rab.	●	●	●	●	X
	pectinalis v. minor (Kütz.) Rab.	●	●	·	·	X
	tenella (Grun.) Hust.	●	·	·	·	X
	valida Hust.	●	·	●	·	X
Fraguaría	leptostauron (Ehr.) Hust.	·	·	·	●	X
Frustulia	rhomboïdes v. saxónica (Rab.) de Toni	●	●	●	·	X
	rhomboïdes v. saxónica fo. capitata Mayer	●	●	·	·	X
	rhomboïdes v. viridula Brèb.	●	·	·	·	·
	vulgaris Thwaites	·	●	·	·	·
	vulgaris v. capitata Krasske	●	·	·	·	·
	vulgaris v. parva Cleve	●	·	·	·	·
Gomphonema	acuminatum v. brebissonii (Kütz.) Cleve	·	●	·	·	·
	angustatum v. producta Grun.	·	●	·	·	·
	constrictum Ehr.	·	●	·	·	X
	constrictum v. capitata (Ehr.) Cleve	·	●	·	·	·
Gomphonema	gracile Ehr.	·	●	·	·	X
	lanceolatum Ehr.	·	●	·	·	X
	longiceps v. subclavata Grun.	·	●	·	●	X
	montanum v. accuminatum Mayer	·	●	·	·	·
	sphaerophorum Ehr.	·	●	·	·	·
Meloslra	roseana Rab.	·	·	●	●	X

TABLE I *(Continuation)*

Islands		T	J	P	F	O
Navícula	cari Ehr.	.	.	.	●	.
	cincia (Ehr.) Kütz.	.	.	●	.	X
	cryptccephala v. exilis (Kütz.) Grün.	.	.	●	.	.
	cryptocephala cfr. v. veneta (Kütz.) Grün.	●	.	.	.	X
	dicephala v. neglecta (Krasske) Hust.	.	●	.	.	.
	cfr. dispersa Mang·.					
	gracilis Ehr.	.	●	.	●	X
	nivalis Ehr.	.	●	.	.	.
	pupula v. elliptica Hust.	.	.	●	.	X
	pupula v. genuina Grün.	.	●	.	.	X
	radiosa Kütz.	.	●	.	●	X
	seminulum Grün.	.	●	.	.	X
	subtilissima Cleve	●	.	.	.	X
Nitzschia	acuta Hantzsch	.	●	.	.	.
	amphibia Grün.	.	●	.	●	X
	dissipata (Kütz.) Grün.	.	●	.	.	X
	linearis W. Smith	.	.	.	●H	X
	palea (Kütz.) W. Smith	.	●	.	.	X
	thermalis Kütz.	.	●	.	.	X
Pinnularia	cfr. appendiculata (Ag.) Cleve	.	.	●	.	X
	gibba Ehr.	.	.	●	.	X
	gibba v. linearis Hust.	.	.	●	.	.
	gibba v. parva (Ehr.) Grün.	.	●	.	.	.
	sublinearis Grün.	.	●	.	.	.
Rhopalodia	gibberula (Ehr.) O. Müll.	.	●	●	●	X
	gibberula v. producta (Grün.) Cleve	.	.	.	●	X
Surirella	linearis W. Smith	.	●	.	.	X
Synedra	acus Kütz.	.	●	.	.	X
	ulna (Nitzsch.) Ehr.	X	●	.	●	X
	vaucheriae Kütz.	.	●	.	●	X
Tabellaría	flocculosa (Koth) Kütz.	●H	●	●	●	X
CHLOROPHYTA						
Chlorophyceae						
Clamydomonas	sp.	.	●	.	.	X
Schizoclamys	gelatinosa A. Br.	.	●	.	.	X
Coccomyxa	confluens (Kütz.) Fott	●	.	.	.	.
Scenedesmus	quadricauda (Turp.) Brèb.	C	.	●C	C	X
Ankistrodesmus	falcatus (Corda) Ralfs	●	●	C	C	X
Radiophilium	flavescens G. S. West	.	●	.	●	.

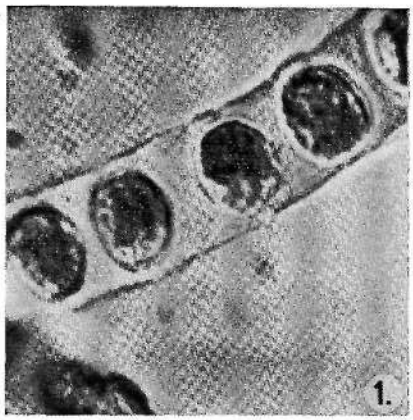
TABLE I *(Continuation)*

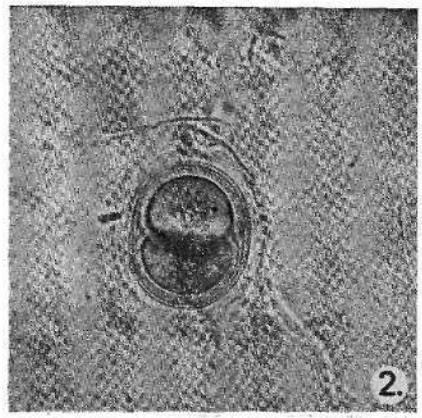
Islands		T	J	P	F	O
Stichococcus	minor Näg.	●	●	.	●	.
Microspora	quadrata Hazen	●	.	.	.	.
	stagnorum (Kütz.) Lagerh.	●	.	.	.	X
	fioccosa (Vauch.) Thur.	●	.	●	.	.
	lauterbornii Schmidle	.	●	.	.	.
	rufescens (Kütz.) Lagerh.	●	.	.	.	.
	loefgrenii (Nordst.) Lagerh.	.	●	.	.	.
	wittrockii (Wille) Lagerh.	.	●	.	.	.
	amoena (Kütz.) Rab.	●	.	.	.	.
	amoena v. gracilis (Wille) de Toni	●	.	.	.	.
Trentepohlia	sp.	●	.	.	.	X
Oedogonium	sp.	C	●	C	●	X
Bulbochaete	sp.	.	.	●	.	X
Conjugatae						
Zygnema	sp.	C	●	●C	.	X
Mougeotia	sp.	C	●	●	.	X
Mesotaenium	micrococcum v. minus (de Bary) Compère	●	.	.	.	.
Tetmemorus	laevis v. minutus (de Bary) Krieger	●C	.	.	.	X
Charophyceae						
Tolypella	prolifera (Ziz.) Leonh.	.	.	.	●	.

PLATES

PIATE I

Fig. 1.—Radiophilium flavescens G. S. West.
Figr. 2. — Chroocoecus turgidus (Kütz.) Näg.
Fig. 3. — Navícula cincta v. heufleri (Grün.) Cleve.
Fig. 4. —Navícula cfr. dispersa Mang.
Fig. 5. — Navicula pupula v. genuina Grün.
Flg. 6.—Rhopalodia gibberula (Ehr.) O. Müll.





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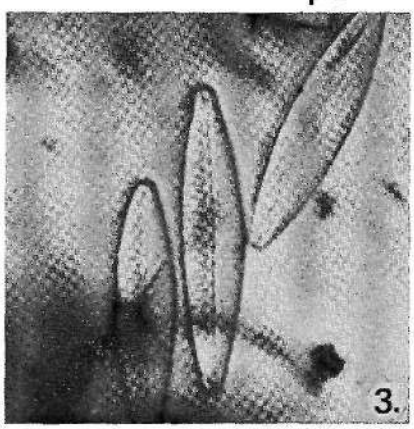

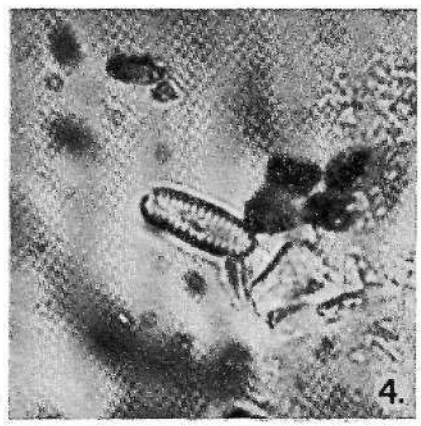

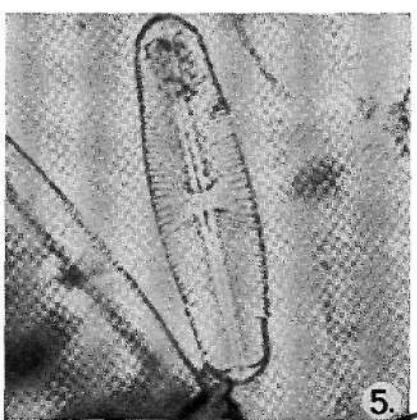

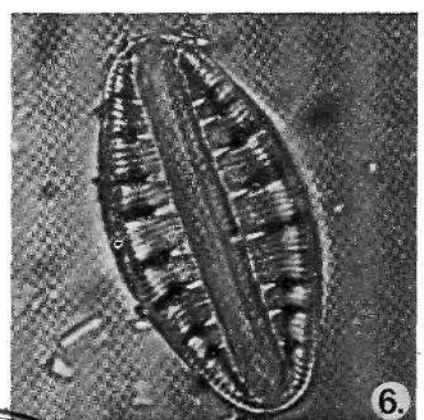

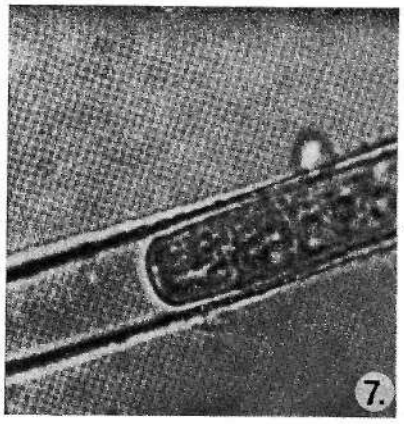

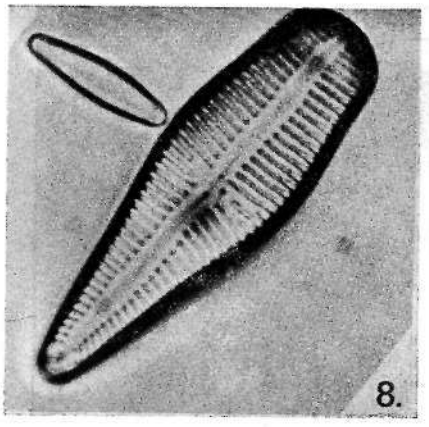

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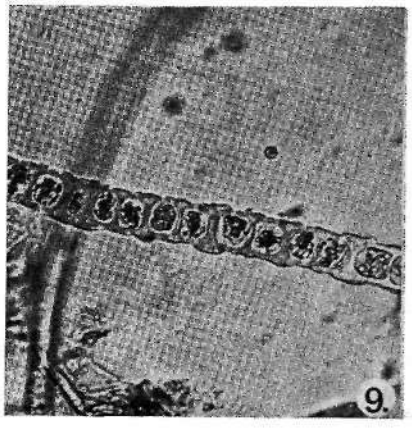

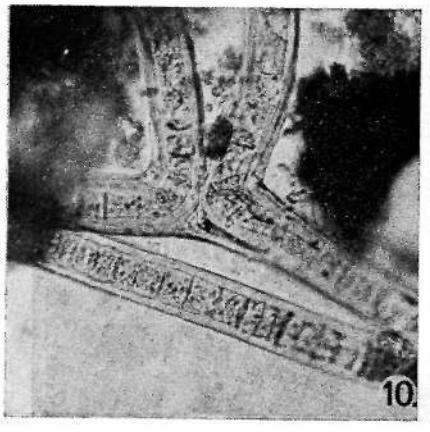

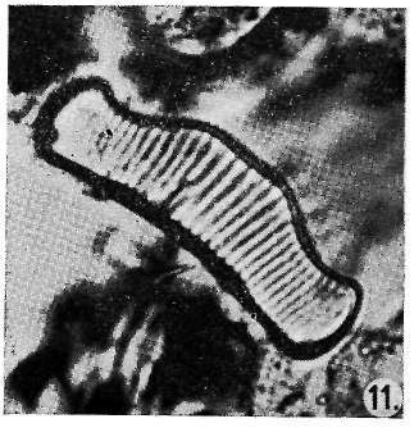

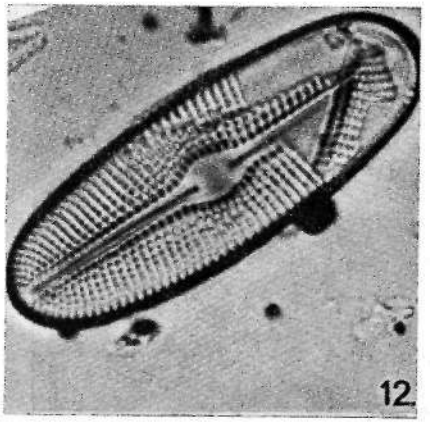

PIATE Π

Fig. 7.—Phormidium retzii Frémy.
Fig. 8. — Gomphonema constrictum Ehr.
Fig. 9. — Microspora lauterbornii Schmidle.
Fig. 10. — Scytonema hofmannii Ag.
Fig. 11. — Eunotia areus Ehr.
Fig. 12. — Diploneis ovalls (Hilse) Cleve.

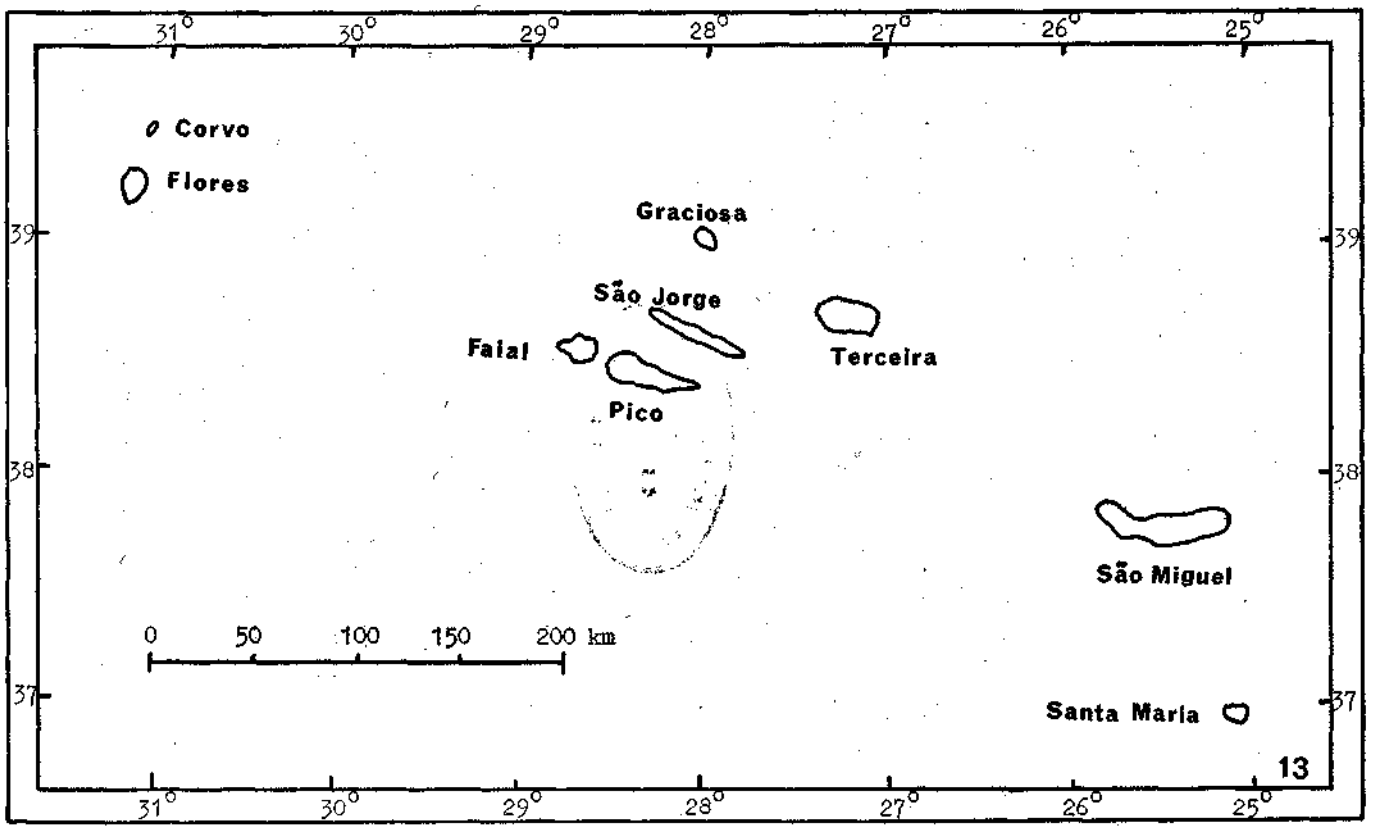

Fig. 13. — The Azorian Islands.

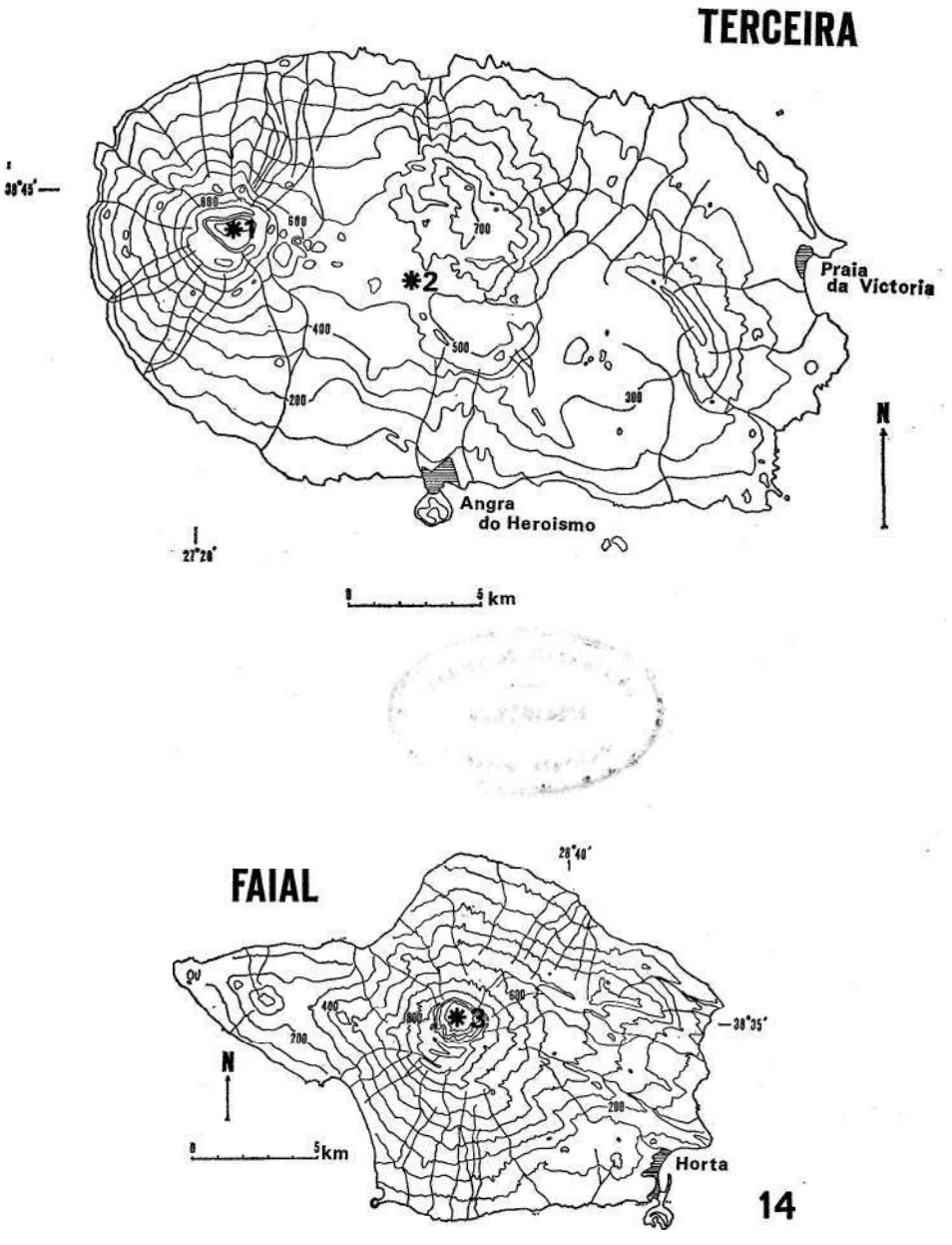

Fig. 14. — Places of collection.

Terceira: 1 — Caldeira de Santa Bárbara; 2 — Furnas do Enxofre.
Faial: 3 — Caldeira.

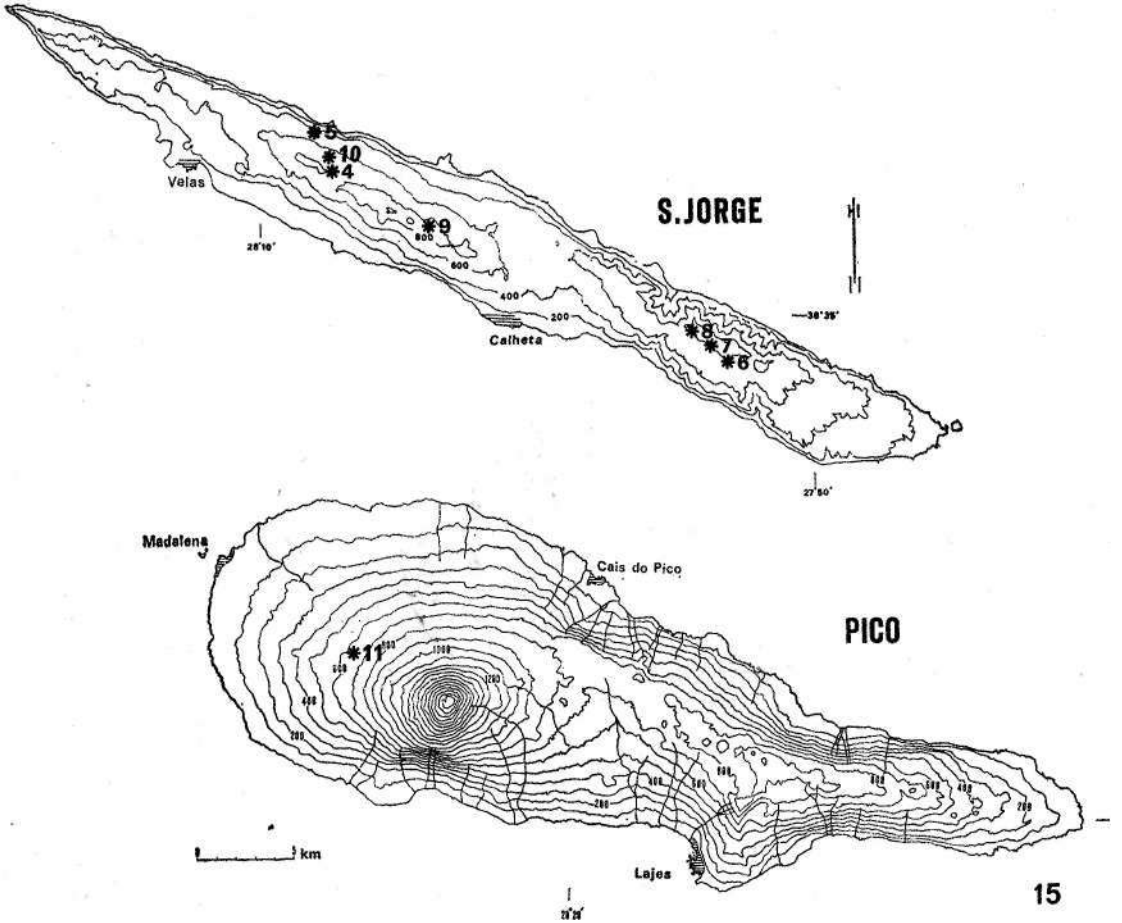

Fig. 15. — Places of collection.

S. Jorge: 4 — Pico das Caldeirinhas; 5 — Ribeira da Ponta Furada; 6 — Ribeira do Cedro; 7 — Ribeira do Cavalete; 8 — Serra do Topo area; 10 — Pico do Areeiro area; 10 — Ribeira da Fonte. Pico: Furna de Frei Matias.

ISOLATION OF A CALLUS FROM POTATO ROOTS

by

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&

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Abstract

Callus induction on root segments of *Solanum tuberosum* was obtained using the Murashighe and Skoog basic medium supplemented with 2 mg/1 2,4-D. Experiments carried out to determine the optimal growth conditions for the isolated callus showed that the best culture medium contains 5 mg/1 2,4-D and 2 mg/1 kinetin. However, kinetin is not essential for growth.

Anatomical studies showed that the callus tissue arises in the pericycle as abnormal lateral roots. The subcultured calluses are extremely friable and they dissociate into cells of varying shapes and sizes.

Chromosome counts in calluses maintained in subculture for two years showed, besides cells with the normal chromosome number (48), aneuploid cells with 44, 82 and 168 chromosomes.

Calluses of the same type as in *Solanum tuberosum* were also obtained in *S. chaooense, S. verrucosum* and in the hybrid S. *chaooense* χ S. *phureja*.

INTRODUCTION

STUDIES on the initiation and growth *in vitro* of callus tissue from potato tubers (STEWARD and CAPLIN, 1951; CHAPMAN, 1955; SHANTZ et al., 1955; WURM, 1960; FELLENBERG, 1963; OKAZWAWA et al., 1967; MIEDEMA, 1973; ANSTIS and NORTHCOTE, 1973), stem segments (LINGAPPA, 1957), and placental tissue (BAJAJ and DIONNE, 1967) have been described.

As far as we know callus from potato roots has not yet been obtained although they have been studied in

several other plants such as *Pisum sativum* (TOEREY and SHIGEMURA, 1957) *Oryza sativa* (YATAZAWA et al., 1967), *Vicia f aba* (GRANT and FUIIJER, 1968) and *Daucus carota* (for the tuberous root, as a classic material, see several authors *in* GAUTHERET, 1959; recently calluses were derived from seedling root segments by SMITH and STREET, 1974).

This paper gives the results of our study of the initiation and growth on different culture media of a root callus from *Solanum tuberosum*, as well as its anatomical origin and its chromosome number after two years of subculture. We also report that the same type of callus can be obtained from roots of other *Solanum* species (S. *chacoense* and *S. verrucosum)* and an hybrid *(S. phureja X S. chacoense).*

MATERIALS AND METHODS

For the induction of calluses, sprouts from *Solanum tuberosum* var. Désiré, were surface sterilized in a 10% calcium hypochlorite solution for 15 minutes and rinsed three times with sterile distilled water. After that each sprout was put aseptically on wet filter paper in a test tube and incubated in the dark at 25° C. From the adventicious roots developed from the sprout in about three days, segments of approximately 1 cm length were cut off and individually transfered to culture tubes, each one containing 20 ml of solid culture medium. In about four weeks each root segment had developed calluses. Such calluses were then subcultured on different media, in the dark at 25° C.

The method described above was also used for the induction of calluses on roots of other *Solanum* species *(S. chacoense* and *S. verrucosum)* and the hybrid *S. chacoense X S. phureja.*

The basic culture medium employed contained the inorganic and organic constituents of the MURASHIGE and SKOOG (1962) medium, omitting kinetin and IAA, and sucrose was added at 2%. The pH of the medium was adjusted to 5.6 with 0.1 N NaOH or 0.1 N HCl before adding Difco-Bacto agar at 0,8 %. The medium was then autoclaved at 120° C during 20 minutes. In these experiments this basic

culture medium was supplemented (see Table I) with the following growth substances: kinetin, 2,4-D and IAA.

Anatomical observations were made on tissues fixed in formalin-acetic acid-alcohol, dehydrated, embedded in paraffin wax and serially cut at 15 μ. The staining was by the safranin-fast green method. For the study of the morphology of the callus, cells, a callus was dissociated, by shaking it in liquid medium in a Petri dish, and the single cells or clumps of cells were photographed. Chromosome counts were made on callus, that had been subcultured regularly every month for 2 years since removal from the root expiant, using the acetic-orcein squash method.

RESULTS

I) Callus induction on root segments

It was found that when root segments were put on the basal medium supplemented with 2 mg/1 2,4-D callus initiation was consistently observed whereas on the basic medium no traces of growth appeared (Plate I, fig. 1). These calluses were visible after about 8 days of culture (Plate I, figs. 2-5) and started as nodule-like structures growing through the cortex of the root. As we shall see later (anatomical study) these nodule-like structures are, morphologically, abnormal lateral roots. After 15 days of culture (Plate I, fig. 6) the nodule-like appearance of the root segment is evident. The poliferation and abnormal growth of the lateral roots transform the root segment in a typical callus in which, after one month of culture (Plate I, fig. 7), the aspect of nodule-like is lost. From these primary calluses, pieces weighting ca. 150 mg, were subcultured on different media (see Table I).

TABLE I

Effect of growth substances on root callus growth (subcultures) in *Solanum tuberosum,*

Media	Substances added (Bg/1)			Callus growth
	Kinetin	2,4-D	IAA	
MS (Basal medium)	–	—		+
MSI	—	0,02	—	+
MS2	—	2	—	+ +
MS3	0,2	—	—	+
MS4	0,2	0,2	—	+
MS5	0,2	2	—	+ + +
MS6	0,2	—	2	+ +
MS7	1	—	—	+
MS8	1	0,1	—	+
MS9	1	1	—	+ +
MS10	1	—	1	+
MS11	2	0,2	—	+
MS12	—	5	—	+ +
MS13	2	5	—	+ + + +
MS14	4	—	—	+

Basal medium (MS) = MURASHIGE & SKOOG (1962) medium without growth substances added. Growth of callus observed after 4 weeks incubation period. + weak; ++ moderate; + + + extensive; + + + + very extensive.

ΠΙ) Growth of Callus

1) **Effect** of **2,4-D and** kinetin

In this study we were mainly interested to find a medium where the callus from *Solanum tuberosum* could grow well. So, no attempts were made to get a detailed quantitative evaluation of the effects on growth of the various growth substances added to the basic medium. However, as shown in Table I, some conclusions are evident. Kinetin is not essential for callus growth (MS_2 and MS_{12} media) and 2,4-D at a low concentration (MS1) has no effect. However the addition of kinetin improves growth, as shown by the effects of the MS_5 medium in comparison

with MS_2 (see also Plate II, fig. 2, e, d). When we compare the ratio 2,4-D/kinetin in the different media the following facts are evident: *a)* for a good callus growth this ratio must be higher than 1 (MS_1, $MS_{8)}$ MS_4 and MS_9 media, in which the ratios are respectively 0.1, 0.1, 1, 1); the best ratio was found to be 2.5 (in MS_{13} medium); *b)* in this ratio it is important the absolute value of the concentration of 2,4-D (for an equal ratio of 1, MS_4 medium promotes lesser growth than MS_9). On comparing the effects of the different media it is concluded that, in order to promote at least a moderate growth, the concentration of 2,4-D must not be inferior to 1 mg/1, the optimal one being 5 mg/1 with Mnetin at 2 mg/1 (MS_{18} medium) (see Plate Π, fig. 2, f).

When the medium contains only Mnetin as a growth substance (MS_3, MS_7, MS14) the growth of the calluses is weaker and they have a brownish colour and a more or less hard texture in contrast to the creamy white colour and soft texture in the MS_5 and MS13 media (see Plate II, fig. 2, a, e, f).

2) Effect of IAA

We have also tested two media with IAA instead of 2,4-D. From Table I it is possible to see that, for equal concentrations, IAA promotes lesser growth than 2,4-D (compare the effect of MS6 with MS_5; and $MS1_0$ with MS_9; see Plate Π, fig. 2, c, e).

3) Callus growth in other Solanum species

As already mentioned we have also induced calluses in the species S. *chacoense, 8. verrucosum* and in the hybrid S. *chacoense* X S. *phureja,* using again the basal medium supplemented with 2 mg/1 2,4-D. With these materials we have only tested its growth on the MS_3 medium, that was the best medium for *8. tuberosum.* As can be seen in Plate Π, fig. 1, all of them have a weaker growth than *8. tuberosum, 8. chacoense* being the one that grows less followed by S. *verrucosum* and *8. chacoense* X *8. phureja.* In terms of fresh weight, *8. tuberosum,* in cultures aged 1 month, shows

a ten-fold increase with respect to cultures of the same age of *S. chacoense;* a five-fold increase with respect to S. *verrucosum* and in relation to *S. chacoense* X *S. phureja* a two-fold increase. It will require further work in order to find the optimal culture medium for these *Solanum* species.

III) Anatomical Study

Sections of same of the root segments presented in Plate I showed that the nodule-like structures are indeed abnormal lateral roots. These roots, as they normally do, arise in the pericycle region (Plate II, fig. 1) but they differ from the normal ones in being densely packed, broad, and of limited growth (Plate III, figs. 2-6). The abnormality of these lateral roots can be seen in Plate *TV,* fig. 1 where it is shown a longitudinal section of a lateral root from the nodule-like root segment represented in Plate I, fig. 6 (15 days of culture). After subculture on MS_{13} medium of these primary calluses, aged one month (Plate I, fig. 7) the lack of organized development is evident (Plate IV, fig. 4) and the calluses are extremely friable. So with subculture, we assist to a complete dedifferentiation of the inicial callus.

When these actively proliferating calluses are agitated in liquid medium they dissociate into single cells and clusters of cells of varying sizes (Plate V, figs. 1-9). The freely suspended cells showed a variety of shapes, with thin and hyaline cell walls. Many cells were observed with buds or papillae (Plate V, figs. 4, 5, 7) ; others were elongated with a sinuous shape (Plate V, fig. 8) and they tended to give rise to aggregations of cells wrapping around each other (Plate V, fig. 6). Some of these elongated cells showed septation, giving rise to a pluricelular filament (Plate V, fig. 3, a, b, c). The clusters of cells were mainly formed by small rounded cells indicative of an intense proliferation (Plate V, figs. 1, 2).

Experiments which are now in progress have demonstrated that this callus from *Solanum tuberosum,* gives good **cell suspension cultures.**

IV) **Differentiation**

We have only made limited observations on the potentialities for differentiation (organogenesis) of the root callus of *S. tuberosum.* As already mentioned when these calluses are subcultured on MS_{13} medium no differentiation occurs. However, calluses cultured on MSn medium (0,2 mg/1 2,4-D + 2 mg/1 kinetin) show the appearance of apparently normal roots (Plate I, fig. 9). An anatomical study of the interior of these calluses show many circular areas with tracheide inside (Plate IV, fig. 3, a, b). Calluses cultured on MS_9 medium (1 mg/1 2,4-D + 1 mg/1 kinetin) show many pear-shaped protuberances (Plate I, fig. 8). Sections made on these protuberances show a structure reminescent of root apical meristems and root caps (Plate IV, fig. 2). In all the media tested (see Table I) no bud formation was observed.

V) Chromosome **number study**

Samples taken at random from all parts of a callus, maintained in subculture for two years, were squashed in acetic-orcein. As shown in Plate VI, figs. 1-3, cells with an hypo-tetraploid chromosome number (44), an hypo-octoploid number (82) and an hypo-16-ploid number (168) were found. As we have not made a quantitative study, it is not possible to say if the higher polyploid cells predominate in the culture, neither to know the full range of chromosome number variation. In many cells it was observed that the chromosomes were contracted and scatered in the cell as in a «C-mitosis» (Plate VI, figs. 2-3).

DISCUSSION

It is well known that one of the effects of 2,4-D as an herbicide is to promote an abnormal growth of the roots expressed in densely packed, stunted laterals (in AUDUS, 1959; in CRAFTS and BOBBINS, 1962). This effect was indeed the same we observed when the root segments of potato

were put on the basal medium supplemented with 2 mg/1 2,4-D and give rise to the calluses.

