

Plants grown with 75 or 750  $\mu\text{M}$  Fe in the nutrient solution exhibited chloroplasts with numerous starch grains and a well-appressed lamellar system, though phytoferritin arrays were already present if plants were grown in the presence of 750  $\mu\text{M}$  Fe. These plants had indistinguishable leaf polypeptide patterns (VARENNES, 1991) and a similar chlorophyll content. Thus, the formation of phytoferritin aggregates in these chloroplasts seemed to act as an efficient buffer for soluble iron, protecting the cell against an overdose of iron which would cause deleterious effects.

If the iron supply was further increased, toxic levels were reached (7500  $\mu\text{M}$  Fe in the nutrient solution). Chloroplasts were filled with phytoferritin crystals and, though they presented high chlorophyll contents, starch grains were absent showing an impaired function. These plants exhibited stress reactions like the accumulation of several low molecular weight acid-extractable polypeptides similar to pathogenesis-related proteins (VARENNES, 1991). They also showed an altered zymogram for leaf aspartate aminotransferase activity (VARENNES & CARVALHO, 1990), a response to micronutrient toxicity frequently observed in white lupin (unpublished observations).

In white lupin roots, plastids were observed to accumulate phytoferritin even in control-plants. The fact that amyloplasts were still present in plants grown with high levels of iron suggests that starch synthesis *per se* is not sensitive to iron. Thus, starch absence in the corresponding chloroplasts is probably the result of impaired photosynthesis.

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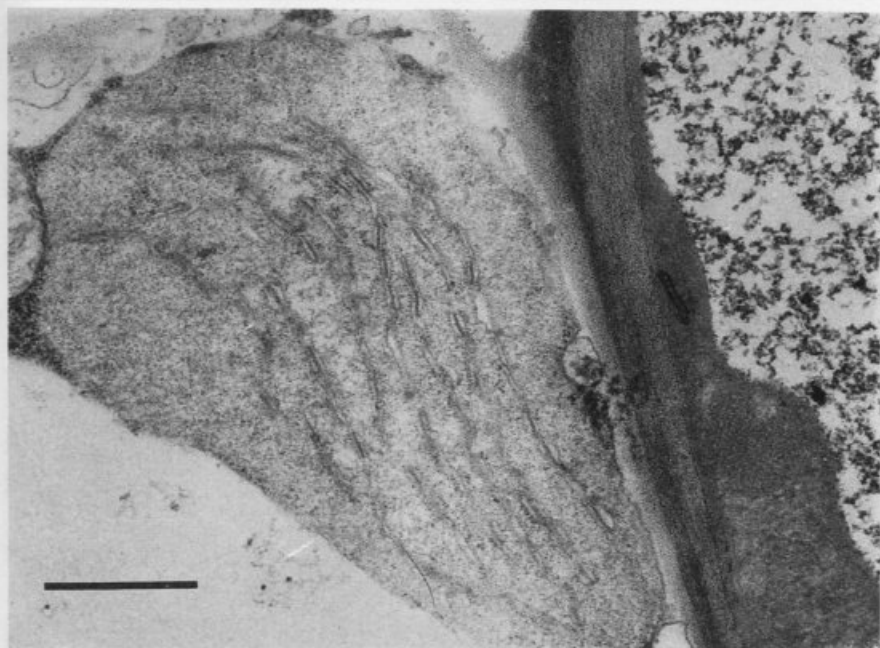
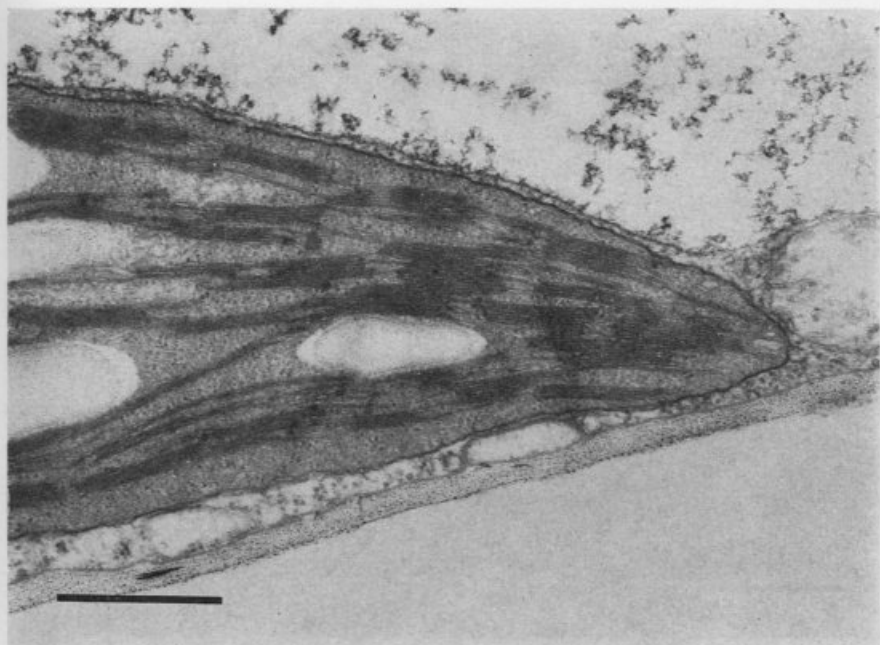
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Leaf levels of iron. Fig. 1: 75  $\mu\text{M}$  Fe; Fig. 2: 0  $\mu\text{M}$  Fe  
Bar = 500 nm

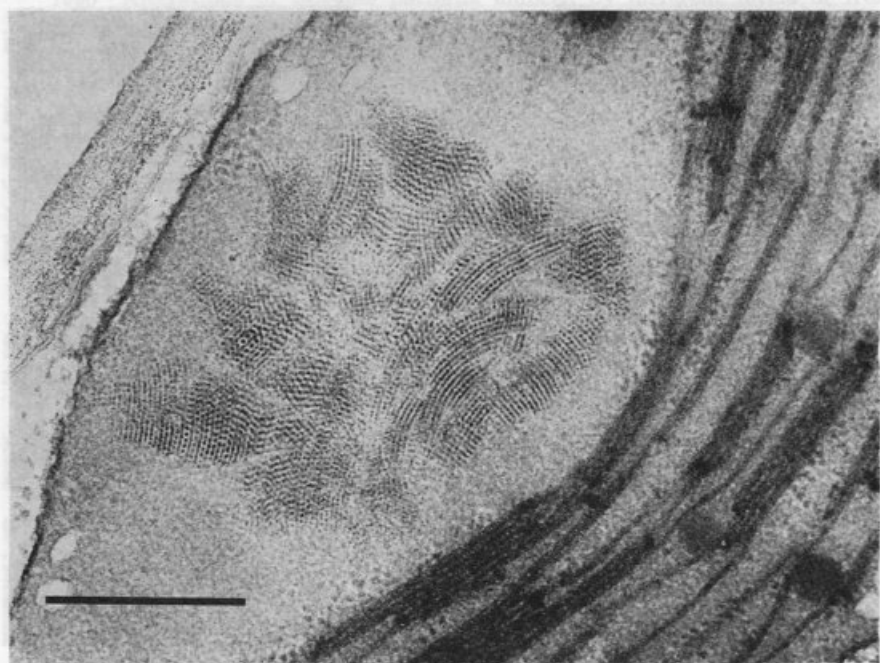
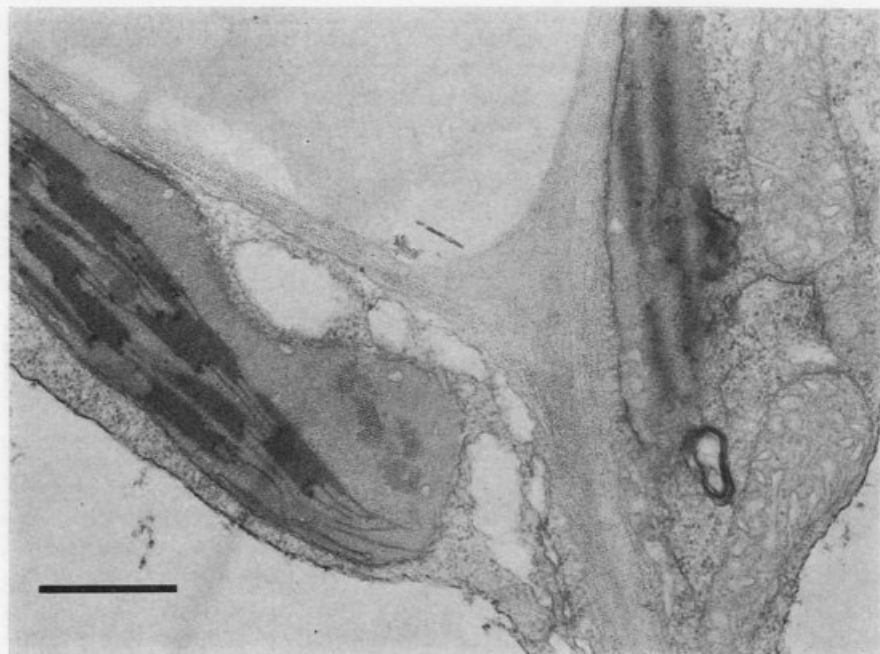
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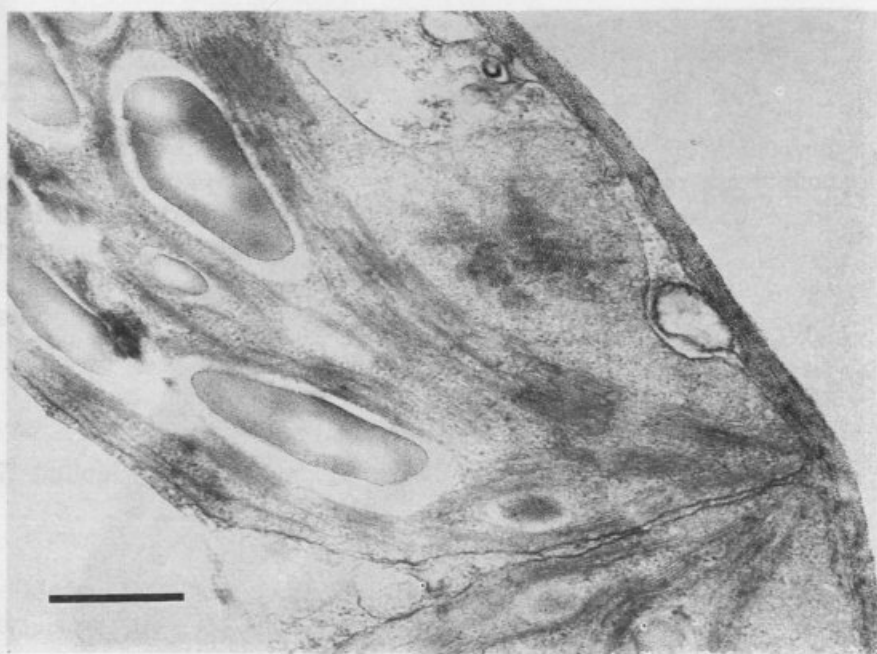
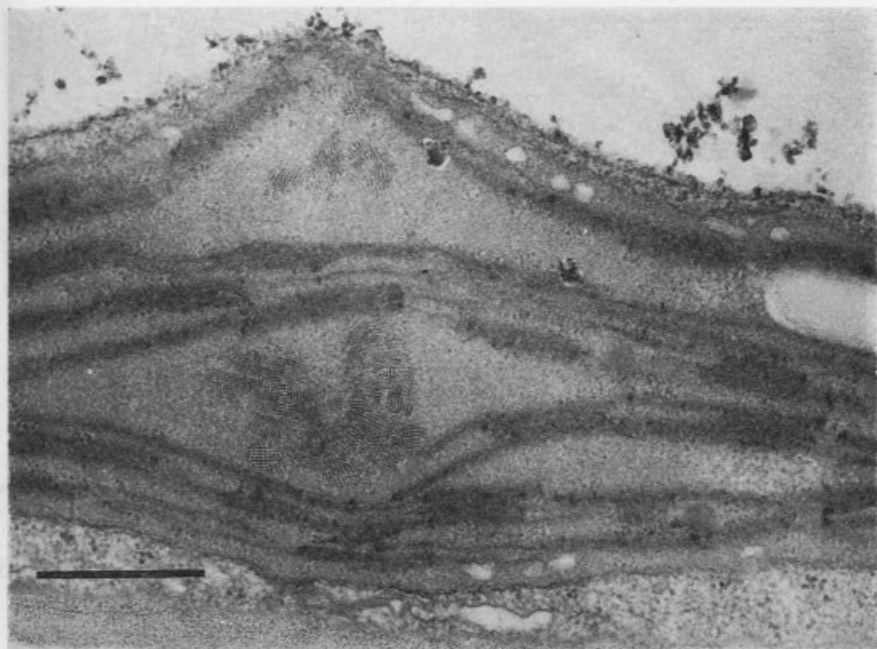
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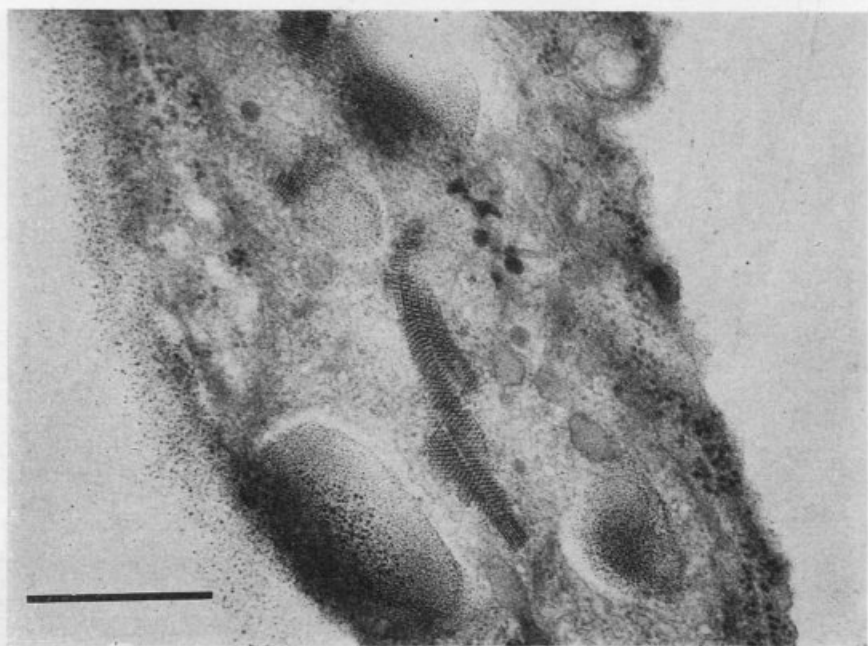
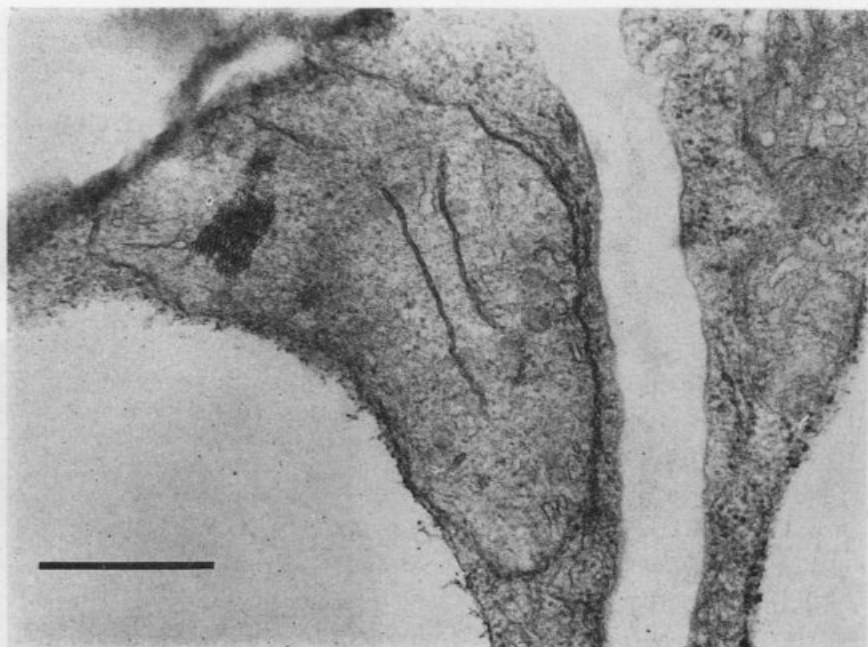
Leaf chloroplasts of white lupin plants grown in nutrient solutions with different levels of iron. Fig. 1:  $75 \mu\text{M Fe}$ ; Fig. 2:  $0 \mu\text{M Fe}$ .  
Bar = 500 nm.



Leaf chloroplasts of white lupin plants grown in nutrient solutions with different levels of iron. Fig. 1 and Fig. 2: 7500  $\mu\text{M}$  Fe.  
Bar = 500 nm.



Leaf chloroplasts of white lupin plants grown in nutrient solutions with different levels of iron. Fig. 1: 7500  $\mu\text{M}$ ; Fig. 2: 750  $\mu\text{M}$  Fe.  
Bar = 500 nm.