Several authors have reported from the work of the *in vitro* culture of roots in different plants the occasional appearance in them of «callus», «nodule-like structures» or «tumor-like structures» both with or without auxins added to the culture media: NÓBÉCOURT (1939) in carrot; JAGENDORF and BONNER (1953) in cabbage; NORTON and BOLL (1954) in *Lycopersicum peruvianum;* TRYON (1955) in *Nicotiana affinis;* TORREY and SHIGEMURA (1957) in *Pisum sativum;* BAJAJ and DIONNE (1968) in potato; MONTEZUMA-DE-CARVALHO (1972) in *Haplopappus gracilis.* Unfortunately, with the exception of the work of MONTEZUMA-DE-CARVALHO, showing that the root callus are developed from cortex tissue, the anatomic origin of these calluses was not studied. The work of BAJAJ and DIONNE is specially concerned with our observations as they have also used potato roots cultured in the MURASHIGE and SKOOG'S medium supplemented with 2,4-D. Their report on the formation of nodule-like structures on the roots, must be interpreted in terms of our anatomic studies, i. e., these nodule-like structures are also abnormal latterai roots, induced by 2,4-D.

As already mentioned, the calluses subcultured on MSi_3 medium are highly friable and easily dissociated into clusters of cells and single cells of a variety of shapes, such as cells with buds or papillae, elongated and sinuous, round, etc. Cells with this same morphology have already been described by STEWARD et al. (1958) and BLAKEIEY (1964) in suspension cultures of carrot cells; Grant and FULLER (1968) in friable callus of *Vicia faba* roots; BAJAJ and DIONNE (1967) in potato callus raised from placental tissue.

Previous studies on the development *in vitro* of potato calluses have used sprouts (LINGAPPA, 1957) very small tuber pieces (STEWARD and CAPLIN, 1951; ANSTIS and NORTHCOTE, 1973) tuber pieces of varying size (SHANTZ et al., 1955; WURM, 1960; FELLENBERG, 1963; OKAZAWA, 1967; MIEDEMA, 1973) or placental tissue (BAJAJ and DIONNE, 1967). So our results on potato root callus growth cannot be directly compared with the work of these authors.

However, it is interesting to see that our finding that the growth of the root callus is independent of the presence of kinetin in the culture medium paralleles the observations of ANSTIS and NORTHCOTE in which their isolated callus tissue does not require kinetin and produces and excrets its own cytokinin(s). As we now know that the plant root system is a major region of cytokinin synthesis (in SKOOG and ARMSTRONG, 1970) our results are even more compreensible. We have found that 2,4-D is superior to IAA for callus growth. In fact all the above mentioned authors have stressed the beneficial growth promoting effects of 2,4-D in their cultures. SHANTZ et al. (1955) and ANSTIS and NORTHCOTE (1973) also reported that the worst growth occurred in the presence of IAA.

Buds and roots had been obtained more or less consistently from potato callus still attached to the tuber expiant (WURM, 1960; FELLENBERG, 1963; OKAZAWA, 1967). Isolated potato callus are more recalcitrant to exhibit organogenesis as only ocasionally they have shown the formation of roots (BAJAJ and DIONNE, 1967; ANSTIS and NORTHCOTE, 1973). In our experiments, although we have only made limited observations, we have found that in a medium with low concentration of 2,4-D (0,2 mg/1) and higher concentration of kinetin (2 mg/1) roots developed from the growing callus. In a medium with 1 mg/1 2,4-D and 1 mg/1 kinetin structures reminescent of roots appeared. With higher concentrations of 2,4-D (2 or 5 mg/1) no root differentiation occurs. These results are in accord with the extensive literature in plant tissue and cell culture showing that high concentrations of 2,4-D induce dedifferentiation. It has been demonstrated (HOWARD, 1964; MIEDEMA, 1967) that under certain experimental conditions intact potato roots can produce buds. As the potato root tissue has this potentiality it is admissible to expect that potato root calluses will be able to produce buds if the correct culture medium is found.

The potato *(Solanum, tuberosum)* is usually considered a natural autotetraploid with 48 chromosomes (SWAMINATHAM and MAGOON, 1961). In our potato calluses, subcultured for two years, we have found besides the normal complement

with 48 chromosomes cells with aneuploid numbers (44; 82; 168). It is known that in long-term cultures the appearance of chromosomal instability is frequent (see revision in SUNDERLAND, 1973). Usually this chromosomal instability consists of polyploidization accompanied by aneuploidy. In our case it seems likely that the existence of cells with hypo-tetraploid number could explain the occurence of hypo-octoploid cells (with 82) and of hypo-16-ploid cells (with 168), if we admit that such hypo-tetraploid cells suffer different levels of polyploidization. But, it is also admissible that loss of chromosomes from euploid cells (octo- and 16-ploid) would produce the same end result. Another possibility is that in the original expiant (root segment) there are already present cells with the octo- and 16-ploid level, which in Culture, by chromosome losses, would produce aneuploiy. In conclusion, it would require further work to assess these possibilities. However, it is known (MOHANDAS and GRANT, 1972) that 2,4-D at high concentrations can induce, in root tips cells of several plants, chromosome breakage, lagging chromosomes and «C-mitosis». Also has been shown (BAYUSS, 1973) that in suspension cultures of *Daucus carota* abnormality of the mitotic spindle leading to multipolar separation, lagging chromosomes and possibly polyploidization, is correlated with the presence of 2,4-D in the culture medium. As we have observed many cells with the chromosomes highly contracted and with an aspect of «C-mitosis» it is very probable that polyploidization by spindle failure may occur in the cultured tissue of *Solanum tuberosum,* under the influence of 2,4-D.

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PLATES

PLATE I

Callus induction in *Solanum tuberosum* root expiants cultured *in vitro* (Figs. 1-7)

Fig. 1.—Root segment cultured on the basal medium (MS). Note the absence of any growth. X 25.

Figs. 2-5. — Root segments after 8 days of culture on MS_3 medium (2 mg/1 2,4-D). Note the formation in all of them of several abnormaly broad and packed lateral roots breaking through the cortex. X 25.

Fig. 6. — Root segment after 15 days of culture on MS, medium. Note the nodule-like appearance of the root segment, X 12.

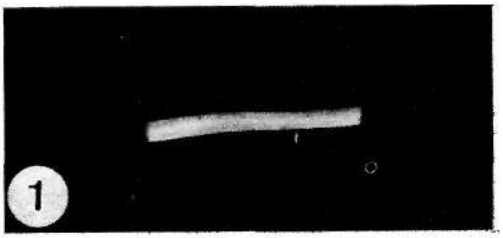
Fig. 7. — A root segment after 1 month of culture on MS, medium. The nodule-like appearance is almost lost. X12.

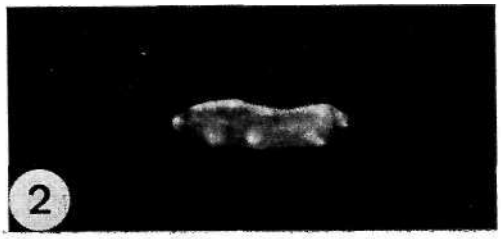
Subculture of root calluses from *Solanum tuberosum* on different media (Figs. 8-10).

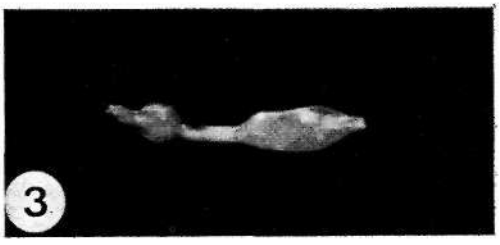
Fig. 8. — Callus growing for 3 months on MS„ medium (1 mg/1 2,4-D + 1 mg /1 kinetin). On the surface of the callus there are several protuberances reminiscent of root primordiums (arrow) (see also Plate IV, fig. 2). X 6.

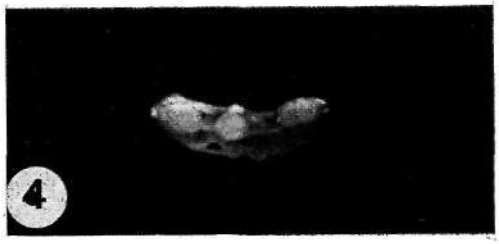
Fig. 9. — Callus growing for 3 months on MS„ medium (0,2 mg/1 2,4-D + 2 mg/1 kinetin). Note the différenciation of normal roots (arrows) on the surface of the callus. X 1.

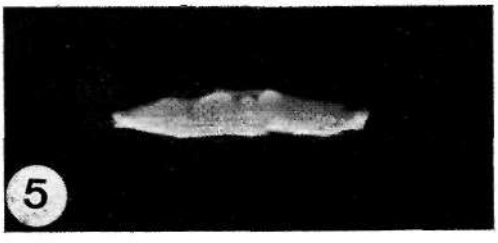
Fig. 10. —- Callus subcultured on MS_5 medium (2 mg/1 2.4-D + 0,2 mg/1 kinetin) during 1 month. Note the absence of any différenciation on the callus. X 1

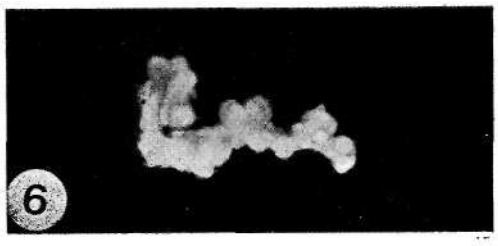


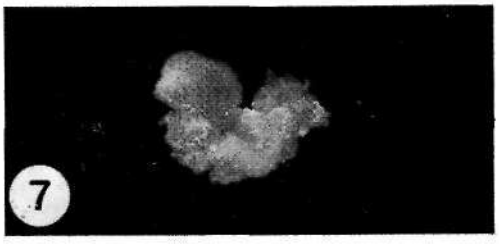


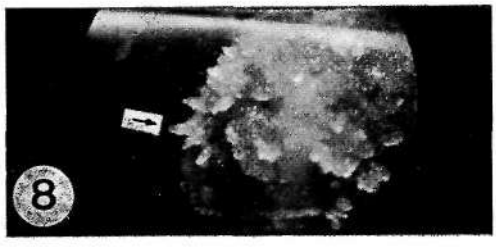


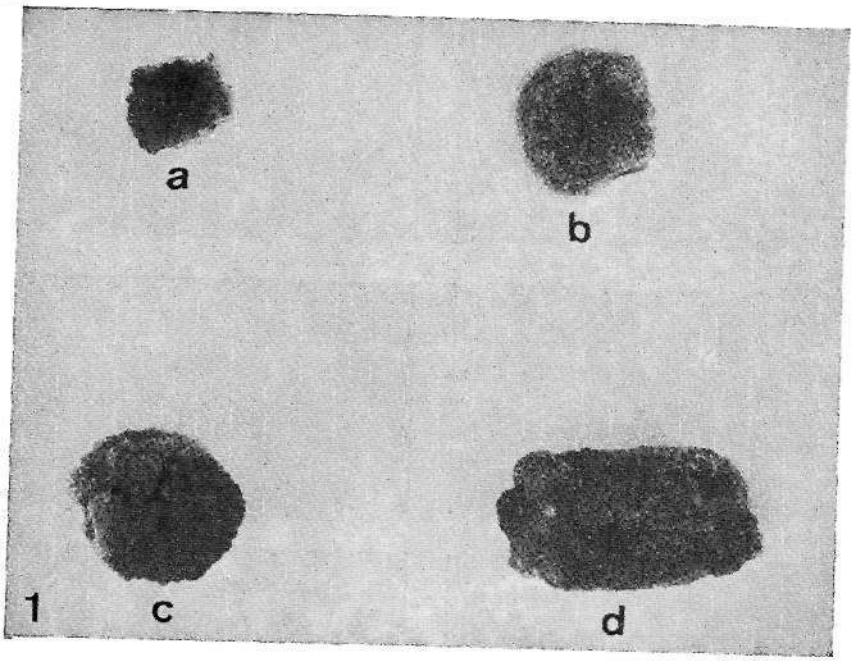








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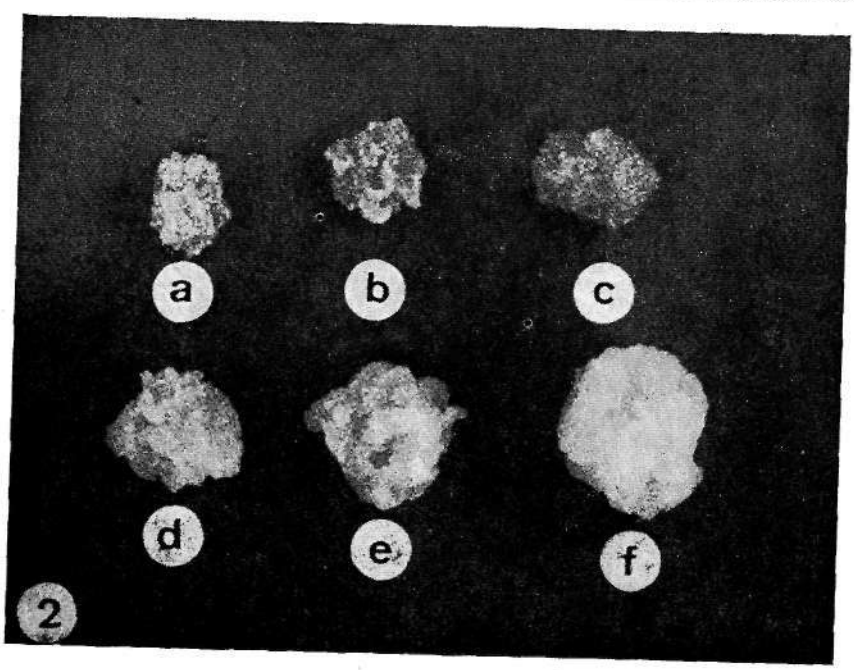
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PLATE II

—Calluses from roots of three species and one hybrid of *Solanum* subcultured on MS13 medium. Note the relative differences in growth after six weeks of culture. X 1.
a = S. *chacoense;* b = S. *verrucosum;* c = *S. chacoense* X S. *phureja;* d = *S. tuberosum.*

— Responses of root calluses from *Solanum tuberosum* to subculture on different media (1 month of culture), a = MS2 medium; b = MS11 medium; c = MS6 medium; d = MS2 medium; e = MS_5 medium; f = MS13 medium. XI.

(for the composition of the media see Table I).

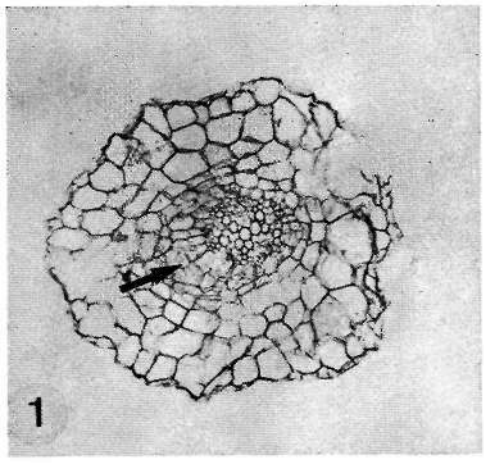
PLATE ΠI

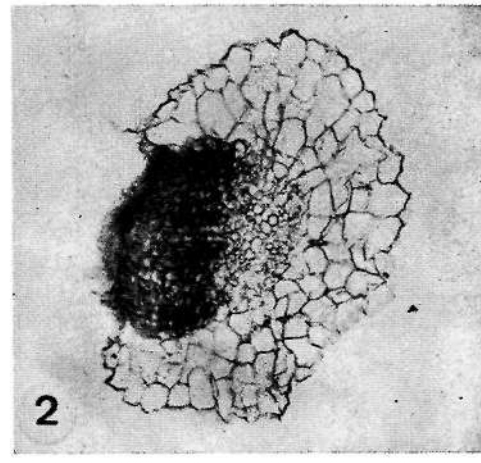
Anatomical study of callus induction in *Solanum tuberosum* root expiants cultured on MS_2 medium (MS + 2mg/l 2,4-D).

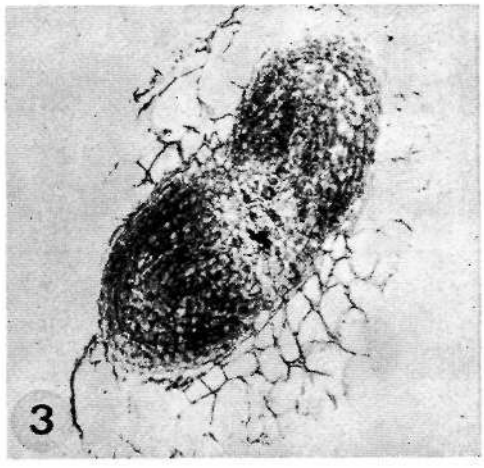
Fig. 1. — Transversal section of a root segment, after 6 days of culture, showing the first stages of the pericycle proliferation (arrow) in a tetrarch stele. X 75.

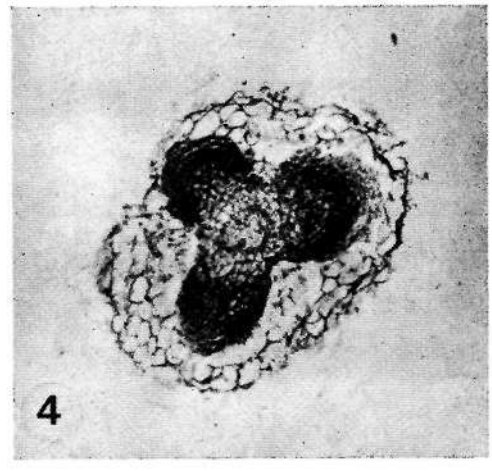
Figs 2-5. — Transversal section of different root segments, after 8 days of culture, showing the development of abnormal lateral roots. (Fig. 4 is a section of the root segment represented in Plate I, fig. 2; fig. 5 is a section of the root segment represented in Plate I, fig. 3). X 75.

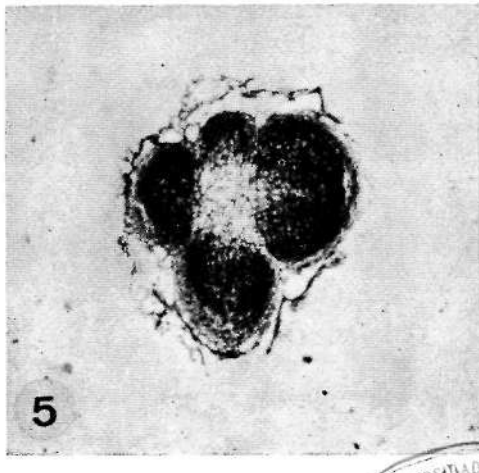
Fig. 6. — Longitudinal section of the root segment represented in Plate I, fig. 4 (8 days of culture). Note that the laterals are abnormally broad and densely packed. X 75.

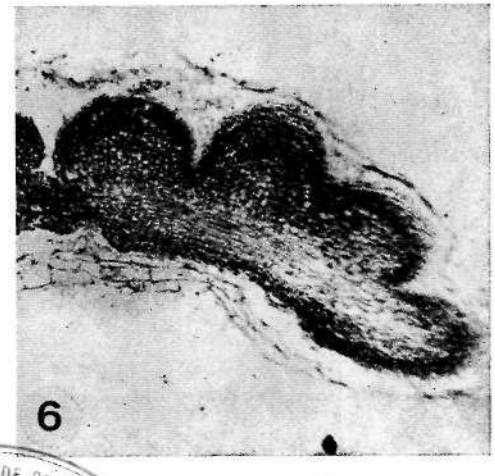


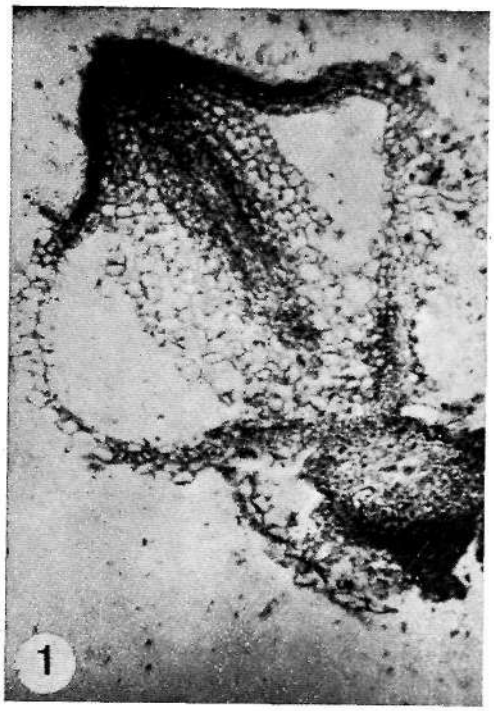








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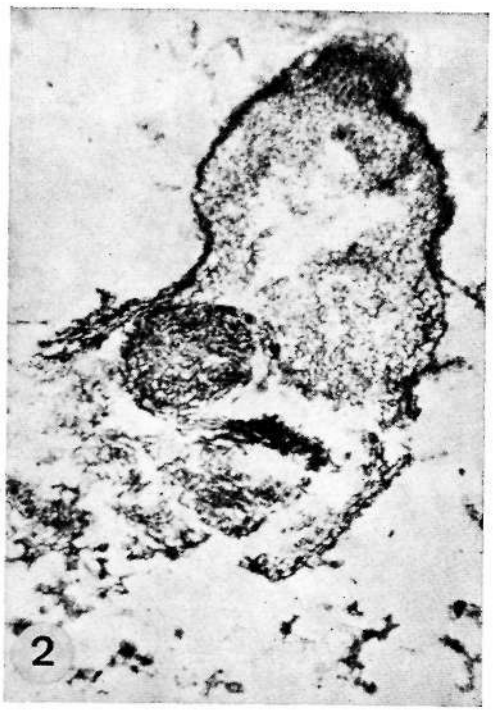
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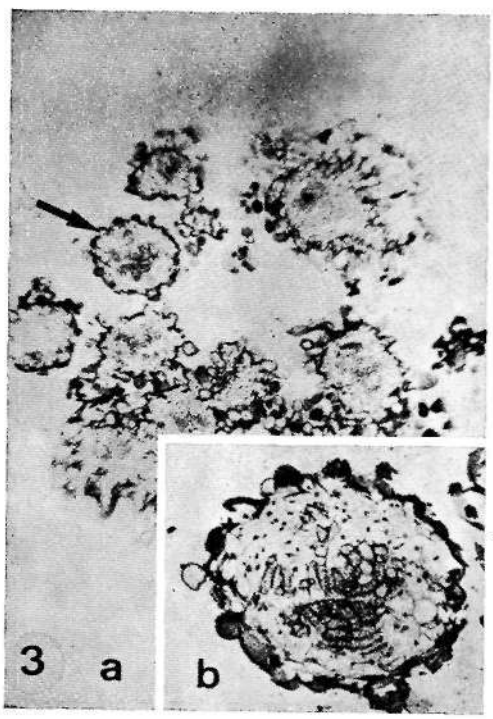
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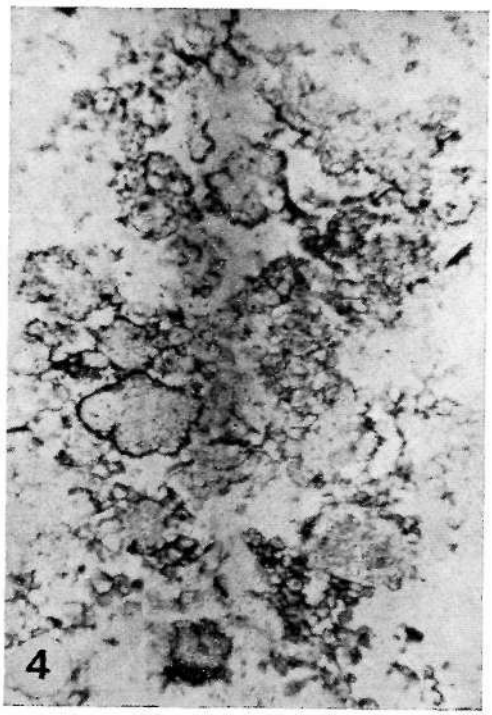
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PLATE IV

Anatomical study of root calluses of *Solanum tuberosum.*

Fig. 1. — Longitudinal section of the nodule-like root segment represented in Plate I, fig. 6, showing an abnormal lateral root. X 75.

Pig. 2.— Longitudinal section of one of the protuberances showed in the callus represented in Plate I, fig. 8. Note the structure reminiscent of a root apical meristem and a root cap (callus subcultured on MS„ medium, aged 3 months). X 75.

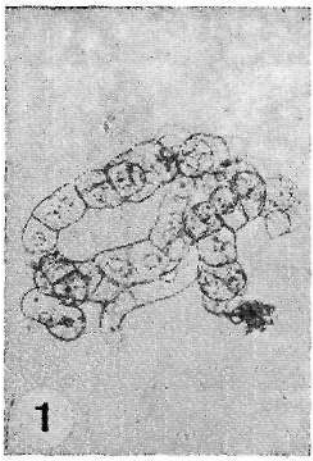
Fig. 3. — (a) Part of a section of the callus represented in fig. 9, Plate I. Note many circular areas with tracheide inside, (b) magnification of the area indicated by the arrow (callus aged 3 months, subcultured on MS11 medium), (a) X 25; (b) X 75.

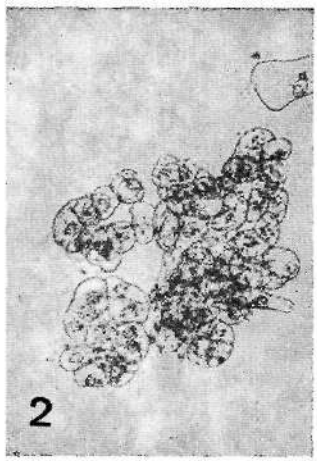
Fig. 4. — Part of a section of the callus represented in fig. 10, Plate I. Note that all traces of organized structure are lost. The callus is extremely friable. X 25.

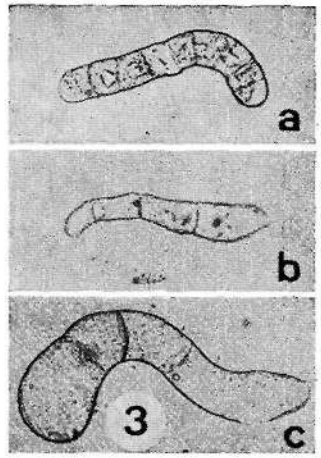
PLATE V

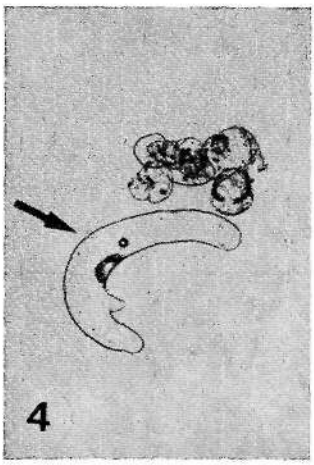
Cell shape in root callus of *Solanum tuberosum* (callus, aged 1 month, with 2 years of subculture on MS13 medium, dissociated in liquid medium). X 75.

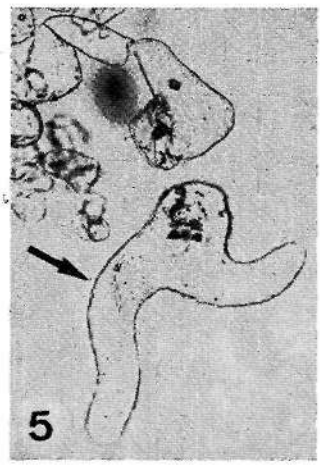
Fig. X, 2.— Clusters of small rounded cells.
Fig. 3, a, b, c — Examples of filaments of cells resulting from septation of single elongated cells.
Fig. 4, 5, 7.— Cells with buds or papillae (arrows).
Fig. 6.—Elongated curved cells wrapping around each other.
Fig. 8. — Elongated cell (arrow) with a sinuous shape.
Fig. 9.—A very large cell.

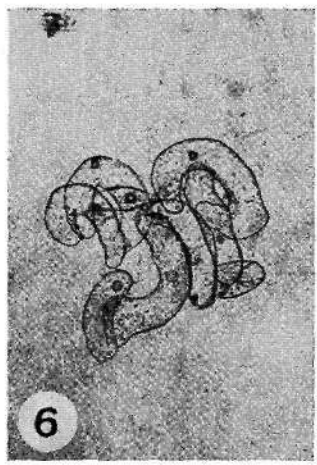


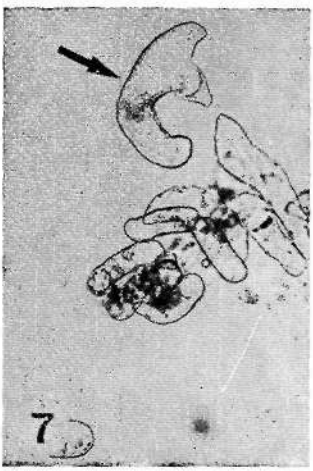


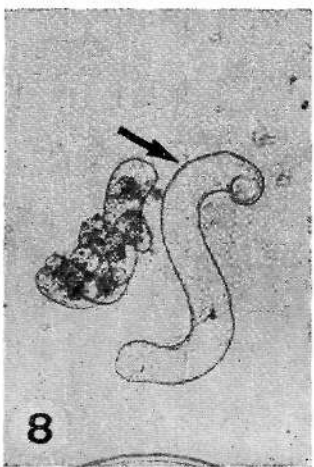


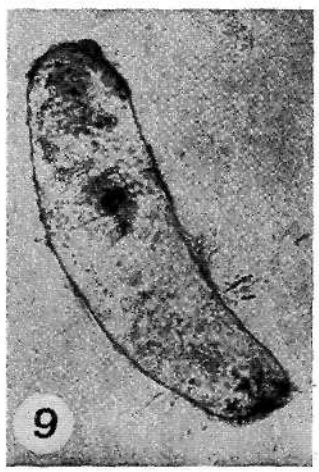


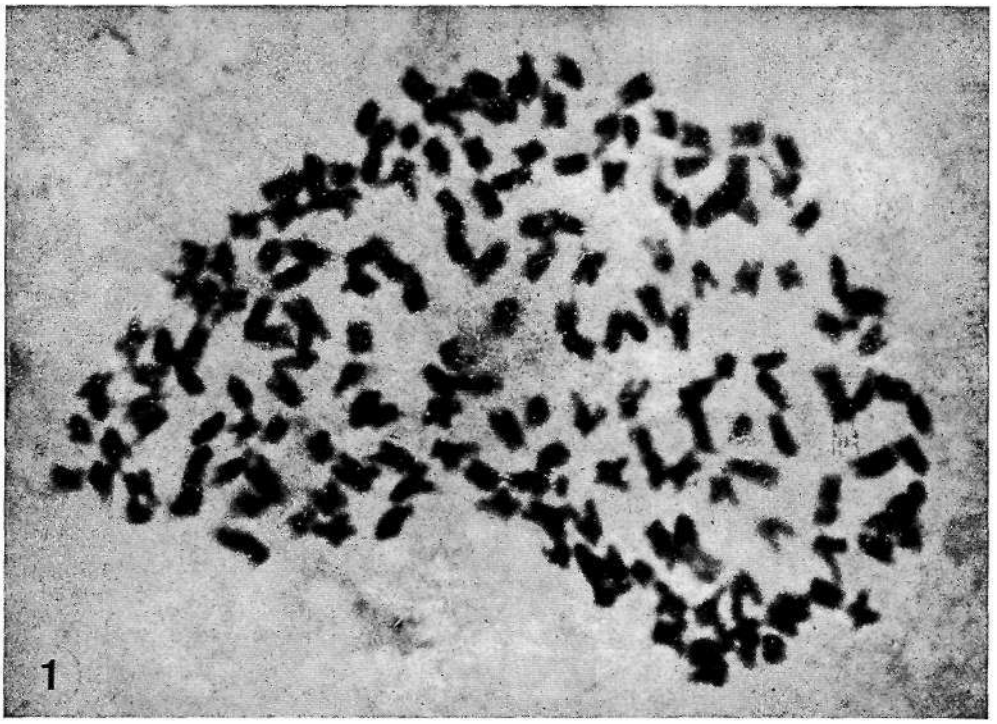








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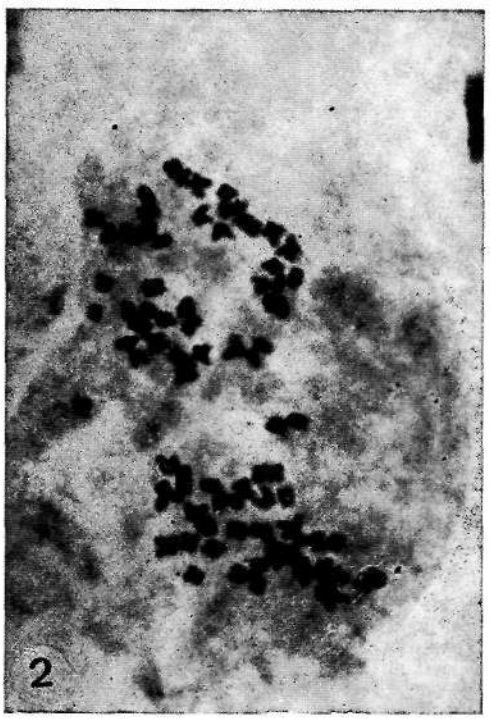
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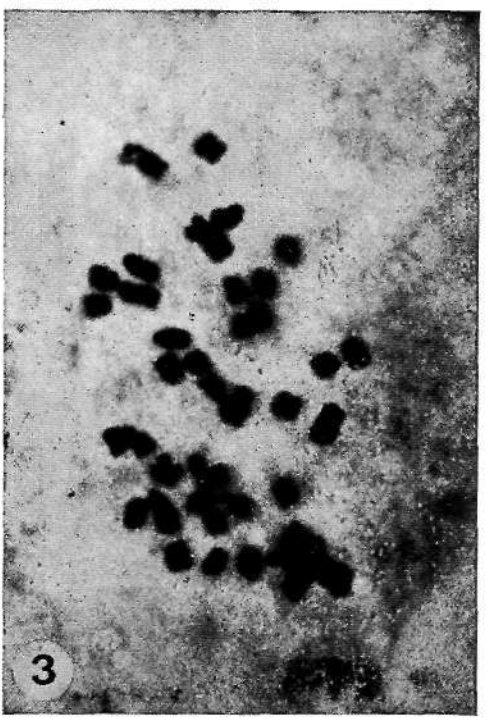
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PLATE VI

Chromosome number variation in root callus of *Solanum tuberosum* (callus subeultured regularly every month, for 2 years, on MS18 medium). Acetic-orcein squashes.

Fig. 1. — An hypo-16-ploid cell with 168 chromosomes. X 750.
Fig. 2. — An hypo-octoploid cell with 82 chromosomes. X 375.
Fig. 3. — An hypo-tetraploid cell with 44 chromosomes (in Figs. 2-3 note the contraction of the chromosomes and the aspect of «C-mitosis»). x 750.

PASSIFLORACEAE AFRICANA E NOVAE VEL MINUS COGNITAE

ROSETTE FERNANDES & A. FERNANDES

Basananthe holmesii, sp. nov.

Herba perennis, erecta, omnino ± adpresse pilosa, caule ramoso, ramis erectis, usque ad 25 cm alto, a rhizomate lignoso orto. *Folia* anguste usque late lanceolata, 2-6 X 0,3-2.4 cm, apice acuta et ± longe acuminata, acumine nigro, margine integra, basi acuta et in petiolum usque ad 4 mm iongum attenuata, utrinque dense cinereo-pilosa, pilis ± adpressis, penninervia, nervis indumento ± absconditis. *Stipulas* lineares, usque ad 12 mm longae, pilosae. *Pseudostipulae* nullae. *Inflorescentiae* 1-2-florae ; pedunculus 1-2 cm longus, hirsutus; bracteolae lineares, circ. 5 mm longae, pilosae. *Flores* pubescentes ; stipites usque ad 7 mm longi, hirsuti. *Hypanthium* 4-5 mm latum. *Sépala* 5, apice obtusa, 14 X 2.5 mm, extus pilosa, intus glabra, 3-nervia. *Pétala* 4, obtusa, 1G X 2 mm, tenuia, sépala breviora. *Coronae exterioris tubus* circ. 2,5 mm altus cum f ilamentis circ. 3,75 mm longis. *Discus* circ. 0,5 mm altus. *Corona interior* cupulata, circ. 1 mm alta. *Stamina* 5 cum f ilamentis circ. 4,5 mm longis et antheris sagittatis, circ. 4,5 X 0,6 mm. *Ovarium* ellipsoideum, 6 X 4 mm post anthesin. *Gynophorum* circ. 1 mm Iongum. *Styli* 3, circ. 6 mm longi, a basi per circ. 1,5 mm connati. *Fructus* ellipsoideus, circ. 2,2 X 1 cm. *Semina* non vidimus.

Fl. & fr.: Nov.

Icon, nostr.: Tab. I et IL

Habitat in Zambia, loco dicto Mwinilunga, «herb up to 2', growing in pockets of soil between limestone rubble and also on red sandy loam derived from limestone», fl. & fr. 7-XI-1955, *Holmes* 1320 (k, holotypus).

Affinis B. pubiflorae de Wilde a qua caule satis ramoso nec simplici; foliis lanceolatis, 2-6 X 0,3-2,4 cm, utrinque dense et = adpresse pubescentibus, margine integris, basin versus in petiolum paulo distinctum et 2-3 mm longum attenuatis neque foliis ellipticis usque elliptico-oblongis, 2-4 X 1,2-2 cm, utrinque tenuiter et breviter pubescentibus, margine subintegris, basi obtusis vel subacutis et petiolo distincto 2,5-b mm longo instruetis; stipulis usque ad 12 mm neque 2-5 mm longis; sepalis circ. 14 mm nec (6)8-11 mm longis; antheris circ, 4,5 mm nec 3-3,5 mm longis; ovario circ. 1 mm longe stipitato; stylis circ. 6 mm neque 3-4,5 mm longis praecipue differt.

Affinis etiam B. hispidulae de Wilde a qua foliis 2-6 X 0,3-2,4 cm nec 1-2,5 X 1-3 mm; stipulis persistentibus usque ad 12 mm longis nec caducis et 1-1,5 mm longis; pedúnculo 1-2 cm neque 0-2 mm longo; bracteolis circ. 5 mm nec 0.5-1 mm longis; hypanthio 4-5 mm nec 2-2,5 mm lato; sepalis usque ad 14 mm neque 5-7 mm longis; coronae exterioris tubo circ. 2,5 mm nec 1-1,25 mm alto; staminum filamentis circ. 4,5 mm neque circ. 3 mm longis; antheris circ. 4,5 mm neque 1,5 mm longis; stylis circ. 6 mm longis a basi connatis neque 3,5-4 mm longis a basi liberis; etc. differt.

Adenia zambesiensis, sp. nov.

Herba scandens, omnino glabra. *Caulis* circ. 1 m longus (vel ultra?), gracilis, sulcatus, a radice tuberosa ortus, internodiis 2,5-7 cm longis. *Folia* (3)5-palmatilobata, ambito late ovata usque ad suborbicularia, 2-5 X 2-5 cm, basi cordata, membranacea, utrinque viridia sed infra pallidiora, (3)5-palmatinervata, retículo inconspicuo; lobi integri vel vix 3-lobulati, apice obtusi vel rotundati, basi ± constricti, penninervi, terminalis usque ad 4X2 cm, intermedii usque ad 2,5 X 1,5 cm et basales satis minoris, usque ad 1 X 0,9 cm; petiolus 1-4 cm longus, supra canaliculars. *Glandulae* laminae basis 2, sessiles, circ. 0,75 mm in diam.; laminae glandulae subcirculare-ovatae, eirc. 0,3-0,5 mm in diam., prope (eirc. 1-2 mm distantes) margines sinuum loborum dispositae. *Cirrhi steriles* axillares, graciles, usque ad 7 cm

longi. *Stipulae* lanceolatae, circ. 1 mm longae. *Inflorescentiae* 1-cirrhiferae et generaliter 1-florae; pedunculus gracilis, 2-3 cm longus. *Bracteolae* circ. 1 mm longae. *Flores* cf 20-25 mm (stipite superne incrassato et 7-9 mm longo incl.) X 3-4 mm. *Hypanthium* circ. 2,5 mm latum. *Catycis tubus* (cum hypanthio) 8-9 mm longus et 3-4 mm latus. *Calycis lobi* 4-5 X 3 mm, tenues, apice ± obtusi, nervis reticulatis instructi. *Pétala* ovata vel late elliptica, 2-2,5 X 1,25-1,5 mm, valde tenuia, apice apiculata vel irregulariter dentata, 1-nervia, circ. 6 mm supra hypanthii basin inserta. *Corona* 0. *Staminum filamenta* circ. 5,5 mm longa, inferne in tubum 1-1,5 mm longum coalita, in basi hypanthii inserta. *Antherae* erectae, circ. 2,5 X 1 mm, apice obtusae, inapiculatae. *Disci ylandulae* spathulatae, circ. 1 mm longae et circ. 1,5 mm supra hypanthii basin insertae. *Ovarii rudimentum* circ. 0,5 mm longum, gynophoro circ. 0.5 mm longo instructum. PL 9 et fr. ignoti.

FL: Jan.

Icon, nostr.: Tab. III et IV.

Habitat in Mossambique, Zambesia Distr., inter Mocuba et Maganja da Costa, alt. circ. 150 m, «Erva com raiz napiforme. Floresta aberta de *Brachystegia spiciformis, B. boehmii, Julbernardia globiflora, Burkea africana, Uapaca* sp., etc; estrato graminoso com *Digitaria* sp., *Panicum* sp.; solos arenosos», fl. *d* 9-1-1968, *Torre & Correia* 16975 (LISC, holotypus).

Affinis A. schliebeni Harms a qua foliis 2-5 X 2-5 cm, glabris, lobis apice obtusis vel rotundatis, marginibus integris nec foliis 5-12 X 4-13 cm, tenuiter praecipue secus nervos pubescentibus, lobis apice acutis, usque ad 0,75 cm longe acuminatis et usque ad 2 mm longe mucronatis; cirrhis brevioribus et gracilioribus; floribus *S* 20-25 mm (stipite 7-9 mm longo incl.) X3-4 mm neque 25-35 mm (stipite 3-6 mm longo incl.) X (6)8-10 mm; calycis tubo (hypanthio incl.) S-9 mm nec (15)18-24 mm longo; calycis lobis ellipticis, 4-5X 3 mm, margine integris neque ovato-triangularibus, 4-5,5 mm longis et margine serrulatis; petalis ovatis vel ellipticis, 2-2,5X 1,25-1,5 mm, margine integris vel irregulariter dentatis, circ.

6 mm supra hypanthii basin insertis neque lanceolatis, 3-5 X 0,75-1(1,5) mm, margine fimbriatis et 11-15 mm supra hypanthii basin insertis; antheris circ. 2,5 X 1 mm, apice obtusis inapiculatis neque 3(4-7) X 0,5-1 mm et breviter apiculatis; staminum filamentis circ. 5,5 mm neque 10-21 mm longis; disci glandulis circ. 1 mm nec 1,5 mm longis differt.

Adenia volkensii Harms — Tab. V

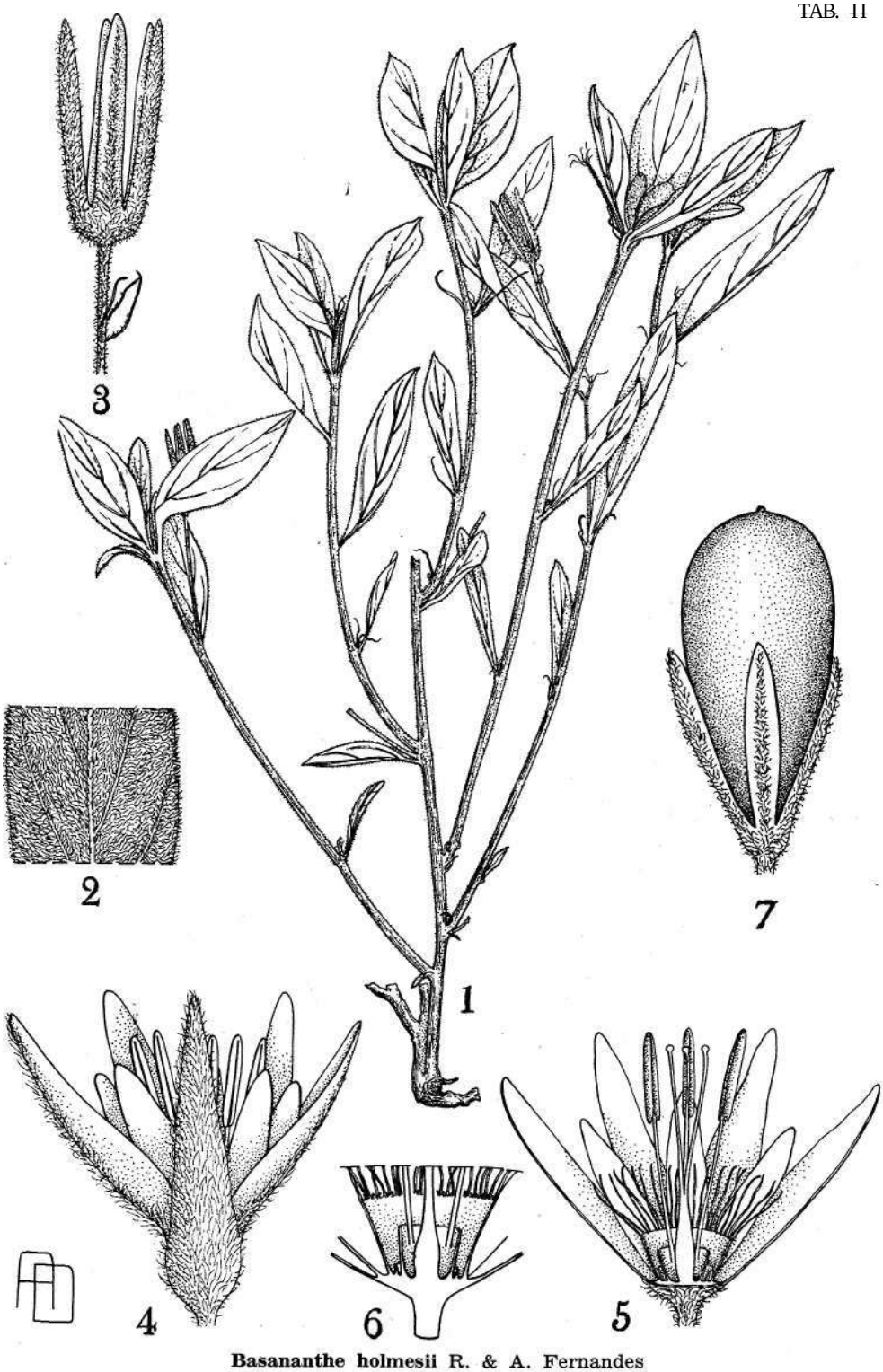
In Engl., Pflanzenw. Ost Afr. 2, C: 281 (1895).— Engl., Pflanzënw. Afr. 3, 2: 606 (1921). — Harms in Engl. & Prantl, Nat. Pflànzenfam; ed. 2, 21: 492 (1925). — Battiscombe, Cat. Trees Shrubs Kenya Col. Spec. n.° H 20. — Verdcourt & Trump, East Afr. Poison. PL, Dicot: 37, fig. 3 (1970).— de Wilde in Meded. Landb. Wag. 71-18: 182, fig. 28-29 (1971).

Haec species etiam in Malawi crescens. Specimen visum (Kusungu Distr., Chamana, alt. 1000 m, 16-I-1959, *Robson* 1227, K) flores hermaphrodites ostentat. Tum speciei descriptio sie amplificanda est:

Flos hermaphroditus late tubulosus, circ. 26 mm (stipite circ. 2 mm longo incl.) X 10 mm, pubeseens. *Pedicelli* usque ad 15 mm longi, pubescentes. *Hypanthium* circ. 8 mm latum. *Calyeis tubus* 18 X 10 mm, dense et breviter lineatus, lineis rubescentibus; caiycis lobi ovatp-triangulares, circ. 8 X 5 mm, apice obtusi, longitudinaliter lineati, lineis rubescentibus, duo lobi margine ciliati, alii tres margine lanate-fimbriati. *Pétala* lineari-lanceolata, 12-13 mm longa, 3-nervata, marginibus dense fimbriata, paulo supra coronam inserta. *Corona* ut in flore. *Staminum filamenta* circ. 9 mm longa, libera, in hypanthii basi inserta. *Antherae* circ. 5,5 X 0,75 mm, usque ad 0,5 mm longe apieulatae, dense punctatae, interdum :rE reductae. *Disci glandulae* 3-3,5 mm longae. *Oynophorum* circ. 4 mm longum. *Ovarium* ellipsoideum vel subglobosum, 4-4,5X3,5mm. *Styli* connati, 3,5mm longi; stigmata capitata, lanata.

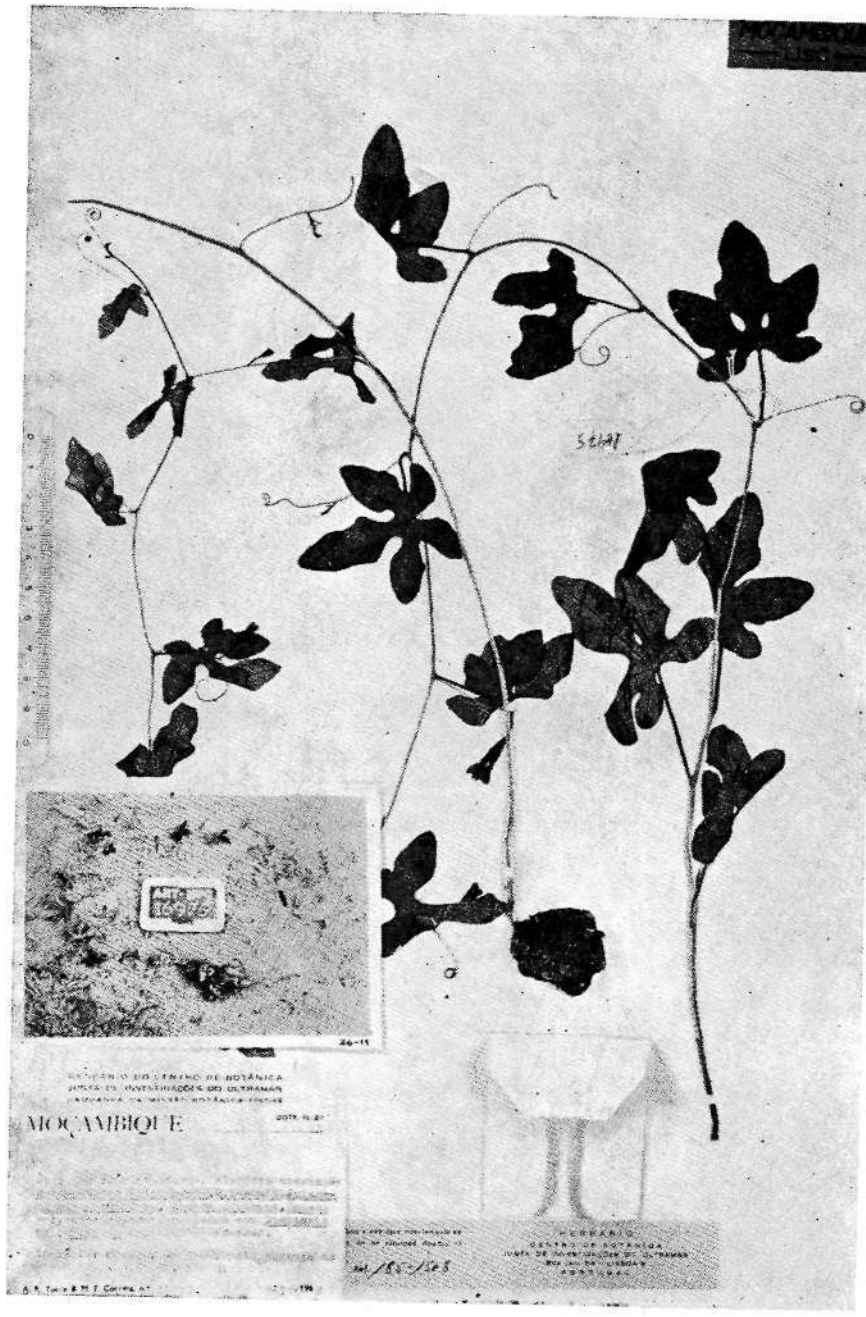
Basananthe holmesii R. & A. Fernandes

Specimen *Holmes* 1320 (K, holotypus)



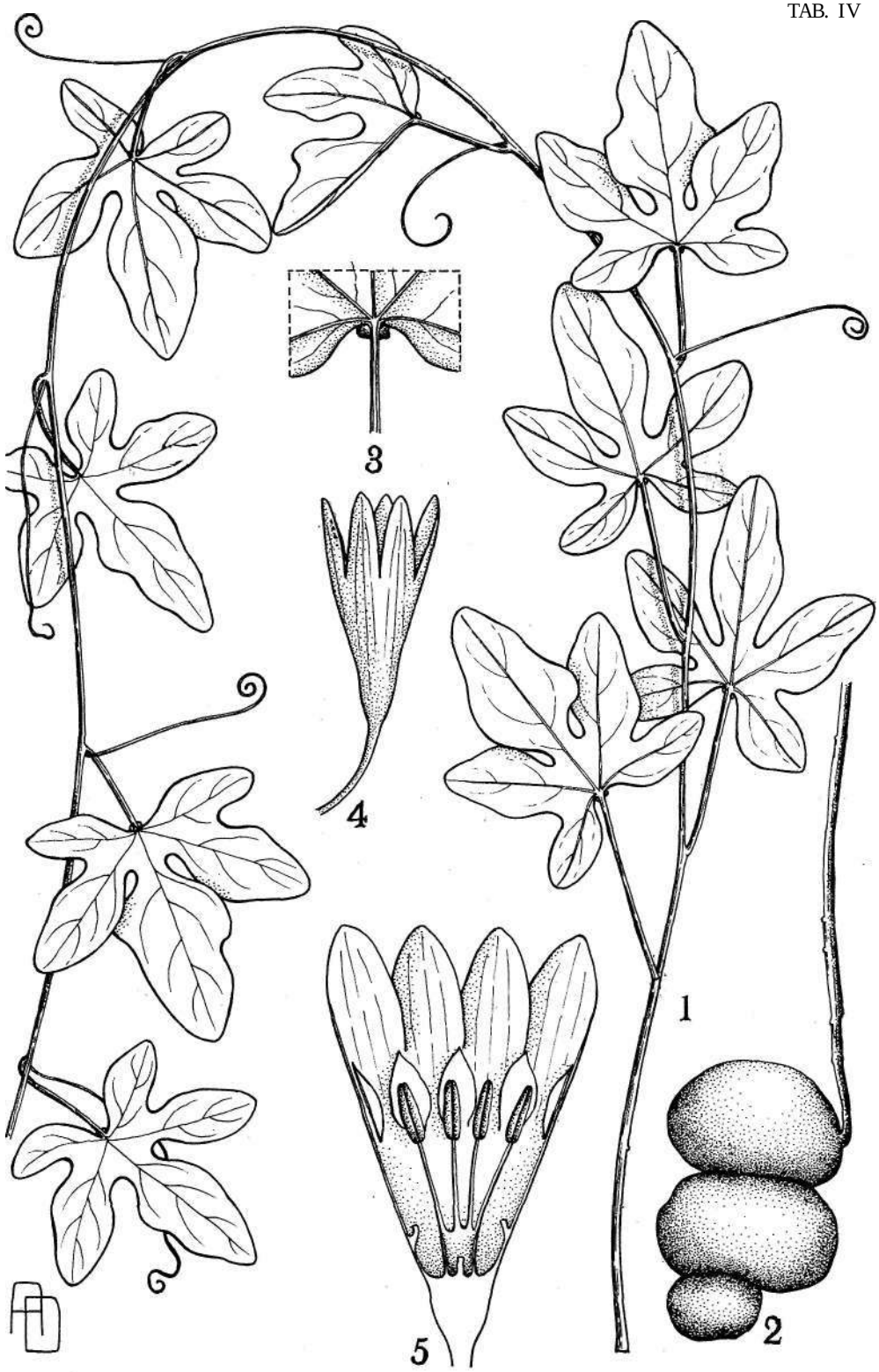
Basananthe holmesii R. & A. Fernandes

1, habitus (× 3/4); 2, pars folii indumentum ostendens (× 2 1/4); 3, calyx cum stipite (× 2 1/4); 4, flos (× 3); 5, sectio longitudinalis floris (× 3); 6, coronae interior et exterior (× 4 1/2); 7, fructus in calyce (× 2 1/4).



Adenia zambesiensis R. & A. Fernandes

Specimen *Torre & Correia* 16975 (LISC, holotypus)



Adenia zambesiensis R. & A. Fernandes

1, habitus (× 3/4); 2, radix tuberosa (× 3/4); 3, folii basis glandulas ostendens (× 1 1/2); 4, flos ♂ (2 1/4); 5, sectio longitudinalis floris ♂ (× 4 1/2).

Adenia volkensii Harms

Specimen *Robson* 1227 (K)

CONTRIBUIÇÕES PARA O CONHECIMENTO DAS ALGAS DE ÁGUA DOCE DE PORTUGAL—V

por

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COM este trabalho tentamos contribuir para o avanço do inventário algológico do nosso País, infelizmente ainda bastante atrasado. Além de novos para Portugal, alguns dos taxa referidos também o são possivelmente para a Europa.

A maior parte das plantas foi colhida por nós na Beira Litoral; os exemplares citados para a Beira Baixa e Trás-os-Montes foram amavelmente cedidos pela nossa colega MARIA CHIESTE ALVES e pelo Sr. Prof. Dr. J. BARBOS NEVES, aos quais expressamos aqui os nossos agradecimentos. Os taxa mencionados para a Beira Alta foram colhidos durante a excursão do VII Simpósio da Flora Europaea, realizado em Coimbra, de 23-31 de Maio de 1972.

A distribuição geográfica apenas é indicada quando, além dos dados contidos neste trabalho, nos foi possível obter indicações concretas sobre ela.

Queremos manifestar o nosso agradecimento ao Ex.mo Sr. Prof. Dr. ABÍLIO FERNANDES e à Ex.ma Sr.a Dr.a ROSETTE FERNANDES pela cuidada revisão do manuscrito, bem como pelas suas sugestões.

CYANOPHYTA

CHROOCOCCALES

Chroococcaceae

Chroococcus turgidus (Kütz.) Näg. var. maximus Nygaard in Vidensk. Medd. Dansk Naturh. Foren. Kjobenh. 82: 201 (1926). —Est. I, fig. 1.

Células esféricas ou elipsoidais, verde-azuladas, reunidas em colónias de 2-4-8, menos vezes isoladas e envolvidas por uma bainha lamelada e hialina, sendo a estratificação mais evidente na zona interna. Dimensões: diâmetro das células 22-45 μ; colónias bicelulares: comp. 56-65 μ; larg. 44-51 μ; colónias com 4-8 células: comp. 73-110 μ; larg. 58-79 μ (nos nossos exemplares: colónia bicelular 74,7-82,5 X 44,8-57,5 μ; colónia de 8 células: 92,5 X 72,5 μ; dimensões das células de uma colónia bicelular, sem bainha 29 X 37,4 μ; com bainha 31,5 X 39 μ).

BEIRA LITORAL: Coimbra, Jardim Botânico, aquários, relativamente abundante, 19-X-1972, *Fátima Santos* 687B (COI); idem, pouco abundante, 3-IV-1973, *Fátima Santos* 705c (coi).

Distribuição geográfica: Ásia.

Nostocaceae

Anabaena cylindrica Lemm. in Ber. Biol. Stat. Plön 4: 186 (1896). —Est. I, fig. 2; Est. XVI, foto 1.

Tricomas direitos, livres e sem bainha evidente, ou reunidos formando uma fina película azulada. Células dolioliformes ou cilíndricas, as apicais cónicas. Heterocistos esféricos ou cilíndricos. Acinetos cilíndricos, com as extremidades arredondadas, isolados ou em série, de ambos os lados do heterocisto, providos de um epísporo incolor. Dimensões: células vegetativas: comp. 3-5μ; larg. 3-4μ; heterocistos: comp. 6-10 μ; larg. 5-6μ; acinetos: comp. 16-60/μ; larg. 5-7μ (nos nossos exemplares: células vegetativas 2,8-5,7 X 3,2-4,1 μ; heterocistos 8,6 X 5,9-6,2 μ; acinetos 10,8-18,5 X 4,8-6,2 μ; célula apical 5,7 X 3,3 μ).

BEIRA LITORAL: pr. cruzamento para Lavariz, pouco abundante, 17-VII-1972, *Fátima Santos. & Celeste Alves* 677B (COI).

Distribuição geográfica: Europa.

Anabaena laxa (Rabenh.) A, Braun ex Bornet & Flanault in Bull. Soc. Bot. Fr. 32: 120 (1885). —Est. I, fig, 3; Est. XVI, fotos 6-8.

Tricomas isolados ou reunidos paralelamente em agregados verde-azulados, sem ou com uma bainha pouco evidente. Células esféricas ou dolioliformes. Heterocistos esféricos ou curtamente elipsoidais. Acinetos cilíndricos, de poios arredondados, isolados ou em série, afastados dos heterocistos; epísporo liso e hialino. Dimensões: largura do tricoma (3)4-6 μ; células vegetativas: comp. 5-6 μ; larg. 4-6 μ ; heterocistos : comp. 6,5-10 *μ',* larg. 4,5-6 *μ;* acinetos: comp. (12)14-20 *μ;* larg. (5)6-8 μ (nos nossos exemplares: células vegetativas 3,3-5,3 X 5,1-5,7 μ; heterocistos 6,9-7,4 X 6,5-7 μ; acinetos 14-17 X 6,3-7 *μ):*

BEIRA LITORAL : pr. cruzamento para Lavariz, em arrozais, pouco abundante, 17-VII-1972, *Fátima Santos & Celeste Alves* 677B (COI).

Distribuição geográfica: Europa e Asia.

Anabaena **lapponica** Borgen in Bot. Not. **1913:** 101 (1913). — Est. II, fig. 1.

Tricomas solitarios, direitos ou encurvados, constituídos por células esféricas, de conteúdo homogéneo ou granuloso e azul-esverdeado. Heterocistos esféricos de dimensões iguais ou superiores às das células vegetativas. Acinetos cilíndricos de poios largamente arredondados, adjacentes aos heterocistos de um ou de ambos os lados, ou deles separados por uma ou duas células; epísporo Uso e hialino. Dimensões: células vegetativas: diam. (5)6-9,5*μ;* heterocistos: diam. 8-11,5 μ; acinetos: comp. 20-85 μ; larg. 9-13 μ (nos nossos exemplares: células vegetativas: 5;8-8 X 6,2-8 μ; heterocistos: 7,4-10 X 7,4-9,5 μ; acinetos: 21-40 X 8,5-10 μ).

BEIRA LITORAL: pr. Vila Franca, á 1,5 km de Arâzede, na estrada para Cantanhede, num charco, abundante, 30-VT--1972, *Celeste Alves* 30 (coi).

BEIRA BAIXA: Travanca, a 10 km de Castelo Branco, numa fonte, muito abundante, 5-V-1973, *Celeste Alves* 75 (coi).

Distribuição geográfica: Europa.

Nota: As figuras 1a, 1b, lc e 1i da Est. II correspondem a exemplares da primeira localidade e as restantes a material de Travanca.

Anabaena saaremaaensis Skuja in Acta Horti Bot. Univ. Latv. 4: 17 (1929). — Est. Π, fig. 2; Est. XVI, fotos 2-5.

Tricomas direitos ou levemente enrolados, reunidos em massas gelatinosas azuladas ou esverdeadas. Células cilíndricas ou dolioliformes, de conteúdo granuloso; heterocistos cilíndricos ou subesféricos; acinetos cilíndricos ou elipsoidais, afastados dos heterocistos, isolados ou em série de 2, 3 ou 4, com membrana incolor, lisa ou levemente pontuada. Dimensões: células vegetativas: comp. 4-6 μ; larg. 5-5,5 μ; heterocistos : comp. 8-14 μ ; larg. 5-7 μ ; acinetos : comp. 15-46*μ;* larg. 7-11 μ (nos nossos exemplares: células vegetativas 3,7-5,7 X 4,9-5,3 μ ; heterocistos 6-10 X 6,5-7,5 μ ; acinetos 12-24(31) X 8-9,8 μ).

BEIRA ALTA : Caramulinho, numa valeta, muito abundante, 30-V-1972, *Fátima Santos* 662B (COI).

Distribuição geográfica: Europa e Ásia.

Nota: Os nossos exemplares apresentam os acinetos, quando totalmente formados, providos de parede espessa por vezes estratificada, hialina ou levemente amarelada, podendo aparecer em série de mais de 4. Seguindo as chaves de STARMACH (1966), julgamos poder incluir o material português nesta espécie.

EUGLENOPHYTA

EUGLENALES

Euglenaceae

Phacus **oscillalis** Klebs — Est. IΠ, fig. 1.

Célula cilíndrico-ovóide, com a extremidade anterior largamente arredondada e emarginada e a posterior em geral

gradualmente atenuada numa cauda curta e romba. Corpo da célula dividido por um sulco longitudinal, apresentando-se a margem direita da célula rectilínea ou levemente còncava e a esquerda convexa. Periplasto estriado da direita para a esquerda. Plastos numerosos, pequenos e discoides. Paramilo sob a forma de um disco, menos vezes dois, geralmente anteriores. Dimensões da célula: comp. 15-33 µ; larg. 5-11 µ (nos nossos exemplares: célula 18-20,5 X 6-8 µ).

BEIRA LITORAL: Montemor-o-Velho, nos arrozais, raro, 23-VI-1971, *Fátima Santos* 587 (coi).

Distribuição geográfica: Europa, Ásia e América do Norte.

Phacus agilis Skuja in Acta Horti Bot. Univ. Latv. 1: 39 (1926). — Est. III, fig. 2.

Célula em forma de grão de café ou elipsoidal, com a extremidade anterior levemente chanfrada ou provida de uma pequena saliência e a posterior estreitamente arredondada ou prolongada em cauda curta e romba. Corpo da célula dividido longitudinalmente por um estreito sulco mediano. Periplasto ornamentado por estrias oblíquas pouco evidentes. Paramilo em duas grandes calotes laterais. Dimensões: comp. 13-17 µ; larg. 8-13 µ; espessura 5-7 µ (nos nossos exemplares: 13,5 X 8,6 µ).

BEIRA LITORAL: S. Facundo, nos arrozais, raro, 1-VII-1970, *Fátima Santos & Celeste Alves* 438 (coi); ponte da Cidreira, num charco, relativamente abundante, 21-VI-1971, *Fátima Santos* 554 (coi).

Distribuição geográfica: Europa e Asia.

Phacus platyaulax Pochm. — Est. III, fig. 3.

Célula de contorno largamente oval com a extremidade posterior mais alargada e contraída em cauda curta e oblíqua, por vezes ligeiramente curva. Corpo da célula provido de um largo sulco inclinado da direita para a esquerda que o divide em porções desigualmente dilatadas e cruzadas

entre si, sendo quase perpendiculares na região apical. Periplast© ornamentado por estrias paralelas às margens do sulco. Paramilo sob a forma de duas grandes calotes ou de anéis laterais. Dimensões da célula: comp. 36-40 *μ;* larg. 23-28 *μ* (nos nossos exemplares: 35-39,6 X 27-28,7 *μ*; cauda **4,1** *μ*),

BEIRA LITORAL: Montemor-o-Velho, nos arrozais, pouco abundante, 23-VI-1971, *Fátima Santos* 587 (coi).

Distribuição geográfica: Europa e Asia.

Phacus anomalus Fritsch et Rich. var. pullus-gallinae Nygaard in Det Kon. Danska Vid. Selsk. Biol. Skr. 7 (1949).— Est. III, fig. 4.

Célula pouco achatada, com a extremidade anterior largamente arredondada e a posterior provida de uma curta cauda obtusa e oblíqua. Corpo da célula dividido em duas porções desiguais mediante um sulco profundo inclinado da direita para a esquerda, originando uma espécie de asa oblíqua. Em vista lateral, mostra-se largamente elíptico enquanto que em vista apical se apresenta constituído por uma porção maior e semicircular e outra menor correspondendo Paramilo sob forma de dois anéis, de discos ou de bastonetes curtos e largos com as extremidades arredondadas, iguais no tamanho ou diferentes, pertencendo o menor à asa. Plastes discoides, pequenos e numerosos. Periplasto estriado longitudinalmente. Dimensões: comp, da célula sem cauda 21,5-26*μ;* larg. 16-20,5*μ;* espessura 14-15*μ;* espessura da asa 7-10 μ; comp, da cauda 2-3 *μ* (nos nossos exemplares: 24,6 X 18,5-21,3 *μ*; espessura 14,4 *μ*; espessura da asa 8,2 *μ*; cauda 2,1μ).

BEIRA LITORAL: S. Facundo, nos arrozais, raro, l-VII-1970, *Fátima Santos & Celeste Alves* 438 (coi).

Distribuição geográfica: Europa (Dinamarca).

Astasiaceae

Astasia pygmaea Skuja in Acta Hört. Bot. Univ. Latv. 11/12: 41 (1939). —Est. III, fig. 5,

Célula hialina, pouco metabòlica, piriforme ou trapezoidal, levemente achatada. Polo anterior largamente oblíquo--arredondado, o posterior contraído em curta cauda obtusa. Flagelo maior que o comprimento do corpo. Periplasto estriado em espiral. Paramilo sob a forma de grossos bastonetes ou de grãos ovoides. Dimensões da célula: comp. 9-16 *μ;* larg. 7-9 *μ;* espessura 3-5 μ (nos nossos exemplares: célula 15-16,4 X 8,6-9,8 *μ;* espessura 6,2-7 *μ).*

BEIRA LITORAL: Coimbra, Jardim Botânico, pouco abundante, 24-V-1974, *Celeste Alves* s. n. (coi).

Distribuição geográfica: Europa (Letónia).

Sphenomonas teres (Stein) Klebs in Zeitschr. Wiss. Zool. 55: 361 (1893). —Est. IV, fig. 1.

Célula não metabolica largamente fusiforme, com o polo anterior prolongado num curto colo obliquamente emarginado e o posterior contraído numa curta cauda obtusa. Periplasto provido de estrias longitudinais muito ténues. Flagelos 2, sendo um bastante maior e atingindo ou excedendo o comprimento da célula. Núcleo anterior. Região mediana ocupada por uma grande massa gelatinosa. Dimensões: comp, da célula 20-40 *μ* ; larg. 8 *μ* (nos nossos exemplares : célula 22,1-27,9 X 7,4-9 μ).

BEIRA LITORAL: Montemor-oVelho, nos arrozais, muito rara, 23-VI-1971, *Fátima Santos* 587 (coi) ; Coimbra, Jardim Botânico, num tanque, muito rara, 21-V-1974, *Fátima Santos* 723 (coi).

Peranemaceae

Fetalomonas mediocanellata Stein var. disomata (Stokes) Lemm. —Est. IV, fig. 2.

Célula ovoide, achatada, provida de um fundo sulco longitudinal nas duas faces. Região anterior obtusa e estreita; região posterior largamente arredondada. Periplasto ornamentado por estrias longitudinais muito finas. Flagelo de tamanho igual ou superior ao comprimento do corpo. Dimensões da célula : comp. 15-29 μ ; larg. 6-16 μ ; espessura 4-8 μ (nos nossos exemplares: 17,2-19,7X10,5-15,6X4,9-7,4 μ).