Root plastids of white lupin plants grown in nutrient solutions with different levels of iron. Fig. 1: 75  $\mu\text{M}$  Fe; Fig. 2: 7500  $\mu\text{M}$  Fe.  
Bar = 500 nm.

## NÚMEROS CROMOSSÓMICOS PARA A FLORA PORTUGUESA. 116-127

por

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Recebido em 27-XI-92.

### 116. *Polygonum hydropiper* L.

Número cromossómico:  $2n = 20$ .

**Material:** Estremadura, Meleças. n.º 6621 (COI). s. d.<sup>1</sup> Det. M. Queirós; Beira Litoral, Matas de Foja. N.º 6975 (COI). 12.10.1972. Det. M. Queirós.

**Observações:** O número somático encontrado está de acordo com as determinações anteriores (QUEIRÓS, 1983) em indivíduos colhidos em outras localidades do País, sob a designação de *Persicaria hydropiper* (L.) Spach.

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### 117. *Fallopia convolvulus* (L.) Á. Löve

Número cromossómico:  $2n = 40$ .

**Material:** Estremadura, Jardim Botânico de Lisboa. N.º 7705 (COI). s. d.<sup>1</sup> Det. M. Queirós.

<sup>1</sup> Sem data de colheita das respectivas sementes.

*Observações:* O número cromossómico encontrado está de acordo com as observações anteriores (QUEIRÓS, 1983), em indivíduos de Quiaios e de Algueirão.

#### 118. *Sisymbrium altissimum* L.

*Número cromossómico:*  $2n = 14$  (fig. 1).

*Material:* Beira Baixa, Vila Velha do Ródão. N.º 5517 (COI). 8.7.1971. Det. M. Queirós.

*Observações:* Referimos para este taxon (QUEIRÓS, 1973)  $2n = 14$ , de acordo com o estudo efectuado por vários autores. Confirmamos, assim, as nossas contagens anteriores para indivíduos de uma nova localidade de Portugal. Achámos conveniente a publicação da fig. 1 por nela se mostrarem 2 cromossomas satelitíferos não observados então.

#### BIBLIOGRAFIA:

- QUEIRÓS, M. 1973 — Contribuição para o conhecimento citotaxonómico das *Spermatophyta* de Portugal. IX. *Cruciferae*. *Bol. Soc. Brot.*, sér. 2, 47: 315-335.

#### 119. *Rorippa sylvestris* (L.) Besser

*Número cromossómico:*  $2n = 32$ .

*Material:* Trás-os-Montes, Alijó. N.º 5162 (COI). 1971<sup>2</sup>. Det. M. Queirós.

*Observações:* Confirmamos os resultados que registámos em 1973 (*op. cit.*) e 1979.

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#### 120. *Diplotaxis catholica* (L.) DC.

*Número cromossómico:*  $2n = 18$ .

<sup>2</sup> Sem indicação do dia e mês de colheita das respectivas sementes.



**Material:** Alto Alentejo, na estrada entre Lavre e Vendas Novas. N.º 3034 (coi). 12.7.1969. Det. M. Queirós.

**Observações:** Confirmamos o resultado mencionado em 1973 (*op. cit.*) em plantas de uma outra localidade do País.

121. **Rapistrum rugosum** (L.) All.

**Número cromossómico:**  $2n = 16$ .

**Material:** Alto Alentejo, Ervedal, próximo de Estremoz. N.º 3037 (coi). 13.7.1969. Det. M. Queirós.

**Observações:** O número somático encontrado está de acordo com as nossas observações anteriores (QUEIRÓS, 1973, *op. cit.*) referentes a plantas portuguesas, embora de outra localidade.

122. **Hypericum undulatum** Schousboe ex Willd.

**Número cromossómico:**  $2n = 16$ .

**Material:** Beira Baixa, Belmonte, na estrada para Maçainhas. N.º 6899 (coi). 25.8.1972. Det. M. Queirós.

**Observações:** Em 1989, registámos  $2n = 16$  para indivíduos do Poço do Inferno, de São Paulo de Frades e da Praia das Maçãs. Confirmamos este número para uma nova localidade portuguesa.

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123. **Micropyrum tenellum** (L.) Link

**Número cromossómico:**  $2n = 14$  (fig. 2).

**Material:** Trás-os-Montes, na estrada de Moncorvo a Freixo de Espada à Cinta, a 7 km desta última localidade. N.º 8050 (coi). 1973<sup>3</sup>. Det. M. Queirós.

<sup>3</sup> Sem indicação do dia e mês de colheita das respectivas sementes.

**Observações:** Confirmamos o número  $2n = 14$  já determinado em estudos anteriores (FERNANDES & QUEIRÓS, 1969; QUEIRÓS, 1973, 1974) sob a designação de *Ctatapodium tenellum* (L.) Trab. Achamos conveniente a publicação da fig. 2 por nela se mostrarem 4 cromossomas satelitíferos não observados então.

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**124. *Dipsacus fullonum* L.**

**Número cromossómico:**  $2n = 18$  (fig. 3).

**Material:** Beira Litoral, Figueira da Foz, Fontela. N.º 866 (COI). 9.11.1965. Det. C. Alves; Beira Litoral, Coimbra, Eiras. N.º 865 (COI). 29.8.1966. Det. C. Alves. Conf. M. Queirós.

**Observações:** Diversos autores, entre eles PALKOVÁ (in MÁJOVSKY & al., 1970), LÖVE & KJELLQVIST (1974), VERLAQUE (1978), COLASANTE (1979) e VERLAQUE (in LÖVE, 1980), atribuem a este taxon  $2n = 18$ , o que é confirmado pelas nossas contagens em indivíduos pertencentes a populações das duas localidades portuguesas acima assinaladas.

Não podemos deixar de referir o número  $2n = 16$  referido por outros autores, entre os quais RISSE (1929) e POUQUES (1948, 1949). No entanto, esta última autora refere, igualmente, para este taxon  $2n = 18$ .

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*num* L. e *Dipsacus ferox* Loisel. *Ann. di Bot.* 38(1): 71-79.

VERLAQUE, R. 1980 (in LÖVE, 1980).

LÖVE, A. 1980 — Chromosome number reports. LXVII. *Taxon* 29(2-3): 347-367.

## 125. *Succisa pratensis* Moench

*Número cromossômico*:  $2n = 20$  (fig. 4).

*Material*: Beira Litoral, Pampilhosa do Botão, Valdoeiro. N.º 1826 (COI). 20.3.1968. Det. C. Alves.

*Observações*: Os dados cariológicos referentes a este taxon são divergentes. RISSE (1926; 1929 — op. cit.) cita o número cromossômico  $2n = 16$ . SUGIURA (1942, 1943) determinou  $2n = 18$ . Diversos autores, entre eles ZUKOWSKI & SLOWINSKA (1979), POGAN & al. (1980), FICINI & al. (1981), VERLAQUE (in LÖVE, 1982), DMITRIEVA (1986) e VERLAQUE (1986), determinaram  $2n = 20$ . Os nossos resultados estão de acordo com os destes autores.

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126. *Pterocephalus diandrus* (Lag.) Lag. (*P. papposus* auct. iber.)

*Número cromossómico*:  $2n = 16$  (fig. 5).

*Material*: Trás-os-Montes, Bragança, entre Guadramil e Deilão. N.º 1427 (COI). 5.8.1967. Det. C. Alves. Conf. M. Queirós.

*Observações*: Confirmamos as contagens ( $2n = 16$ ) efectuados anteriormente por DEVESA (1981) em material de Huelva, as de VERLAQUE (in LÖVE, 1980) em populações portuguesas dos arredores de Coimbra e, ainda, as do mesmo autor (in LÖVE, 1982) em material de Cáceres. Em 1986, VERLAQUE cita de novo  $2n = 16$  para este taxon, considerando-o endémico da Península Ibérica.

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127. *Dactylorhiza maculata* (L.) Soó

Número cromossômico:  $2n = 80$  (fig. 6).

**Material:** Trás-os-Montes, Castro Daire, próx. do rio Balsemão. N.º 1202 (COR). 6.8.1967. Det. C. Alves. Conf. M. Queirós.

**Observações:** Confirmamos o número  $2n = 80$  determinado por FERNANDES (1950) referente a exemplares portugueses colhidos entre S. Bento da Porta Aberta e Còvide (Serra do Gerês). Este resultado está de acordo com a determinação de outros autores, entre os quais JAGIELLO & MRÓR (1986-87), GATHOYE & TYTEKA (1989), JAGIELLO & al. (1989) e SEMERENKO (1989).

O número  $2n = 40$  mencionado, igualmente, por diversos autores, entre os quais LÖVE & LÖVE (1944), SKALINSKA & al. (1957) e VAN LOON & DE JONG (in LÖVE, 1978), corresponderia a *D. fuchsii* e *D. saccifera* (GATHOYE & TYTEKA, 1989). Segundo estes últimos autores, o número cromossômico  $2n = 60$  atribuído igualmente a *D. maculata* por alguns autores, entre os quais GROLL (1965), VAUCHER (1966), VÖTH & GREILHUBER (1980) e AVERYANOV & al. (1982) corresponderia a plantas de origem híbrida resultantes do cruzamento de *D. maculata* e *D. fuchsii*.

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DISTRIBUCIÓN COROLÓGICA  
DEL GÉNERO *ORONTITES* EN LA PROVINCIA



Fig. 1. — *Sisymbrium altissimum* L. ( $2n = 14$ ); Fig. 2. — *Micropyrum tenellum* (L.) Link ( $2n = 14$ ); Fig. 3. — *Dipsacus fullonum* L. ( $2n = 18$ ); Fig. 4. — *Succisa pratensis* Moench ( $2n = 20$ ); Fig. 5. — *Pterocephalus diandrus* (Lag.) Lag. ( $2n = 16$ ); Fig. 6. — *Dactylorhiza maculata* (L.) Soó ( $2n = 80$ ).  $\times 3000$ .

El género *Orontites*, que comprende 10 especies, se halla bien representada en Andalucía, y especialmente en la provincia de Granada, en donde hemos podido constatar la existencia de 6 taxones, según se deduce del estudio que hemos realizado de los pliegos existentes en los Herbarios de las Facultades de Farmacia (GDA) y de Ciencias (GDAC) de la Universidad de Granada, así como de la consideración y comprobación de numerosas citas bibliográficas y de nuestras propias herborizaciones y trabajos personales.

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Fig. 1 — *Stigmaphyllon dilatatum* L. (2n = 14); Fig. 2 — *Microglossum tenuissimum* (L.) Link (2n = 14); Fig. 3 — *Diplocentrus fulvum* L. (2n = 18); Fig. 4 — *Succisa pratensis* Moench (2n = 20); Fig. 5 — *Pterocarya lutea* (L.) Lag. (2n = 16); Fig. 6 — *Dactylorhiza maculata* (L.) Sob (2n = 60). X 3000.



## DISTRIBUCIÓN COROLÓGICA DEL GÉNERO *ODONTITES* EN LA PROVINCIA DE GRANADA

### ECOLOGIA Y FITOSOCIOLOGIA

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Recibido el 22-XII-92.

#### RESUMEN

Se realiza un estudio de seis especies del género *Odontites*, presentes en la provincia de Granada. Indicamos su corología y su comportamiento ecológico y fitosociológico

#### SUMARY

We have studied six species of genera *Odontites* are living in the province of Granada. In this paper we indicated their corology and their ecology and phitosociology also.

#### INTRODUCCIÓN

**E**L género *Odontites*, interesante taxon de la Familia Escrofulariáceas, y del que la Flora Europea cita para España 10 especies, se halla bien representada en Andalucía, y especialmente en la provincia de Granada, en donde hemos podido constatar la existència de 6 taxones, según se deduce del estudio que hemos realizado de los pliegos existentes en los Herbarios de las Facultades de Farmacia (GDA) y de Ciencias (GDAC) de la Universidad de Granada, así como de la consideración y comprobación de numerosas citas bibliográficas y de nuestras propias herborizaciones y trabajos personales.

## DISPERSIÓN COROLÓGICA DEL GÉNERO ODONTITES

Se trata de unas plantas herbáceas, en su mayoría anuales, dominando los biotipos terofíticos, y a veces camefíticos, con frecuencia glanduloso-pubescentes; hojas generalmente enteras, lineares, estrechas y casi sentadas; flores en espigas laxas, con corolas bilabiadas, amarillas o rojas, de tubo generalmente largo y saliente; cápsula comprimida, obtusa y con numerosas semillas fusiformes y estriadas.

Corológicamente estos táxones se encuentran y son característicos de la provincia Bética, abundando especialmente en los sectores Nevadense, Malacitano-Almijareense y Albujarro-Gadoreense. Ya con menos frecuencia, y de manera más dispersa y esporádica, aparecen en la parte N.E. de la provincia granadina, en donde la flora bética contacta y se entremezcla con la castellano-manchega.

Las especies que a continuación vamos a estudiar, indicando su corología, ecología y comportamiento fitosociológico, son las siguientes:

*O. granatensis* Boiss.

*O. longiflora* (Vahl) Webb.

*O. purpurea* (Desf.) G. Don fil

*O. verna* (Bellardi) Dumort subsp. *serotina* (Dumort) Corb.

*O. viscosa* (L.) Clairv. subsp. *hispanica* (Boiss. et Reuter) Rothm.

*O. viscosa* L. subsp. *viscosa*

**Odontites granatensis** Boiss.

Planta anual de pequeña talla, no sobrepasa los 15-20 cm de altura; hojas enteras, lanceoladas y agudas, ligeramente peloso-viscosas; espigas flojas con flores pequeñas rojizas.

Su dispersión corológica dentro de la provincia de Granada es muy puntual y localizada, circunscribiéndose solamente al sector malacitano-almijareense (ladera N.O. de Sierra Nevada) en el llamado Collado de las Sabinas (2000-2150 m de altitud).

Herbario GDA

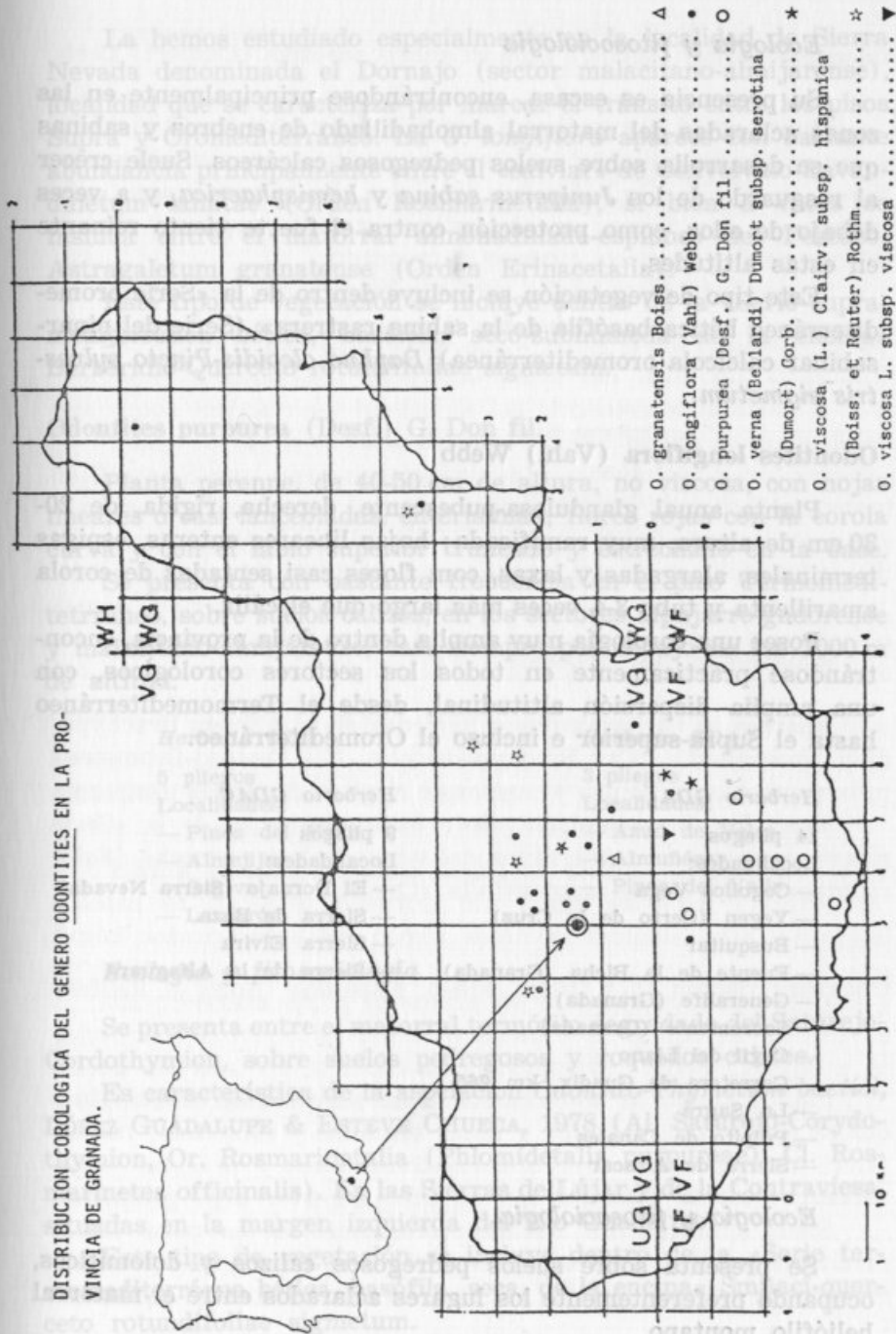
Un solo pliego

Localidad: Güejar-Sierra

(Collado de las Sabinas

30SVG60

DISTRIBUCION COROLOGICA DEL GENERO ODONTITES EN LA PROVINCIA DE GRANADA.



- △ *O. granatensis* Boiss. ....
- *O. longiflora* (Vahl) Webb. ....
- *O. purpurea* (Desf.) G. Don fil. ....
- *O. verna* (Bellardi) Dumort subsp. serotina (Dumort) Corb. ....
- ★ *O. viscosa* (L.) Clairv. subsp. hispanica (Boiss. et Reuter) Rothm. ....
- ★ *O. viscosa* L. subsp. viscosa .....

*Ecología y fitosociología*

Su presencia es escasa, encontrándose principalmente en las zonas aclaradas del matorral almohadillado de enebros y sabinas que se desarrolla sobre suelos pedregosos calcáreos. Suele crecer al resguardo de los *Juniperus sabina* y *hemisphaerica*, y a veces debajo de ellos, como protección contra el fuerte viento reinante en estas altitudes.

Este tipo de vegetación se incluye dentro de la «Serie oromediterránea bética basófila de la sabina rastrera» (Serie del pinar-sabinar calcícola oromediterránea) *Daphno oleoidis-Pineto sylvestris sigmetum*.

**Odontites longiflora** (Vahl) Webb

Planta anual glandulosa-pubescente, derecha, rígida, de 20-30 cm de altura, muy ramificada; hojas lineares enteras, espigas terminales, alargadas y laxas, com flores casi sentadas de corola amarillenta y tubo 3-4 veces más largo que el cáliz.

Posee una corología muy amplia dentro de la provincia, encontrándose prácticamente en todos los sectores corológicos, con una amplia dispersión altitudinal, desde el Termomediterráneo hasta el Supra-superior e incluso el Oromediterráneo.

*Herbario GDA*

- 14 pliegos  
Localidades:  
— Cogollos Vega  
— Yegen (Cerro de la Cruz)  
— Busquitar  
— Fuente de la Bicha (Granada)  
— Generalife (Granada)  
— Sacromonte (Granada)  
— Cagil del Llano  
— Carretera de Guadix, km 265  
— La Sagra  
— Pulpito de Canales  
— Sierra de Alfacar

*Herbario GDAC*

- 9 pliegos  
Localidades:  
— El Dornajo (Sierra Nevada)  
— Sierra de Baza  
— Sierra Elvira  
— Sierra de la Alfaguara

*Ecología y fitosociología*

Se presenta sobre suelos pedregosos calizos y dolomíticos, ocupando preferentemente los lugares aclarados entre el matorral heliófilo montano.

La hemos estudiado especialmente en la localidad de Sierra Nevada denominada el Dornajo (sector malacitano-almijareense), localidad que se caracteriza por marcar el tránsito entre los pisos Supra y Oromediterráneo. La *O. longiflora* aparece con bastante abundancia principalmente entre el «salviar» de Convolvulo-Lavanduletum lanatae (Orden Rosmarinetalia), si bien a veces se insinúa entre el matorral almohadillado-espinoso del Festuco-Astragaletum granatense (Orden Erinacetalia).

Este tipo de vegetación se incluye dentro de la «Serie supra-mediterránea bética, basófila, seco-subhúmeda de la encina» Berberidio-Querceto rotundifoliae sigmetum.

### **Odontites purpurea** (Desf.) G. Don fil.

Planta perenne, de 40-50 cm de altura, no viscosa, con hojas lineares o casi lanceoladas, enterísimas; flores rojas con la corola curva y con el labio superior truncado y estrechado en la base.

Se presenta con bastante frecuencia en el piso Termomediterráneo, sobre suelos calizos, en los sectores alpujarro-gadoreense y malacitano-almijareense, casi siempre por debajo de los 1000 m de altitud.

#### *Herbario GDA*

5 pliegos

#### Localidades:

- Pinos del Valle
- Almejijar
- Orgiva
- Lanjarón

#### *Herbario GDAC*

3 pliegos

#### Localidades:

- Azud de Velez
- Almuñécar
- Pinos del Valle

### *Ecología y fitosociología*

Se presenta entre el matorral termófilo degradado del Saturejo-Cordothymion, sobre suelos pedregosos y roquedos calizos.

Es característica de la asociación *Odontito-Thymetum baetici*, LÓPEZ GUADALUPE & ESTEVE CHUECA, 1978 (Al. Saturejo-Cordothymion, Or. Rosmarinetalia (Phlomidetalia purpureae), Cl. Rosmarinetea officinalis). En las Sierras de Lújar y de la Contraviesa, situadas en la margen izquierda del Río Guadalfeo.

Este tipo de vegetación se incluye dentro de la «Serie termomediterránea bética, basófila, seca, de la encina» Smilaci-querceto rotundifoliae sigmetum.

**Odontites verna** (Bellard) Dumort subsp. **serotina** (Dumort) Corb.

Planta anual, peloso-áspera, con tallos rectos de hasta 30-40 cm y ramas abiertas y patentes; hojas sentadas, lanceolado-lineares; flores en espigas unilaterales provistas de brácteas algo más cortas que las flores; corola vellosa, rosado-purpúrea, con el labio superior un poco cóncavo y truncado.

Se presenta muy escasa y circunscrita a las zonas húmedas de montaña, en los sectores malacitano-almijareense y nevadense de Sierra Nevada, entre los 1000-1500 m de altitud.

*Herbario GDA*

3 pliegos

Localidades:

— Quentar (Barranco del Polvorista)

— Río Trevélez

— Acequia de Trevélez

*Ecología y fitosociología*

Aparece en herbazales muy húmedos de la Alianza *Brachypodium phoenicoidis* (Or. *Brachypodietalia*, Cl. *Festuco-Brometea*) próximos a ríos, acequias y canales de riego de las zonas montañas.

Este tipo de vegetación puede incluirse dentro de la «Serie riparia meso-supramediterránea del sauce de hoja estrecha [*Salix eleagnos* Scop. subsp. *angustifolia* (Cariot) Rech. fil.]».

**Odontites viscosa** (L.) Calirv. subsp. **hispanica** (Boiss et Reuter)

Rothm.

Planta anual, de 40-50 cm de alta, pubescente, viscoso-glandulosa en la parte superior y hojas sentadas y estrechas; racimo floral corto y denso, con flores pedunculadas y brácteas empizarradas; corola amarilla con el albio superior algo pestañoso.

Suele aparecer con relativa frecuencia en los pisos Mesomediterráneo horizonte superior y Supramediterráneo, y en los sectores guadiciano-bacense, malacitano-almijareense y subbético.

*Herbario GDA*

2 pliegos

Localidades:

- Dehesa del Camarate
- Lugros

*Herbario GDAC*

5 pliegos

Localidades:

- Sierra de Baza
- Dehesa del Camarate
- Sierra de Alfacar
- Encinar de Sierra Elvira

*Ecología y fitosociología*

Se presenta en suelos calizos y a veces sobre esquistos metamórficos ultrabásicos, formando parte del matorral degradado que ocupa los claros y bordes de los encinares montanos.

PÉREZ RAYA, en su tesis doctoral (1987), la cita en la localidad de Güejar-Sierra (sector malacitano-almijareense de Sierra Nevada) como compañera en la Asociación Helicotricho filifolii-Festucetum scariosae, M. Parras, Peinado y Alcaraz, festucetosum elegantis Pérez Raya (Al. Festucion scariosae, Or. Lygeo-Stipetalia, Cl. Lygeo spartii-Stipetea tanacissimae).

Esta vegetación constituye un «lastonar» de degradación del encinar supramediterráneo calizo. Serie: Berberidio-Querceto rotundifoliae sigmentum.

***Odontites viscosa* L. subsp. *viscosa***

Planta perenne de hasta 30 cm de altura, pubescente y muy viscosa en la parte superior, con hojas sentadas y estrechas; flores pedunculadas con corola amarilla, lampiña, más larga que el estilo.

Su presencia en la provincia de Granada es muy puntual y localizada, circunscribiéndose solamente al sector nevadense de Sierra Nevada, en la localidad denominada, Tajos Colorados de Capileira, a 2550 m de altitud.

*Herbario GDA*

Un solo pliego

Localidad:

Tajos Colorados de Capileira  
(Sierra Nevada) 30SVF6997

*Ecología y fitosociología*

Aparece en pequeñas terreras y rellanos situados en cortados y taludes terrosos, sobre suelos de micasquistos con abundantes afloramientos de mármoles.

La vegetación de estos lugares está constituida por un pequeño matorral almohadillado-espinoso, discontinuo, de *Siderito-Arenarietum pungentis* (*Ptilotrichion purpureae* — *Festucetalia indigestae* — *Juncetea trifidi*) presentando una facies rica en elementos básicos.

Esta vegetación se incluye en la «Serie oromediterránea nevadense silicícola del enebro rastrero (*Juniperus nana*)» *Genisto baeticae-Junipereto nanae sigmetum*.

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## ON THE BIOLOGICAL ORIGIN OF DEPSIDONES

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Received, 19-III-1993.

### RESUMO

Depsidones são compostos químicos produzidos exclusivamente por líquenes, com dois fenóis condensados pela formação de uma banda ester na posição 1 e 4' e uma banda suplementar nas posições 2 e 5'. Uma hipótese muito comum e aceita, sugere que as depsidonas podem ser produzidas a partir dos dépsides, que reduzem a reacção de hidroxil-substituinte C<sub>2</sub>. E que a banda éter pode ser enzimaticamente reduzida. Este facto é oposto às ideias de CULBERSON & ELIX que afirmam hipoteticamente sobre hidroxilação na posição 5' nos dépsides seguido de uma migração acil e rearranjos tipo «smiles».

Na possibilidade de que as bandas éter forem hidrolizadas enzimaticamente por extratos de algumas espécies de líquens, está de acordo com a hipótese de SALA & SARGENT sobre o envolvimento anidrido phtálico como precursor das depsidonas. De acordo com este caminho biosintético as bandas de éter devem ser produzidas antes da reacção de esterificação.

### INTRODUCTION

**D**EPSIDONES are unique compounds produced by lichens in which two monocyclic phenols condense by formation of an ester bond between 1 and 4' positions and a supplementary ether bond between 2 and 5' positions. A commonly accepted hypothesis suggests that depsidones could be produced from depsides through a reducing reaction involving the hydroxyl-

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substituted C<sub>2</sub>. However, ether bond cannot be enzymatically reduced. This is in opposite to CULBERSON and ELIX hypothesis about a hydroxylation at 5' position in the depside followed by acyl migration and smiles re-arrangement. The possibility that the ether bond was hydrolyzed by enzymatic extracts from several lichen species is in agreement with the hypothesis of SALA and SARGENT about the involvement of a phtalic anhydride as precursor of depsidones. According to this pathway, ether bond would be produced before esterification reaction.

### Hypothesis derived from biomimetic organic synthesis

The origin of depsidones remains uncertain. Depsidones are bicyclic phenolic compounds, produced by lichens, in which the linkage includes both ester and ether bonds. Ester bond is achieved by depside hydrolases which esterify the free carboxyl group in «A» rig with the 4'-hydroxyl substituent in «B» ring (MOSBACH, 1973). The existence of couples depside-depsidone in the same lichen species, such as olivertoric-physodic acids in *Cetraria ciliaris* (CULVERSON, 1964), indicates the possibility that the depsidone was produced from its depside through a dehydrogenase-catalyzing reaction forming an ether bond between the two phenolic units (Fig. 1).

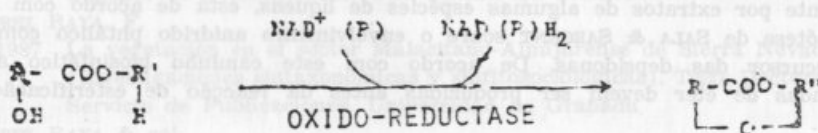


Fig. 1. — Hypothesis of depsidone biosynthesis from a depside precursor through dehydrogenase reaction.

However, this hypothesis has two main problems. Firstly, it is very rare for the pair depside-depsidone to occur in the same lichen species (ELIX *et al.*, 1984; ROGERS, 1989). Secondly, several attempts to cleave the ether linkage by the action of a hypothetical oxido-reductase, coupled to NADH, HADPH or FADH<sub>2</sub> failed.

To explain the occurrence of iso-structural depside-depsidone pairs, ELIX *et al.* (1987) and CULBERSON & ELIX (1989) suggested that C-hydroxylation of a para-depside in the 5' position would

be followed acyl migration and subsequent smiles rearrangement of the meta-depside formed, to lead to the corresponding orcinol-depsidones (Fig. 2). However, this implies that  $R_2$  group in «B»

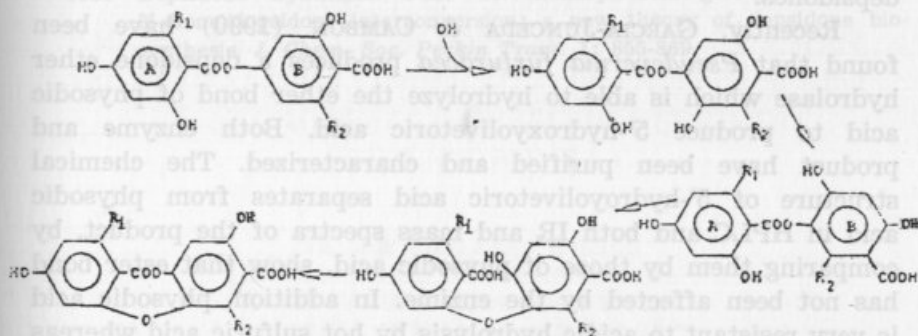


Fig. 2. — Hypothesis of CULBERTSON & ELIX (1989) about the synthesis of depsidones from depside precursors.

ring could be and hydrogen and, so, ether linkage might be sensitive to enzymatic reduction. As above we say, this enzymatic reduction fails by using the most common cofactors of oxidoreductases.

### Organic chemistry versus biological processes

Alternatively, SALA & SARGENT (1981) proposed that this ether bond could be produced by de-halogenation of a phthalic anhydride derivative, followed by its coupling to a phenol and intramolecular arrangements (Fig. 3). This proposal is explained

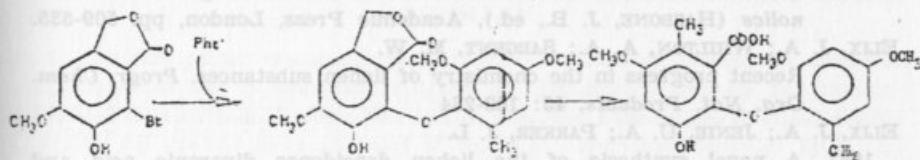


Fig. 3. — Hypothesis about depsidone biosynthesis involving a phthalic anhydride as precursor.

on the basis of several studies on organic synthesis of depsidones isolated from *Buellia canescens*. Since dehalogenation can be achieved by hydrolysis, the consequence would be that ether bond

could be formed between two hydroxy-substituted carbons. So, enzymatic hydrolysis rather than reduction could be the biological process implied in the reversible breakage of the bond-defining depsidones.

Recently, GARCIA-JUNCEDA & CAMBON (1990) have been found that *Pseudevernia furfuracea* produces a depsidone ether hydrolase which is able to hydrolyze the ether bond of physodic acid to produce 5'-hydroxyolivetoric acid. Both enzyme and product have been purified and characterized. The chemical structure of 5'-hydroxyolivetoric acid separates from physodic acid in HPLC and both IR and mass spectra of the product, by comparing them by those of physodic acid, show that ester bond has not been affected by the enzyme. In addition, physodic acid is very resistant to acidic hydrolysis by hot sulfuric acid whereas the product is not. Acidic hydrolysis of 5'-hydroxyolivetoric acid produced two well distinguishable peaks in HPLC, the polarity of which is higher than that of the product before hydrolysis. This implies that ester bond, the only one that lies the two monocyclic units in the product, is sensitive to acidic hydrolysis and both units separate whereas they remain together after acid treatment of physodic acid, since ether bond remains unaltered.

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EM  
SELAGINELLA CONVOLUTA (ARNOTT) SPRING  
E SELAGINELLA MUSCOSA SPRING  
(PTERIDOPHYTA)

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RESUMO

A morfologia dos aparelhos estomáticos em *Selaginella brynesii*, *S. convoluta* e *S. muscosa* é descrita neste trabalho. As micrófilas laterais e medianas são anfistomáticas em *S. brynesii*; são hipostomáticas em *S. convoluta* e *S. muscosa*. As esporófilas são epistomáticas nas três espécies estudadas. Os aparelhos estomáticos são do tipo anisocítico. As densidades são de 0,4; 2,5 e 1,4 células por campo de 5  $\mu\text{m}^2$ , respectivamente em *Selaginella brynesii*, *S. convoluta* e *S. muscosa*. As espículas são frequentes nas epidermes adaxiais das micrófilas laterais e medianas e frequentes nas epidermes abaxiais das esporófilas de *S. brynesii* e *S. muscosa*, sendo ausentes em *S. convoluta*.