BEIRA LITORAL: S. Facundo, num arrozal, rara, l-VII-1970, *Fátima Santos & Celeste Alves* 438 (coi) ; Montemor-o-Velho, nos arrozais, muito rara, 23-VI-1971, *Fátima Santos* 587 (coi) ; Coimbra, Jardim Botânico, num vaso, muito rara, 15-V-1973, *Fátima Santos* 710D (COI).

Distribuição geográfica: Europa (Letónia) e América do Norte.

Petalomonas dubosquii Holl. in Arch. Zool. Exp. et Gen. 83: 78 (1942). —Est. IV, fig. 3.

Célula estreitamente ovòide, com a extremidade anterior estreita e a posterior arredondada ou chanfrada, consoante a posição da célula. Núcleo com nucléolo volumoso. Reservatório dorsal. Face esquerda da célula provida de um largo sulco e a direita de duas quilhas ligeiramente oblíquas, com as margens onduladas. Secção transversal triangular. Dimensões da célula: comp. 25-28μ; larg. 10-12μ,; núcleo: diam. *i μ;* nucléolo diam. *2 μ* (nos nossos exemplares: célula 25,4 X 11,1$_{\mu}$) .

BEIRA LITORAL : Montemor-o-Velho, nos arrozais muito rara, 23-VI-1971, *Fátima Santos* 587 (coi).

Distribuição geográfica: Europa (França).

Petalomonas asymmetrica Shawh. & Jahn in Trans. Amer. Micr. Soc. 66: 188 (1947). — Est. IV, fig. 4.

Célula incolor, levemente achatada, de contorno oval, com a extremidade posterior largamente arredondada. Corpo da célula provido de profundo e largo sulco oblíquo, situado na margem esquerda, que a divide em duas porções diferentes, sendo a dorsal mais espessa e comprida; grânulos numerosos de ambos os lados do sulco. Flagelo com cerca de vez e meia c comprimento da célula. Dimensões da célula: comp. 18-25 μ (nos nossos exemplares: 18,6-20,5 X 12,3-15,6 μ; espessura 8,6-12,3 μ).

BEIRA LITORAL: Coimbra, Jardim Botânico, num tanque, rara, 21-V-1972, *Fátima Santos* 723 (coi).

Petalomonas abscissa (Duj.) Stein var. **parallela** Klebs in Zeitschr. Wiss. Zool. 55: 381 (1893). — Est. IV, fig. 5.

Célula de contorno largamente elipsoidal, achatada, com a face ventral convexa e a dorsal provida de duas quilhas paralelas. Flagelo ligeiramente mais longo que o comprimento do corpo. Dimensões da célula : comp. 27-30 μ ; larg. 16-19 μ ; espessura 7-10 μ (nos nossos exemplares: 26,7-27,5X18,5--19,3 μ; espessura *8 μ).*

BEIRA LITORAL: Montemor-o-Velho, num arrozal, muito rara, 23-VI-1971, *Fátima Santos* 587 *(voi).*

PYRROPHYTA

CRYPTOPHYCEAE

CRYPTOMONADALES

Cryptomonadaceae

Chroomonas coerulea (Geitler) Skuja in Symb. Bot. Ups. 9, 3: 350 (1948). — Est. V, fig. 1.

Célula elipsoidal a cilíndrica com uma margem fortemente convexa e a outra plana ou côncava. Extremidade anterior obliquamente truncada, provida de dois flagelos

menores que a célula, a posterior largamente arredondada. Plasto verde-azulado, dorsal, provido de um grande pirenóide equatorial ou ligeiramente posterior; estigma ovoide submediano; tricocistos em duas séries paralelas; vacùolo contráctil anterior. Dimensões da célula: comp. 8-12 μ; larg. 4-6 μ (nos nossos exemplares: 8-9 X 4,9-5μ).

BEIRA LITORAL: Coimbra, Jardim Botânico, num tanque, muito abundante, 29-V-1974, *Fátima Santos* s. η. (σοι).

Distribuição geográfica: Europa.

DINOPHYCEAE

DINOCOCCALES

Cystodiniaceae

Cystodinium brevipes Geitler in Arch. Prot. 61, 1 (1928).— Est. V, fig. 2; Est. XVII, fotos 3 e 4.

Célula reniforme, ovòide ou largamente elipsoidal, fixa por um curto e largo pedúnculo; polos largamente arredondados e providos de um espinho curto e forte. Plasto amarelo-acastanhado, irradiando do centro, onde por vezes se pode observar um pirenóide; dois vacúolos grandes, situados nos polos, sendo ainda frequente a ocorrência de uma volumosa mancha pigmentar alaranjada, em regra mediana. Dimensões da célula: comp. 18-30μ; lárg. 11-20μ (nos nossos exemplares: comp, com espinhos 32-35,7μ; comp, sem espinhos 29,5-32,8 ja; larg. 16,4-18,9^).

BEIRA LITORAL: Coimbra, Jardim Botânico, aquários, pouco abundante, 3-Γ7-1973 e 20-III-1976, *Fátima Santos* 705A e 795 (coi).

Distribuição geográfica: Europa (Áustria).

CHRYSOPHYTA

CHRYSOPHYCEAE

PHAEOPLACALES

Sphaeridiothricaceae

Sphaeridiothrix globulosa Pascher—Est. V, fig. 3; Est. XVII, foto 2.

Filamentos não ramificados, envolvidos por bainha gelatinosa e constituídos por uma série de células esféricas ou levemente comprimidas, justapostas ou afastadas. Plasto geralmente pequeno, ocupando a zona central da célula, sem pirenóides. Multiplicação por divisão celular. Dimensões: diâmetro das células 7μ (nos nossos exemplares: células 6,7-7,4 X 6-6,9 μ ; espessura da bainha ca. 20 μ).

BEIRA LITORAL: Coimbra, Jardim Botânico, num tanque, pouco abundante, 26-XI-1974, *Fátima Santos* 757B (COI).

Distribuição geográfica: Europa.

CHROMULINALES

Chrysococcaceae

Bicoeca kepneri Reynolds in Trans. Amer. Micr. Soc. 46: 54 (1927). —Est. V, fig. 4.

Lorica incolor, ovòide ou raramente cilíndrica, fixa por um fino pedúnculo. Célula incolor, provida de dois flagelos, um anterior, motor, e outro dirigido para trás e fixo à lorica; núcleo mediano; um vacùolo contráctil posterior. A parte apical do citoplasma pode estender-se em lábio amibóide. Dimensões da lorica: comp. 10-13 μ; larg. 6μ (nos nossos exemplares: 8,5-9,5 X 4-5,5 u.).

BEIRA LITORAL: Coimbra, num tanque do Jardim Botânico, raro, 24-VII-1972, *Fátima Santos* 684 (coi).

Distribuição geográfica: América, Europa (França).

Bicoeca **petiolata** (Stein) Pringsh. in Trans. Roy. Soc. London, Ser. B, 232: 588 (1946). — Est. V, fig. 5.

Células loricadas, reunidas em colonias arborescentes, fixas. Lorica incolor ou acastanhada, campanulada, de bordos divergentes e base afilada onde a parede é mais espessa, terminando em longo pedicelo que se fixa no interior da lorica mãe. Citoplasma ovóide, com um lábio anterior e lateral e dois vacúolos contrácteis basais, fixo à base da lorica pelo flagelo posterior. Dimensões : lorica : comp. 10-25 *μ ;* larg. 6-10/*μ* ; pedúnculo 10-60-100 *μ* ; citoplasma sem o lábio: comp. 6-10*μ;* larg. 5-8*μ* (nos nossos exemplares: lorica 14-18 X 6-7μ; citoplasma 5,5-6,5 X 4-5*μ)*.

BEIRA LITORAL: Coimbra, Jardim Botânico, num tanque, rara, 24-VII-1972, *Fátima Santos* 684 (coi).

Distribuição geográfica: Europa.

ISOCHRYSIDALES

Isochrysidaceae

Pseudodendromonas vlkii Bourr. in Österr. Bot. Zeitschr. **100** : 537 (1953).—-Est. VI, fig. 1; Est. XVII, foto 1.

Colònia fixa, corimbiforme, com os pedúnculos espessos, rígidos e incolores, ramificados dicotomicamente, apresentando uma célula em cada extremidade. Células incolores, piriformes ou cónicas, com o ápice plano ou côncavo e oblíquo em relação ao eixo, provido de dois flagelos subiguais, inseridos lateralmente. Vacúolos contrácteis anteriores em número de 2 e um parabasal. Dimensões: comp, da colónia **200***μ;* larg. 100*μ;* células: comp. **6-10***μ*.; larg. 4-6 ju.; flagelos do comprimento do corpo (nos nossos exemplares: células: 8-9,5 X 4-5,5*μ)*.

BEIRA LITORAL: Coimbra, Jardim Botânico, num tanque, pouco abundante, 19-X-1972, *Fátima Santos* 683 (coi).

Distribuição geográfica: Europa.

Spongomonas uvella Stein, Der Organ, der Infusionsth. 3, 1 (1878) —Est. VI, fig. 2; Est. XVII, fotos 5-7.

Colonia fixa, acastanhada, constituída por uma geleia de aspecto granuloso, inicialmente com a forma de almofada, tornando-se por fim multilobada e apresentando uma célula em cada lobo. Células ovóides, com um vacùolo contráctil e dois flagelos 2 a 3 vezes maiores que o corpo. Dimensões: comp, da célula 8-12 *μ* (nos nossos exemplares: célula 6,5-8X4-6*μ)*.

BEIRA LITORAL: Coimbra, Jardim Botânico, em vasos de cultura, rara, 31-V-1973 e 7-XΠ-1973, *Fátima Santos* 714B e 716 (coi).

Distribuição geográfica: cosmopolita.

MONOSIGALES

Monosigaceae

Monosiga varians Skuja var. vagans Skuja in Symb. Bot. Upsal. 9, 3: 300 (1948). —Est. VI, fig. 3.

Célula nua, largamente ovóide, livre ou fixa por um curto e fino pedicelo plasmático, provida de um colar que rodeia o flagelo e de dois vacúolos contrácteis basais. Dimensões: célula sem colar: comp. 5,5-8*μ;* larg. 7-10*μ;* colar: comp, até 10*μ;* larg. 7-8*μ;* flagelo: 3 a 4 vezes o comprimento do corpo (nos nossos exemplares: célula sem colar 6 X 5-6*μ;* colar 5,5 X 4,1 *μ)*.

BEIRA LITORAL: ponte da Cidreira, num charco, muito raro, 30-VT-1972, *Celeste Alves* 6 (coi) ; Coimbra, Jardim Botânico, num tanque, muito raro, 24-VII-1972, *Fátima Santos* 684 (coi).

Distribuição geográfica: Europa (Suécia).

Salpingoecaeeae

Salpingoeca gracilis Clark — Est. VI, fig. 4.

Lorica incolor, em forma de vaso alongado, com os bordos divergentes e a base atenuada em forte estipe. Citoplasma alongado, ocupando apenas $^1/_4$-$^1/_3$ da lorica; núcleo anterior; em regra, dois vacúolos contrácteis posteriores; numerosos grânulos de reserva ou de assimilação dispersos no citoplasma. Flagelo ligeiramente maior que a célula. Dimensões: comp, da lorica 31,7-50μ (nos nossos exemplares: lorica 35-45 X 6-7,5 μ; abertura 8,6 µ; citoplasma 14,7-15,1 X 4,5-4,9,.).

BEIRA LITORAL: Cidreira, num charco, pouco abundante, 17-VII-1972, *Fátima Santos & Celeste Alves* 669 (coi); Coimbra, Jardim Botânico, num tanque, rara, 24-VII-1972, 19-X-1972 e 16-III-1976, *Fátima Santos*, 684, 689 e 794 (coi).

Distribuição geográfica: Europa.

Salpingoeca gracilis Clark var. abbreviata Skuja in Acta Horti Bot. Univ. Latv. 11/12: 64 (1939).—Est. VI, fig. 5.

Esta variedade difere do tipo pelas menores dimensões e pelo estipe filiforme e bastante longo. Dimensões: comprimento da lorica 16-27μ; larg. 4-5 μ; abertura 6-7 μ (nos nossos exemplares: lorica 26-29 X 6 μ; abertura 7 μ^citoplasma 17 X 6 μ.).

BEIRA LITORAL: Coimbra, Jardim Botânico, num tanque, rara, 24-VII-1972, *Fátima Santos* 684 (coi).

Distribuição geográfica: Europa (Letónia).

Biploeca flava (Korsch.) Bourr. in Rev. Alg., Mém. Hors Sér. 1: 326 (1957). —Est. VII, fig. 1.

Lorica esférica ou hemisférica com a base achatada, de parede espessa e acastanhada, provida no ápice de um

colo hialino, cilindrico, levemente alargada na extremidade. Célula incolor, provida de um colar citoplasmàtico e de um só flagelo. Dimensões: altura da lorica com o colo 10-12 μ; larg. 6-10 μ; altura do colo 5 μ; largura da extremidade do colo 3-4 μ (nos nossos exemplares: largura da lorica 6-12 μ; altura da lorica sem colo 4,5-6,2 μ; altura do colo 4-4,5 μ; abertura do colo 2,8-3,2*μ)*.

BEIRA LITORAL: Coimbra, num tanque do Jardim Botânico, muito rara, 2-III-1973, *Fátima Santos* 706 (coi).

Distribuição geográfica: França e Rússia.

XANTHOPHYCEAE

VAUCHERIALES

Vaucheriaceae

Vaucheria aversa Hassall, Hist. Brit. Freshw. Alg.: 54 (1845). — Est. VII, fig. 2.

Filamentos ásperos, livremente ramificados, com 35--131 μ de largura. Oogónios sésseis ou muito curtamente pedicelados, geralmente aos pares, ovóides e oblíquo-divergentes, com os poros na extremidade de uma papila no polo distai. Anterídios subcilíndricos, encurvados e subparalelos ao eixo, um junto de cada oogónio. Zigoto globoso, não enchendo o oogónio e provido de uma parede 3-lamelada. Dimensões: oogónio: comp. 180-250 μ; larg. 100-125 μ; diâmetro do zigoto : 75-120 μ ; largura do anterídio 30-40 μ (nos nossos exemplares: largura do filamento 82,5 μ; oogónio 150-212 X 120-137,5 μ; diâmetro do zigoto 112,5-125 *μ)*.

TRÁs-os-MONTES: entre Miranda do Douro e Duas Igrejas, a 3 km de Miranda, muito abundante, 12-III-1973, *Barros Neves* s. n. (COI).

Distribuição geográfica: Europa, Asia e América.

CHLOROPHYTA

CHLOROCOCCALES

Hydrodictyaceae

Pediastrum arenosum (Racib.) G. M. Smith in Bull. Torr. Bot. Club 43: 476 (1916;.—Est. XV, fig. 2.

Cenobio inteiro, constituído por células hexagonais no interior e pentagonais à periferia, apresentando estas a face externa emarginada e provida de 2 curtos lobos. Parede hialina e reticulada. Dimensões: diâmetro das células 15-32 μ (nos nossos exemplares: colónia com 32 células 110-216 X 97-216μ; células periféricas 21-30,7 X 21-28,7μ; células centrais 16-24,6 X 23-30 μ).

BEIRA LITORAL: Mira, viveiros dos Serviços Florestais, raro, 21-VI-1971, *Fátima Santos* 570A (COI).

Distribuição geográfica: América do Norte.

CHAETOPHORALES

Chaetophoraceae

Stigeoclonium subsecundum (Kütz.) Kütz. var. **tenue** Nordst. emend. Nurul Islam in Beih. Nova Hedw. 10: 85 (1963). — Est. VIII, fig. 1.

Filamentos pouco ramificados em especial na região inferior. Ramos principais constituídos por células cilíndricas pouco ou nada constrictas nos septos, geralmente 4-9(12) vezes mais compridas que largas, menos frequentemente com o comprimento apenas 2-3 vezes maior que a largura. Ramificações dicotômicas ou alternas, por vezes os ramos unisseriados, sendo as células produtoras dos ramos semelhantes ou ligeiramente menores que as restantes. Ramos secundários por vezes atenuados na extremidade em longos pêlos. Plasto maciço, de tipo ulotricoide, ocupando quase toda a célula, de margens lisas ou lobadas. Dimensões: diâmetro das células 7-10-12 μ (nos nossos exemplares: células dos ramos

principais 18,6-54 X 8,3-10 μ; células das extremidades dos ramos 8,3-19,9 X 7,5 µ).

BEIRA LITORAL: Amieiro, num charco, pouco abundante, 4-III-1972, *Fatima Santos* 639 (coi).

Distribuição geográfica: Europa, Ásia, América do Norte, Nova Zelândia e Antartico.

OEDOGONIALES

Oedogoniaceae

Oedogonium rivulare (Le Clerc) A. Braun in Abh. Akad. Wiss. Berlin 1855: 23 (1855). —Est. VIII, fig. 2.

Macrândrico, diòico. Oogónios 1-7, obovóides, abertos superiormente por um poro. Zigotos obovóides, elipsóides ou subglobosos, não enchendo o oogónio no sentido longitudinal; parede lisa. Anterídios em número variável até 13, produzindo dois anterozoides por divisão horizontal. Dimensões: células vegetativas do filamento feminino: comp. 75--350μ; larg. 35-45μ; células vegetativas do filamento masculino: comp. 120-288 µ; larg. 30-36 μ; oogónios: comp. 130--160 μ ; larg. 70-85 μ ; zigotos : comp. 65-100 μ ; larg. 55--70 µ; anterídios: comp. 14-26 μ; larg. 21-28.µ (nos nossos exemplares : células vegetativas 185 X 45 μ ; oogónios 150 X 78 µ; zigotos 92 X 75µ).

BEIRA LITORAL: Coimbra, Choupal, num charco, muito raro, 17-VII-1972, *Fátima Santos & Celeste Alves* 665 (coi).

Distribuição geográfica: Europa, Africa e América.

Oedogonium crenulato-costatum Wittrock var. cylindricum (Hirn) Tiffany in N. Amer. Flora 11, 1 (1937).— Est. VIII, fig. 3.

Filamento macrândrico, diòico. Oogónios solitários ou em série de 2 a 6, cilíndricos, oblongos ou elipsoidais, abertos por um poro superior. Zigoto da mesma forma do oogónio, enchendo-o quase completamente; episporo e endós-

poro lisos e mesósporo provido de pregas longitudinais levemente erenuladas. Dimensões: células vegetativas: comp. 44-50 μ ; larg. 11-16 μ ; oogónios: comp. 42-81 μ ; larg. 30--36μ; zigotos: comp. 40-65μ; larg. 27-34μ (nos nossos exemplares : células vegetativas : 93-150 X 11,5-16 μ ; oogónios 60-70 X 33-36 μ; zigotos 54-65 X 30-35,5 μ; células sufultórias 78 X 17/0.

BEIRA LITORAL: Mira, viveiros dos Serviços Florestais, muito raro, 21-VI-1971, *Fátima Santos* 570A (COI).

Distribuição geográfica: norte de Africa, América do Norte, Ásia.

Oedogonium boseii (Le Clerc) Wittrock in Öfv. K. Sv. Vet. Akad. Forh. 3 (1870).—Est. IX, fig. 1.

Macrândrico, diòico. Oogónios solitários, raramente aos pares, longamente elipsoidais, abertos por um poro superior. Zigoto elipsoidal, não enchendo o oogónio; exósporo e mesósporo providos de 27-35 pregas longitudinais, geralmente não anastomosadas. Anterídios 1-8, em regra na região superior do filamento, produzindo 2 anterozoides por divisão vertical do protoplasto. Dimensões: células vegetativas femininas: comp. 42-138 μ; células vegetativas masculinas: comp. 52-108μ; larg. 13-18 μ; oogónios: comp. 75-110 µ; larg. 39-51 μ; zigotos: comp. 56-70μ; larg. 36-43μ; anterídios: comp. 6-16 μ; larg. 13-14 μ (nos nossos exemplares: células vegetativas femininas 102-152 X 16,2-18,2 μ ; célula suf ultória 148 X 20 μ; oogónios 70-89 X 41-55 µ; zigotos 45-65,5 X 32-45,6 μ).

BEIRA LITORAL: Mira, viveiros dos Serviços Florestais, muito raro, 21-VI-1971, *Fátima Santos* 570A (COI).

Distribuição geográfica: Europa, Ásia, África e América.

ZYGNEMATALES

Zygnemataceae

Zygnemopsis minuta Rhand. in Proc. Ind. Acad. Sci. 5: 312 (1937). —Est. X.

Filamentos constituidos por células cilíndricas, separadas por septos planos por onde se dissociam frequentemente. Plastes, dois por célula, irregularmente circulares por vezes quase estrelados, com um pirenóide. Zigotos globosos ou quadrado-globosos, formados por isogamia entre duas células livres; exósporo hialino, mesósporo espesso e de cor castanho--chocolate. Aplanósporos frequentes, assimetricamente ovóides ou globosos. Dimensões : células vegetativas : comp. 30-70 μ ; larg. 8-12 μ; zigotos: comp. 25-30 μ; larg. 22-25 μ ; aplanósporos : comp. 18-31 μ ; larg. 18-20 μ (nos nossos exemplares : células vegetativas 50-65 X 7,8-10 µ ; zigotos 30-32 X 30 μ; aplanósporos 25-44 X 17,4-21 μ).

BEIRA LITORAL: Coimbra, num tanque do Instituto Antropológico, muito abundante, 23-VII-1970, *Fátima Santos* s. n. (coi) ; pr. Vila Franca, a 1,5 km de Arazede, na estrada para Cantanhede, num charco, relativamente abundante, 30-VI-1972, *Celeste Alves* 30A (COI).

Distribuição geográfica: Ásia (Índia).

Nota: As figuras b, d, e, f, h, i da Est. X correspondem ao material colhido no tanque do Instituto Antropológico, material em que não foram observados zigotos; as restantes figuras são referentes aos exemplares da segunda localidade.

Mougeotia recurva (Hassall) De Toni, Sylloge Alg. 1: 714 (1889). —Est. IX, fig. 2.

Células vegetativas providas de 4-8 pirenóides dispostos em série no piaste. Conjugação escalariforme. Zigoto globoso, formado no tubo, de parede castanha e Usa. Aplanósporos globosos ou cilíndrico-ovóides. Dimensões: células vegetativas: comp. 50-180 μ; larg. 10-18 µ; diâmetro dos zigotos

22-33/ι; aplanósporos: comp. 29 μ; larg. 34 μ (nos nossos exemplares: células vegetativas 64-190 X 15-19 μ; zigotos 33-36,9 X 31-35 μ).

BEIRA ALTA: Serra da Estrela, Lagoa do Lageado, num charco, pouco abundante, 28-V-1972, *Fátima Santos* 647B (COI).

Distribuição geográfica: Europa, Ásia, América e Austrália.

Mougeotia quadrangulata Hassall in Ann. Mag. Nat. Hist. **11:** 434 (1843). —Est. IX, fig. 3.

Células vegetativas providas de um piaste com 8-16 pirenóides. Conjugação escalariforme, apresentando-se os gametângios geniculados. Zigoto dividindo ambos os gametângios, quadrangular, com as margens direitas, levemente côncavas ou convexas e os ângulos truncados; parede hialina com pontuações. Partenósporos elipsoidais, com poios truncados. Dimensões: células vegetativas: larg. 7-13 μ; diâmetro do zigoto 28-40 μ; partenósporos: comp. 36-44 μ; larg. 20-21 μ (nos nossos exemplares: células vegetativas 100-202 X 12--15 μ; zigotos 36-47 X 35-43 μ; pontuações da parede: diâmetro 1-2,5 μ; profundidade 2 μ).

BEIRA ALTA: Serra da Estrela, Lagoa do Lageado, num charco, pouco abundante, 28-V-1972, *Fátima Santos* 647B (COI).

Distribuição geográfica: Europa, Ásia, África e América.

Mougeotia laetevirens (A. Braun) Wittrock in Bot. Not. **1877:** 23 (1877). —Est. XI, fig. 1; Est. XVIII, foto 1.

Células cilíndricas, providas de um piaste com vários pirenóides, distribuídos irregularmente. Conjugação escalariforme no tubo. Zigoto curtamente cilíndrico, comas margens côncavas, as bases convexas e a membrana amarelo-acastanhada, lisa. Aplanósporos ovóides. Dimensões: células vegetativas: comp. 65-350 μ; larg. (22) 27-41 μ; zigotos: comp.

45-75*μ;* larg. 36-60 *μ* (nos nossos exemplares: células vegetativas: 190-265 X 26 μ; zigotos 48-56 X 40-52 ja).

BEIRA LITORAL: Mira, viveiros dos Serviços Florestais, pouco abundante, 21-VI-1971, *Fátima Santos* 570A (coi).

Distribuição geográfica: Europa, Asia, Africa, América do Sul e Australia.

Mougeotia rava Transeau in Ohio Jour. Sci. 44: 244 (1944). — Est. XI, fig. 2.

Células vegetativas providas de plasto com uma série de 4-8 pirenóides. Reprodução por aplanósporos geralmente formados fora do esporangio, que se torna curvo; membrana lisa, cinzento-acastanhada. Dimensões: células vegetativas: comp. 32-120μ; larg. 9-12*μ* diâmetro dos aplanósporos 16-20 *μ* (nos nossos exemplares: células vegetativas 50-122,5X 10-11,5*μ;* diâmetro dos aplanósporos 20*μ)*.

BEIRA LITORAL: Semede, num poço, relativamente abundante, 4-III-1972, *Fátima Santos* 636 (ora).

Distribuição geográfica: América do Norte.

Mougeotia ventricosa (Wittrock) Collins in Tufts College Studies 3: 76 (1912).—Est. XI, fig. 3.

Células muito alongadas com plasto provido de 4 pirenóides dispostos em série. Reprodução por aplanósporos elipsoidais ou subglobosos e oblíquos, de membrana lisa e amarelo-acastanhada. Dimensões: células vegetativas: comp. 100-140 *μ* ; larg. 5-9 *μ* ; aplanósporos : comp. 16-34 *μ* ; larg. 12-24 *μ* (nos nossos exemplares : células vegetativas 137 X 6,9 *μ;* aplanósporos 27,5 X 20μ).

BEIRA LITORAL: pr. Vila Franca, a 1,5km de Arazede, na estrada para Cantanhede, num charco, muito rara, 30-VT--1972, *Celeste Alves* 29 (coi).

Distribuição geográfica: Europa e América do Norte.

Spirogyra gracilis (Hassall) Kütz., Sp. Alg.: 438 (1849).— Est. XII, fig. 1; Est. XVIII, foto 2.

Células providas de um plasto enrolado em 1/2-4 espiras. Conjugação escalariforme, apresentando-se o gametângio feminino dilatado na face de conjugação; tubo formado pelos dois gametângios. Zigotos elipsoidais com os polos arredondados e mesósporo liso, amarelo-acastanhado. Dimensões: células vegetativas: comp. 50-100 μ; larg. 16-24 µ; zigotos: comp. 40-75μ.; larg. 23-34μ (nos nossos exemplares: células vegetativas 45-135 X 24-28,3$_{\mu}$; zigotos 50-70 X 29-33/μ).

BEIRA LITORAL: Coimbra, Jardim Botânico, muito abundante, 31-V-1973 e 21-V-1974, *Fátima Santos* 714B e 721A (COI).

Distribuição geográfica: Europa, Ásia, Àfrica e América do Norte.

Spirogyra submaxima Transeau in Amer. Journ. Bot. 1: 295 (1914). —Est. XIII, fig. 1.

Células vegetativas com 8-9 plastos quase direitos ou fazendo uma só espira. Conjugação escalariforme, permanecendo os gametângios cilíndricos ou levemente alargados e contribuindo ambos para a formação do tubo. Zigotos lenticulares, de epísporo fino e liso; mesósporo espesso, castanho e liso. Dimensões : células vegetativas : comp. 100-300 μ ; larg. 70-110 μ; zigotos: diâmetro 70-110 μ; espessura 50-75 μ (nos nossos exemplares: células vegetativas 60-120 X 85--110 µ; zigotos 70-88 X 70-75 µ; espessura 48-55μ).

BEIRA LITORAL: S. João do Campo, num charco, muito rara, 17-VII-1972, *Fátima Santos* 673E (COI); Cidreira, num charco, relativamente abundante, 17-VII-1974, *Fátima Santos & Celeste Alves* 728A (COI).

Distribuição geográfica: América do Norte e do Sul, Ásia (Índia, China) e África.

Spirogyra pseudomajuscula Gauthier-Lièvre in Beih. Z. Nov. Hedw. 20: 146 (1965). —Est. XIII, fig. 2.-

Conjugação escalariforme, sendo o tubo formado pelos dois gametângios que permanecem cilíndricos ou levemente dilatados na face interna. Zigoto lenticular, de epísporo fino, liso e incolor e mesósporo espesso (4-5*μ),* amarelo-acastanhado, provido de escrobieulações irregularmente distribuídas; linha de deiscência longitudinal nítida. Dimensões: células vegetativas: comp. 50-125 μ; larg. 90-100 *μ* ; zigotos: comp. 50-65*μ;* larg. 72-80μ (nos nossos exemplares: células vegetativas 55-145 X 87,5-100 *μ;* zigotos: diâmetro 72-77,5 *μ*; espessura 52,5-60*μ;* espessura do mesósporo ca. 5*μ)*.

BEIRA LITORAL: S. João do Campo, num charco, rara, 17-VII-1972, *Fátima Santos* 673E (COI).

Distribuição geográfica: África.

Nota: Observámos nesta espécie alguns filamentos onde ocorria conjugação anormal, pois que um gametângio c? emitia dois tubos de conjugação e fecundava dois gametângios ? adjacentes (Est. XΠI, fig. 5b). Este fenómeno foi também já referido por RIETH (1972) em *Spirogyra quadrilaminata* Jao.

Spirogyra heeriana Näg., Sp. Alg.: 442 (1849). —Est. XIV, fig. 1; Est. XVIII, fotos 3 e 4.

Células vegetativas cilíndricas, providas de cerca de 8 plastos, dando 1/2-l volta. Conjugação escalariforme, concorrendo os dois gametângios para a formação do tubo e permanecendo cilíndricos. Zigotos lenticulares, de epísporo fino, incolor e liso; mesósporo espesso e castanho, ornamentado por papilas castanho-escuras, irregularmente distribuídas. Dimensões: células vegetativas: comp. 130-250 *μ*; larg. 130-150*μ;* diâmetro dos zigotos 120-150*μ;* espessura 80-98*μ* (nos nossos exemplares: células vegetativas: 160--245 (300) X 142-154 *μ;* zigotos 120-212 X 120-142 *μ* ; espessura 87,5 *μ)*.

BEIRA, LITORAL: S. João do Campo, num charco, muito rara, 17-VII-1972, *Fátima Santos* 673E (COI).

Distribuição geográfica: Europa e África.

Nota: Os nossos exemplares apresentam um número variável de plastos, podendo estes atingir 12, sendo por vezes quase direitos.

Spirogyra semiornata Jao in Sinensia 6: 604 (1935).— Est. XIV, fig. 2.

Células vegetativas com paredes transversais replicadas, providas de um plasto enrolado em 2-6 espiras. Conjugação escalariforme e lateral, havendo contribuição dos dois gametângios na formação do tubo; gametângio feminino ligeiramente dilatado pelo zigoto. Zigotos elipsoidais, de polos arredondados; mesósporo liso, amarelo-acastanhado. Aplanósporos subglobosos, subelipsoidais ou ovoides. Dimensões: células vegetativas : comp. 96-245 μ ; larg. 25-33 μ ; zigotos : comp. 60-110 (180) µ; larg. 30-46*µ;* aplanósporos: comp. 35-51 µ; larg. 32-38 µ (nos nossos exemplares: células vegetativas 120-228 X 27,5-33*µ;* zigotos 84-107,5 X 32,5-43 *µ)*.

BEIRA LITORAL: S. João do Campo, num charco, muito rara, 17-VII-1972, *Fátima Santos* 673E (COI).

Distribuição geográfica: Europa, Ásia (China), África e América do Norte.

Desmidiaceae

Staurastrum bibrachiatum Reinsch emend. Grönblad & Scott in Acta Soc. F. Fl. Fenn. 72, 6: 3 (1955). —Est. XV, fig. 1.

Células pequenas, podendo apresentar três aspectos diferentes consoante o número de apófises, as quais são serreadas, com 2-4 espinhos terminais. Semicélulas sem ou com 2 apófises apicais, um pouco divergentes e 2 basais, horizontais, ligeiramente inclinadas, afastando-se do istmo,

ou apenas 2 apófises apicais mais estreitas e compridas do que no tipo anterior; outras células apresentam 6 apófises por coexistirem os 2 tipos referidos. Dimensões: comp, com apófises 25,5-42 μ ; comp, sem apófises 11-12 μ ; larg. com apófises 28-61 μ (nos nossos exemplares : células com apófises 28,7-32 X 23-30,7 µ; comp, sem apófises 10,5-12 μ; istmo 5,7µ).

BEIRA LITORAL: pr. Vila Franca, a 1,5 km de Arazede, na estrada para Cantanhede, num charco, pouco abundante, 27-VI-1973, *Celeste Alves* **107** (coi).

Distribuição geográfica: Europa (Itália) e América.

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ESTAMPAS

ESTAMPA I

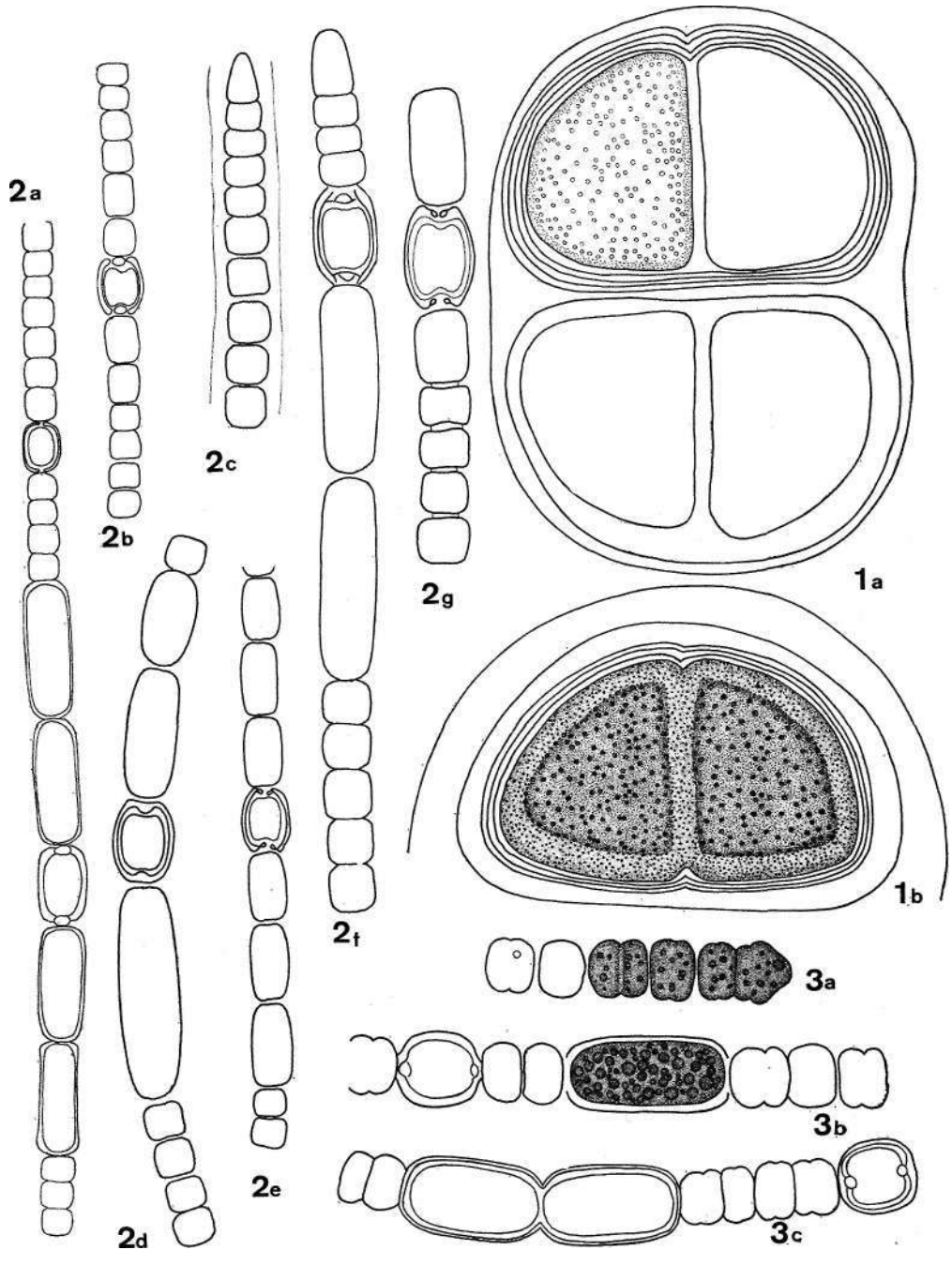
Fig. 1. — a, b — *Chroococcus turgidus* (Kütz.) Näg. var. *maximus* Nygaard
a — colónia
b — pormenor das células.