SUMMARY

In this paper the morphology of the stomata of *Selaginella brynesii*, *S. convoluta* and *S. muscosa* is described. *Selaginella brynesii* has amphistomatal microphylls, *S. convoluta* and *S. muscosa* have hypostomatal microphylls. All species have anisocytic stomata. Stomatal densities by 5  $\mu\text{m}^2$  are 0.4; 2.5 and 1.4 in *S. brynesii*, *S. convoluta* and *S. muscosa*, respectively. The adaxial epidermis of the microphylls and the abaxial epidermis of the sporophylls usually present spicules; in *S. convoluta* they are lacking.

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APARELHOS ESTOMÁTICOS  
EM *SELAGINELLA BREYNEII* SPRING ET CHAR.,  
*SELAGINELLA CONVOLUTA* (ARNOTT) SPRING  
E *SELAGINELLA MUSCOSA* SPRING  
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## INTRODUÇÃO

A família Selaginellaceae é representada por dois gêneros: *Selaginella* Beauv. e *Selaginellites*, gênero extinto, conhecido no Carbonífero Superior, do qual são conhecidas cerca de 6 espécies, segundo SMITH (1955).

*Selaginella* tem distribuição cosmopolita, com cerca de 700 espécies e aproximadamente 270 na América Tropical, muitas crescendo em florestas úmidas (TRYON & TRYON, 1982). Em estudos sobre a distribuição geográfica para o Estado de Pernambuco, 10 espécies foram registradas (BARROS *et al.*, 1988).

O gênero é representado por espécies terrestres, rupícolas ou raramente epífitas; caule delgado, ramificado, rastejante ou com ramos ascendentes; **micrófilas** (folhas diminutas com uma única vênula) cerca de 0,5-10 mm de comprimento, dispostas em espiral fechado ou quatro filas alternadas: duas maiores laterais e duas medianas menores; **esporófilas** bem diferenciadas com um esporângio largo nascido na região basal da epiderme adaxial, formando **estróbilos**; **heterosporada** (o megasporângio normalmente na região basal do estróbilo, em geral com quatro **macrósporos** e o microsporângio, com muitos **micrósporos**); número de cromossomos:  $n = 8, 9, 10, 12, 18, 96$  e  $2n = 14, 16, 18, 20, 24, 36, 49-50, 60$  (TRYON & TRYON, 1982).

*Selaginella* difere das demais Pteridófitas por apresentar **rizóforos** e internamente no caule estruturas denominadas **trabéculas** (SMITH, 1955).

O conhecimento sobre aparelhos estomáticos sempre esteve centrado em Angiospermas (METCALFE & CHALK, 1950), os primeiros trabalhos referidos para a morfologia dos estômatos em Pteridófitas (Filicopsida) são os de VAN COTTEN (1970a, 1970b e 1973) onde foram encontrados tipos distintos dos padrões descritos e receberam denominações específicas (Fig. 1).

Este trabalho tem como objectivo contribuir para o estudo dos aparelhos estomáticos em Pteridófitas e fazer uma correlação dos ambientes de ocorrência com os tipos dos estômatos, sua localização na lâmina foliar e densidade em *Selaginella breyneii* Spring et Char., *S. convoluta* (Arnott) Spring e *S. muscosa* Spring (as duas primeiras espécies são procedentes de mata e a última de caatinga).



Nº Células Subeidiárias.	Tipo de estômatos.	Desenho esquemático.	Nº Células Subs.	Tipo de estômatos.	Desenho esquemático.
2, 3, 4, 2n	CICLOCÍTICO		2	EPICÍTICO	10
> 4	ACTINOCÍTICO	2		DIACÍTICO	11
> 3	HELICOCÍTICO		1 x 2	HIPOCÍTICO	12
> 3	ALELOCÍTICO			DESLOCÍTICO	13
> 3	A. DIALELOCÍTICO.			PERICÍTICO:	14 A
> 3	B. PARALELOCÍTICO.			A. EUPERICÍTICO	14 B
2, 4, 6, 2n	PARACÍTICO			B. COPERICÍTICO	14 C
> 6	HEXACÍTICO		C. DUPLOPERICÍTICO.	15 A	
> 4	ESTAUROCÍTICO		POLOCÍTICO:	A. PARIETOCÍTICO.	15 B
> 4	TETRACÍTICO		B. AXILOCÍTICO	15 C	
> 3	ANISOCÍTICO		C. COPARIETOCÍTICO.	15 D	
			D. COAXILOCÍTICO.	16	
			HEMPRACÍTICO	16	
			0	ANOMOCÍTICO	17

Fig. 1. — Classificação de estômatos segundo VAN COTTHEM (1970a).

**MATERIAL E MÉTODOS**

O material examinado encontra-se depositado no Herbário Geraldo Mariz da Universidade Federal de Pernambuco/UFP — *Selaginella breynei* (031616), *Selaginella convoluta* (06034) e *Selaginella muscosa* (08193).

A pesquisa foi realizada no Laboratório de Criptógamos no Departamento de Botânica do Centro de Ciências Biológicas da UFPE.

Foram isoladas micrófilas laterais, medianas e esporófilas; utilizada a técnica de diafanização segundo STRITTMATTER (1973), modificada; preparadas lâminas semipermanentes (guardadas no laminário do Mestrado de Botânica da Universidade Federal Rural de Pernambuco/UFRPE).

Para a observação dos estômatos foi utilizado microscópio óptico com câmara clara, através do qual foram realizadas ilustrações.

A classificação dos tipos de estômatos foi feita de acordo com VAN COTTEN (1970a e b). Para o estudo da densidade estomática foi utilizada a metodologia de LABOURIAU *et al.* (1961) com projecção de um retículo da lâmina micrométrica, com con-

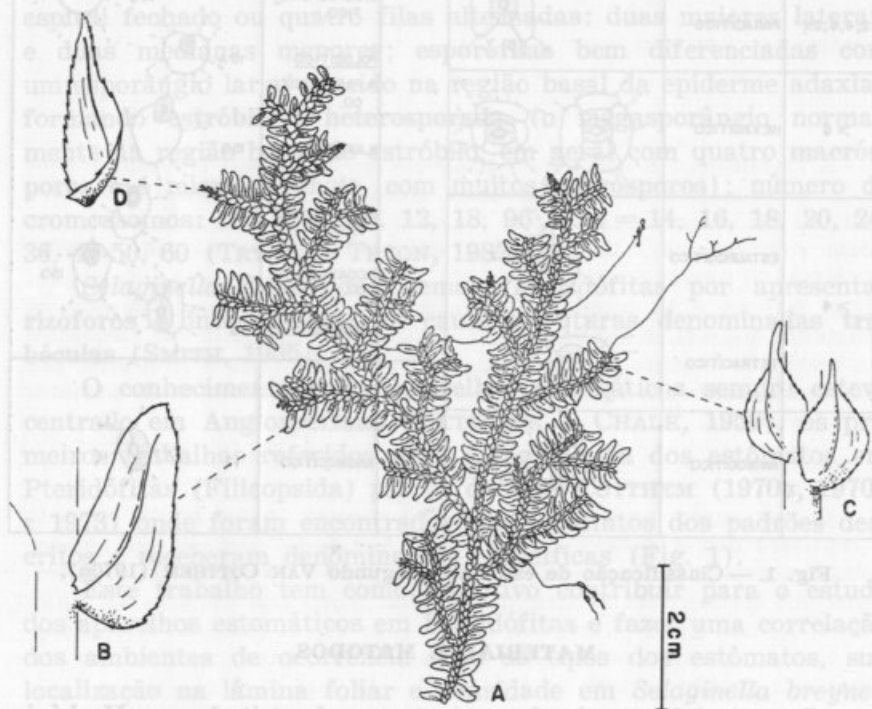


Fig. 2. — *Selaginella breynei* Spring ex bhar 03616. A — Hábito; B — Micrófila lateral, 32 ×; C. — Esporófila, 32 ×; D — Micrófila central, 32 ×.

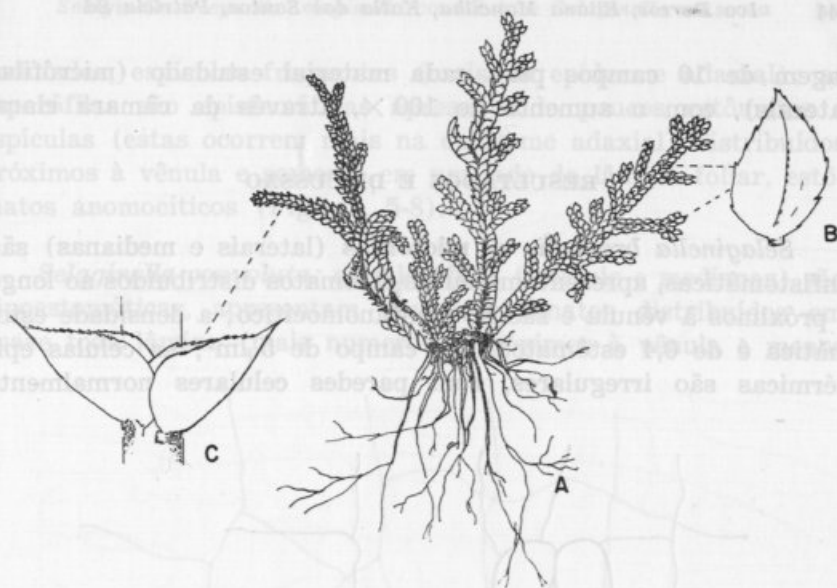


Fig. 3. — *Selaginella covoluta* (Arn.) Spring 06279. A — Aspecto geral. B — Micrófila, 32 X; C — Esporófila, 32 X.

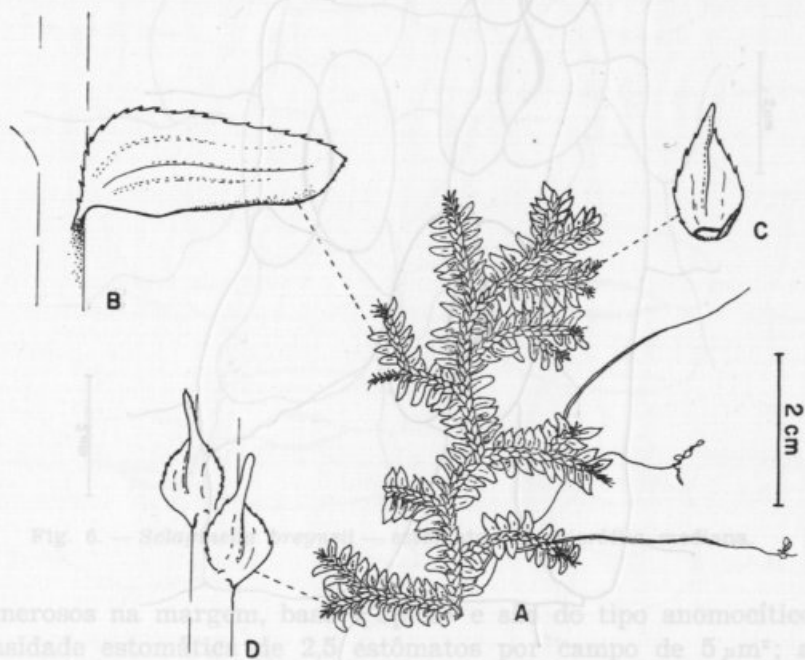


Fig. 4. — A-D — *Selaginella muscosa*. A — Aspecto geral; B — Micrófila lateral, 32 X; C — Esporófilo, 32 X; D — Micrófila central, 32 X.

tagem de 10 campos para cada material estudado (micrófilas laterais), com o aumento de 100 $\times$ , através da câmara clara.

### RESULTADOS E DISCUSSÃO

*Selaginella breynei*: as micrófilas (laterais e medianas) são anfistomáticas, apresentam poucos estômatos distribuídos ao longo e próximos à vênula e são do tipo anomocítico; a densidade estomática é de 0,4 estômatos, por campo de 5  $\mu\text{m}^2$ ; as células epidérmicas são irregulares, com paredes celulares normalmente

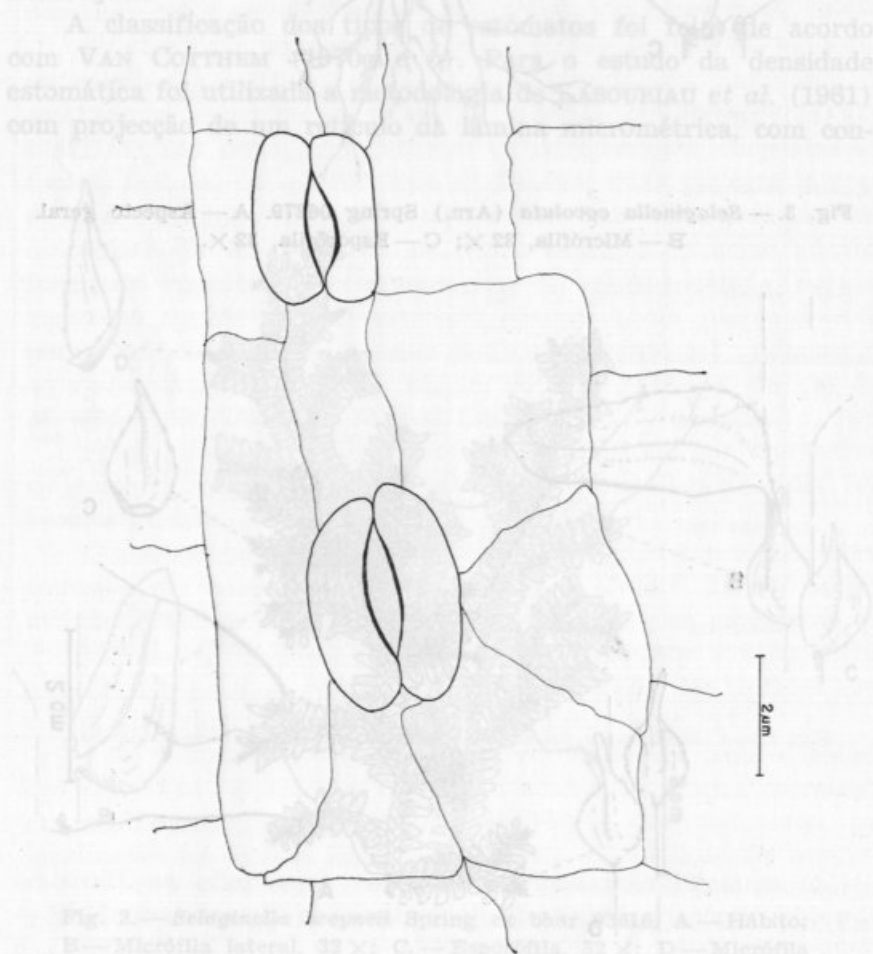


Fig. 5 — *Selaginella breynei* — estômatos da micrófila lateral.

onduladas, espículas frequentes (mais na epiderme adaxial); as **esporófilas** são epistomáticas apresentando poucos estômatos e espículas (estas ocorrem mais na epiderme adaxial) distribuídos próximos à vênula e somente em um lado da lâmina foliar, estômatos anomocíticos (Figs. 2, 5-8).

*Selaginella convoluta*: as **micrófilas** (laterais e medianas) são hipostomáticas, apresentam muitos estômatos distribuídos em quase toda lâmina (mais numerosos próximos à vênula e menos

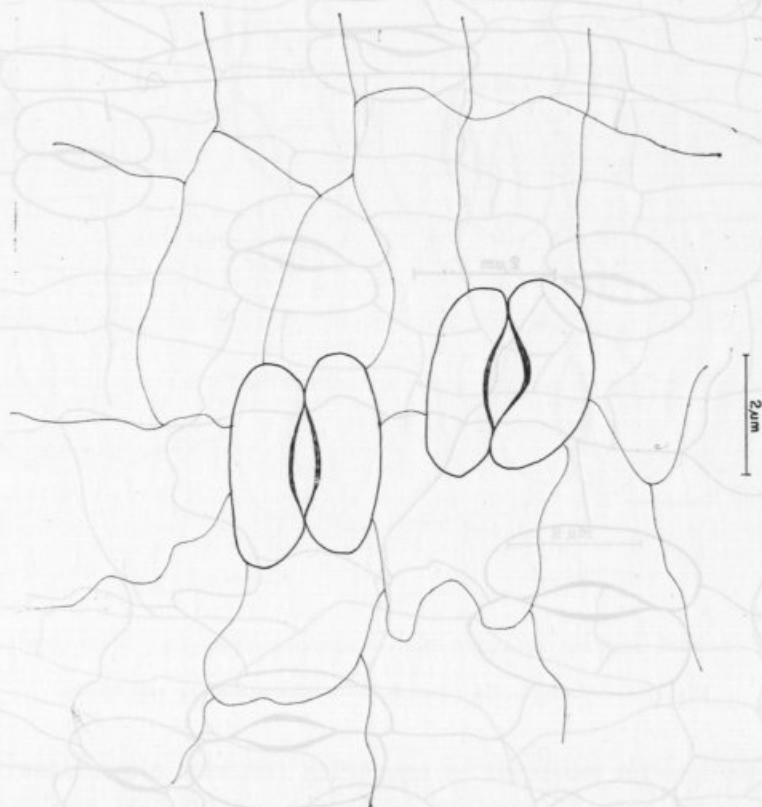


Fig. 6. — *Selaginella breynei* — estômatos da micrófila mediana.

numerosos na margem, base e ápice) e são do tipo anomocítico; densidade estomática de 2,5 estômatos por campo de  $5\mu\text{m}^2$ ; as células epidérmicas são irregulares com paredes celulares normalmente retas, não apresentando espículas; as **esporófilas** são

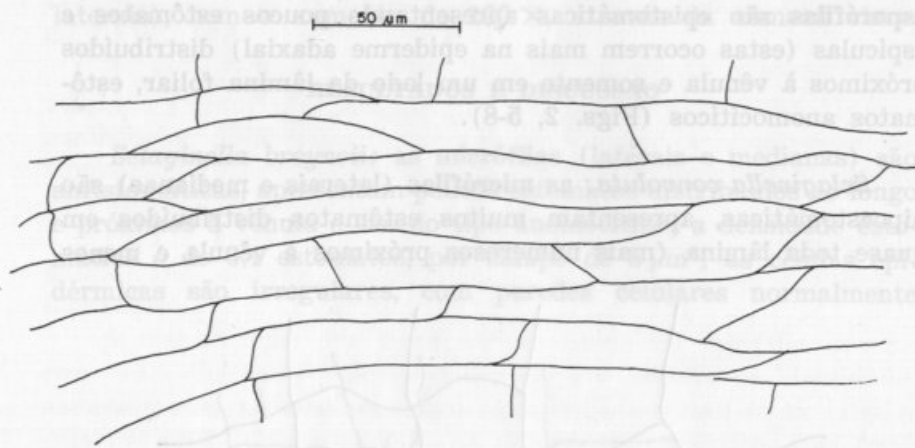


Fig. 8. — *Selaginella breyniei* — epicalles na epiderme foliar.

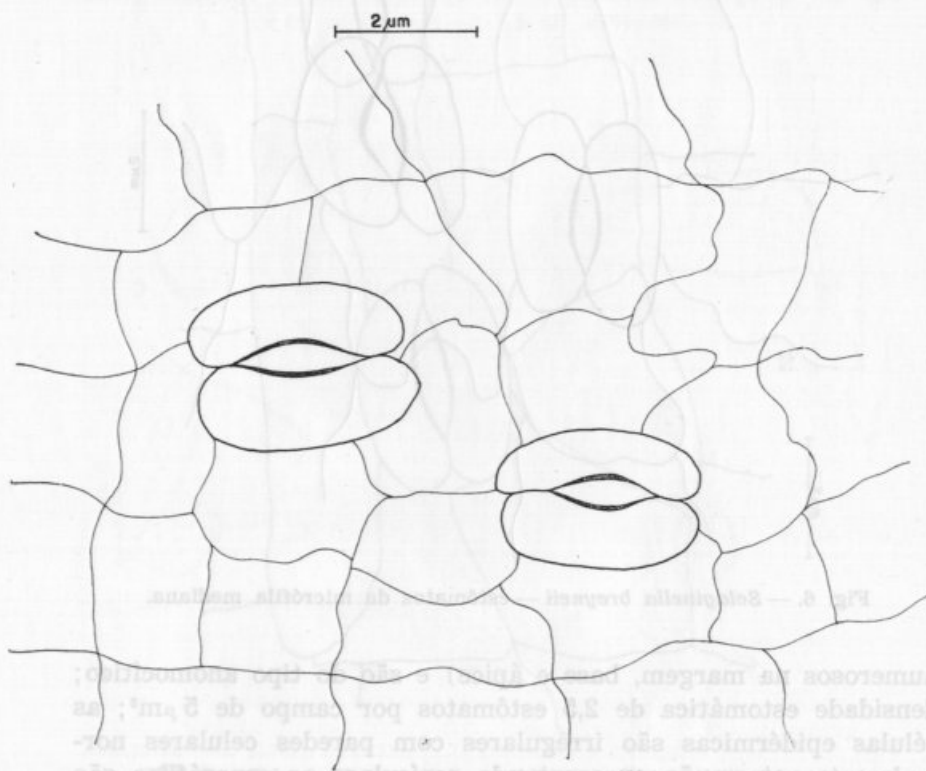


Fig. 7. — *Selaginella breyniei* — estômatos da esporófilia.

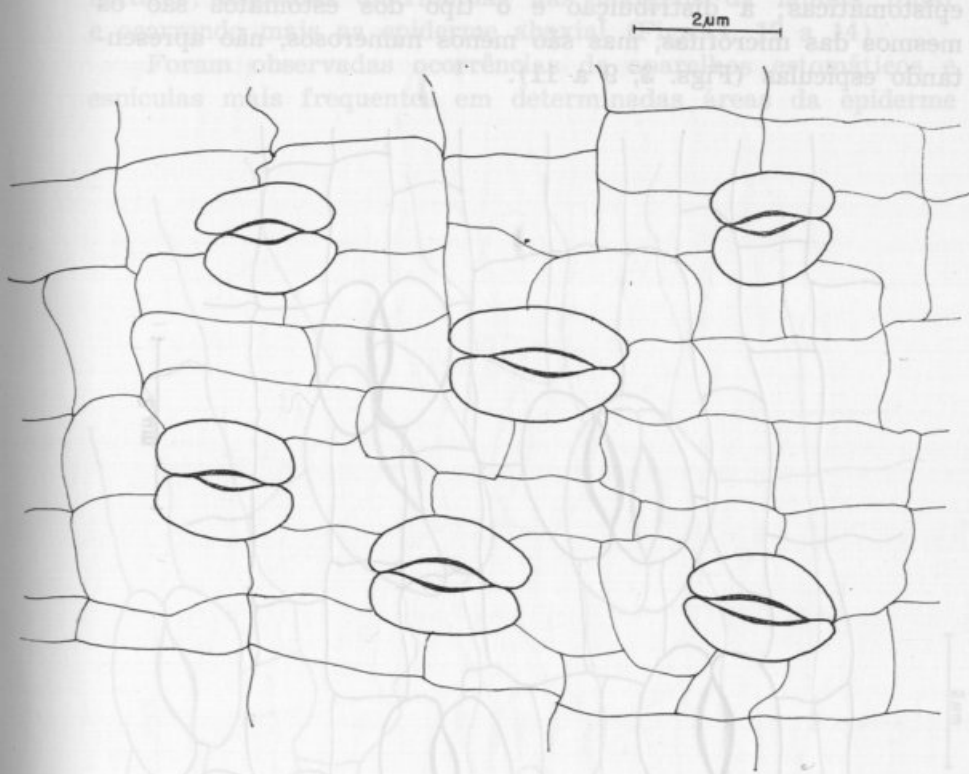


Fig. 10. — *Selaginella convoluta* — estômatos da micrófila mediana.

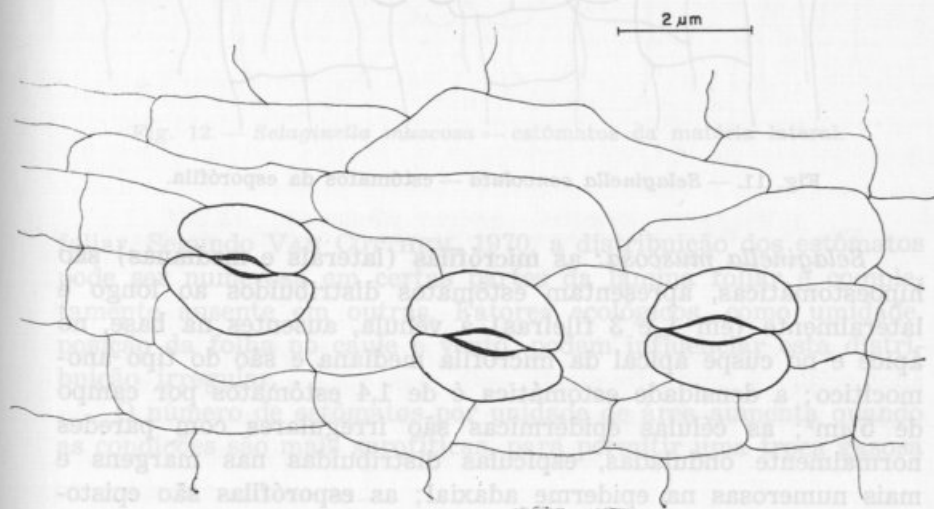


Fig. 9. — *Selaginella convoluta* — estômatos da micrófila lateral.

epistomáticas; a distribuição e o tipo dos estômatos são os mesmos das micrófilas, mas são menos numerosos, não apresentando espículas (Figs. 3, 9 a 11).

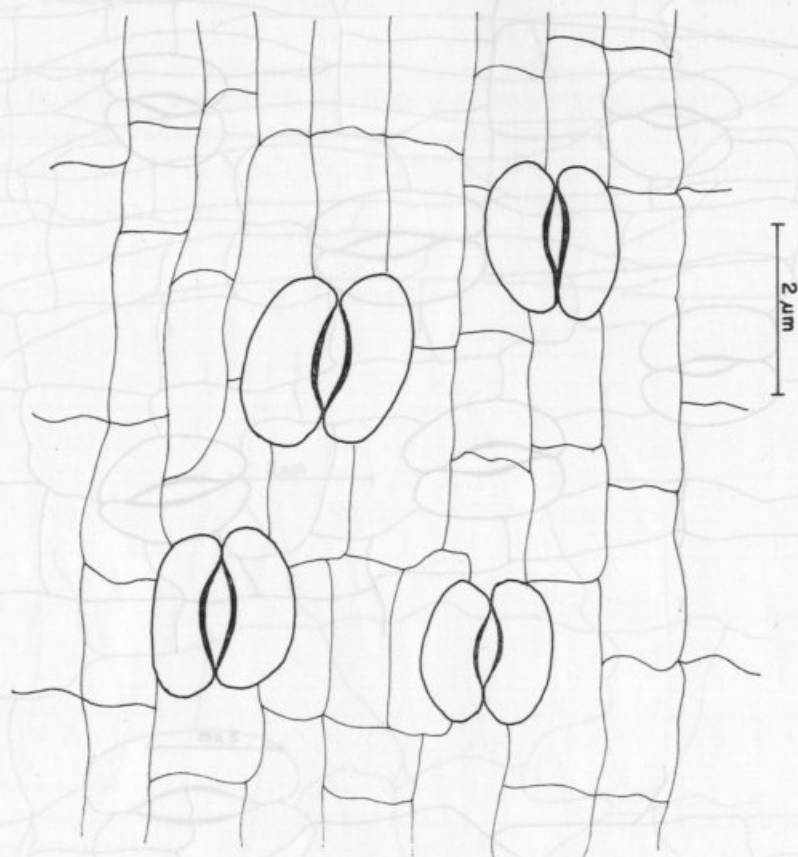


Fig. 11. — *Selaginella convoluta* — estômatos da esporófila.

*Selaginella muscosa*: as micrófilas (laterais e medianas) são hipoestomáticas, apresentam estômatos distribuídos ao longo e lateralmente (em 1 e 3 fileiras) à vênula, ausentes na base, no ápice e no cuspe apical da micrófila mediana e são do tipo anocítico; a densidade estomática é de 1,4 estômatos por campo de  $5 \mu\text{m}^2$ ; as células epidérmicas são irregulares com paredes normalmente onduladas, espículas distribuídas nas margens e mais numerosas na epiderme adaxial; as esporófilas são episto-



máticas; espículas distribuídas nas margens da lâmina foliar e ocorrendo mais na epiderme abaxial (Figs. 4, 12 a 14).

Foram observadas ocorrências de aparelhos estomáticos e espículas mais frequentes em determinadas áreas da epiderme

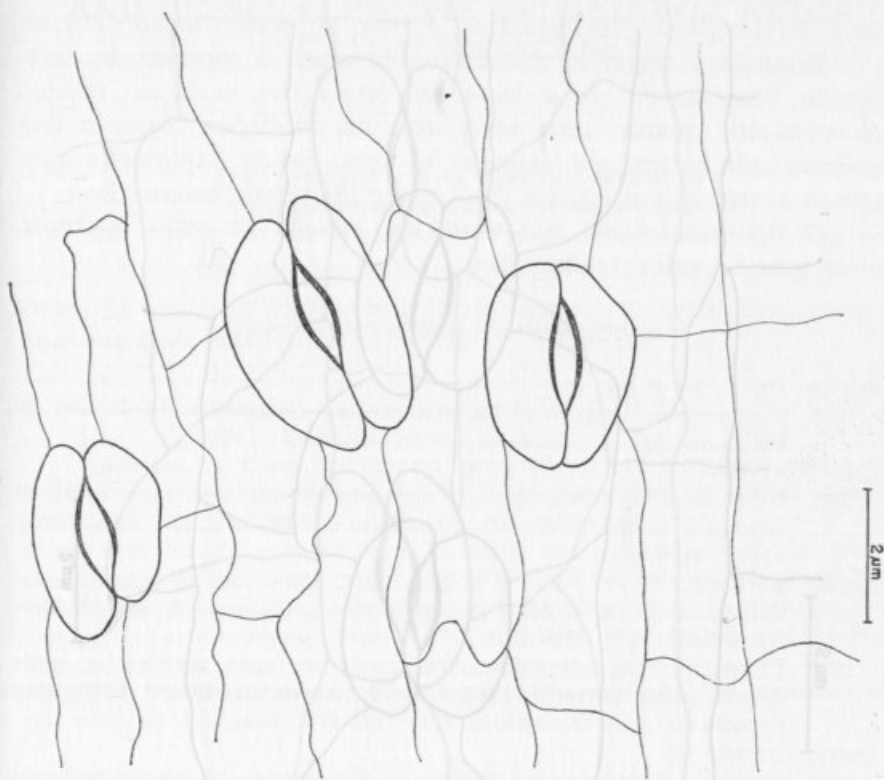


Fig. 12. — *Selaginella muscosa* — estômatos da matéria lateral.

foliar. Segundo VAN COTTEN, 1970, a distribuição dos estômatos pode ser numerosa em certas partes da lâmina foliar e completamente ausente em outras. Fatores ecológicos, como umidade, posição da folha no caule e vento, podem influenciar esta distribuição irregular.

O número de estômatos por unidade de área aumenta quando as condições são mais xerofíticas, para permitir uma troca gasosa na epiderme foliar.

mais eficiente nos períodos em que a umidade relativa é alta, quando o perigo de desidratação excessiva é mínimo (LLERAS, 1977) esta afirmação está de acordo com os resultados apresentados.

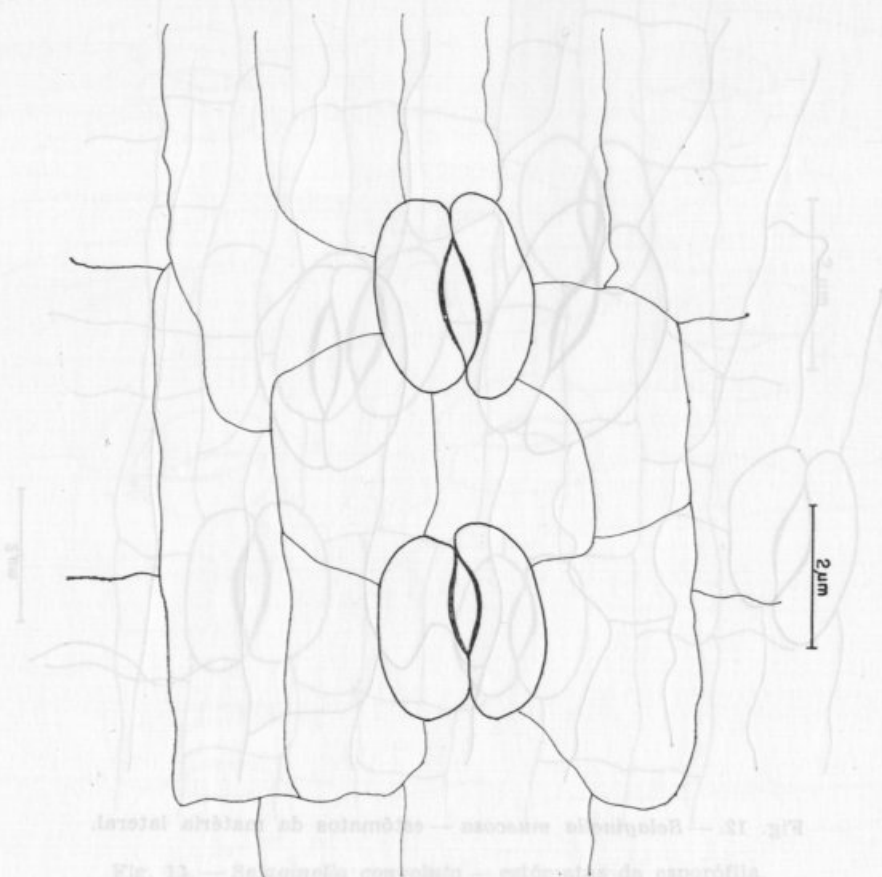


Fig. 13. — *Selaginella muscosa* — estômatos da micrófila mediana.

Segundo LLERAS (1977a e b) o aparelho estomático do tipo anomocítico é muito frequente, está presente em 85 gêneros estudados, não estando presente em Marattiaceae Loxomaceae ou Davalliaceae e, nas Polypodiaceae, só nos fetos Gramniticos.