2.— (a-g)—*Anabaena cylindrica* Lemm.
a, b, d, e, g— porções medianas do filamento mostrando acinetos e heterocistos
c, f — porções terminais com a célula terminal cónica.

3.— (a-c)—*Anabaena laxa* (Rabenh.) A. Braun
a — extremidade do filamento e pormenor do conteúdo celular
b, c — porções medianas do filamento com acinetos e heterocistos.

Ampliações

Fig. 1 a, b (X 1350).
Fig. 2 a, b, c (X1000).
Fig. 2 d, e (X 1380).
Fig. 2 f, g (X 2000).
Fig. 3 a, b, c (X 1650).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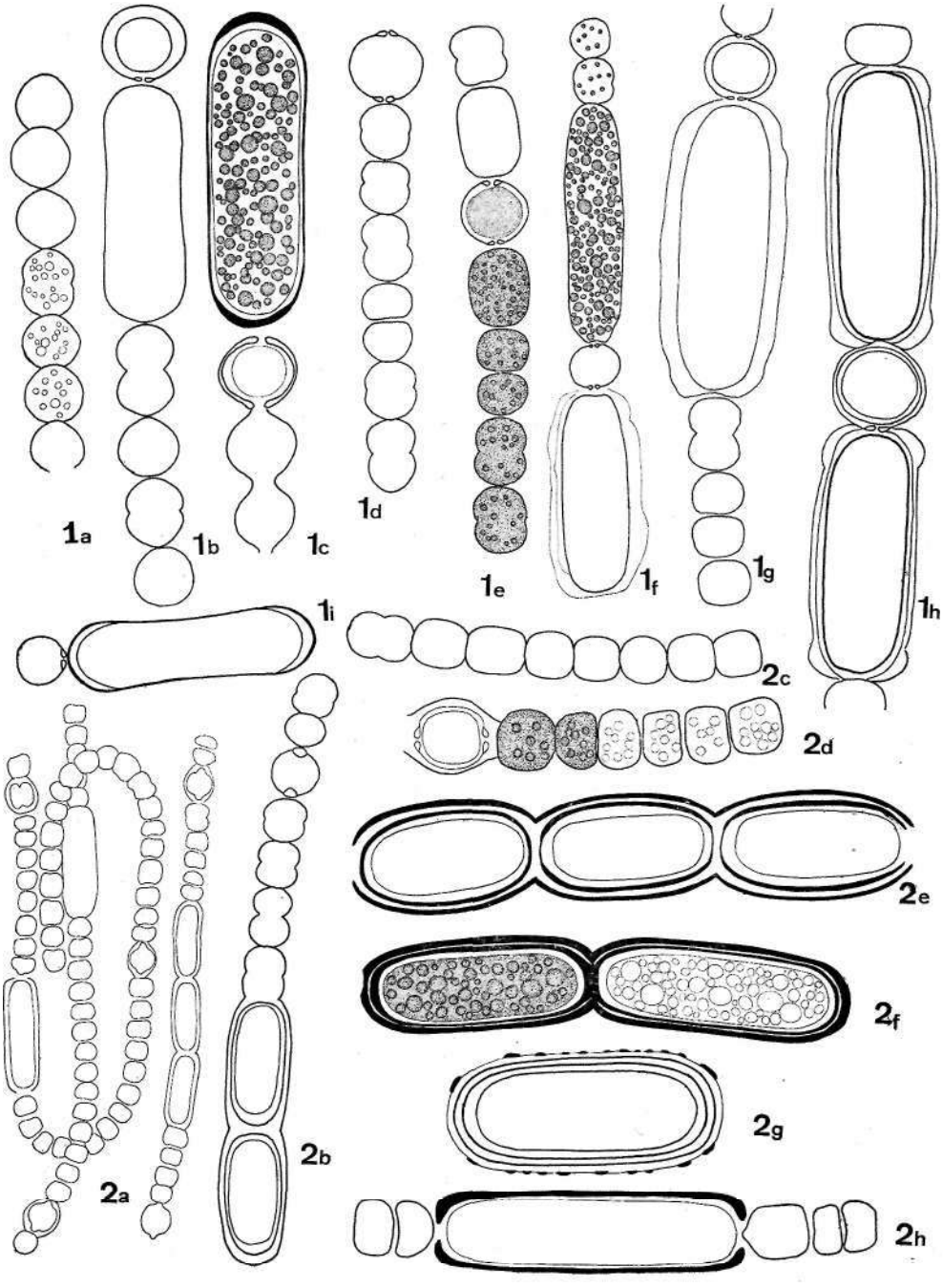
ESTAMPA II

— (a-i)—*Anabaena lapponica* Borgen
a, b, c, i — vários aspectos de exemplares colhidos próximo de Vila Franca
d-h — porções de filamentos colhidos em Travanca.

— (a-h) — *Anabaena saaremaaensis* Skuja
a—-tricomas: enrolado e direito
b, c — pormenor da forma das células
d — porção de um filamento mostrando o conteúdo celular granuloso
e-h — alguns aspectos apresentados pelos acinetos.

Ampliações

Fig. 1 a, b, d, e, g, h, i; 2 c (X 1340).
Fig, 1 c; 2 d-h (X 2000).
Fig. 1 f; 2 b (X 980).
Fig. 2 a (X 550).



ESTAMPA III

Fig. 1. — (a-f)—*Phacus oscillans* Klebs
a-e — aspectos da célula em várias posições; em e e e mostram a ornamentação do periplasto
f — vista apical (esquemática)

2. — (a-e) —*Phacus agilis* Skuja
a-d — vários aspectos da célula
e — pormenor do conteúdo celular.

3.— (a-d)—*Phacus platyaulax* Pochm.
a — figura mostrando as calotes de paramilo e a ornamentação do periplasto
b — desenho mostrando o sulco oblíquo
c — célula em posição lateral
d — vista apical.

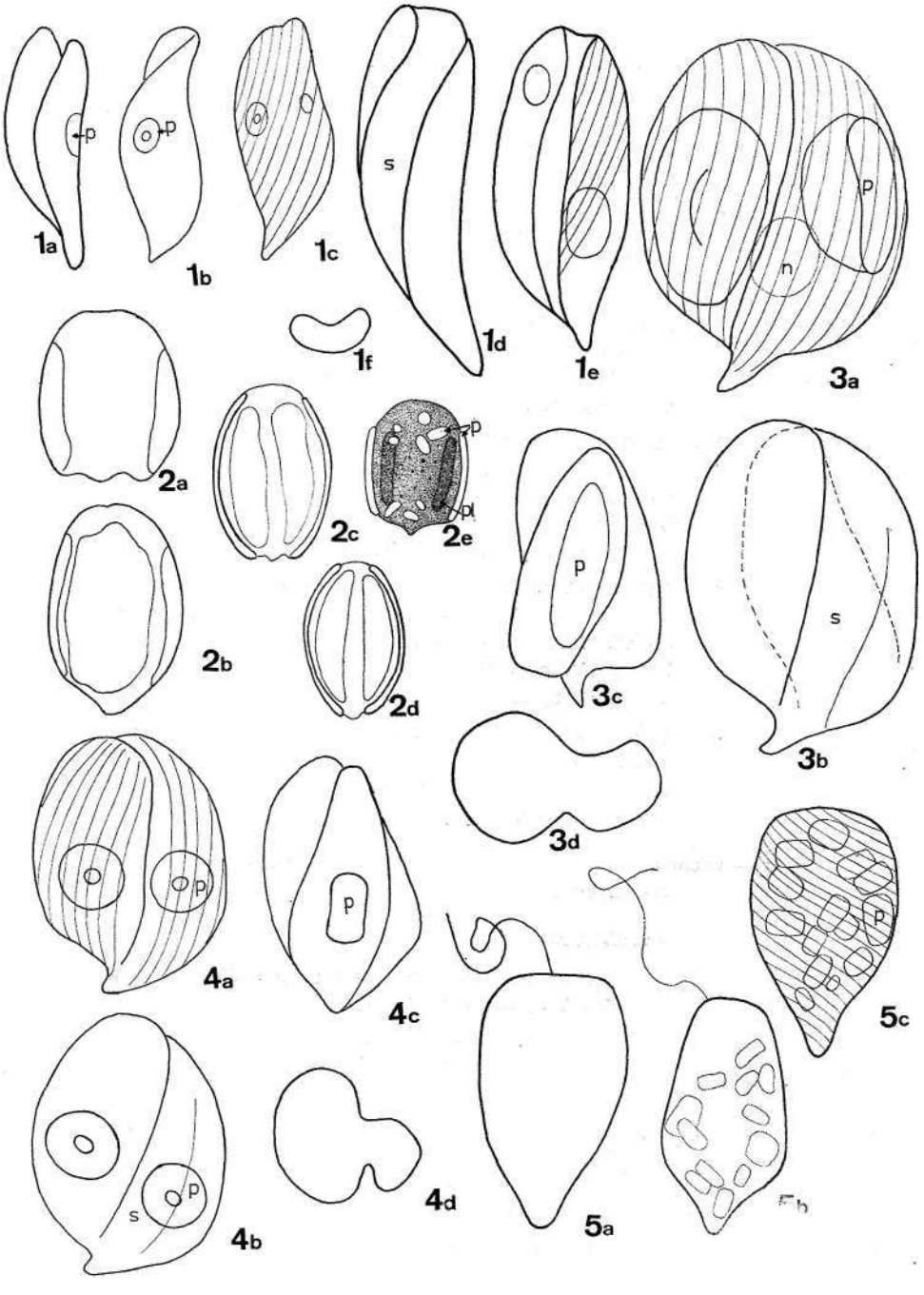
4. — (a-d) —*Phacus anomalus* Fritsch et Rich. var. *pullus--gallinae* Nygaard
a — periplasto estriado e anéis de paramilo
b — sulco oblíquo e paramilo
c — vista lateral
d—vista apical.

5.— (a-c)—*Astasia pygmaea* Skuja
a — forma normal da célula
b — célula modificada pelo metabolismo
c — periplasto estriado e bastonetes de paramilo.

ρ — paramilo; pl — plastos; s — sulco; n — núcleo.

Ampliações

Fig. 1 a-c; 2 e; 3 a, b; 4 a-c (X 1350).
Fig. 1 d, e; 2 a, b; 5 a-c (X 2025).
Fig. 2 c, d (X1620).
Fig. 3 c (X 1080).
Fig. 3 d; 4 d (X 1025).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ESTAMPA IV

Fig. 1. — a, b — *Sphenomonas teres* (Stein) Klebs
a — aspecto do conteúdo celular
b — periplasto estriado longitudinalmente.

2.— (a-c)—*Petálomonas meãiocanellata* Stein var. *disumata* (Stokes) Denim.
a — célula mostrando o sulco mediano profundo
b — vista apical
c — vista lateral.

3.— (a-e)—*Petalomonas dubosquii* Holl.
a — célula mostrando uma quilha mediana e o núcleo com o nucléolo volumoso
b-d — aspectos da célula noutras posições
e — vista apical (esquemática).

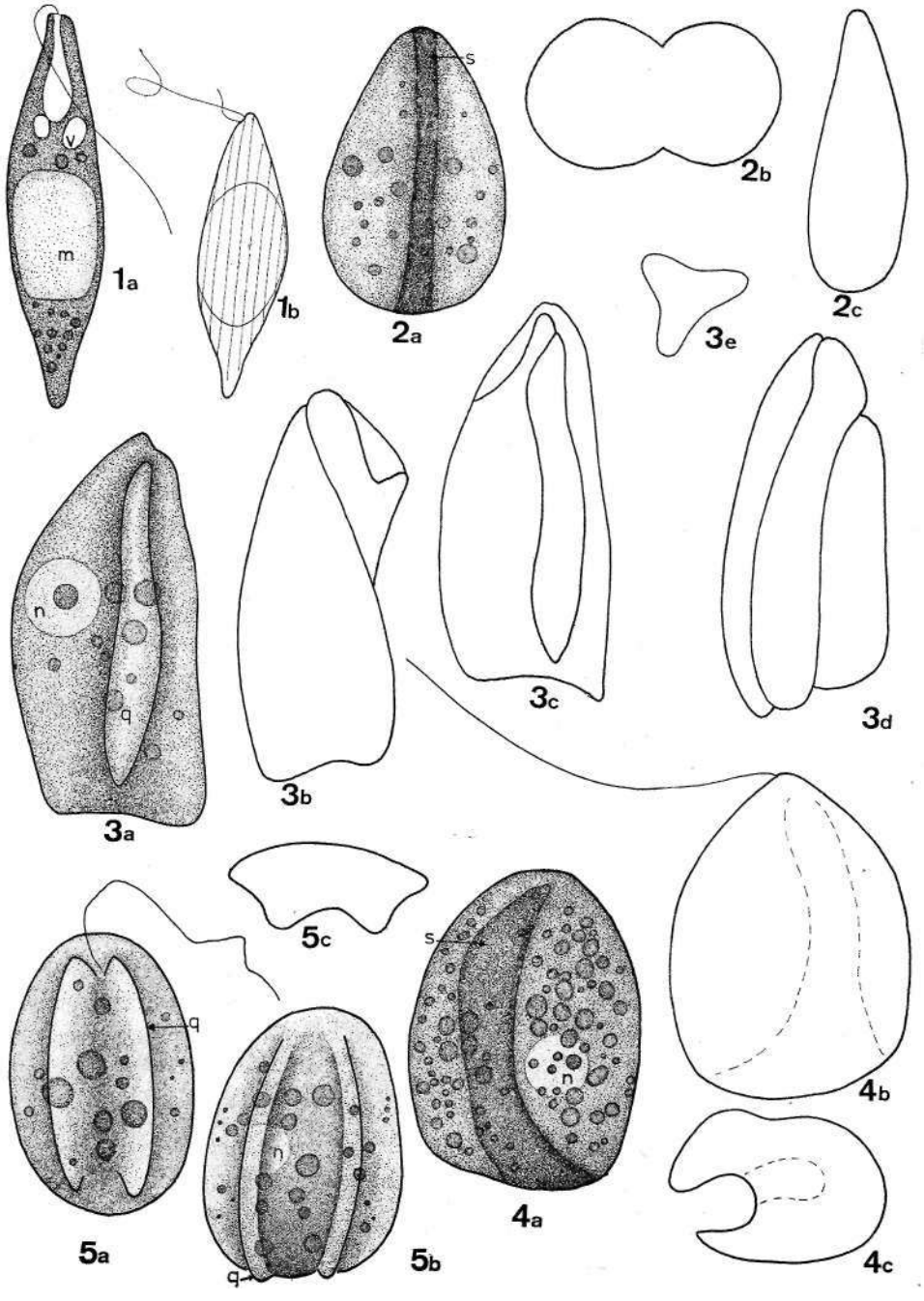
4.— (a-c)—*Petalomonas asymetria* Shawh. et Jahn
a — figura mostrando o sulco oblíquo, as granulações do citoplasma e o núcleo
b—-célula com flagelo
c—-vista apical.

5. — (a-c) —*Petalomonas abscissa* (Duj.) Stein var. *parallela* Klebs
a, b — aspectos da célula com as duas quilhas
c —vista apical.

ν — vacùolo; m — massa gelatinosa; s — sulco; q — quilha; n — núcleo.

Ampliações

Fig. 1 a; 2 a-c; 3 a-d; 4 a-c (X2025).
Fig. 1 b; 5 a-c (X 1350).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ESTAMPA V

1. — *Chroomonas coerulea* (Geitler) Skuja,

2. — (a-e) — *Cystodinium brevipes* Geitler
a-c, e — células vegetativas
d — célula com zoosporo.

3. — *Sphaeridiothrix globulosa* Pascher

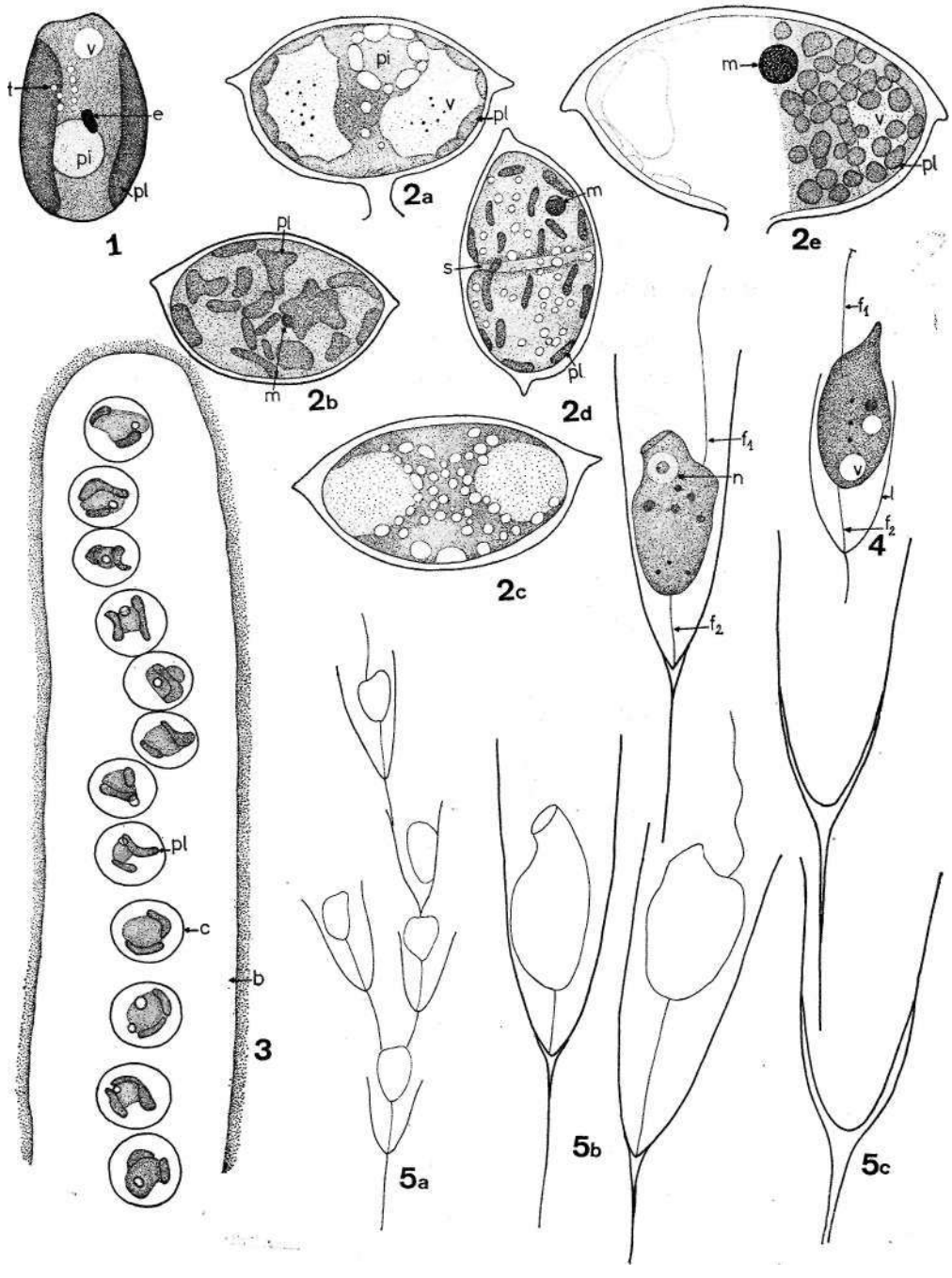
4. — *Bicoeca kepneri* Reynolds

5. — (a-c)—*Bicoeca petiolata* (Stein) Pringsh.
a — aspecto da colonia
b — porção da colonia com pormenor de uma célula
e — lorica.

plasto; pi — pirenóide; ν — vacùolo; m — mancha pigmentar; s — sulco transversal; η — nucléolo; f1 — flagelo anterior; f_2 — flagelo posterior; 1 — lorica; b — bainha posta em evidencia com tinta da china; e — célula; t — tricocistos; e — estigma.

Ampliações

Fig. 1; 4; 5 b, c (x 3000).
Fig. 2 a-d; 3(X 1350).
Fig. 2 e (X2000).
Fig. 5 a (X1275).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ESTAMPA VI

Fig. 1. — a, b — *Pseudodendromonas vlkii* Bourr.
a —colónia
b — pormenor das células.

2. — (a-c) — *Spongomonas uvella* Stein, porções da colònia mostrando a matriz granulosa e as células biflageladas.

3. — (a-c) — *Monosiga varians* Skuja var. *vagarìs* Skuja

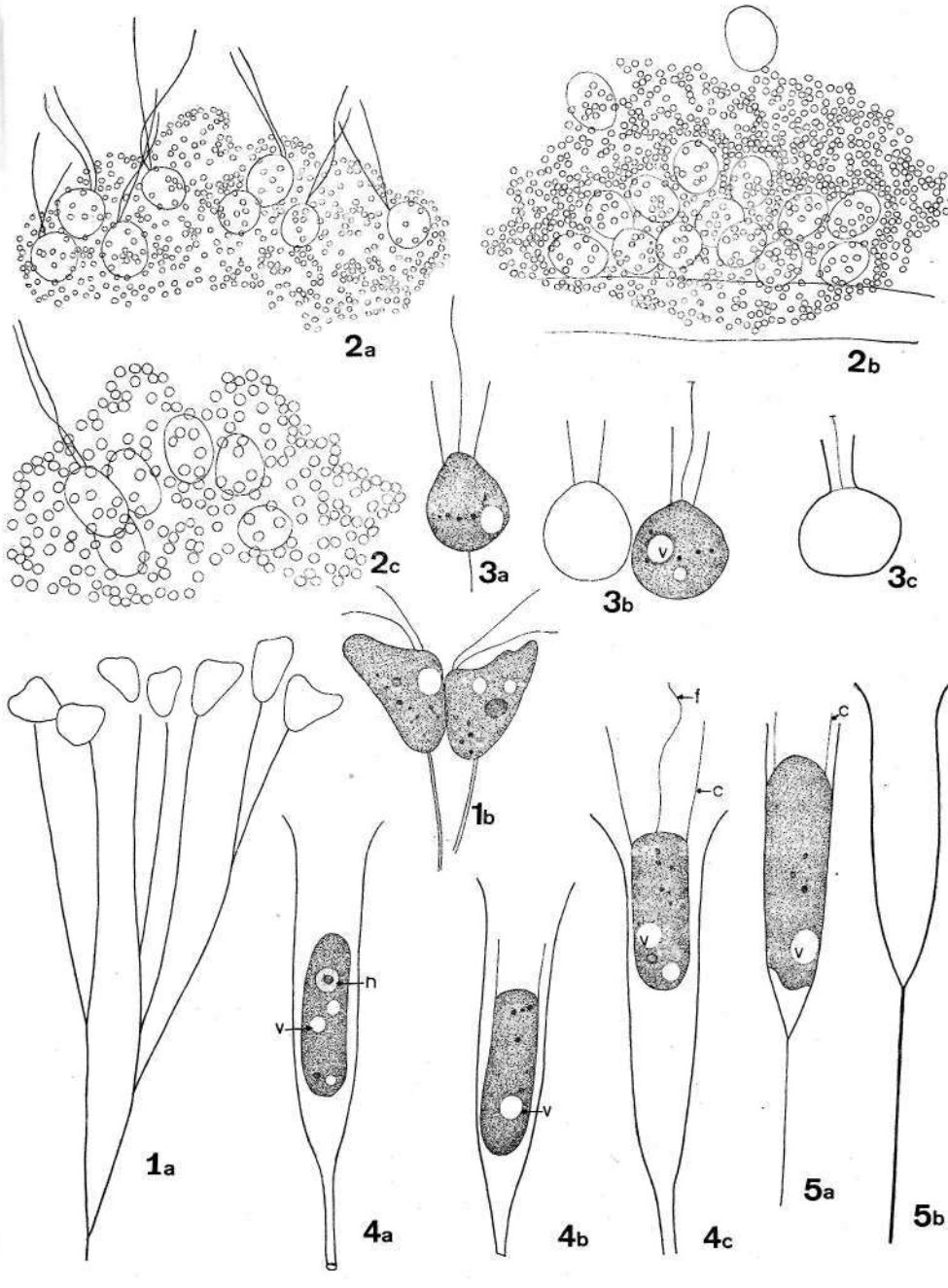
4. — (a-c) — *Salpingoeca gracilis* Clark

5. — a, b — *Salpingoeca gracilis* Clark var. *abbreviata* Skuja
a — lorica e citoplasma
b — lorica.

v — vacùolo; n — nucléolo; f—flagelo; c—colar citoplasmàtico.

Ampliações

Pig. 1 a (X 1840).
^ Fig. 1 b; 3 a-c (X 3000).
Fig. 2 c; 4 a-c; 5 a, b (X 2000).
Fig. 2 a, b (X 1350).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ESTAMPA VII

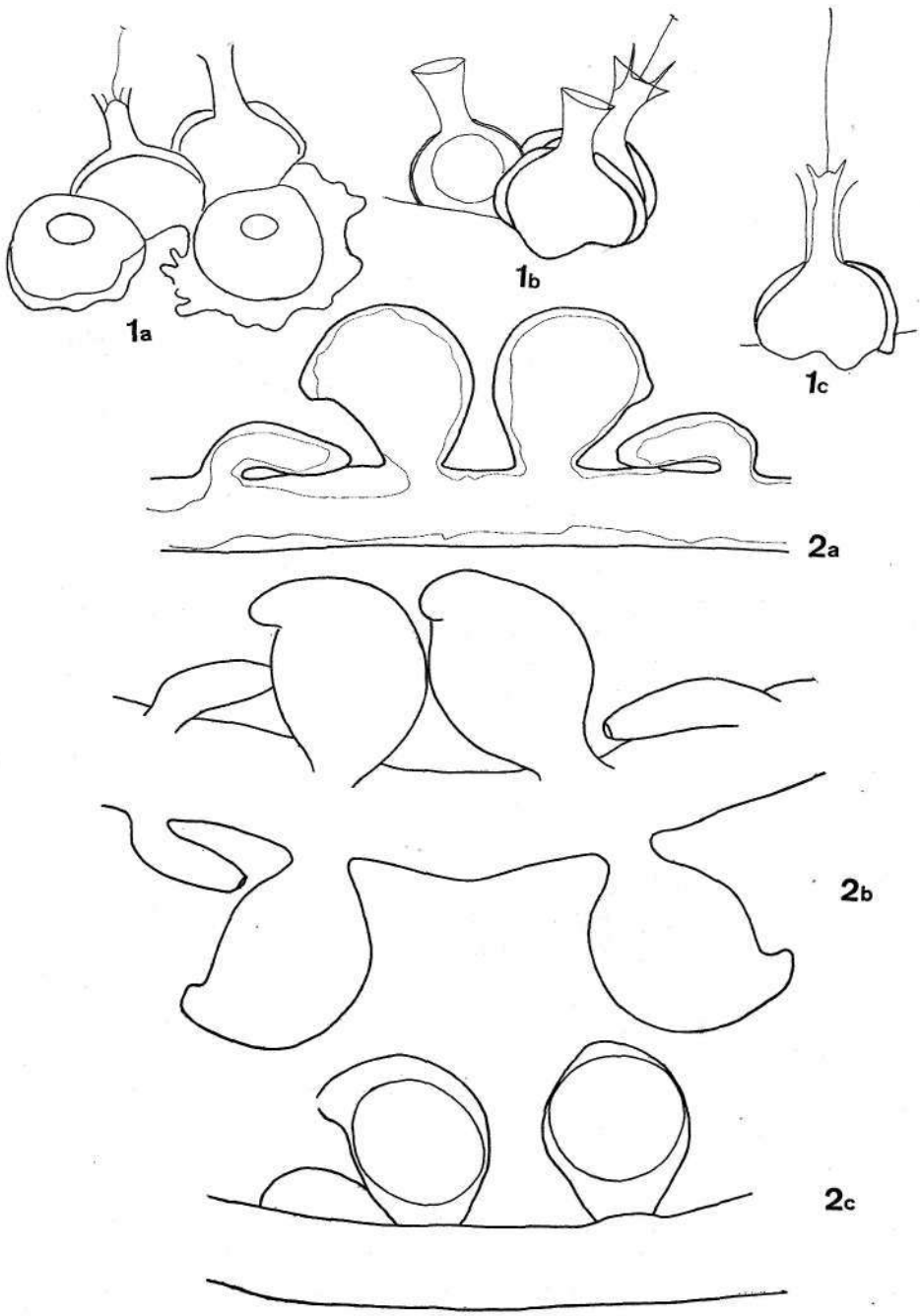
Fig[1]. 1. — (a-c)—*Diploeca flava* (Korsch.) Bourr.

2. — (a-c) — *Vaucheria aversa* Hassall
a, b — porção do filamento com oogónios e anterídios
e — zigotos.

f — flagelo; 1 — lorica; e — colar citoplasmàtico.

Ampliações

Fig. 1 a-c (X 3000).
Fig. 2 a-c (X215).


1a
1b
1c
2a
2b
2c

ESTAMPA VIII

Fig·. 1.— (a-d)—*Stigeoclonium subsecundum* (Kütz.) var. *tenue* Nordstedt
a — porção terminal de um filamento
b — porção basal com rizoide
e — região mediana
d — pormenor das células.

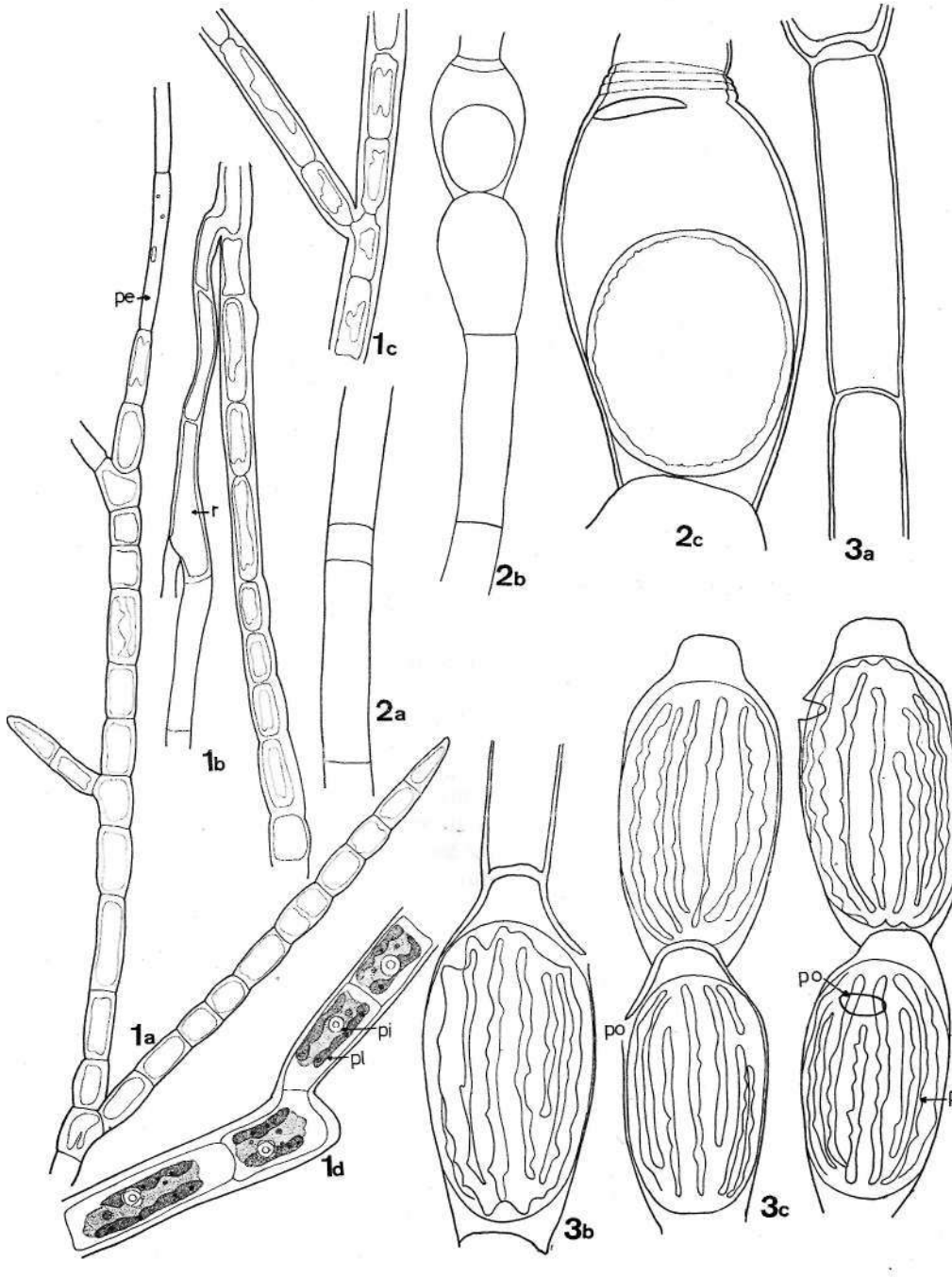
2.— (a-c)—*Oedogonium rivulare* (Le Clerc) Al. Braun
a — filamento masculino
b-c — filamentos femininos com zigotos.

3.— (a-c)—*Oegogonium crenulato-costatum* Wittrock var. *cylinäricum* (Him) Tiffany
a — célula sufultória e parte de célula vegetativa
b, c — zigotos.

r — rizoide; pe — pêlo terminal; pl — plasto; pi — pirenóide; po — poro do oogónio; pr — prega longitudinal.

Ampliações

Fig. 1 a-c; 2 c(X 550).
Fig. 1 d (X 1350).
Fig. 2 a, b (X215).
Fig. 3 a-c (X850).


pe
r
1a
1b
1c
1d
2a
2b
2c
3a
3b
3c
pi
pl
po
po
pr

ESTAMPA IX

Fig·. 1. — (a-c)—*Oedogonium boscii* (Le Clerc) Wittrock
a — porção do filamento com células vegetativas, oogónio e zigoto
b, c — pormenor do zigoto.