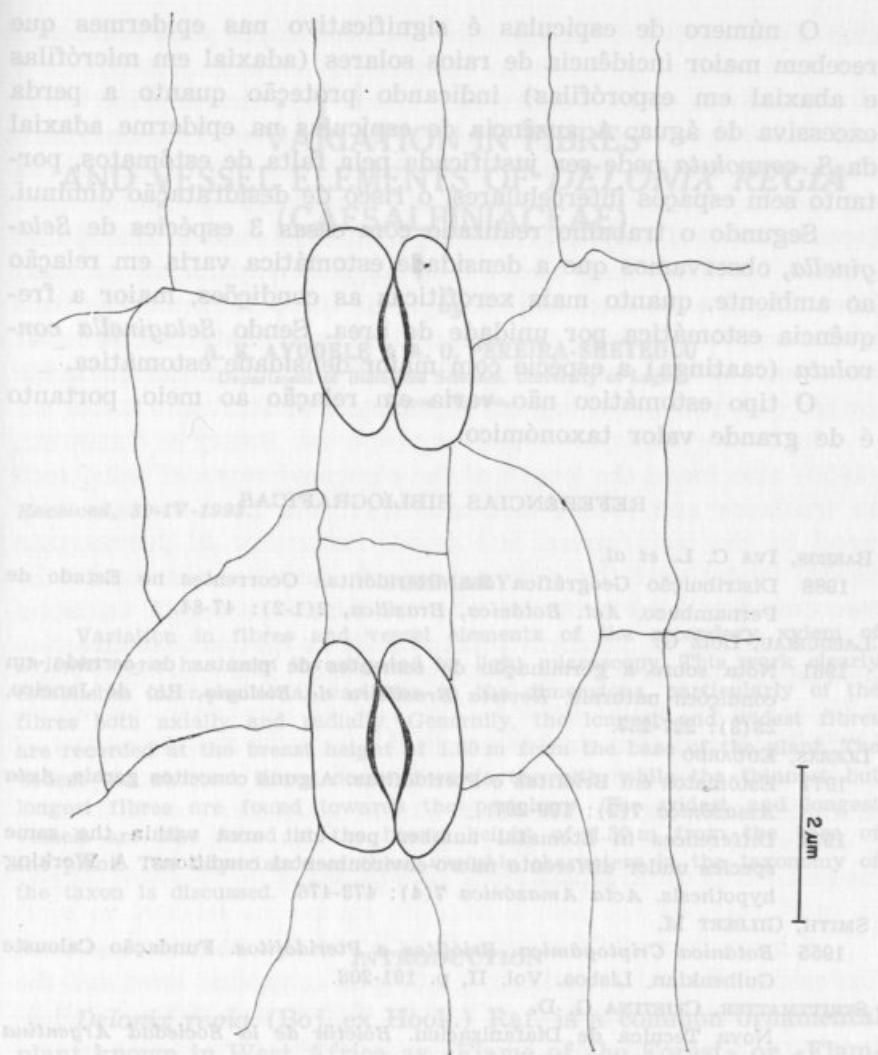


Fig. 14. — *Selaginella muscosa* — estômatos da esporófila.

#### CONCLUSÕES

A distribuição dos estômatos e espículas nas micrófilas (laterais e medianas) é semelhante na mesma espécie, mas é diferente entre espécies; quanto às esporófilas, a distribuição é semelhante entre as espécies. As esporófilas diferem das micrófilas da mesma espécie pela ocorrência dos estômatos e espículas na epiderme foliar.



O número de espículas é significativo nas epidermes que recebem maior incidência de raios solares (adaxial em micrófilas e abaxial em esporófilas) indicando proteção quanto a perda excessiva de água. A ausência de espículas na epiderme adaxial da *S. convoluta* pode ser justificada pela falta de estômatos, portanto sem espaços intercelulares, o risco de desidratação diminui.

Segundo o trabalho realizado com essas 3 espécies de *Selaginella*, observamos que a densidade estomática varia em relação ao ambiente, quanto mais xerofíticas as condições, maior a frequência estomática por unidade de área. Sendo *Selaginella convoluta* (caatinga) a espécie com maior densidade estomática.

O tipo estomático não varia em relação ao meio, portanto é de grande valor taxonômico.

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## VARIATION IN FIBRES AND VESSEL ELEMENTS OF *DELONIX REGIA* (CAESALPINIACEAE)

by

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### SUMMARY

Variation in fibres and vessel elements of the secondary xylem of *Delonix regia* has been investigated by light microscopy. This work clearly establishes inter-positional variation in the dimensions, particularly of the fibres both axially and radially. Generally, the longest and widest fibres are recorded at the breast height of 1.30 m from the base of the plant. The widest but shortest fibres occur towards the pith while the thinnest but longest fibres are found towards the periphery. The widest and longest vessels are also found at the breast height of 1.30 m from the base of the plant. The importance of these variable characters in the taxonomy of the taxon is discussed.

### INTRODUCTION

*Delonix regia* (Boj. ex Hook.) Raf. is a common ornamental plant known in West Africa as «Flame of the Forest» or «Flamboyant» but now subsponaneous in many localities (HUTCHINSON & DALZIEL, 1954). The plant is a widely grown decorative tree and a native of Madagascar but now grown in all parts of the tropics. The leaves are compound bipinnate with small and feathery leaflets. Flowers are bright scarlet mixed with yellow in colour, and comprise of 10 stamens with long, red filaments (IRVINE, 1961). Timber obtained from this plant is reported to be fairly resistant to moisture and insects. Also, the stem exudes yellow or reddish-brown latex which is a source of watersoluble

gum (IRVINE, 1961). Furthermore, the bark of the stem is reputed for its medicinal purposes in Indo-China (BURKILL, 1935).

Studies in the cell dimensions of wood particularly the fibres and vessel elements have shown that the cells vary in their dimensions with respect to their positions in the plant. The works of HARTIG, STAUFFER, as well as, PRITCHARD & BAILEY (cf. DINWOODIE, 1961) lay credence to this fact. In some of the species studied by them, they observed a progressive decrease in wood fibre length with increase in height of the plant from the base of the trunk to the apex. BISSET & DADSWELL (1949) showed that fibres increase in length from the centre of the trunk outwards within one tree of *Eucalyptus regnans* F. V. M.

In both gymnosperms and angiosperms, BISSET & DADSWELL (1950) also found the length of the elongated types of cells, such as tracheids and fibres, increases from the first formed early wood to the last formed late wood. PHILIPSON & BUTTERFIELD (1967) stated that the shorter elements found at the top of the tree occur as a result of the trend of horizontal length variation from cambium to periderm which varies in the vertical direction to such an extent that a reduction in cell length occurred along with enhanced radial growth. The aim of this work, therefore, is to investigate the variation which occurs in the dimension of fibres and vessel elements, radially and axially in *D. regia*, as a contribution to the taxonomy of the species.

#### MATERIALS AND METHOD

A large tree of *D. regia* was felled at the Obafemi Awolowo University, Ile-Ife, Nigeria, by cutting it at ground level and the stem marked at intervals of 0.20 m, 1.30 m and 2.60 m (breast height), and 3.00 m, 3.60 m, 4.10 m and 4.40 m from the ground level on the bole towards the highest branch. Wood discs of about 7.50 cm thick were sliced-off from these marked points and used to determine the radial variation in the cell dimensions. The discs were marked at three points, viz. 1.50 cm, 3.50 cm and 5.50 cm, from the periphery towards the pith. Pieces of wood were chipped off from these points, cut into thin slices and macerated in SCHULTZE's fluid [2% Potassium trioxonitrate (V) dissolved in concentrated trioxonitrate (V) acid]. For the maceration technique, the materials were placed in glass vials and

the macerating liquid added and the set-up were kept in the fume chamber in the laboratory.

Pieces of wood were also taken from other marked points and macerated so as to determine variation along the height of the stem. After about 45 minutes, the materials were removed from the macerating liquid, washed thoroughly in several changes of distilled water and stored in 70 % ethanol. For cell measurements, small pieces of macerated wood were stained in Toluidine blue and mounted in 25 % glycerol. For each sample, 50 wood fibres and 20 vessel elements were chosen randomly and measured using a micrometer eye-piece. For each quantitative character, the mean, standard deviation and standard error were determined.

### RESULTS AND DISCUSSION

The results obtained from the method of investigation reveal a number of interesting features. Fibre size and cellwall thickness vary considerably through the entire plant. At point 1.50 cm from the periphery, the longest fibre of 1121.90  $\mu\text{m}$  is recorded at point B, which is 1.30 m from the base of the plant (Fig. 1). But the shortest fibre of 924.50  $\mu\text{m}$  occurs at point D, which is 3.00 m from the base (Table 1). Similar results are obtained at point 3.50 cm from the periphery as the longest and shortest fibres of 1069.40  $\mu\text{m}$  and 855.40  $\mu\text{m}$  respectively occur at points B and D. However, at point 5.50 cm from the periphery, the longest fibre of 1060.80  $\mu\text{m}$  occurs at point B but the shortest fibre of 716.20  $\mu\text{m}$  is recorded at point E (Table 1).

At point 1.50 cm from the periphery, the widest fibre of 33.40  $\mu\text{m}$  occur at point B while the smallest fibre of 23.90  $\mu\text{m}$  occurs at point E (Table 1). Similarly, at point 3.50 cm from the periphery, the widest and the narrowest fibres of 33.40  $\mu\text{m}$  and 23.40  $\mu\text{m}$  respectively occur at points B and E. However, at point 5.50 cm from the periphery, the widest fibre of 37.00  $\mu\text{m}$  occurs at point B while the narrowest fibre of 25.20  $\mu\text{m}$  occurs at point D (Table 1). At point 1.50 cm from the periphery, the thickest fibre wall of 7.50  $\mu\text{m}$  occurs at point C while the thinnest fibre of 5.50  $\mu\text{m}$  is recorded at point B (Table 1). At point 3.50 cm from the periphery, the thickest wall of 7.30  $\mu\text{m}$  is recorded at point F while the thinnest 5.50  $\mu\text{m}$  occurs at point G.

At point 5.50 cm from the periphery, the thinnest wall of  $6.20 \mu\text{m}$  occurs at point G while the thickest wall of  $7.50 \mu\text{m}$  is recorded at point F (Table 1).

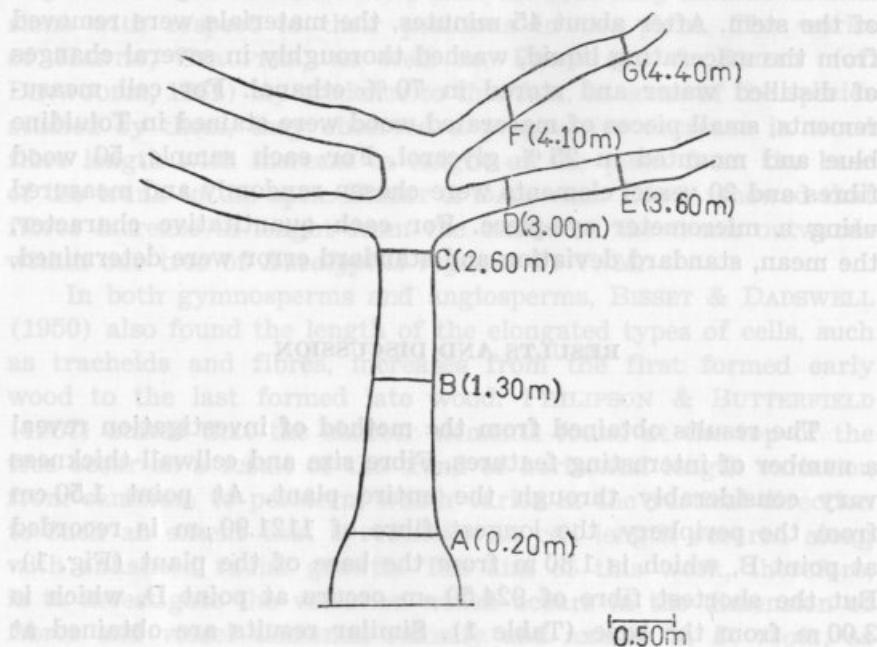


Fig. 1. — Semi-diagrammatic sketch of *D. regia* showing the different sampling points on the stem and branches.

The size of vessels also vary considerably at the different points examined. The variation recorded is from  $251.60 \mu\text{m}$  at point B to  $148.80 \mu\text{m}$  recorded at point D. Similarly, the width varies from  $28.20 \mu\text{m}$  at point D to  $98.50 \mu\text{m}$  at point B (Table 2). It would be observed that the fibre length decreases from the base of the plant upwards to the top. This is in conformity with the proposition of PHILIPSON & BUTTERFIELD (1967). They attributed this to changes that occur in the cambium leading to the formation of the periderm. Also, this is in agreement with the suggestion of BISSET & DADSWELL (1950) that elongated types of cells increase in length from the first formed early wood (those at the periphery) to the last formed late wood.



**TABLE 1**  
Variation in fibre sizes and cellwall thickness

Height from plant base (metres)	Distance from periphery (cm):								
	1.50		3.50		5.50				
	F. L. $\pm$ S. E.	F. W. $\pm$ S. E.	C. T. $\pm$ S. E.	F. L. $\pm$ S. E.	F. W. $\pm$ S. E.	C. T. $\pm$ S. E.			
G (4.40)	993.60 $\pm$ 33.40	26.00 $\pm$ 0.60	6.80 $\pm$ 0.40	880.30 $\pm$ 36.90	26.00 $\pm$ 1.60	5.50 $\pm$ 0.30	901.00 $\pm$ 53.70	26.30 $\pm$ 1.40	6.20 $\pm$ 0.40
F (4.10)	965.80 $\pm$ 42.20	27.80 $\pm$ 1.10	7.00 $\pm$ 0.40	964.80 $\pm$ 21.10	27.30 $\pm$ 1.10	7.30 $\pm$ 0.50	937.00 $\pm$ 52.30	30.90 $\pm$ 1.50	7.50 $\pm$ 0.60
E (3.60)	974.40 $\pm$ 49.50	23.90 $\pm$ 0.90	5.90 $\pm$ 0.40	913.00 $\pm$ 44.50	23.40 $\pm$ 1.10	5.70 $\pm$ 0.40	716.20 $\pm$ 37.40	27.00 $\pm$ 1.00	6.80 $\pm$ 0.40
D (3.00)	924.50 $\pm$ 39.90	26.00 $\pm$ 0.50	6.20 $\pm$ 0.40	855.40 $\pm$ 61.60	25.20 $\pm$ 0.40	6.50 $\pm$ 0.70	721.90 $\pm$ 46.40	25.20 $\pm$ 0.70	6.80 $\pm$ 0.40
C (2.60)	1106.50 $\pm$ 23.70	29.10 $\pm$ 0.60	7.50 $\pm$ 0.20	1027.00 $\pm$ 18.90	27.10 $\pm$ 0.30	6.80 $\pm$ 0.20	1014.70 $\pm$ 19.20	28.90 $\pm$ 0.40	6.80 $\pm$ 0.20
B (1.30)	1121.90 $\pm$ 28.20	33.40 $\pm$ 0.60	5.50 $\pm$ 0.20	1069.40 $\pm$ 25.00	33.40 $\pm$ 0.80	5.90 $\pm$ 0.20	1060.80 $\pm$ 20.70	37.00 $\pm$ 0.60	6.60 $\pm$ 0.20
A (0.20)	1035.50 $\pm$ 24.40	27.90 $\pm$ 0.50	6.80 $\pm$ 0.20	1020.30 $\pm$ 20.60	28.70 $\pm$ 0.50	7.00 $\pm$ 0.20	970.10 $\pm$ 17.80	29.50 $\pm$ 0.60	7.10 $\pm$ 0.30

F. L.—Fibre length; F. W.—Fibre width; C. T.—Cell wall thickness.

The concentration of the longest and widest fibres at the breast (1.30 m) may not be unconnected with the burden of the load of the vegetative and floral parts shouldered by that portion of the plant. However, the age of a tree as in *D. regia* is likely to influence the dimensions and general structure of

TABLE 2  
Variation in vessel sizes

Height from plant base (metres)	Mean vessel size $\pm$ STD error ( $\mu\text{m}$ )	
	Length	Width
G (4.40)	154.20 $\pm$ 15.20	46.60 $\pm$ 4.20
F (4.10)	153.10 $\pm$ 12.30	35.00 $\pm$ 2.00
E (3.60)	168.30 $\pm$ 16.30	69.20 $\pm$ 11.50
D (3.00)	148.80 $\pm$ 21.60	28.20 $\pm$ 2.40
C (2.60)	163.70 $\pm$ 10.90	39.70 $\pm$ 1.90
B (1.30)	251.60 $\pm$ 19.90	98.50 $\pm$ 17.00
A (0.20)	203.70 $\pm$ 21.60	60.40 $\pm$ 11.70

the individual elements in the tree. This work has clearly established interpositional variation in the dimensions particularly of the fibres both axially and radially. Variation in fibre length and width along the tree axially and radially is generally accepted to be connected with the changes in size of the fusiform initials of the cambium (DINWOODIE, 1961). This is because the secondary xylem is always affected by the irregular activity of the cambium, a factor which is in itself dictated by season.

#### ACKNOWLEDGEMENTS

The authors wish to thank Dr. O. A. OLATUNJI and Dr. W. M. KPIKPI of the Department of Botany, Obafemi Awolowo University, Ile-Ife, for their useful contributions and suggestions.

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#### SUMARIO

Foram observadas as formas de reprodução sexuada e as células iniciais de *Cocconeis placentale* Ehr. e de *Amphora vesicis* Kütz. em populações diatomológicas naturais recolhidas em Murta, uma das estações de amostragem do Rio Cértima.

Para além das duas espécies referidas, na comunidade diatomológica natural, dominava ainda uma outra espécie, *Schumannia hungarica* Grun.

Os auxósporas de *Cocconeis placentale* bastante mais numerosas do que os de *Amphora vesicis*, são largamente elípticas e revestidas por uma espessa camada mucilaginosa. A ornamentação frutular da célula inicial é muito diferente da das células vegetativas. Nesta, ambas as valvas são acridóides e diferentemente ornamentadas.

Os auxósporas de *Amphora vesicis*, largamente elípticos, apresentam um ténue e fino revestimento mucilaginoso e as células iniciais possuem uma frústula cuja ornamentação é muito semelhante à das células vegetativas, excepto na área central.

#### ABSTRACT

Sexual reproductive stages and initial cells of *Cocconeis placentale* Ehr. and *Amphora vesicis* Kütz. were observed in natural diatomologic population. The samples were taken from Murta, one of the sampling locals on Cértima river.

*Schumannia hungarica* Grun. is another species that is dominant in natural community.

The auxospores of *Cocconeis placentale* were more abundant than those of *Amphora vesicis*. They are largely elliptic and they are covered by a thick mucilaginous layer.

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Sample	Length (mm)	Width (mm)
A (0.5)	0.91 ± 0.13	0.71 ± 0.03
B (1.5)	0.91 ± 0.13	0.71 ± 0.03
C (2.5)	0.91 ± 0.13	0.71 ± 0.03
D (3.0)	0.91 ± 0.13	0.71 ± 0.03
E (3.5)	0.91 ± 0.13	0.71 ± 0.03
F (4.1)	0.91 ± 0.13	0.71 ± 0.03
G (4.4)	0.91 ± 0.13	0.71 ± 0.03

the individual elements in the tree. This work has clearly established interpositional variation in the dimensions particularly of the fibres both axially and radially. Variation in fibre length and width along the tree axially and radially is generally accepted to be connected with the changes in size of the fusiform initials of the cambium (DIXWOOD, 1961). This is because the secondary xylem is always affected by the irregular activity of the cambium, a factor which is in itself dictated by season.

#### ACKNOWLEDGEMENTS

The authors wish to thank Dr. O. A. OLATUNJI and Dr. W. M. KIKKI of the Department of Botany, Obafemi Awolowo University, Ile-Ife, for their useful contributions and suggestions.

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## CONTRIBUIÇÃO PARA O CONHECIMENTO DA REPRODUÇÃO SEXUADA EM *BACILLARIOPHYCEAE*

AUXÓSPOROS E CÉLULAS INICIAIS DE *COCCONEIS*  
*PLACENTULA* EHR. E *AMPHORA VENETA* KUTZ.

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Recebido em 28-V-1993.

### SUMARIO

Foram observadas as formas de reprodução sexuada e as células iniciais de *Cocconeis placentula* Ehr. e de *Amphora veneta* Kutz. em populações diatomológicas naturais recolhidas em Murta, uma das estações de amostragem do Rio Cértima.

Para além das duas espécies referidas, na comunidade diatomológica natural, dominava ainda uma outra espécie, *Achnanthes hungarica* Grun.

Os auxósporos de *Cocconeis placentula* bastante mais numerosos do que os de *Amphora veneta*, são largamente elípticos e revestidos por uma espessa camada mucilaginosa. A ornamentação frutular da célula inicial é muito diferente da das células vegetativas. Nela, ambas as valvas são arrafideas e diferentemente ornamentadas.

Os auxósporos de *Amphora veneta*, longamente elípticos, apresentam um ténue e fino revestimento mucilaginoso e as células iniciais possuem uma frústula cuja ornamentação é muito semelhante à das células vegetativas, excepto na área central.

### ABSTRACT

Sexual reproductive stages and initial cells of *Cocconeis placentula* Ehr. and *Amphora veneta* Koetz. were observed in natural diatomic population. The samples were taken from Murta, one of the sampling locals on Cértima river.

*Achnanthes hungarica* Grun. is another specie that is dominant in natural community.

The auxospores of *Cocconeis placentula* were more abundant than those of *Amphora veneta*. They are largely elliptic and they are covered by a thick mucilaginous layer.

The initial cell ornamentation is very different from the vegetative cells. The valves of initial cells are both araphids but with different valvar ornamentation.

The *Amphora veneta* auxospore are extensively elliptic and covered by a thin mucilaginous wall of polysaccharide material. The ornamentation of both, the initial and the vegetative cells is very similar, but the central area ornamentation is quite distinct.

## INTRODUÇÃO

COMO consequência do trabalho de investigação que temos vindo a efectuar sobre a ecologia das Diatomáceas da região Centro-Litoral de Portugal, temos observado, com relativa frequência, a divisão celular vegetativa destas Algas.

O trabalho de rotina tem-nos permitido analisar numerosas formas de reprodução assexuada de diversificadas espécies, nomeadamente de *Achnanthes hungarica* Grunow, *Achnanthes lanceolata* de Brébisson, *Achnanthes saxonica* Krasske, *Caloneis bacillum* (Grun.) Mereschkowsky, *Peronia fibula* (de Brébisson & Arnott) Ross, *Rhoicosphenia curvata* (Kutz.) Grunow, etc.

Contrastando com a frequência e a quase «vulgaridade» com que examinamos este tipo de reprodução, a reprodução sexuada das Diatomáceas nunca tinha por nós sido observada a não ser em *Melosira varians* C. A. Agardh.

Uma amostra, particularmente rica em *Cocconeis placentula*, *Amphora veneta* e *Achnanthes hungarica*, deu-nos a rara oportunidade de conhecer as formas de reprodução sexuada destas espécies, em populações naturais.

Como é do conhecimento geral, a reprodução das Diatomáceas faz-se fundamentalmente por reprodução celular vegetativa. Este processo leva, a uma gradual redução do tamanho celular, uma vez que, as novas valvas (hipovalvas) são sempre formadas por dentro das valvas maternas herdadas (CRAWFORD, 1981; MIZUNO, 1987).

Segundo MANN (1984), quando as dimensões das frústulas atingem dimensões que se encontram dentro de determinados limites mínimos, variáveis de espécie para espécie, ocorrem transformações fisiológicas que levam a célula a «produzir» gâmetas.

As condições ambientais que induzem as células vegetativas a transformarem-se em potenciais gametângios são, particular-

mente, no caso das Pennales, desconhecidas para a maior parte das espécies (DREBES, 1977).

Após alguns anos de estudo de populações naturais e de análise dos mais relevantes e comuns parâmetros físico-químicos ambientais, acreditamos, que os factores responsáveis por aquela indução, sejam, não só, de natureza biótica (associações específicas definidas, etc.) mas predominantemente de natureza física (foto-período, incidência luminosa, temperatura, etc.).

Neste trabalho referimos alguns aspectos relacionados com a formação do zigoto (auxósporo), das valvas iniciais e das valvas secundárias das espécies *Cocconeis placentula* e *Amphora veneta*.

#### MATERIAL E MÉTODOS

As colheitas foram feitas em Murta, rio Cértima (RINO & GIL, 1987), respectivamente nos dias 13 e 10 de Setembro de 1989 e 1990.

As Diatomáceas epífitas foram colhidas, por espremedura do rizobentos da margem direita. Na colheita das Diatomáceas potamoplanctónicas foi utilizada uma rede de plâncton, em nylon, de 25  $\mu$  de malha.

Para o estudo da comunidade diatomológica usámos preparações definitivas obtidas por incineração. Esta, é efectuada sobre uma placa polida de ferro fundido, aquecida ao rubro, durante 45 minutos. As lamelas, contendo o material biológico, são colocadas sobre a placa depois de secas à temperatura ambiente.

Estas preparações, que não desagregam as colónias nem ocasionam a desarticulação das valvas, são particularmente favoráveis a este tipo de observações.

O estudo morfológico das valvas iniciais de *Cocconeis placentula* e de *Amphora veneta* foi fundamentalmente feito usando material incinerado. As células gametângiais e os auxósporos foram predominantemente observados e estudados em material não tratado conservado com formol a 5%.

Todas as observações foram feitas num microscópio LABORLUX S equipado com uma objectiva NPL FLUOTAR 100  $\times$  e com uma câmara fotográfica WILD MPS 52 e um exposímetro automático WILD MPS 46.

A determinação dos mais relevantes parâmetros físico-químicos ambientais também foi efectuada mas, os resultados obtidos serão oportunamente publicados.

### RESULTADOS E DISCUSSÃO

Ambas as espécies estudadas são alogâmicas (GEITLER, 1973) e o par de células vegetativas, que funcionam como gametângios, coloca-se adjacente ou com ligeira obliquidade, produzindo depois um nítido invólucro mucilaginoso, que é muito mais espesso em *C. placentula* do que em *A. veneta*.

As duas células vegetativas conjugantes são, em ambos os casos, e tanto quanto pudemos observar, no material conservado, de tamanhos diferentes (Fotos n.ºs 1 e 2) sendo uma delas sempre bastante maior que a outra.

Ambas as espécies originam por plasmogamia e cariogamia dos dois gametas um único zigoto (auxósporo) que sofre expansão (ROUND, 1990), se torna livre (sem associação às tecas gametangiais) e predominantemente epifítico embora também possa ser potamoplanctónico.

Não temos a certeza, porque não observámos o material vivo, nem efectuámos os necessários estudos citológicos, em culturas, se os zigotos são formados segundo a variante do tipo Ib2 ou Ia1 da nomenclatura de GEITLER (1973).

#### Formação do auxósporo e morfologia da frústula inicial em *Cocconeis placentula*

Da observação efectuada, em material conservado, parece poder inferir-se que o zigoto (Foto n.º 3) se forma no interior de uma das frústulas gametangiais após a parcial desarticulação das suas valvas.

Este, depois de libertar-se das valvas da frústula gametangial, sofre uma expansão bipolar e torna-se largamente elíptico, de contornos simétricos ou assimétricos, evidenciando, neste último caso, uma maior convexidade de um dos lados (Fotos n.ºs 4 e 5). O auxósporo expandido pode atingir dimensões da ordem dos  $50 \times 17,5 \mu$ .

A construção da frústula inicial começa com a formação da epivalva que é muito menos convexa que a hipovalva. A epivalva



apresenta-se ornamentada por estrias pontuadas, que sendo quase paralelas na área central se tornam convergentes para os polos (Foto n.º 6). O pseudorrafe, bem definido, pode ser rectilíneo (Foto n.º 7), tal como o das células vegetativas, ou apresentar um trajecto sigmoide (Foto n.º 8). Os bordos valvares do manto são espessos e possuem uma ornamentação constituída por linhas pontuadas paralelas.

As cinturas conectivas não são observáveis em microscopia óptica; elas estão somente demarcadas, na zona de contiguidade das duas valvas, por uma muito estreita área hialina (Foto n.º 9).

A hipovalva, muito mais acentuadamente convexa que a epivalva, é ornamentada no bordo valvar por linhas pontuadas paralelas (ou aproximadamente paralelas) que se prolongam pelo bordo da área valvar (Foto n.º 10). Perto da área axial, as linhas ornamentais tornam-se convergentes mas distribuem-se de modo irregular. Na hipovalva inicial (Foto n.º 11) não existem rafe nem pseudorrafe definidos.

As dimensões das 30 frústulas iniciais observadas variaram entre  $50,0 \times 21,2 \mu$  e  $41,5 \times 26,5 \mu$ . O tamanho médio das frústulas iniciais é de  $44,2 \times 24,0 \mu$ .

As segundas frústulas, formadas por divisão celular vegetativa da célula inicial (Foto n.º 12), possuem as características das células vegetativas normais, isto é, a hipovalva já apresenta rafe definido (Fotos n.ºs 13-16).

### Formação do auxósporo e morfologia da frústula inicial em *Amphora veneta*

O estudo efectuado sobre material conservado tornou evidente que os gâmetas se libertam das duas frústulas gametangiais (Foto n.º 17), que entretanto se destacam da camada orgânica envolvente (Foto n.º 18).

Por expansão bipolar, o zigoto formado pela fusão dos dois gâmetas, torna-se longamente elíptico mas com duas pronunciadas convexidades centrais.

As duas valvas da frústula inicial parecem não serem formadas em simultâneo; tanto quanto pudemos observar, ocorre primeiro a formação de uma das valvas e só depois desta concluída, se inicia a construção da outra (Foto n.º 19).

A ornamentação das valvas iniciais, pouco definível ao microscópico óptico, é constituída por estrias que resultam da contiguidade de pontos muito próximos uns dos outros.

Para além das dimensões, a principal diferença existente entre a frústula inicial e a frústula das células vegetativas reside na ornamentação da área central. Na frústula inicial as estrias ornamentais da área central são tão espessadas e tão robustas quanto as dos polos.

As dimensões médias das valvas iniciais são da ordem dos  $36,5 \times 12,5 \mu$ ; algumas formas maiores podem atingir as dimensões de  $41,0 \times 15,0 \mu$ .

#### DISCUSSÃO

Consoante as raças de *Cocconeis placentula*, o auxósporo (zigoto) pode formar-se por isogamia, anisogamia ou ainda por partenogénese (GETTLER, 1982 in ROUND, 1990).

Todo o estudo efectuado parece indicar que a raça que constitue a população natural recolhida, seja anisogâmica, como acontece com a var. *pseudolineata* (GETTLER, 1930). Esta nossa afirmação baseada apenas na observação do zigoto não expandido, mas sempre incluído no interior de uma das frústulas gametangiais, necessita ainda de ser confirmada por estudos citológicos a realizar, quer em populações naturais quer em culturas laboratoriais.

As valvas das frústulas iniciais são arraffídeas; enquanto a epivalva possui, para além, de um nítido pseudorrafe igual ao das células vegetativas, um a vários fragmentos de pseudorrafes contorcidos, a hipovalva apresenta apenas, e somente em alguns casos, um pseudorrafe contorcido e muito mal definido.

Estas características da hipovalva inicial de *Cocconeis placentula* são nitidamente diferentes das de *Cocconeis placentula* var. *pseudolineata* Geitler que, se conhece, apresentar um pseudorrafe direito e bem definido (GETTLER, 1958a in MANN, 1984). A epivalva inicial, da espécie estudada, também é diferente da de *Cocconeis pediculus* Ehrenb., visto, esta última, apresentar dois ou mais pseudorrafes contorcidos (MANN, 1984).

As valvas com rafe são originadas unicamente após divisão celular vegetativa da célula inicial contrariamente ao que se

observa, em geral, no género *Achnanthes* (MANN, 1984) onde uma das valvas da frústula inicial possui sempre rafe.

Sobre a formação do auxósporo em *Amphora veneta* apenas podemos adiantar que ele parece resultar de uma união isogâmica, após as duas células gametangiais se terem libertado das frústulas. Não sendo do nosso conhecimento, qualquer estudo deste tipo efectuado no género *Amphora*, é absolutamente indispensável a realização de estudos citológicos em populações naturais ou em culturas laboratoriais que possibilitem observações mais conclusivas e, se possível, definitivas.

Do estudo ecológico que temos vindo a efectuar sobre estas duas espécies pudemos perceber que têm uma actividade de auxosporulação bem sincronizada. Esta ocorre predominantemente durante o mês de Setembro mas as valvas iniciais só podem ser detectadas na população apenas durante uma semana. Como a quantidade de auxóporos é muito variável de ano para ano, e é em geral muito pequena, em 1991 não detectámos nas populações naturais nenhuma forma auxosporangiais que nos permitissem concluir e confirmar, como desejávamos, as nossas observações.

#### AGRADECIMENTOS

A realização deste trabalho foi possível graças ao apoio financeiro do I. N. I. C. o qual agradecemos.