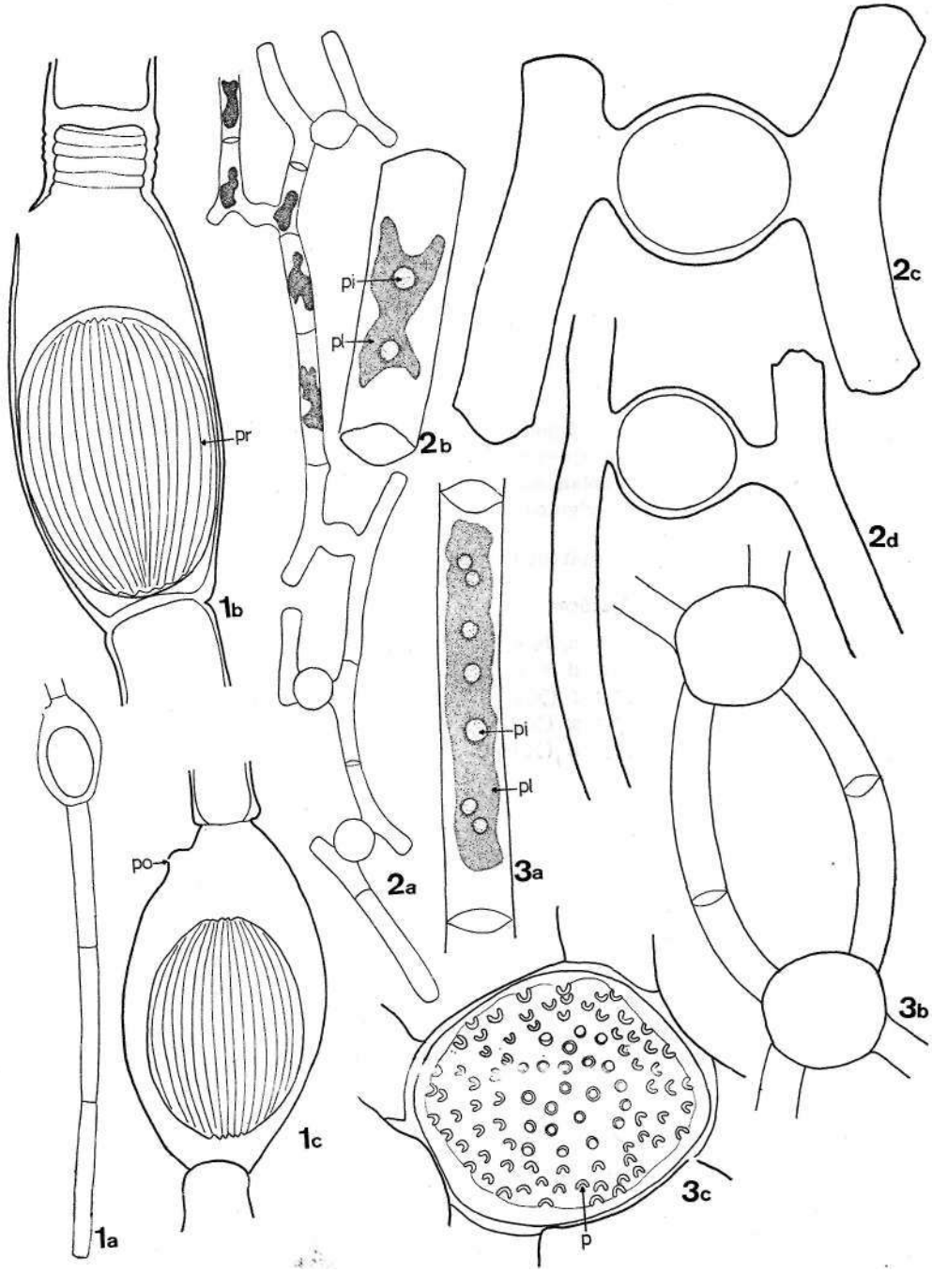
2.— (a-d)—*Mougeotia recurva* (Hassall) De Toni
a — filamentos em conjugação
b — célula vegetativa
c, d — zigotos.

3.— (a-c)—*Mougeotia quadrangulata* Hassall
a — célula vegetativa
b — zigotos
c — parede do zigoto com pontuações.

po — poro; pr — pregas longitudinais; pi — piaste; pi — pirenóide; ρ — • pontuações.

Ampliações

Fig. 1 a; 2 a (X 215).
Fig. 1 b, c (X 650).
Fig. 2 b, c; 3 a (X850).
Fig. 2 d; 3 b (X 550).
Fig. 3 c (X 1350).


pi
pl
pr
2b
2c
2d
1b
pi
pl
3a
po
2a
1c
3b
3c
p
1a

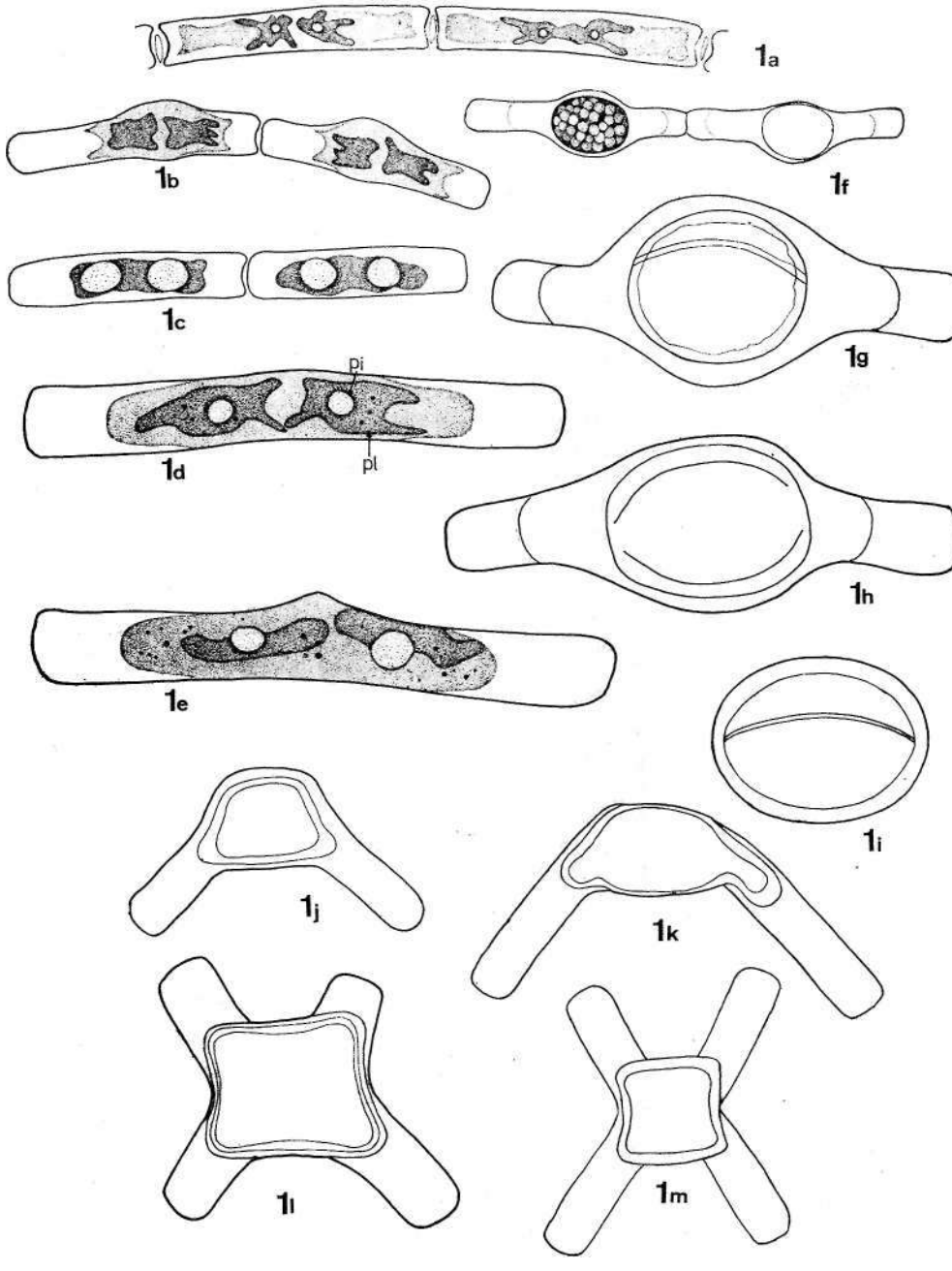
ESTAMPA X

Fig. 1. — (a-m)—*Zygnemopsis minuta* Rhand.
a-e — pormenor dás células
f -k — aplanósporos
1, m — zigotos.

pi — plasto; pi —· pirenóide.

Ampliações

Fig. a, b, c, j, l, m (X 850).
Fig. d, e, h, i (X 1080).
Fig. f (X 425).
Fig. g (X 1225).
Fig. k (X 650).


1a
1b
1f
1c
1g
pi
1d
pl
1h
1e
1i
1j
1k
1l
1m

ESTAMPA XI

Fig[1]. 1.— (a-e)—*Mougeotia laetevirens* (A. Braun) Wittrock
a — célula vegetativa
b, e — zigotos.

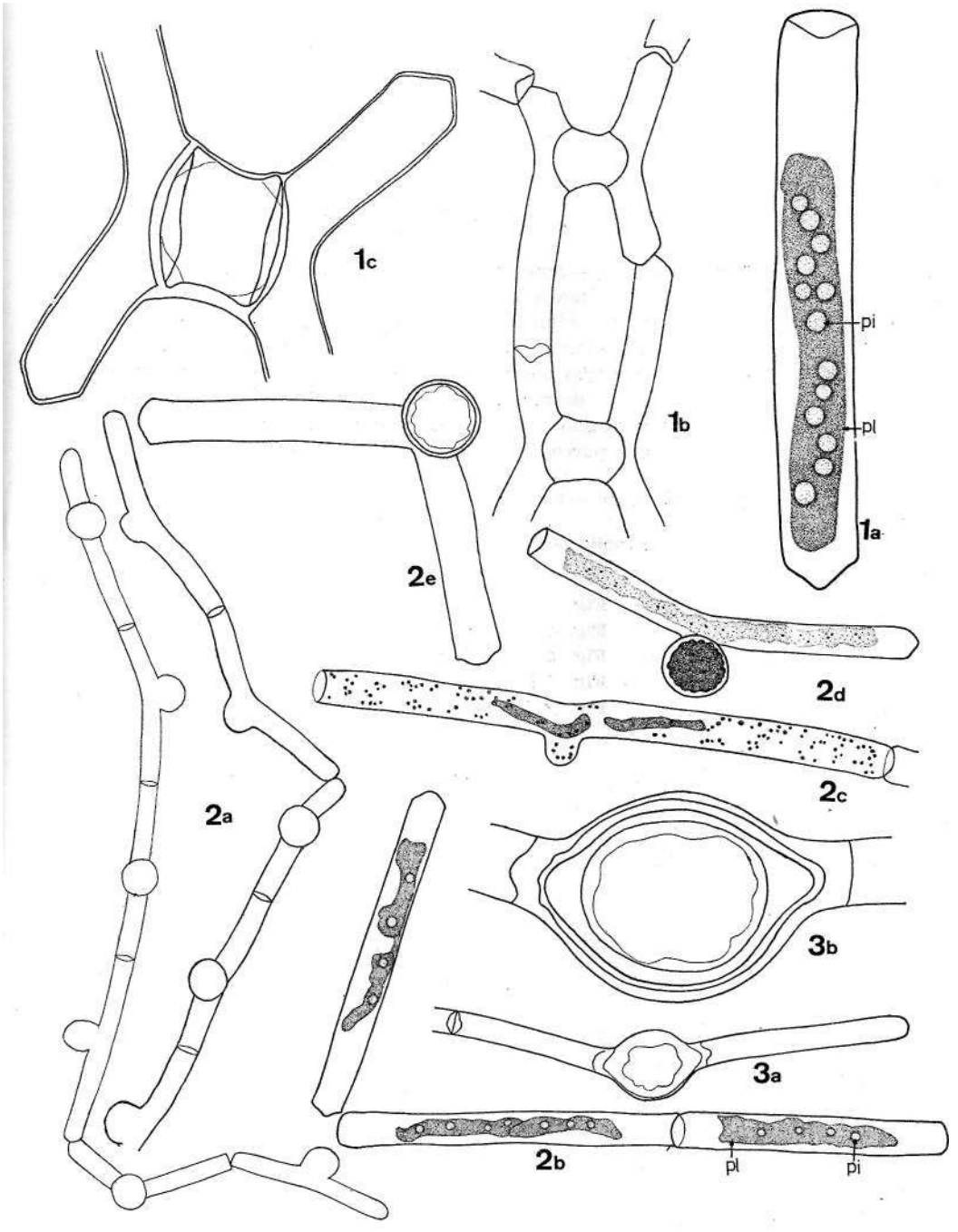
2. — (a-e) — *Mougeotia rava* Transeau
a — filamentos com aplanósporos
b — células vegetativas
c — célula diferenciando urn aplanósporo
d, e — aplanósporos.

3.— (a-b)—*Mougeotia ventricosa* (Wittrock) Collins
a, b — aplanósporos.

pi—plasto; pi — pirenóide.

Ampliações

Fig. 1 a, e; 2 e (χ 510).
Fig. 1 b (X 280).
Fig. 2 a (X 215).
Fig. 2 b-d (X435).
Fig. 3 a (X 650).
Fig. 3 b (X2000).


1c
1b
pi
pl
1a
2e
2d
2c
2a
3b
3a
2b
pl
pi

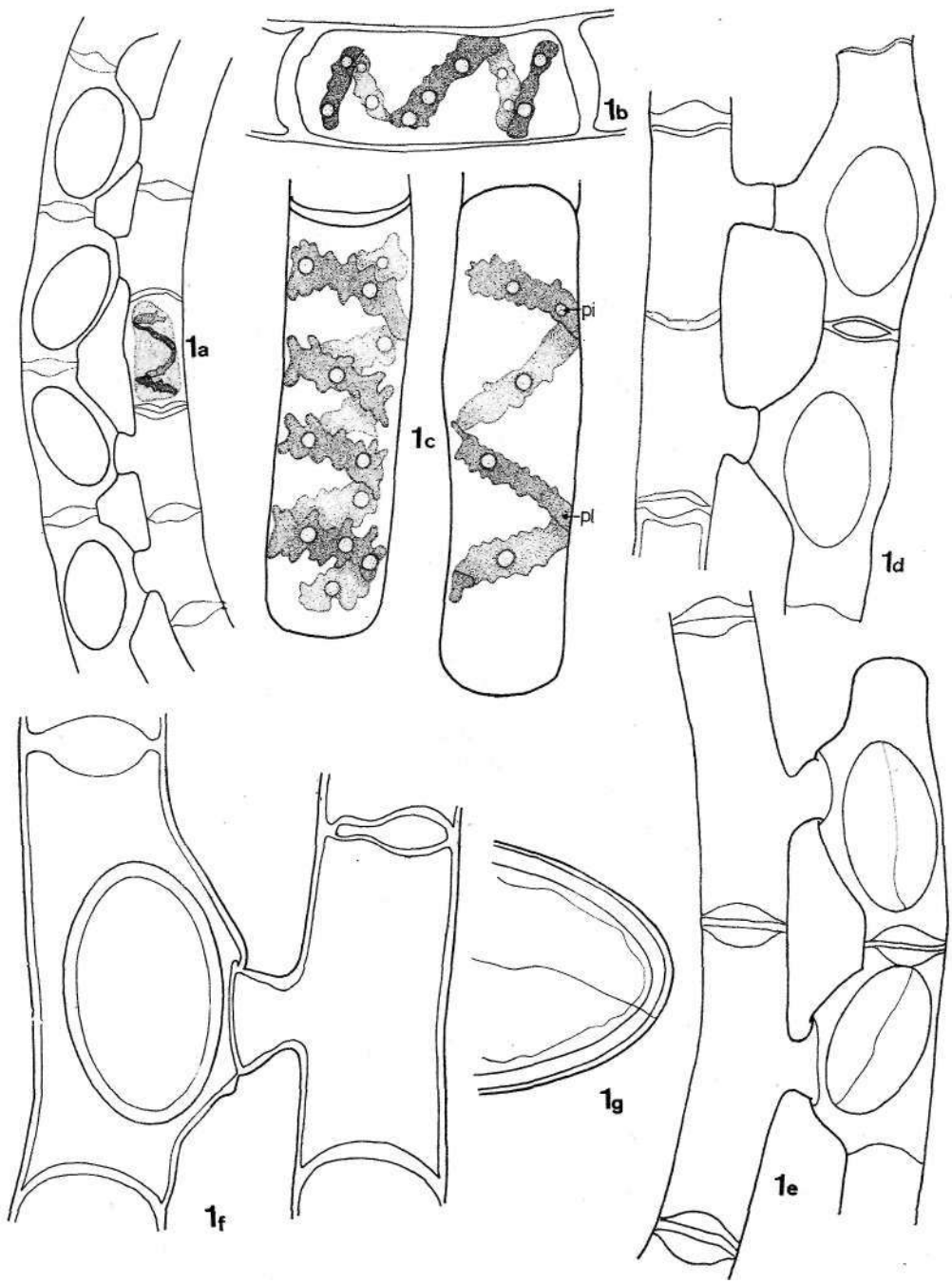
ESTAMPA XII

Flg. 1. — (a-g) — *Spirogyra gracilis* (Hassall) Kütz.
a — filamentos em conjugação
b, c — células vegetativas
d — filamentos em conjugação, sendo evidente a contribuição dos dois gametângios na formação do tubo
e, f — outros aspectos, mostrando a dilatação do gametângiò feminino na face de conjugação e zigotos
g — parede do zigoto.

pi — plasto; pi — pirenóide.

Ampliações

Pig. a (X 280).
Fig. b (X 550).
Fig. c (X 820).
Fig. d, e (X 410).
Fig. f (X 930).
Fig. g (X 1020).


1a
1b
1c
pi
pl
1d
1e
1f
1g

ESTAMPA XIII

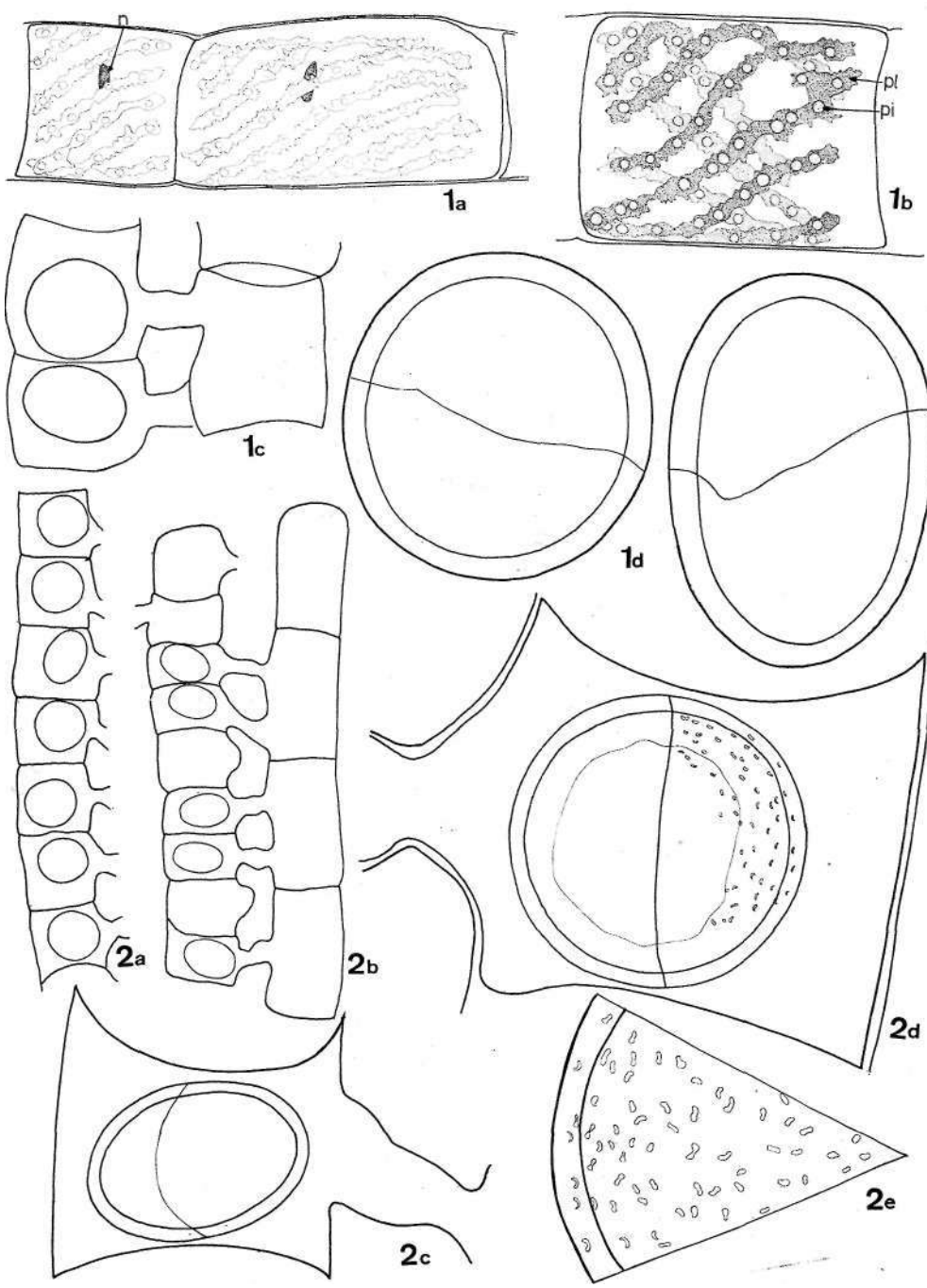
1. — (a-d) —*Spirogyra submascima* Transeau
   a, b — células vegetativas
   c, d — zigotos.

2.— (a-c)—*Spirogyra pseudomajuscula* Gauthier-Lièvre
   a — filamento com zigotos
   b — conjugação anormal (o conteúdo de um gametângio fecunda dois gametângios adjacentes)
   c, d — zigotos
   e — parede do zigoto.

plasto; pi — pirenóide; n — núcleo.

Ampliações

Fig. 1 a, c (X 280).
Fig. l b ; 2 c (X 410).
Fig. 1 d (X850).
Fig. 2 a, b (X 140).
Fig. 2 d (X 550).
Fig. 2 e (X 1350).


n
1a
pl
pi
1b
1c
1d
2a
2b
2c
2d
2e

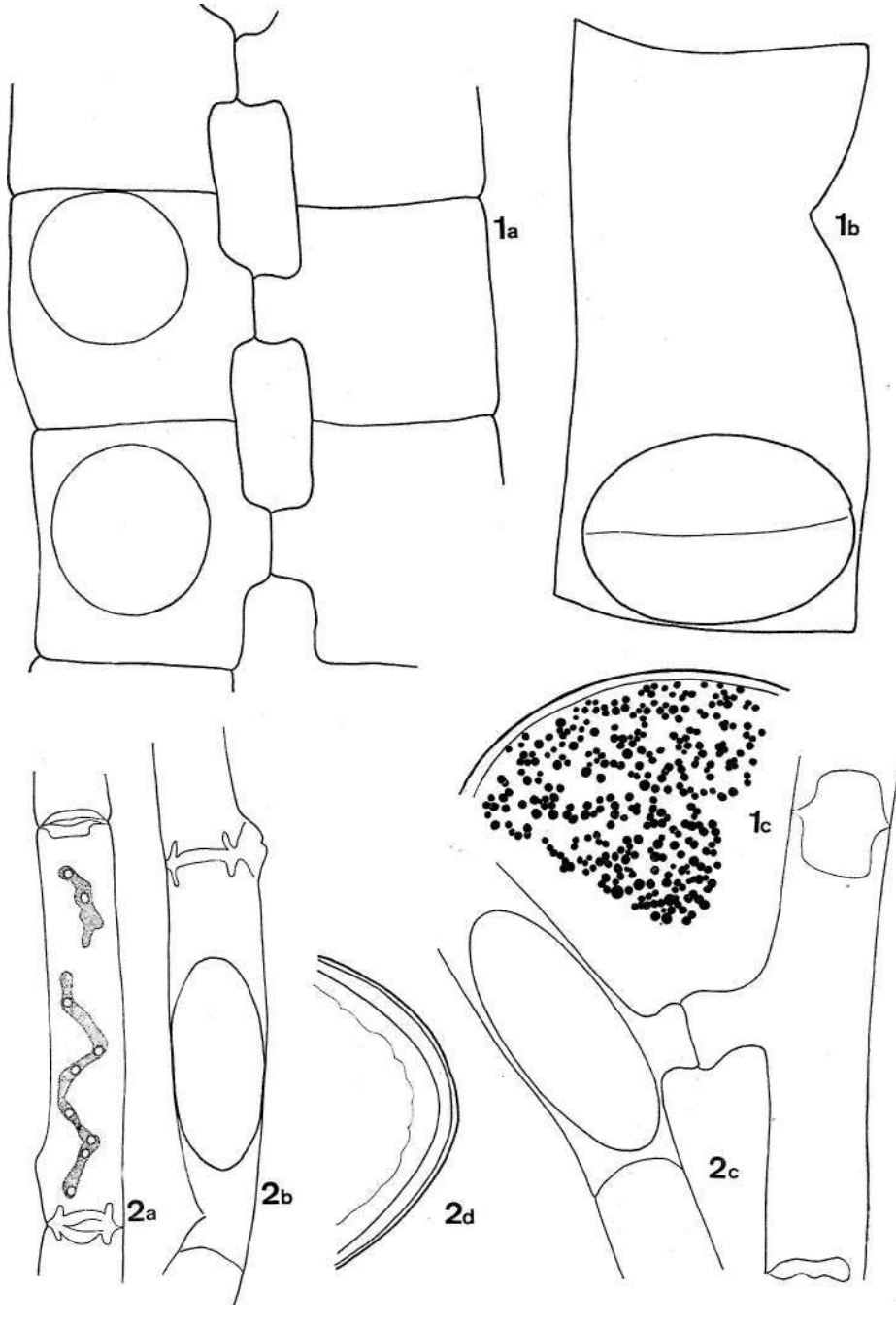
ESTAMPA XIV

Fig. 1. — (a-c) — *Spirogyra heeriana* Näg.
a — filamentos em conjugação
b — zigoto
e — parede do zigoto.

2. — (a-d) — *Spirogyra semiornata* Jao
a — célula vegetativa
b — filamento onde ocorreu conjugação lateral
e — conjugação escalariforme
d — parede do zigote

Ampliações

Fig. 1 a (X 175).
Fig. 1 b (X 280).
Fig. 1 c (X 850).
Fig. 2 a, b (X 410).
Fig. 2 c (X 550).
Fig. 2 d (X 2000).


1a
1b
1c
2a
2b
2c
2d

ESTAMPA XV

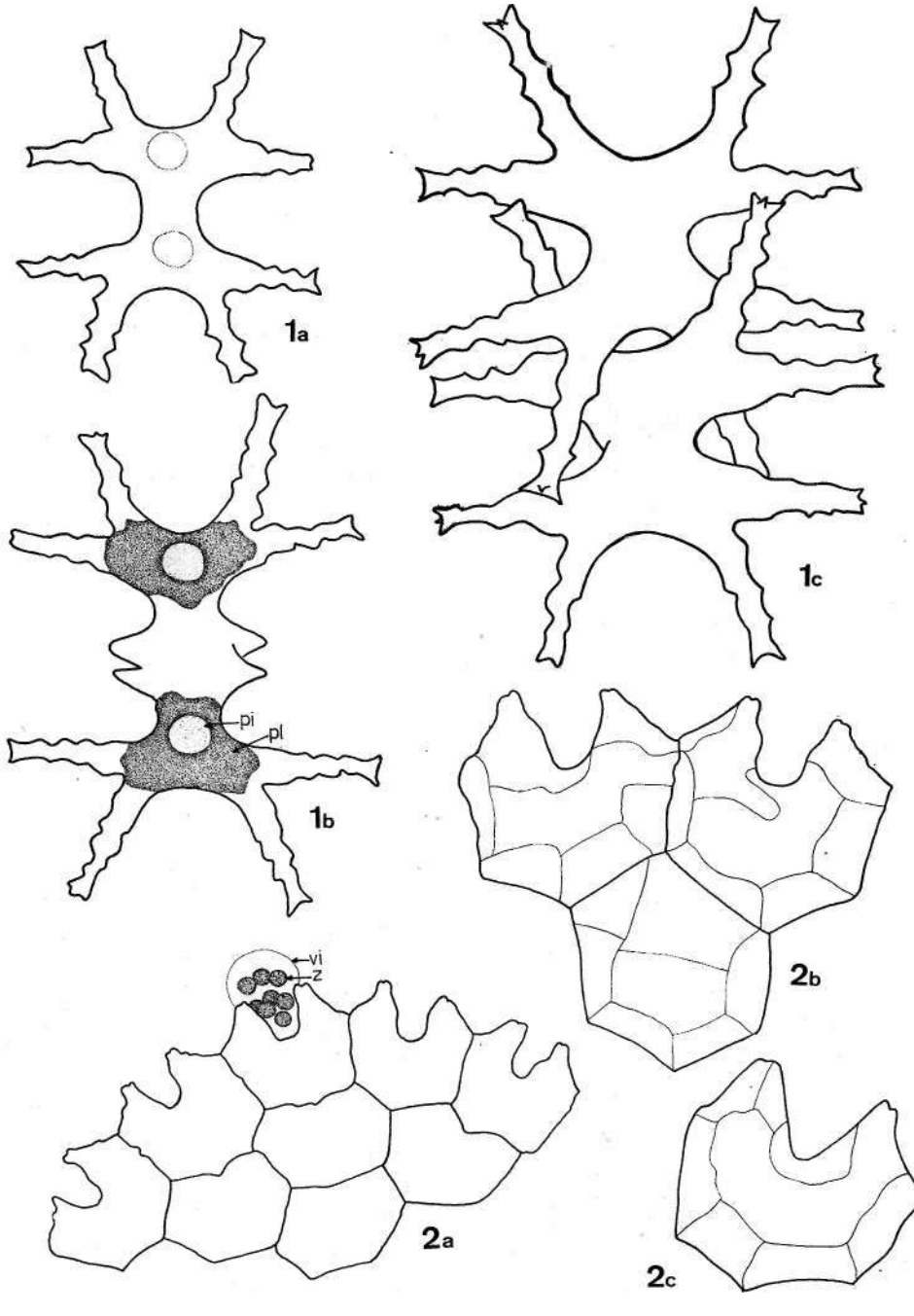
Fig. 1. — (a-c) — *Staurastrum bibrachiatum* Reinsch
a — célula
b — célula em divisão
c — células permanecendo juntas após a divisão.

2.— (a-c)—*Pediastrum arenosum* (Racib.) G. M. Smith
a — porção do cenobio com uma célula produzindo zoosporos
b, c — pormenor da parede.

pi — plasto; pi — pirenóide; ζ— zoosporos; ve — vesícula.

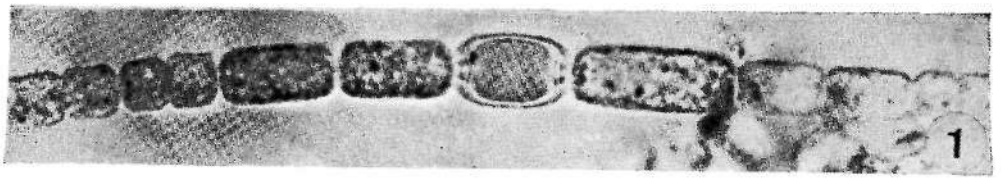
Ampliações

Fig. 1 a (X 1000).
Fig. 1 b, c; 2 b, c (X 1350).
Fig. 2 a (X 850).


1a
1b
pi
pl
1c
2b
vi
z
2a
2c

ESTAMPA XVI

Foto 1. — *Anabaena cylindrica* Lemm.
2-5. — *Anabaena saaremaaensis* Skuja
6-8.—*Anabaena laxa* (Rabenh.) A. Braun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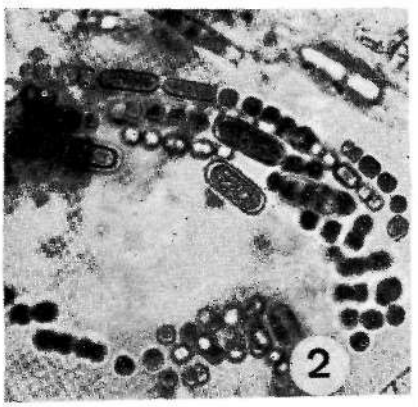
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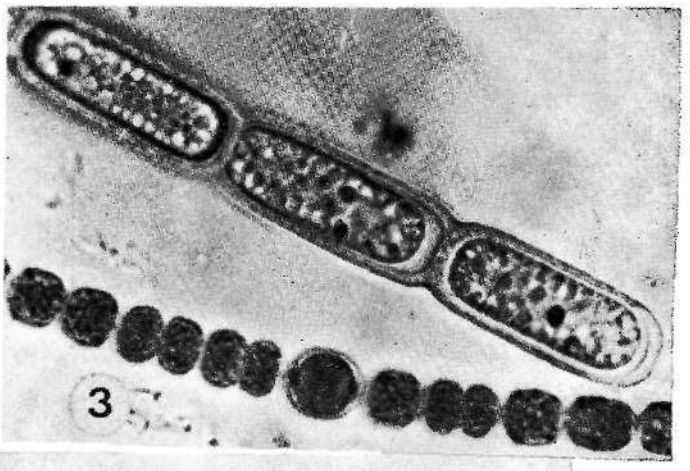
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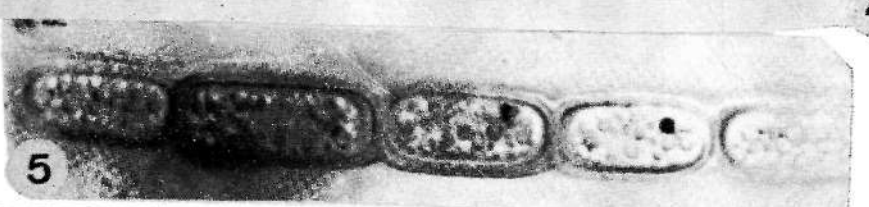
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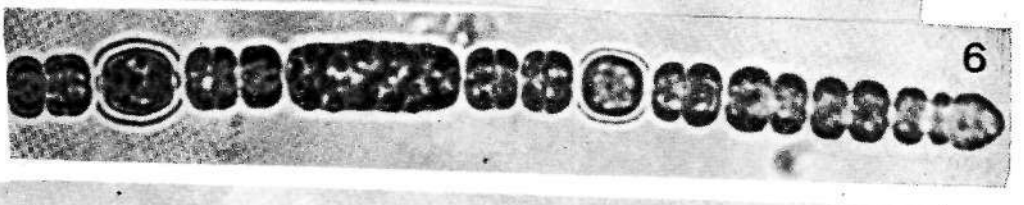
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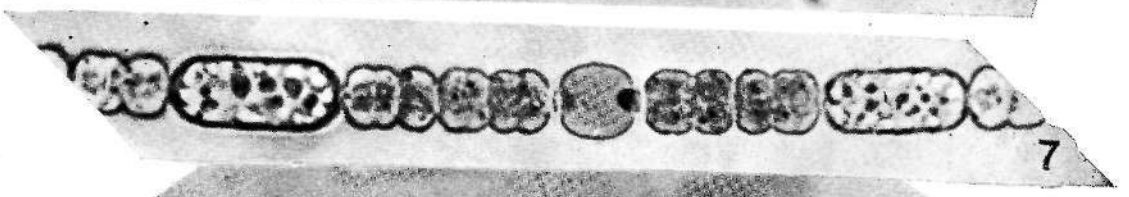
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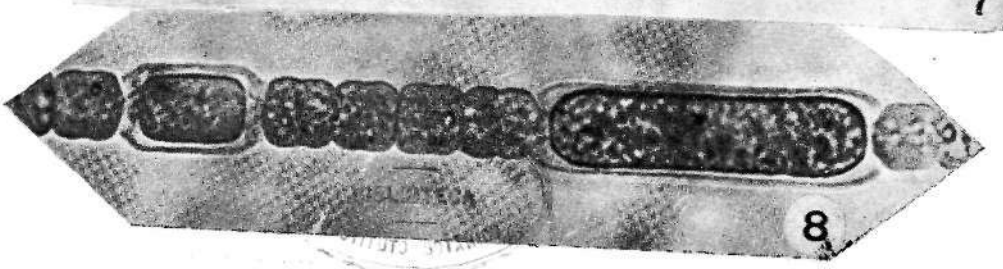
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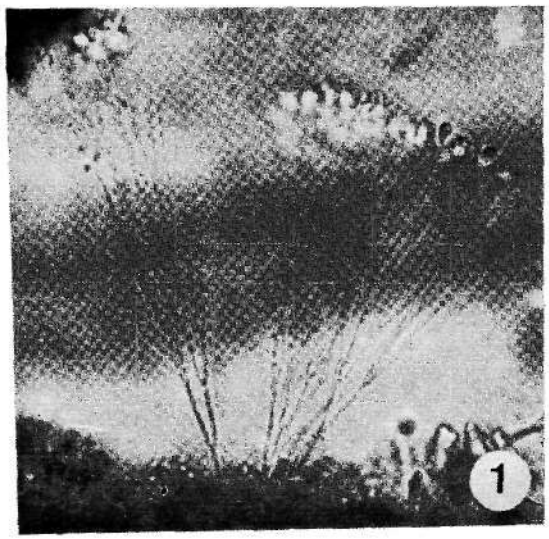
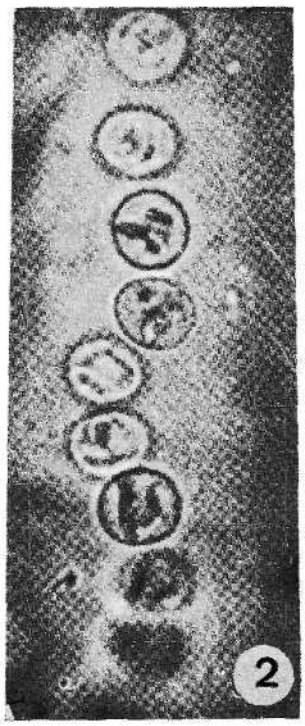

1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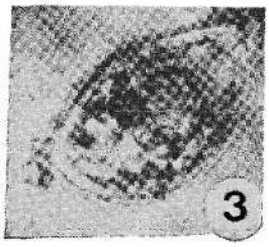
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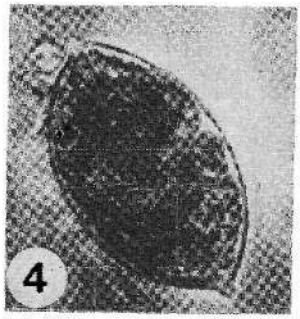
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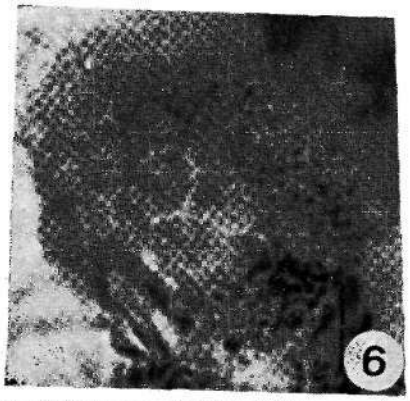

6


7

ESTAMPA XVII

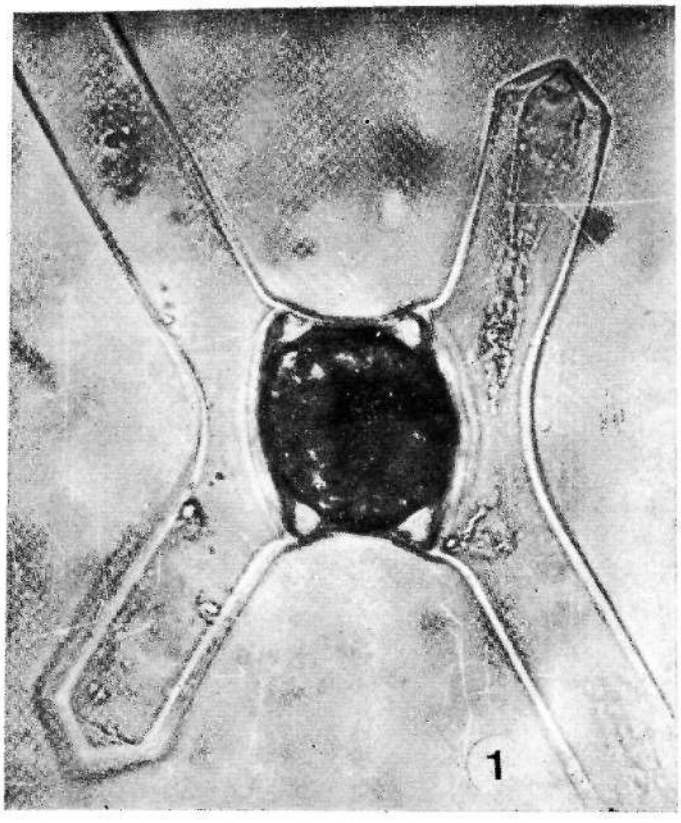
Foto 1. — *Pseudodendromonás vlkìi* Boürr.
2. — *Sphaeridiothrix globulosa* Pascher
3, 4. — *Cystodinium brevipes* Geitler
5-7. — *Spongomonas uvella* Stein

ESTAMPA XVIII

Foto 1, — *Mougeotia laetevirens* (A. Braun) Wittrock

2. — *Spirogyra gracilis* (Hassall) Kütz.

3-4. — *Spirogyra heeriana* Nä.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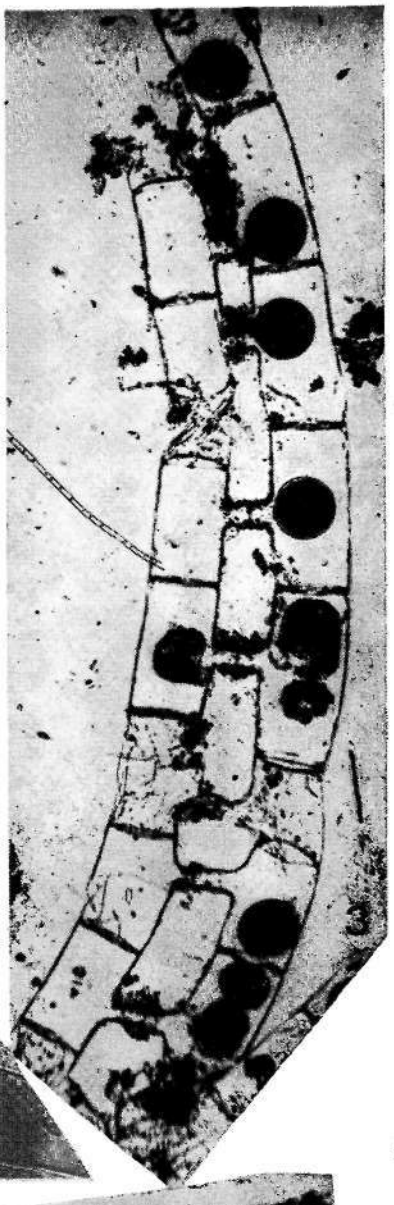
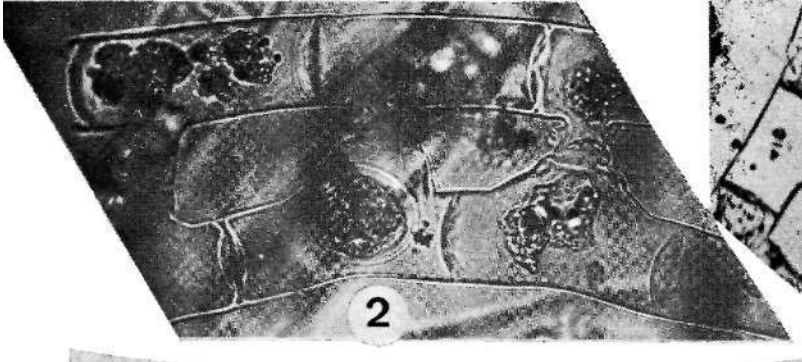
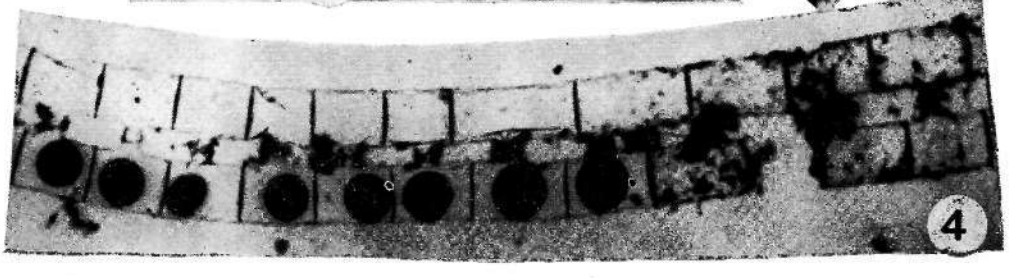



2


4

CONTRIBUIÇÃO PARA O CONHECIMENTO CITOTAXONÓMICO DAS *SPERMATOPHYTA* DE PORTUGAL

XIII. GERANIACEAE *

por

MARIA CELESTE ALVES & MARIA TERESA LEITÃO
Instituto Botânico da Universidade de Coimbra

INTRODUÇÃO

COM O fim de se elaborar o atlas cromossómico da Flora Portuguesa e procurar resolver problemas de Taxonomía com os dados fornecidos pelo nùmero e morfologia dos cromossomas, foram há alguns anos encetados no Instituto Botânico da Universidade de Coimbra estudos cariológicos nas *Spermatophyta.* No prosseguimento desses estudos, debruçámo-nos neste trabalho sobre alguns taxa da família das *Geraniaceae.*

Algumas das plantas estudadas foram colhidas no estado espontâneo e cultivadas em vasos no Jardim Botânico e outras foram obtidas por germinação de sementes colhidas em plantas espontâneas.

Os taxa em questão foram identificados pela Flora Europaea, sendo a sua ordenação em géneros feita de acordo com a classificação de H. SCHOLZ in Syllabus der Pflanzenfamilien, ed. 12 (1964), enquanto que, dentro de cada género, as espécies foram ordenadas segundo a Flora Europaea.

Todas as observações se efectuaram em preparações de cortes transversais, com a espessura de 18 μ, de vértices vegetativos de raízes, fixados no líquido de Navachine (modificação de Brunn) e coradas pelo violeta de genciana.

Os desenhos foram executados à câmara clara com uma ampliação de ca. de 3 000 X.

* Trabalho subsidiado pelo Projecto de Investigação CB1 do INIC.

OBSERVAÇÕES

Geranium pyrenaicum Burra. — Castro de Avelãs, Bragança (n.° 3001).

Para esta espécie determinámos o número cromossómico 2n = 26. Um par de cromossomas é satelitífero (fig. 1a).

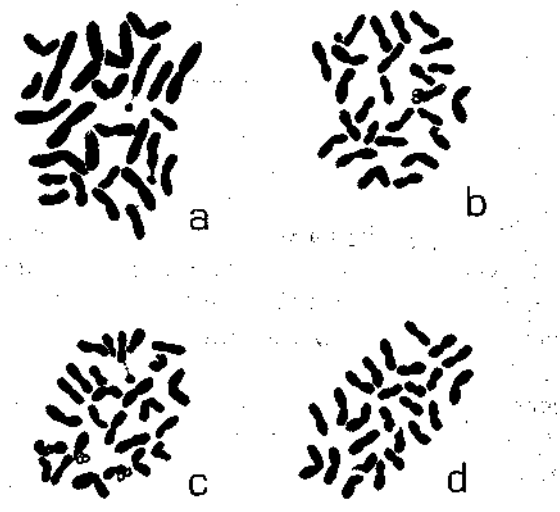


Fig. 1.—*a, Geranium pyrenaicum* (n.° 3001) 2n = 26. *b, Geranium rotundifolium* (n.° 896) 2n = 26. *o, Geranium molle* (n.° 894) 2n = 26. *d, Geranium pusillum* (n.° 5812) 2n = 26.

Confirmámos, assim, as determinações feitas por GAUGER (1937), LOEVKVIST (1963) e MURIN (1973). Entretanto, WARBURG (1938) e VAN DOON et al. (1971) indicaram 2n=28. CHATTERJEE & SHARMA (1970) determinaram 2n = 20. A contagem 2n = 22-24 foi feita por HEIΓZ (1962) e referida por TISCHLER (1934).

Geranium rotundifolium L. — Coimbra, Fornos (n.° 896) ; Ansião (n.° 4518) ; Estrada Batalha-Rio Maior, próximo do ramal para Ataija de Cima (n.° 3226) ; Serra de Monsanto (n.° 2467); Alcochete (n.° 2143).

WARBURG (1938), LARSEN (1956), DAHLGREN, KARLSON & LARSEN (1971), STRID (1971) e VAN LOON, GADELLA & KLIPHUIS (1971) estabeleceram para esta espécie 2n = 26.

Confirmámos essa determinação (fig. 1b), tendo verificado a existência de um par de cromossomas satelitíferos.

Geranium molle L. — Vila Nova de Gaia, Serra do Pilar (n.° 3937) ; Igrejas, estrada de S. Pedro do Sul-Arouca (n.° 894); Coimbra, Eiras (n.° 5937); Algueirão (n.os 2144 e 7828) ; Estrada de Portimão a Faro, a 30 km de Faro (n.° 4516).

Alguns autores (GAUGER, 1937; WARBURG, 1938; LOVE & LOVE, 1956; BÖCHER & LARSEN, 1958; MULLIGAN, 1959; GADELLA & KLIPHUIS, 1966; TAYLOR & MULLIGAN, 1963) determinaram para esta espécie 2n = 26.

Confirmámos esse número em todas as plantas das localidades acima mencionadas (fig. 1c).

Notámos a existência na guarnição de um par de cromossomas satelitíferos.

Geranium pusilum L. — Guarda, próximo da Estação do *C,* F. (n.° 5812).

GAUGER (1937), FRITSCH (1937), MURIN (1937), LÖVE & LOVE (1944), POLYA (1950), JACKSON (1951), SHAW (1952), LÖVE & LÖVE (1956), BÖCHER & LARSEN (1958), MULLIGAN (1959), LOEVKVIST (1963), GADELLA & KLIPHUIS (1966) indicaram para esta espécie 2n = 26 e WARBURG Í1938) determinou 2n = 34.

Verificámos a existência de 26 cromossomas pequenos e isobraquiais nas plantas estudadas (fig. *lã).*

Geranium columbinum L. — Bragança, Vilarinho (n.° 7825); Coimbra, S. Paulo de Frades (n.° 5968) ; Condeixa (n.° 891); Ansião (n.° 3225); Sintra, Algueirão (n.° 6491).

GAUGER (1937), WARBURG (1938), LOVE & LÖVE (1944), LOEVKVIST (1963), STRID (1971), VAN LOON, GADELLA &

KLIPHUIS (1971) e MURIN (1973) citam para esta espécie 2n = 18.

As nossas contagens concordam com a dos autores referidos. Um par de cromossomas é satelitífero (fig. 2a).

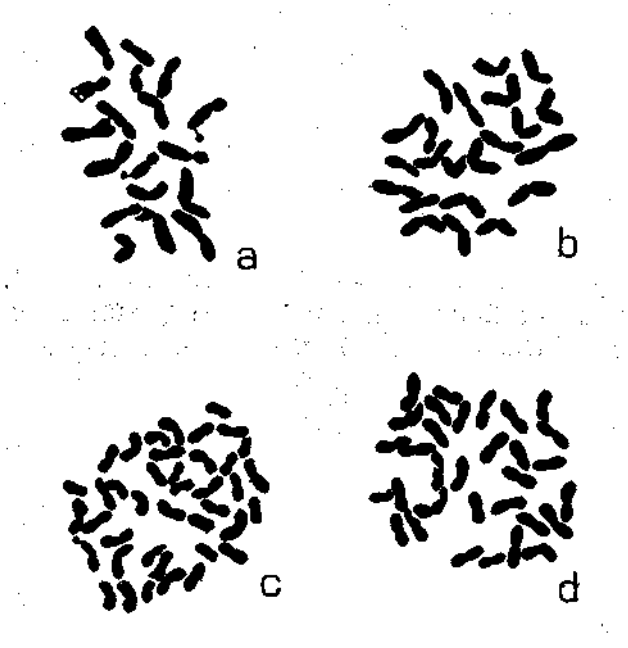


Fig. 2.—-*a, Geranium columbinum* (n.° 891) 2n = 18. *b, Geranium dïssectum* (n.° 892) 2n=22. c, Geranium *lucidum* (n.° 893) 2n=40. d, Geranium purpureum (n.° 4517) 2n = 32.

Geranium dissectum L. — Maia, Moreira (n.° 8592) ; Coimbra, Alcarraques (n.° 892); Sintra, Algueirão (n.^{os} 2442 e 6492); E. A. N., Oeiras (n.° 7826).

Todos os autores que se ocuparam desta espécie (GAUGER, 1937; WARBURG, 1938; LOVE & LOVE, 1944; LOEVKVIST, 1963; GADELLA & KLIPHUIS, 1966; TAYLOR & MULLIGAN, 1968; UHRIKOVA, 1974) determinaram 2n = 22, estando este número de acordo com o determinado por nós. Os cromossomas são todos isobraquiais ou quase (fig. 26).

Geranium lucidum L. — Entre Tábua e Midões (n.° 893).

Para esta espécie, WARBURG (1938) citou 2n = 20.

Nas plantas da localidade acima indicada, verificámos ε existência de 40 cromossomas somáticos (fig. 2c). As plantas examinadas são, portanto, tetraplóides. No entanto, só observámos um par de cromossomas satelitífero.

Geranium purpureum Vill. — Ferreira do Zêzere, Lagar do Gato (n.° 4517).

Determinámos para esta espécie 2n=32 (fig. 2d), confirmando, assim, os resultados apresentados por alguns autores (WARBURG, 1938; BÖCHER, 1947; BAKER, 1949; POLYA, 1950; FAHMY, 1951; BÖCHER & LARSEN, 1955 e LARSEN, 1960). Segundo as contagens destes autores, *G. robertianum* tem 2n = 64, enquanto *G. purpureum* possui 2n = 32. O número determinado para este material é uma prova a favor da ideia de que *G. purpureum* e *G. robertianum* devem considerar-se espécies distintas. A primeira seria tetraplóide de número básico 8 e a segunda octoplóide.

Erodium chium (Burm. f.) Willd. — Serra de Monsanto (n.° 2465).

Esta espécie foi estudada por autores que determinaram 2n = 20 (LARSEN, 1960; GUTTONNEAU, 1964, 1965), enquanto que WARBURG (1938) contou 2n = 40 e LARSEN (1960) 2n = 80.

Confirmámos o número 2n = 20 e verificámos que a guarnição (fig. 3α) era constituída somente por cromossomas isobraquiais.

Erodium laciniatum (Cav.) Willd. — Portinho da Arrábida (n.° 3230).

GUITTONNEAU (1967) estabeleceu para *E. laciniatum* (Cav.) Willd. subsp. *laciniatum* 2n = 20.

Determinámos 2n = 40, o que mostra que a planta estudada é tetraplóide.

Todos os cromossomas são isobraquiais, tendo observado somente um par satelitífero (fig. *Sb).*

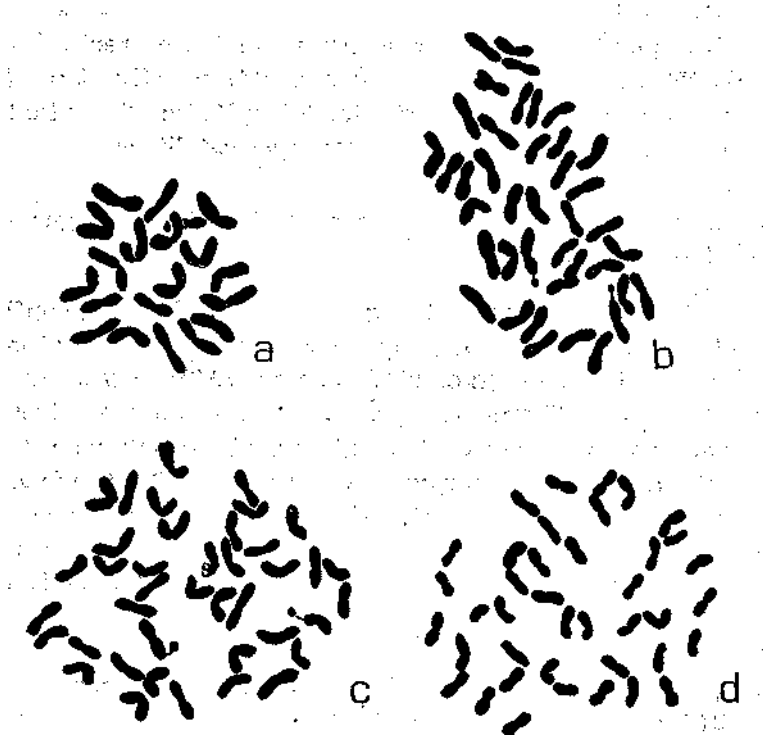


Fig. 3.— *a, Erodium chium* (n.° 2465) 2n = 20. *b, Eroäium laciniaium* (n.° 3230) 2n = 40. *c, Eroäium malacoiäes* (n.° 888) 2n = 40. *d, Erodium botrys* (n.° 2464) 2n = 40.

Erodium malacoides (L.) L'Hérit. — Coimbra, Choupal (n.° 888) ; E. A. N., Oeiras (n,° 3707) ; Faro (n.° 4509) ; Ilha de Faro (n.° 2280).

WARBURG (1938), DIEBS (1961), GUITTONNEAU (1965, 1966, 1967) e FERNANDES & QUEIRÓS (1971) determinaram para esta espécie 2n = 40. Confirmámos essa determinação. As plantas estudadas são, pois ,tetraplóides, sendo a guarnição constituída por cromossomas com a constrição cinética mediana ou submediana (fig. 3c).

Erodium botrys (Cav.) Bertol. — Coimbra, Vila Franca (n.° 5933) ; Arruda de Azeitão (n.° 2464) ; Castro Verde (n.^{0} 2140).

As placas metafásicas mostraram a existência de 2n=40 (fig. *3d),* o que concorda com as contagens de HEISER & WHITAKER (1948), GUITTONNEAU (1955, 1957) e BORGEN (1970).

Erodium cieutarium (L.) L'Hérit subsp. cicutarium — Andados 12 km de Bragança para Valpaços (n.° 1428) ; Maia, Crestine (n.° 5269); Matosinhos, Guifões (n.° 1039).

Considerando a espécie num sentido amplo, podemos dizer que HEΓΓZ (1926), GAUGER (1937), WARBURG (1938), LOVE & LOVE (1942, 1944), HEISER (1948), STEBBINS (1948), MATTICK (1950), ROTTGARDT (1950), BAKER (1955), LOVE & LOVE (1956b), LABSEN (1958), GARAJOVÁ (1959, 1970), LARSEN (1960), DIERS (1961), GUITTONNEAU (1964, 1965a, 1965b, 1966a, 1968b, 1967), PODLECH (1969), DAHLGREN, KARLSON & LARSEN (1971), NOWAK (1971), SKALINSKA, JANKUN & Wcislo (1971), STRIE- (1971) e ROTTGARDT (1956) determinaram 2n = 20, 36, 48, 54 e 56.

No tipo da espécie contámos 2n = 40 (fig. 4α), sendo os cromossomas isobraquiais.

Erodium cicutarium (L.) L'Hérit subsp. bipinnatum Tourlet — Ovar (n.° 889) ; Coina (n.° 4194).

GUITTONNEAU (1965b), que considera este taxon como espécie distinta, indica 2n = 40, número que confirmámos. Os cromossomas são isobraquiais, havendo pelo menos um par satelitífero (fig. 46).

Erodium cicutarium (L.) L'Hérit subsp. jacquinianum (Fischer, Meyer & Avé-Lall.) Briq. — Costa da Caparica, areias junto da praia (n.° 1429) ; Portinho da Arrábida (n.° 3229).

GUTTONNEAU (1964, 1966, 1967), que considera este taxon como espécie distinta sob o nome de *E. aetiopicum*, indica 2n = 40. As nossas contagens levaram-nos a estabelecer a existência de 20 cromossomas isobraquiais, sendo um par satelitífero (fig. 4c).

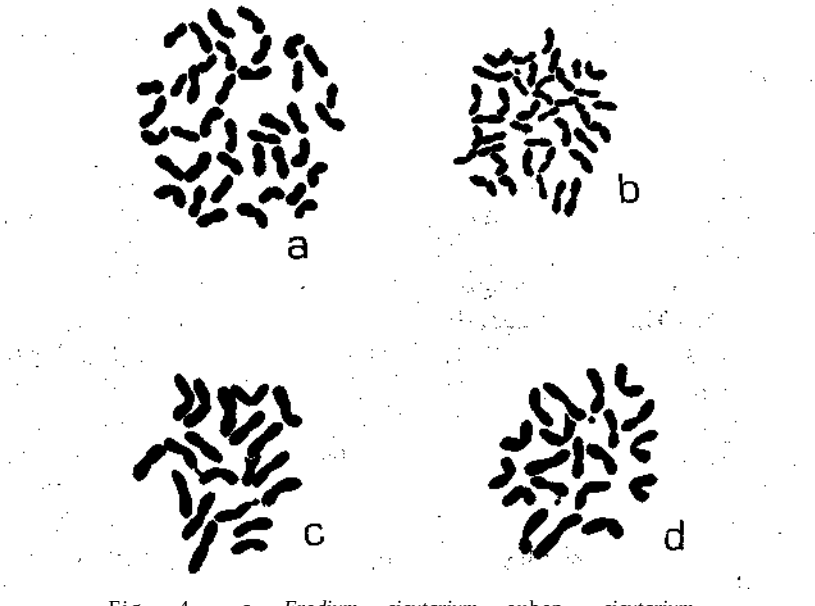


Fig. 4. — *a, Erodium cicutarium* subsp. *cicutarium* (n.° 5269) 2n = 40. *b, Erodium cicutarium* subsp. *bipinnatum* (n.° 889) 2n = 40. *c, E. cicutarium* subsp. *jacquinianum* (n.° 1429) 2n = 20. *d, E. moschatum* (n.° 890) 2n = 20.

Ê de admitir que um estudo mais pormenorizado deste taxon possa revelar que alguma das subspecies se possa considerar espécie distinta.

Erodium moschatum (L.) L'Hérit. — Vila Nova de Gaia, Quebrantões (n.° 1040); Ílhavo, Gafanha da Nazaré (n.° 4506); Coimbra, Cruz de Morouços (n.° 890); Coimbra, S. Paulo de Frades (n.° 5934); Sintra, Algueirão (n.° 2141); Monsanto (n.° 7640); Jardim Botânico de Lisboa (n.° 4745); Lagos (n.° 4508).

QUADRO I

Números de cromossomas determinados em *Geraniaceae* de Portugal

	Anual ou bienal	Vivaz	2n	2x	4x	6x	Números determinados por outros autores
Geranium, χ = 9, 10, 11, 13 e 16							
G. pyrenaicum Burnì.		+	26	+			20, 26, 28
G. rotundifolium L.	+		26	+			26
G. molle L.	+		26	+			26*
G. pusillum L.	+		26	+			26, 34
G. columbinum L. , ...	+		18	+			18
G. dissectum L.	+		22	+			22
G. lucidum L.	+		40		+		20
G. purpureum Vili.	+		32	+			32
Erodium, χ = 9, 10							
E. chium (Burm. f.) Willd. subsp. chium			20	+			20, 40, 80
E. laciniatum (Cav.) Willd.	+		40		+		20
E. malacoides (L.) Willd.	+		40		+		40*
E. botrys (Cav.) Bertol.	+		40		+		40*
E. cicutarium (L.) L'Hérit. subsp. cicutarium.	+		40		+		20, 36, 40, 48, 54, 56
E. cicutarium (L.) L'Hérit. subsp. bipinnatum Tourlet.	+		40		+		40*
E. cicutarium (L.) L'Hérit. subsp. jaquinianum (Fischer, Meyer & Avé Lall.) Briq.	+		20	+			40
E. moschatum (L.) L'Hérit.	+		20	+			20*

Alguns autores referiram para esta espécie 2n = 20 (GAUGER, 1937; GADELIA & KLIPHUIS, 1970; WARBURG, 1938; STEBBINS, 1948) e 2n = 40 (GUITTONNEAU, 1964, 1965a, 1965b, 1967; FERNANDES & QUEIRÓS, 1971).

Confirmámos o número 2n = 20 em todas as placas metafásicas por nós observadas. Um par de cromossomas é satelitífero (fig. 4d). Entre as plantas de Portugal parece, pois, haver diplóides e tetraplóides.

CONCLUSÕES

Os resultados deste trabalho estão reunidos no quadro junto, onde, para cada taxon, figura o nome, a duração de vida, os números cromossómicos somáticos por nós determinados e os encontrados por outros autores. Os números assinalados por um asterisco foram estabelecidos em plantas provenientes de sementes colhidas em Portugal: *Geranium molle* (2n = 26), determinado por BOCHER & LARSEN (1958), e os restantes por GUITTONNEAU (1965) e FERNANDES & QUEIRÓS (1971).

Não há a assinalar números determinados por nós pela primeira vez.

Os números encontrados estão de acordo com os indicados por outros autores, à excepção de *Geranium lucidum* para o qual nenhum autor havia anteriormente assinalado a existência de tetraploidia.

Os dados referentes à duração de vida foram obtidos a partir da Flora de Portugal ed. 2 de P. COUTINHO e da Flora Europaea (vol. 2).

A análise do quadro mostra que há 10 taxa diplóides e 6 tetraplóides (estes pertencentes na quase totalidade a *Erodium),* números aos quais corresponde a percentagem de 62,5 % e 37,5% respectivamente. Há, portanto, uma maior percentagem de taxa diplóides.

Não se verifica qualquer correlação entre o grau de poliploidia e a duração de vida dessas plantas, uma vez que apenas uma das espécies *(Geranium pyrenaicum)* é vivaz.

A análise do quadro mostra, ainda, que os principais processos evolutivos dentro dos géneros estudados são a aneuplóidia e a poliploidia. Ê provável que 8 seja também um número básico do género *Geranium.*

AGRADECIMENTOS

Agradecemos ao Senhor Professor Dr. ABÍLIO FERNANDES toda a colaboração prestada na elaboração deste trabalho e a todos aqueles que de algum modo contribuíram para a sua realização.

RESUMO

São referidos os números cromossómicos de 16 taxa de *Geraniaceae* da Flora de Portugal.

SUMMARY

The chromosome numbers of 16 taxa of *Geraniaceae* from Portugal have been reported.

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CONTRIBUIÇÃO PARA O CONHECIMENTO CITOTAXONÓMICO DAS *SPERMATOPHYTA* DE PORTUGAL

XIV. CISTACEAE *

por

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INTRODUÇÃO

PROSSEGUINDO OS trabalhos citotaxonómicos encetados há alguns anos no Instituto Botânico da Universidade de Coimbra com o fim de futuramente ser elaborado o Atlas dos números cromossómicos das *Spermatophyta* de Portugal, iniciámos o estudo das *Cistaceae* da flora portuguesa. Apresentamos aqui o resultado das observações efectuadas em 24 taxa distribuídos pelos géneros *Cistus, Hálimium, Helianthemum, Fumana* e *Tuberaria,* que existem no nosso país segundo a *Flora Europaea* (vol. 2, 1968).

Nesta exposição, ordenamos as tribos e os géneros segundo a classificação de H. MELCHIOR (in ENGLER,. Syllabus der Pflanzenfamilien, 1964) e as espécies dentro dos géneros segundo a *Flora Europaea.*

MATERIAL E TÉCNICA

Colhidas as sementes de plantas que crescem no estado espontâneo no nosso País, foram semeadas no Jardim Botânico. Das plantas desenvolvidas foram retirados os meristemas radiculares, que foram fixados em Navachine (modificação de Brunn). Os respectivos cortes transversais,

* Trabalho subsidiado pelo Projecto de Investigação CB1 do INIC.

feitos com uma espessura de 18 μ, foram corados pelo violeta de genciana. Os desenhos das placas metafásicas foram executados ä câmara clara e com uma ampliação de cerca de 3 000.

OBSERVAÇÕES

Trib. CISTEAE

Cistus albidus L. — Figueira de Castelo Rodrigo, Barca de Alva, Quinta da Pedriça (n.° 7129); Azeitão (n.° 3648).

Numerosos investigadores (CHIARUGI, 1925; DANSERAU, 1940; BOWDEN, 1940, 1945; LA COUR, 1945; A. LOVE & KJELLQVIST, 1964; NILSSON & LARSEN, 1971 e VAN LOON, GADELLA & KLIPHUIS, 1971) observaram esta espécie sob o ponto de vista cariológico, tendo contado $\eta = 9$ nas células mães dos grãos de pólen e no gametófita feminino e 2n=18 nos vértices vegetativos da raiz. Determinámos igualmente o último número nas plantas portuguesas (fig. 1a), tendo verificado que a guarnição corresponde à representada por BOWDEN (1940) e LOVE & KJELLQVIST (1964).

Cistus crispus L. — Souselas (n.° 832) ; Castelo de Vide, Montosa (n.^{os} 5226 e 7133).

Tal como os nossos antecessores (DANSEREAU, 1940 e RODRIGUES, 1954) observámos 2n = 18 (fig. 1&). O idiograma apresentado por RODRIGUES e representado pela fórmula 2n=18=6LL + 4LP + 411 + 4IP1 corresponde à nossa observação. Verificámos, porém, a existência de um par de cromossomas satelitífero do tipo 11.

Cistus monspeliensis L. — Coimbra, Baleia (n.° 836) ; Alvalade, Baixo Alentejo (n.° 4997).

A primeira determinação feita para esta espécie data de 1952. Efectivamente, CHIARUGI determinou então 9 cromossomas no gametófita feminino. Este número foi depois confirmado por SIMONET (1937) nas células mães dos grãos de pólen. LA COUR (1945), LOVE & KJELLQVIST (1964),

FAGIOLI & FABRI (1971), NILSSON & LARSEN (1971) e VAN LOON, GABELLA & Kliphus (1971) contaram 18 cromossomas nas metafases somáticas.

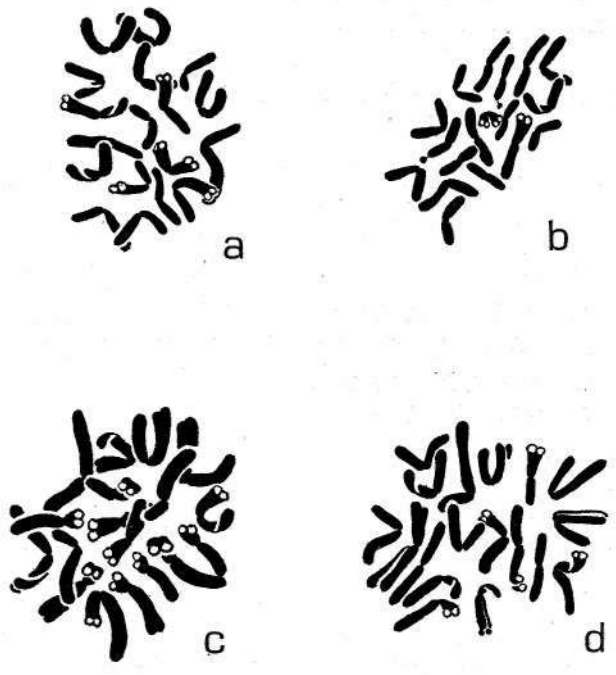


Fig. 1.—*a, Cistus albidus* n.° 831 (2n = 18). *b, Cistus crispus* n.° 7133 (2n = 18). *o, Cistus monspeliensis* n.° 836 (2n = 18). *d, Cistus psilosepálus* n.° 1020 (2n = 18).

Em plantas portuguesas encontrámos o mesmo número de cromossomas cuja morfologia corresponde à representada por LOVE & KJELLQVIST (fig. lc).

Cistus psilosepálus Sweet — Porto, Lordelo do Ouro (n.° 1020) ; Guarda, Souto do Bispo (n.° 5794) ; Vendas de Galizes (n.° 6805); Coimbra, Vila Franca (n.° 1834); Torres Vedras (n.° 6415) ; Mercês, Pinhal do Escouto (n.° 3650); Rio de Mouro (n.° 2913); Mata de Queluz (n.° 4996).

SIMONET (1937) determinou n = 9 e LA COUR (1945) 2n — 18. Confirmámos o número somatico nas plantas portuguesas estudadas. Os cromossomas são longos, quase isobraquiais, destacando-se dois pares maiores e com constrição sub-mediana (fig. 1*d).*

Cistus salvifolius L.— Estrada Chaves-Braganga a 1km de Assureira de Baixo (n.° 1359) ; Vila do Conde, Arvore (n.° 1021) ; Estrada de Castelo Branco-Malpica a 5 km de Malpica (n.° 5485) ; Montijo, Canha, Herdade de Monte Silves (n.° 8127).

2n = 18 foi o número determinado por SEMONET (1937), DANSEREAÜ (1940), LA COUR (1945), ATSMON & FEINBRUN (1960), A. LOVE & KJELLQVIST (1964), GADELLA & al. (1966), DAHLGREN, KARLSSON & LARSEN (1971) e NILSSON & LARSEN (1971). Confirmamos estas determinações, tendo detectado apenas 1 cromossoma satelitífero (fig. 2α).

Cistos populifolius L. — Vinhais (n.° 1358) ; Foz do Caneiro (n.° 3450).

Contámos 2n = 18 (fig. *2b),* confirmando, assim, as determinações anteriores de CHIARUGI (1937), SIMONET (1937), DANSEREAÜ (1940) e A. LOVE & KJELLQVISTT (1964).

Cistus laurifolius L.—Bragança, Monte de S. Bartolomeu (n.° 1357).

Diversos investigadores indicam para esta espécie 2n = 18 (CHIARUGI, 1925; SMONET, 1937; BOWDEN, 1940, 1945; LA COUR, 1945; A. LOVE & KJELLVIST, 1964). Nas nossas observações confirmámos este número, assim como a guarnição representada por LOVE & KJELLQVIST. Apenas observámos um cromossoma satelitífero (fig. 2c).

Cistus ladanifer L. — Serra da Arrábida, pr. de Sesimbra (n.° 3651).

Nesta espécie determinámos 2n = 18 (fig. *2d),* enquanto n = 9 foi encontrado por CHIARUGI (1937) e DANSEREAU

(1940) nas células mães dos grãos de pólen da variedade *macúlatus* Dunal.

Os cromossomas são longos e com constrições primárias medianas, à excepção de dois pares que se apresentam nitidamente heterobraquiais. Apenas notámos a presença de um cromossoma portador de satélite.

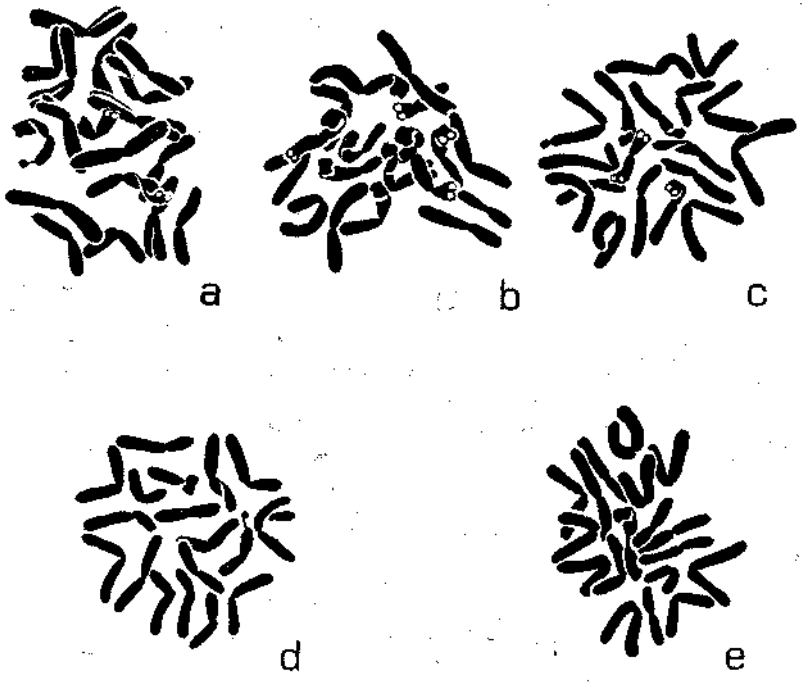


Fig. 2. — *a, Cistus salvifolius* n.° 1021 (2n = 18). *b, Cistus populifolius* n.° 1358 (2n = 18). *c, Cistus laurifolius* n.° 1357 (2n = 18). *d, Cistus ladanifer* n.° 3651 (2n = 18). *e, Cistus palhinhae* n.° 7555 (2n = 18).

Cistus palhinhae Ingram — Cabo de S. Vicente (n.° 7555).

Contámos 2n = 18 (fig. 2e). Este número confirma as determinações anteriormente feitas por RODRIGUES (1954) e A. FERNANDES & QUEIRÓS (1970). A guarnição por nós observada concorda também com a figurada por estes autores.

Halimium ocymoides (Lam.) Willk. — Coimbra, Eiras (n.° 3454).

Confirmando a determinação feita por PROCTOR (1955) cm plantas provenientes de Portugal, encontrámos 2n = 18. 6 pares apresentam constrições medianas ou quase, enquanto que um par é heterobraquial e outro cefalobraquial (fig. 3α).

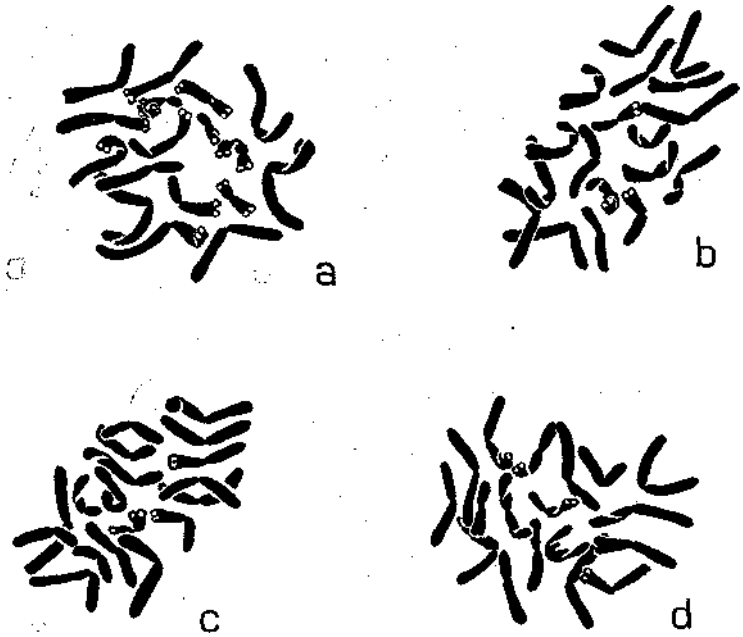


Fig. 3. — *a, Halimium ocymoides* n.« 4180 (2n = 18). *b, Halimium alyssoides* n.° 4396 (2n = 18). *c, Halimium lasianthum* ssp. *lasianthum* n.° 2401 (2n = 18). *d, Halimium halimifallium* ssp. *halimifolium* n.° 4129 (2n=18).

Halimium alyssoides (Lam.) C. Koch — Montalegre, Padornélas (n.° 4396); Porto, Sobreirinho (n.° 2316).

Contámos 18 cromossomas (fig. 3b), entre os quais existem elementos longos e outros mais curtos, mas todos eles com as constrições primárias medianas ou quase. Pensamos que este número é aqui apresentado pela primeira vez, visto não termos encontrado na bibliografia qualquer referência a esta espécie.

Halimium lasianthum (Lam.) Spach ssp. lasianthum—A 1km de Vendas Novas para Lavre (n.° 3213); próximo de Azeitão (n.° 2401).

Confirmamos a citação de 2n = 18 feita por SNOAD (in DARLINGTON & WYLIE, 1955).

Além disso, verificámos que os cromossomas são semelhantes aos da espécie anterior, isto é, todos isobraquiais ou quase (fig. 3c).

Halimium halimifolium (L.) Willk. ssp. halimifolium — Entre Azeitão e a Serra de Arrábida (n.° 4129).

CHIARUGI (1925) e PROCTOR (1955) citam para a espécie 2n — 18. As nossas contagens concordam com as destes autores (fig. *3d).*

Halimium umbellatum (L.) Spach — Estrada Chaves-Bragança, Assureira de Baixo (n.° 1364).

O número encontrado para esta espécie foi 2n = 18, o que confirma uma determinação anterior feita por PROCTOR (1955) em plantas obtidas de sementes provenientes também do nosso País. Detectámos um par de cromossomas satelitífero (fig. 4o).

Halimium verticillatum (Brot.) Sennen — Guarda, Souto do Bispo (n.° 860); Estrada de Castelo Branco-Malpica, a 2 km de Castelo Branco (n.° 5488).

Não encontrámos na bibliografia referências a esta espécie, pelo que pensamos que o número 2n = 18 é apresentado aqui pela primeira vez. Os cromossomas, idênticos aos da espécie anterior, são uns mais longos e outros mais curtos, mas todos eles com constrições primárias medianas ou quase (fig. 4b).

Halimium comutatum Pau—Charneca da Caparica (n.° 7559).

Tal como para a espécie anterior, determinámos 2n=18 (fig. 4c) e notámos a presença de um par satelitífero. Este número é também citado aqui pela primeira vez.

Tuberaria lignosa (Sweet) Samp.—Valongo, Senhora das Chãs (n.° 5229); Santana Ferreira, Matas de Fôja (n.° 3455).

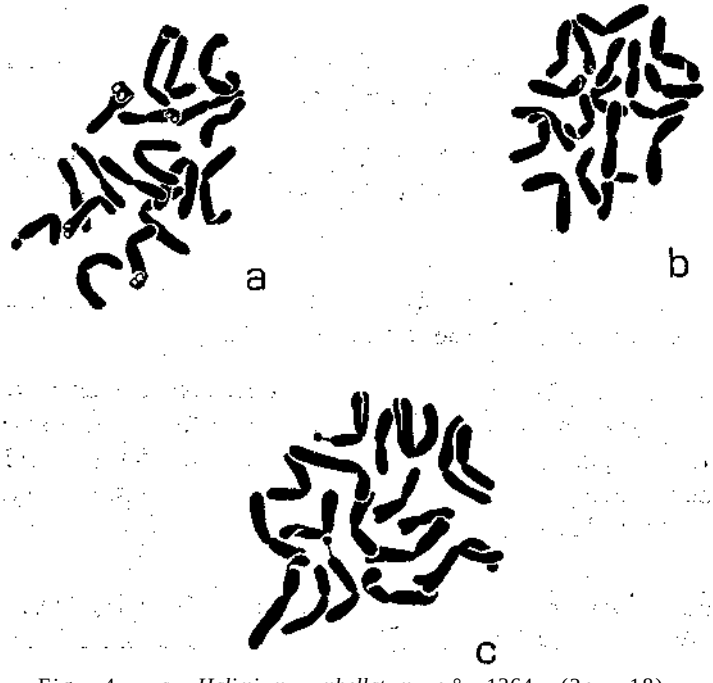


Fig. 4.— *a, Halimium umbellatum* n.° 1364 (2n = 18). *b, Halimium verticiïlatum* n.° 6860 (2n=18). *c, Halimium comutatum* n.° 7559 (2n = 18).

À contagem de 14 cromossomas em placas metafásicas dos meristemas radiculares (fig. 5α) confirma as determinações anteriores feitas por PROCTOR (1955) em plantas portuguesas e por ARRIGONI e MORI (1971) em material da flora italiana. Um cromossoma mostrou-se satelitífero, não tendo sido encontradas figuras que mostrassem os dois simultaneamente.

Tuberaria guttata (L.) Fourr. — Coimbra, Cruz de Morouços (n.° 842); a 2 km de Castelo Branco para Malpica (n.° 5486).

Esta espécie foi anteriormente estudada por CHIARUGI (1925), que contou 2n = 48, PROCTOR (1954, 1955, 1960), que determinou 2n = 36, e por ATSMON & FREINBRUN (1960) e MARKOVA (1972), que encontraram 2n = 24. O número por nós estabelecido foi de 2n=36, de acordo com as observações de PROCTOR. Pensamos que os exemplares observados por este autor e por nós são hexaplóides de número básico 6.

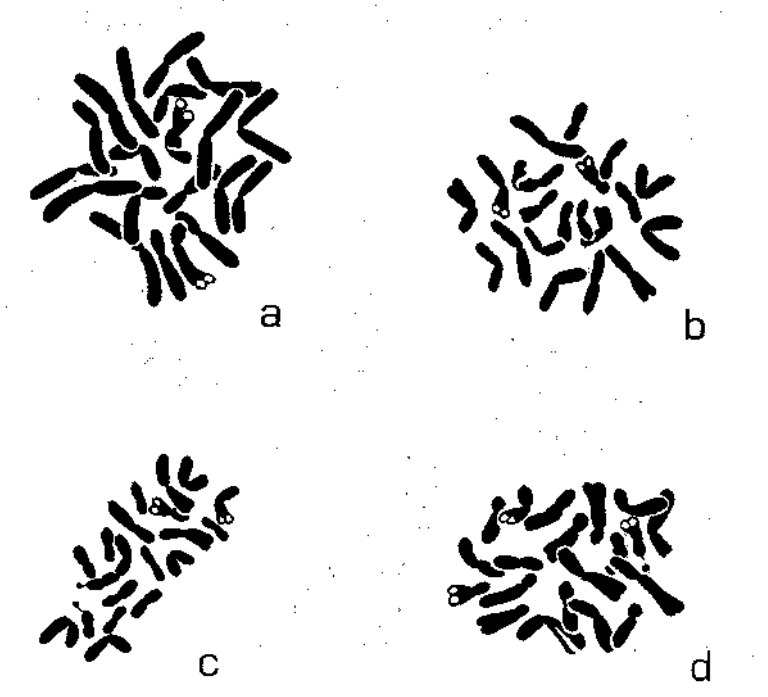
Fig. 5. — *a*, *Tuberaria lignosa* n.° 5229 (2n = 14). *b*, *Tuberaria guttata* n.° 842 (2n = 36).

Helianthemum nummularium (L.) Miller ssp. nummularium — Trás-os-Montes, Mouradela entre Tourim e Covelas (n.° 4447); Serra da Freita (n.° 839).

Diversos investigadores estudaram esta espécie, tendo determinado os números 20 e 32. Tal como LOVE & LOVE (1944), MURIN (1967), LEVEQUE & GORENFLOT (1969), MAJOVSKY & al. (1970), MURIN & SHEICH (1971), contámos 2n = 20 (fig. 6α). BowDEN (1940 e 1945) e MATTICK (in TISCHLER, 1950) determinaram 2n = 32. Pensamos que a guarnição será constituída por 20 cromossomas, mimerò que é indicado para as espécies deste género pela maioria dos autores.

Helianthemum ledifolium (L.) Miller — Sintra, Algueirão (n.° 5001) ; pr. de Estremoz, junto ao monumento do Ameixial (n.° 3214).

Esta espécie já tinha sido estudada, tendo os nossos antecessores determinado, respectivamente, os números 2n=16 (CHIARUGI, 1925), 2n=20 (PROCTOR, 1955; A. LOVE & KJELLQVIST, 1964; GADELLA& ai., 1966; BJORQVIST, BOTHMER, NILSSON & NORDENSTAM, 1969; MURIN & SHEIKH, 1971) e 2n = 40 (MARKOVA, 1972).



Pig. 6. — *a, Helianthemum nummularium* asp. *nummularium* n.° 839 (2n = 20). *b, Helianthemum ledifolium* n.° 3214 (2n = 20). *c, Helianthemum salicifolium* n.° 5487 (2n = 20). *d, Helianthemum aegyptiacum* n.° 2226 (2n = 20).

Determinámos 2n = 20 (fig. 6b), correspondendo a guarnição à representada por LOVE & KJELLQVIST (1964).

Helianthemum salicifolium (L.) Miller — Vila Velha de Ródão (n.^{0} 5487).

De acordo com ATSMON & FEINBRUN (I960), A. LOVE & KJELLQVIST (1964), MURIN & CHAUDLARI (1970) e MARKO VA (1972) encontrámos 2n = 20 (fig. 6c). A forma dos cromossomas corresponde à apresentada pelos dois primeiros autores, embora tenhamos posto em evidência um par de cromossomas curtos providos de satélites.

Helianthemum aegyptiacum (L.) Miller — Castro Verde (n.° 2226).

Como em todas as outras espécies deste género, contámos 2n = 20 cromossomas (fig. 6d), dos quais 8 pares são cefalobraquiais e dois mais longos com as constrições primárias submedianas. Observámos apenas um cromossoma com satélite ligado ao braço curto de um dos cromossomas com constrição primária subterminal. Dado que não encontrámos qualquer referência a esta espécie, julgamos ser este número apresentado aqui pela primeira vez.

Fumana ericoides (Cav.) Gand. — Coimbra, Alto de Santa Clara (n.° 840).

O número 2n = 32 (fig. 7α) concorda com o indicado por NILSSON & LARSEN (1971).

Os cromossomas mais pequenos e o número básico 8 justificam inteiramente a separação desta espécie do género *Helianthemum.*

Fumana thymifolia (L.) Webb — Souselas (n.° 847): a 13 km de Almancil, pr. de Poço da Boliqueira (n.° 4448). .

A primeira determinação para esta espécie foi feita por ATSMON & FEINBRUN (1960) que estudaram a variedade *laevis* (Cav.) Gross. Este número foi mais tarde confirmado por A. LOVE & KJELLKVIST (1964), ao estudarem material

correspondente ao tipo. VAN LOON, GADELLA & KLIPHTJIS (1971) contaram igualmente 2n = 32. Confirmamos estas determinações (fig. *7b).*

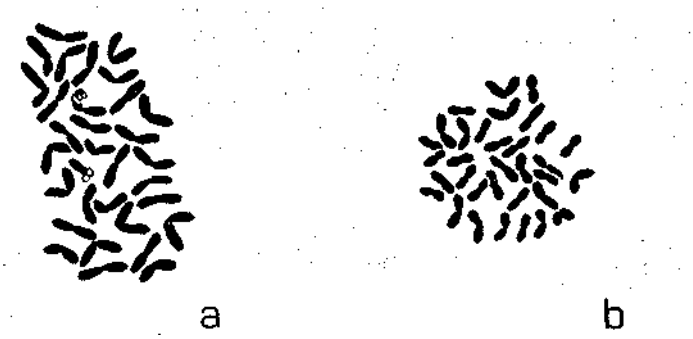


Fig. 7.— *a, Fumana ericoides* n.° 840 (2n = 32).
b, Fumana thymifolia n.° 847 (2n = 32).

CONCLUSÕES

O quadro junto, onde as tribos e os géneros estão dispostos pela classificação de MELCHIOR (loc. cit.) e as espécies dentro dos géneros segundo a *Flora Europaea* (vol. 2, 1968), mostra que foram por nós estudadas sob o ponto de vista cariológico 24 espécies de *Cistaceae* da flora de Portugal. O citado volume da *Flora Europaea* menciona a existência em Portugal de 38 espécies pelo que foram estudadas apenas cerca de 63 % das existentes. Ä medida que o material seja obtido, esperamos publicar outros resultados.

Assinala-se ainda no quadro, para cada taxon, os dados relativos à duração de vida — anuais ou bienais e perenes —, o grau de poliploidia e os números determinados por outros autores. Estão marcados com um asterisco os números estabelecidos por estes em material português.

O quadro mostra ainda que os números cromossómicos das espécies *Halimium ályssoides, Halimium verticülatum, Halimium, comutatum* e *Helianthemum aegiptiacum* são mencionados aqui pela primeira vez, encontrando-se os números determinados enquadrados nos números básicos já estabelecidos para os respectivos géneros. Os números dos

QUADRO I

Numeros de cromossomas determinados em algumas *Cistaceae* de Portugal

Nome do taxon	An. ou Bian.	Per.	2n	2x	4x	6x	N.os determinados por outros autores
Cistaceae							
Cistus, χ = 9							
C. albidus L.		+	18	+			9, 18
C. crispus L.		+	18	+			18
C. monspeliensis L.		+	18	+			9, 18
C. psilosepalus Sweet		+	18	+			9, 18
C. salvifolius L.		+	18	+			9, 18*
C. popullfolius L.		+	18	+			9, 18
C. laurlfolius L.		+	18	+			9, 18
C. ladanifer L.		+	18	+			18
C. palhinhae Ingram		+	18	+			18*
Halimium, x = 9							
H. ocymoides (Lam.) Willk.		+	18	+			18*
H. alissoides (Lam.) C. Koch		+	18	+			N
H. lasianthum (Lam.) Spach ssp. lasianthum		+	18	+			18
H. halimifolium (L.) Willk. ssp. halimifolium		+	18	+			18*
H. umbellatum (L.) Spach		+	18	+			18*
H. vertlcillatum (Brot.) Sennen		+	18	+			N
H. comutatum Pau		+	18	+			N
Tuberaria, χ = 6, 7							
T. lignosa (Sweet) Samp.		+	14	+			14*
T. guttata (L.) Pourr.	+		36			+	24, 36*
Helianthemum, χ = 10							
H. numularium (L.) Miller ssp. numularium		+	20	+			20*, 22, 32
H. ledifolium (L.) Miller	+		20	+			16, 20*, 40
H. salicifolium (L.) Miller	+		20	+			20
H. aegyptiacum (L.) Miller	+		20	+			N
Fumana, χ = 8							
F. ericoides (Cav.) Gand.		+	32		+		32
F. thymifolia (L.) Webb		+	32		+		32

outros taxa estudados estão de acordo com determinações feitas por outros autores em material de outras proveniências.

Embora apenas 63% de *Cistaceae* da flora portuguesa tenha sido estudada, a análise dos números permite-nos concordar com PROCTOR (1955) que atribui a esta família um baixo grau de poliploidia. Apenas *Tuberaria guttata* é hexaplóide e as duas espécies de *Fumana* são tetraplóides. Segundo os dados obtidos, parece não existir qualquer correlação entre o grau de poliploidia e a duração de vida.

Parece-nos correcta a separação de *Halimium* e *Fumana* de *Helianthemum*, proposta em 1856 por WILLKOMM, pois são diferentes morfològica e cariologicamente.

O género *Tuberaria* apresenta mais do que um número básico, cada um correspondente a sua secção: $\chi = 6$ na secção *Scorpioides* e $x = 7$ na secção *Tuberaria*. Também aqui os dados cariológicos apoiam o estabelecimento das duas secções mencionadas, as quais se distinguem, além disso, pela morfologia externa e pela duração de vida.

Comparando cariológica e morfologicamente *Cistus ladanifer* e *Cistus palhinhae* somos levados a apoiar a opinião de FERNANDES & QUEIRÓS (1971) que consideram o último como forma ecológica de *Cistus ladanifer* induzida pela acção do mar.

AGRADECIMENTOS

Ao Ex.mo Senhor Prof. Doutor ABÍLIO FERNANDES expressamos o nosso reconhecimento por toda a amável colaboração prestada. Os nossos agradecimentos são também extensivos a todos os que contribuíram para a realização deste trabalho.

SUMÁRIO

Foram determinados os números cromossómicos de 24 taxa de *Cistaceae* da Flora de Portugal, sendo apresentados pela primeira vez os das espécies: *Halimium alyssoides*, *Halimium verticillatum*, *Halimium comutatum* e *Helianthemum aegptiacum*.

SUMMARY

The chromosome numbers of 24 taxa of *Cistaceae* from Portugal are reported.

The chromosome counts from *Halimium atyssoides, Halimium verticillatum, Hálimium comutatum* and *Helianthemum aegptiacum* are reported here for the first time.

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CONTINUOUS CLONAL CULTURE OF EXCISED POTATO ROOTS

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ABSTRACT

CONTINUOUS clonal culture of potato roots (*Solanum tuberosum* var. Désiré) has been achieved using the MURASHIGE & SKOOG medium without growth substances added. Cultures have been maintained in this medium, with no loss of vigor, for a period of sixteen months and through sixteen transfers.

A comparison of the pattern of growth between shoot attached roots growing in sand and cultured roots is made.

INTRODUCTION

Although the roots of a great number of species have been cultured for limited periods, the roots of only very few species have been successfully maintained in continuous culture (BUTCHER & STREET, 1964). WHITE (1938) tried to obtain continuous growth of excised potato roots but without success. Later, CHAPMAN (1956) and BAJAJ & DIONNE (1968) obtained continuous growth of roots by using a modified WHITE'S medium fortified with a tuber extract. The present paper reports that it is possible to achieve continuous clonal cultures of potato roots by using a culture medium of defined composition.

We also found interesting to compare the pattern of growth of excised roots with those remaining attached to the shoot and growing in sand.

MATERIALS AND METHODS

Sprouts from *Solanum tuberosum*,«var.,/Désiré were surface sterilized in a 10% calcium hypochlorite solution for 15 minutes and rinsed three times with sterile distilled water. After that each sprout was put aseptically on wet filter paper in a test tube and incubated in the dark at 25° C. From the adventicious roots developed from the sprout in about three days, approximately 1 em. Of root including a growing point, was excised and transferred, singly, to 100 ml Erlenmeyer flasks containing 50 ml of sterile nutrient medium. The root cultures were incubated at 25° C in complete darkness. The employed culture medium contained the inorganic and organic constituents of the MTJRASHIGE & SKOOG (1962) medium without growth substances. Sucrose was added at 2% and the pH of the medium was adjusted to 5.6 with 0.1 N NaOH or 1.0' N HCL The medium Was then autoclaved at 120° C during 20 minutes.

After one month of culture we selected the best root culture and from it a clone was established by propagating the excised 1 cm. apical tips of its primary laterals. Continuous clonal culture was maintained by subculturing lateral tips. Duration of the incubation period (passage length) was of one month.

The following criteria for the growth of the roots were used: length of the main axis; number of emergent primary laterals; length of primary laterals ; distance between primary laterals; number of emergent secondary laterals.

For an accurate measure of the roots the following method was used: each root, after one month of subculture, was disposed on a wet glass plate "and its main axis and laterals carefuly disentangled; after that an- outline Of the root was made on the back of the glass plate with an instant dry drawing pencil. This outline was thereafter accurately measured. From these measures ä diagram of the root was constructed (see Fig. 1 and Fig. 2)." ~;

For studihg attached roots, sprouts With a little piece of tuber were put in pots with sand. The pots were ' *main*-tained in a greenhouse at a temperature 20-25º C. After ohe

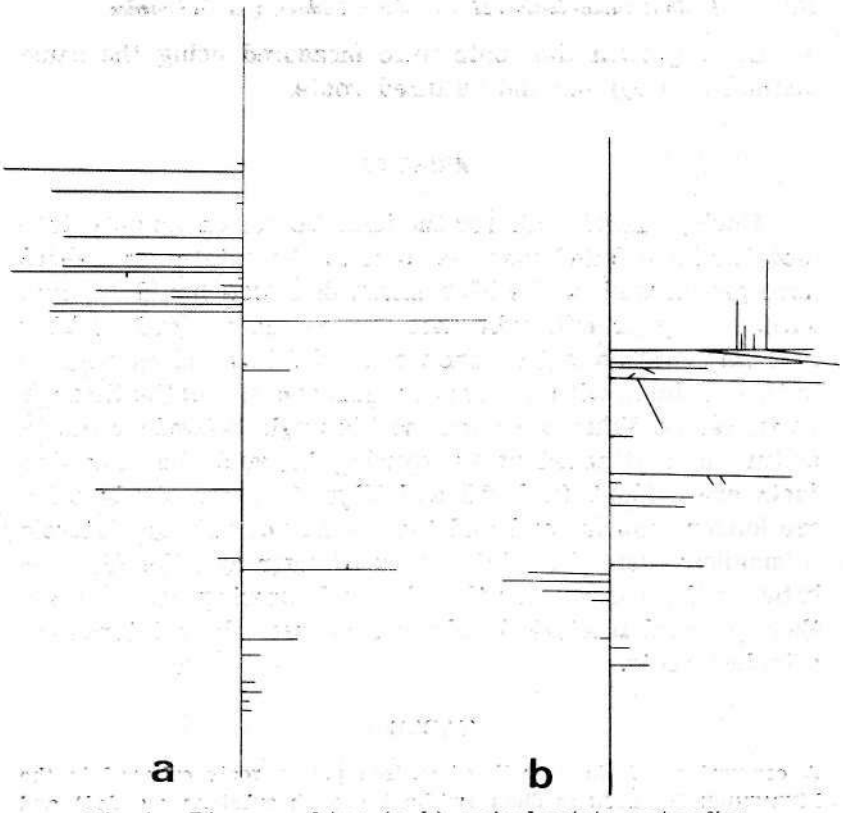

Fig. 1 — Diagram of two (a, b) excised potato roots after one month of culture (twelfth transfer). × ⅓.

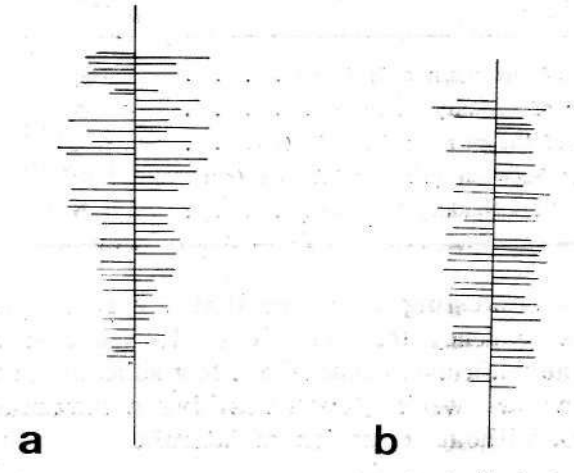

Fig. 2 — Diagram of two (a, b) shoot-attached potato roots growing during a month in sand. × ⅓.

month of growth the roots were measured using the same method as used for the cultured roots.

RESULTS

During experiments for the induction of callus on potato roots we have found that the roots of the potato var. Désiré have grown well in the MURASHIGE & SKOOG liquid medium without any growth substances or extracts added. Taking advantage of this fact a clone was established and maintained in this medium, with no loss of vigor, for 16 months through 16 transfers. When a comparison is made between roots in culture and attached roots growing in sand the following facts are evident (Table I and Figs. 1, 2) : roots *in vitro* are longer than in sand and the number of primary laterals is smaller; also, these laterals are longer and the distance between them is greater than in sand growing roots; finaly there is a characteristic absence of secondary laterals in attached roots.

TABLE I

A comparison of the growth of excised potato roots cultured in the MURASHIGE & SKOOG medium (without growth substances added) and of attached roots growing in sand. (All roots with one month of growth).

	Cultured (mean of 17 roots)	Sand (mean of 7 roots)
Length of the main axis (cm.)	29,60	18,54
	10,94	55,57
Length of the primary laterals (cm.) . .	5,04	1,62
Distance between primary laterals (cm.)	2,33	0,30
Number of secondary laterals	3,55	—

It is interesting to notice that for roots growing in culture a tendency for the laterals to accumulate on one side of the main axis occurs. In a few subcultures the roots showed a very weak growth reaching a maximum length of 3-7 cm. without formation of laterals.

DISCUSSION

CHAPMAN (1956) and later BAJAJ & DIONNE (1966) were able to establish continuous cultures of excised potato roots using a modified WHITE'S medium supplemented with a tuber extract. Our results show that it is possible to obtain continuous clonal culture of excised potato roots by using a medium of defined composition, the MURASHIGE & SKOOG medium (1962) without addition of growth substances. This fact has some importance in order to understanding the physiology of root growth *in vitro,* as it is essential that unidentified substances, like those present in tuber extracts, are not included in the culture medium.

CHAPMAN used the potato variety Triumph and BAJAJ & DIONNE the variety Netted Gem. It would be of interest to see if the roots of these and other varieties are able to grow in the same medium we have used. However, negative results are expected as a great intraspecific, intrastrain and intraclonal variation exists in excised root cultures (STREET, 1957).

Concerning the exuberance of the roots growth we can conclude from the data of CHAPMAN (maximum root length 10 cm. and maximum number of laterals 5, after 45 days of culture) that our results are indicative of a much greater growth rate. BAJAJ & DIONNE do not give a quantitative estimation of the roots growth; so, it is not possible to make a comparison, although, as judged by the photographs, it seems rather weak.

As shown in Table I there is a marked difference in growth pattern between the excised roots in culture and the attached roots growing in sand. It is known (STREET, 1966) that differences between excised roots and intact roots exist. Excised roots do not normally show secondary thickening, and it has also been found that excised roots may differ significantly in chemical composition from seedling roots. Recently ABBOTT (1972), working with pea roots, has found that attached roots tips had fourfold quantities of RNA and thereefold quantities of DNA compared with the tips of cultured roots. Although these facts do not bear

a direct relationship with our. observations we can tentatively assume that the greater linear growth of the cultured roots is due to a greater cell enlargement and/or a greater increase in number of cells.

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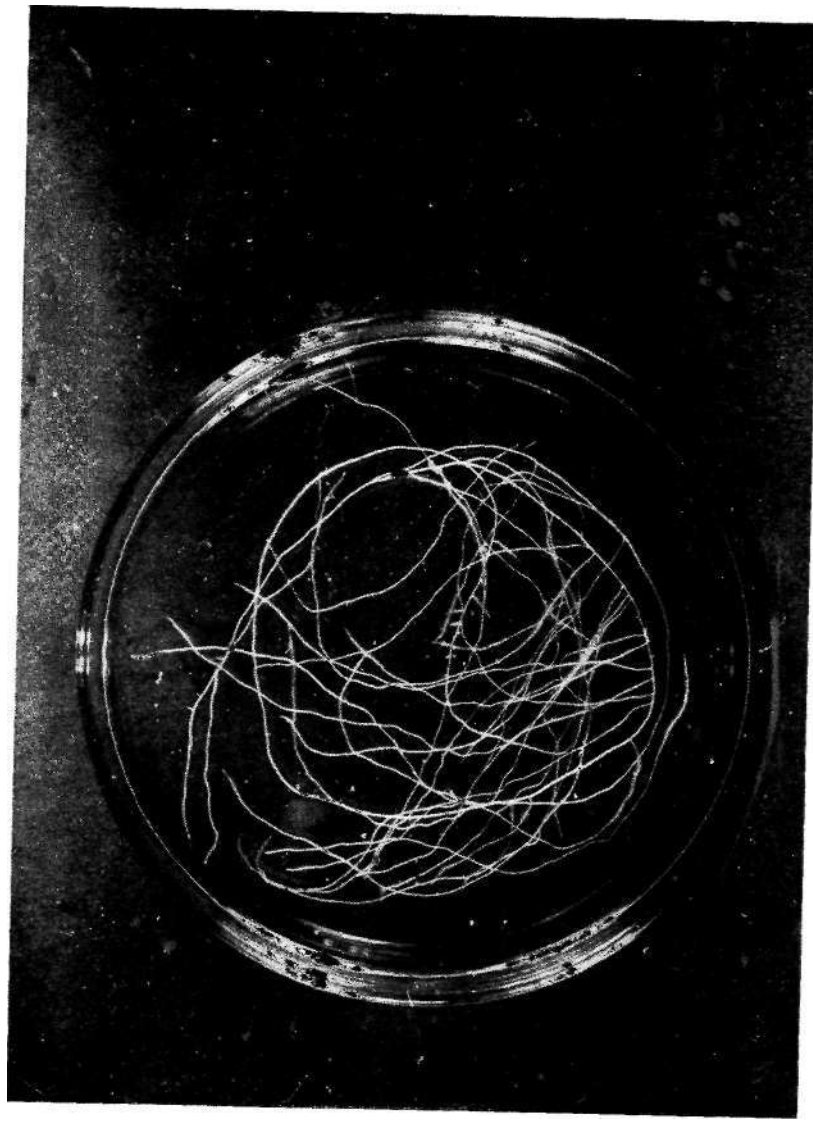

A vigorous root subculture, one month old. (twelfth transfer· for photographing the root has been placed in a Petri dish). XI.

INDICE