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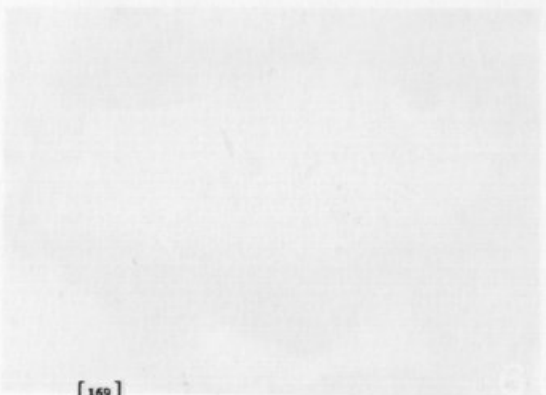
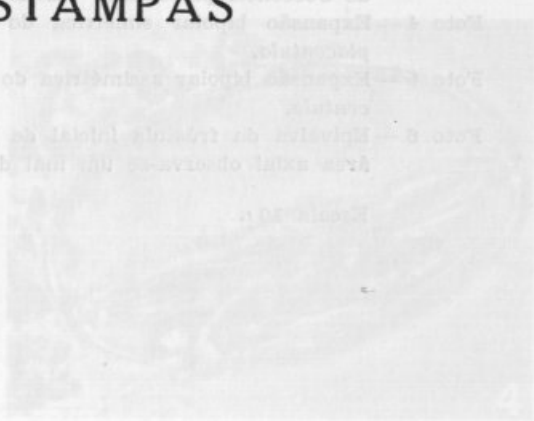
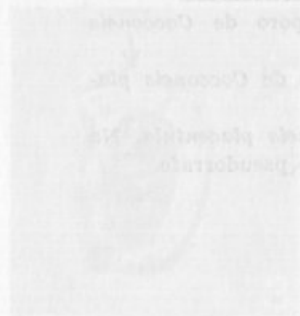
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Foto 1.—Células vegetativas conjuntas de *Coconus* pinnatis. Foto 2.—Células vegetativas conjuntas de *Coconus* pinnatis, revestidas por un invólucro mucilaginoso. Foto 3.—Ejeto formado en el interior de una fructula pinnatis. Foto 4.—Ejeto formado en el interior de una fructula pinnatis. Foto 5.—Ejeto formado en el interior de una fructula pinnatis. Foto 6.—Ejeto formado en el interior de una fructula pinnatis. Foto 7.—Ejeto formado en el interior de una fructula pinnatis. Foto 8.—Ejeto formado en el interior de una fructula pinnatis. Foto 9.—Ejeto formado en el interior de una fructula pinnatis. Foto 10.—Ejeto formado en el interior de una fructula pinnatis.

**ESTAMPAS**

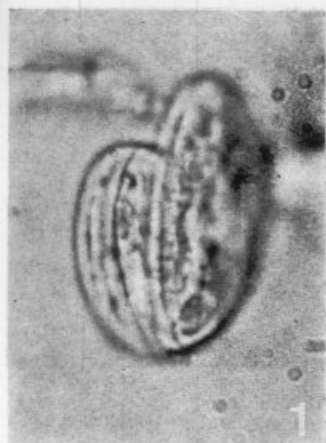


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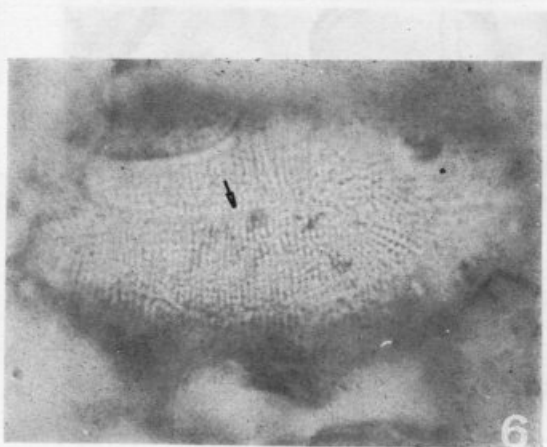
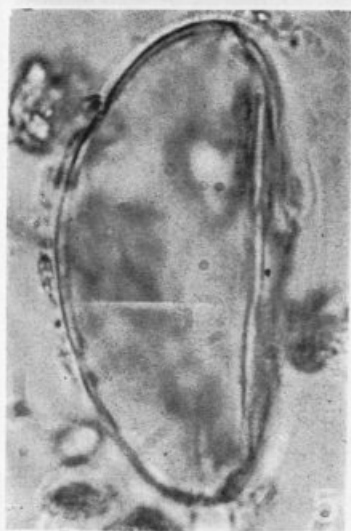
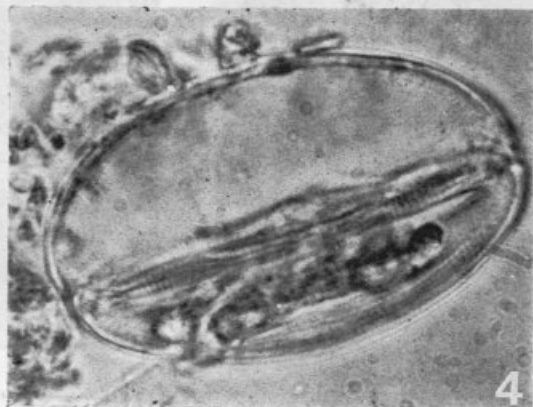
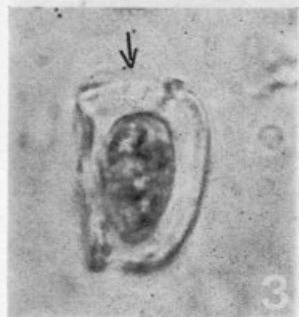
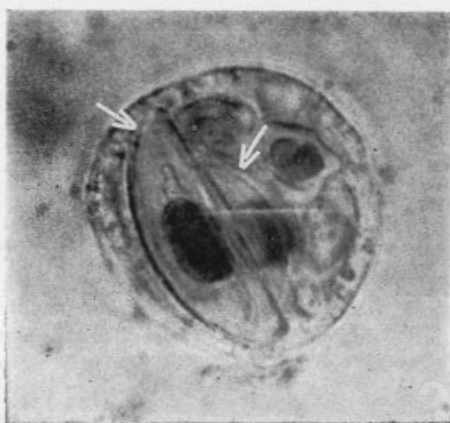
## ESTAMPA I

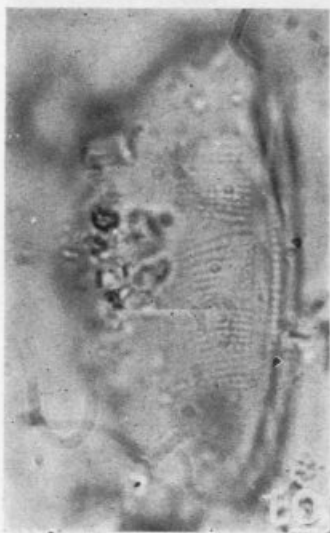
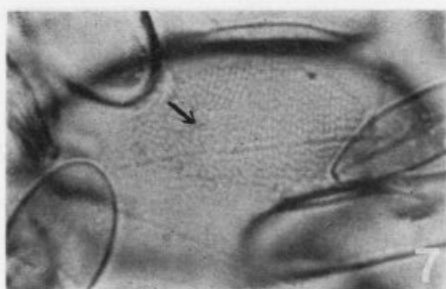
- Foto 1 — Células vegetativas conjugantes de *Cocconeis placentula*.
- Foto 2 — Células vegetativas conjugantes de *Amphora veneta* já revestidas por um invólucro mucilaginoso.
- Foto 3 — Zigoto formado no interior de uma frústula gametangial de *Cocconeis placentula*. Valvas já desarticuladas.
- Foto 4 — Expansão bipolar simétrica do auxósporo de *Cocconeis placentula*.
- Foto 5 — Expansão bipolar assimétrica do zigoto de *Cocconeis placentula*.
- Foto 6 — Epivalva da frústula inicial de *Cocconeis placentula*. Na área axial observa-se um mal definido pseudorrafe.

Escala 10  $\mu$ .

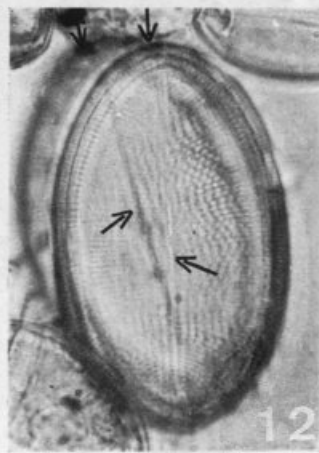
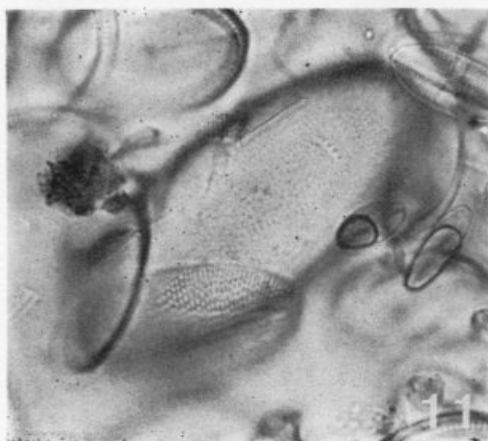


10  $\mu$





10μ —————





## ESTAMPA II

- Foto 7 e 8 — Epivalva da frústula inicial de *Cocconeis placentula* evidenciando um pseudorrafe rectilíneo (7) ou sigmoide (8). Note-se o fragmento de pseudorrafe e as estrias a ele associadas (seta) na foto 7.
- Foto 9 — Hipovalva acentuadamente convexa de *Cocconeis placentula*. Entre as duas valvas não é possível observar cinturas conectivas (seta).
- Foto 10 — Estrias ornamentais do bordo valvar da hipovalva de *Cocconeis placentula*.
- Foto 11 — Ornamentação desordenada na área axial da hipovalva *Cocconeis placentula*.
- Foto 12 — Frústulas secundárias de *Cocconeis placentula* originadas por divisão celular vegetativa da célula inicial. A hipovalva de cada frústula secundária possui as características da espécie, apresenta rafe.

Escala 10  $\mu$ .

## ESTAMPA III

- Foto 13 e 14 — Epivalva e hipovalva de uma das frústulas secundárias de *Cocconeis placentula* originada a partir de uma determinada célula inicial.
- Foto 15 e 16 — Hipovalva e epivalva da outra das frústulas secundárias de *Cocconeis placentula* originada a partir da mesma célula inicial anteriormente referenciada.
- Foto 17 — Gâmetas de *Amphora veneta* já libertos da frústula gametangial.
- Foto 18 — Valva da frústula gametangial de *Amphora veneta* destacando-se da camada orgânica envolvente.
- Foto 19 — Formação de uma das valvas da frústula inicial de *Amphora veneta*.
- Foto 20 — Frústula inicial de *Amphora veneta*. Note-se a convexidade da área central e a ornamentação valvar (seta).

Escala 10  $\mu$ .

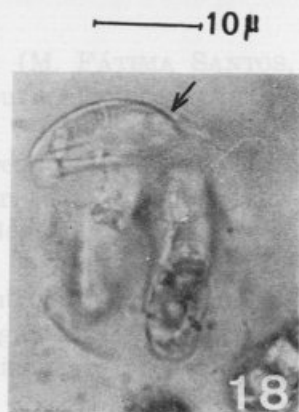
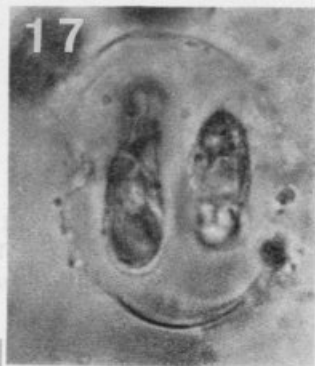
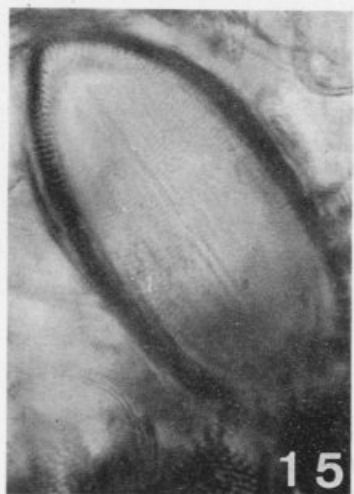
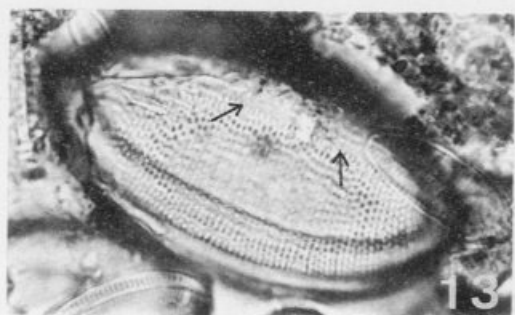




Foto 17 - Ganchos  
facial

Foto 18 - Valva da



101 —————



# ACOI—THE CULTURE COLLECTION OF ALGAE OF THE DEPARTMENT OF BOTANY UNIVERSITY OF COIMBRA

## SUPPLEMENT — II

by

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### ABSTRACT

This is a supplement to our previous lists (M. FÁTIMA SANTOS & J. F. MESQUITA, 1986 and M. FÁTIMA SANTOS, 1988). New 194 *taxa* are added to our «Algoteca» and some corrections to names are introduced.

### 1 — INTRODUCTION

SINCE the publication of the latter list (M. FÁTIMA SANTOS, 1988) we have been incorporated into our «Algoteca» (ACOI) 194 new *taxa* distributed by 4 Divisions, 7 Classes, 17 Orders and 102 genres. A great part of these cultures have been collected, isolated and identified during the last four years. The majority of those *taxa* are belonging to our phycological flora; some of them were collected in others countries.

Unfortunately the cultures now presented are still contaminated by bacteriae and other nonalgal organisms. Specification for culture conditions and media preparations are the same as previously published.

2.1. — LIST

Algae collected, isolated and identified in ACOI

Reference	Name of the taxon	Collector and date of isolation	Culture medium	Origin: (harvest place)
<b>Order Euglenales</b>				
<b>PHACUS Dujardin</b>				
614	<i>P. orbicularis</i> Hübner fo. <i>communis</i> Popowa	SANTOS, M. F.; CARVALHO, G. (1993)	L-S2T2	Sintra (Monserrate Park)
659	<i>P. splendens</i> Pochmann	SANTOS, M. F.; CARVALHO, G. (1994)	L-S2T2	Coimbra (Mondego river)
<b>Order Chlorococcales</b>				
<b>ANKISTRODESMUS Corda</b>				
400	<i>A. spiralis</i> (Turn.) Lemmermann	CARVALHO, G.; LOURENÇO, O. (1988)	L-M7	Quilais (Lagoa das Braças)
682	<i>A. spiralis</i> (Turn.) Lemmermann	FERNANDES, J. (1994)	M7	Quilais (Lagoa da Vela)
<b>BRACTEACOCOCUS Tereg</b>				
529	<i>B. giganteus</i> Bisch. & Bold	CARVALHO, G. (1989)	M7	Coimbra (airborne alga)
419	<i>B. grandis</i> Bisch. & Bold	LOURENÇO, O. (1989)	S2T2	Ereira
<b>CHLORELLA Beijerinck</b>				
516	<i>C. emersonii</i> Shih. & Krauss	PAIVA, J. (1991)	L-M7	S. Tomé
621	<i>C. fusca</i> Shih. & Krauss v. <i>fusca</i>	PAIVA, J. (1993)	L-S2T2	Madeira
454	<i>C. reisingli</i> S. Watanabe	LOURENÇO, O. (1991)	M7	Serra da Estrela (Lagoa Comprida)
620	<i>C. reisingli</i> S. Watanabe	PAIVA, J. (1993)	M7	Madeira
531	<i>C. zofingtensis</i> Dönnz	CARVALHO, G. (1989)	Lc	Coimbra (airborne alga)
<b>CHLOROCOCCUM Meneghini</b>				
513	<i>C. foebastum</i> Eui & Gärtnert	PAIVA, J. (1991)	L-Lc	S. Tomé
<b>DIVISION EUGLENOPHYTA</b>				
<b>CLASS EUGLENOPHYCEAE</b>				
<b>DIVISION CHLOROPHYTA</b>				
<b>CLASS EUCHLOROPHYCEAE</b>				

CHORICYSTIS (Skuja) Fott

586 C. hindakii Tell

CORONASTRUM Thompson

473 C. aestivale Thompson

CRUCIGENIA Morren

492 C. mucronata (G. M. Smith) Komárek

CRUCIGENIELLA Lemmermann

474 C. pulchra (W. & G. S. West) Komárek

DACTYLOSPHAERIUM Steinecke

478 D. jurisii Hindák

DESMATRACTUM W. & G. S. West

467 D. bipyramidalatum (Chod.) Pascher

DICTYOCHLOROPSIS Gettler

455 D. splendida Gettler

KIRCHNERIELLA Schmidle

641 K. danubiana Hindák

640 K. rotunda (Kors.) Hindák

NEOSPONGIOCOCCUM Deason

525 N. gelatinosum (Archibald & Bold) Ettl

& Gärtner

OOCYSTIS Nägeli

434 O. heteromucosa Hegewald

PALMODICTYON Kützing

462 P. varium (Näg.) Lemmermann

PEDIASTRUM Meyen

658 P. biradiatum Meyen v. biradiatum

394 P. boryanum (Turp.) Menegh. v. longitorne

Reinsch

SANTOS, S. (1993)	S2T2	Coimbra (Mondego river)
CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Ditch of Maiorca (Montemor-o-Velho)
CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Ditch of Maiorca (Montemor-o-Velho)
CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Ditch of Maiorca (Montemor-o-Velho)
CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Near S. Martinho de Arvore (Mondego river)
LOURENÇO, O. (1991)	S2T2	Serra da Estrela (Lagoa Comprida)
PAIVA, J. (1991)	M7	Serra do Gerês (Pincães)
SANTOS, S. (1993)	M7	Coimbra (Mondego river)
SANTOS, S. (1993)	M7	Coimbra (Mondego river)
LOURENÇO, O. (1991)	Lc	Serra da Estrela (Lagoa Comprida)
MONTEZUMA DE CARVALHO (1990)	M7	Açores
LOURENÇO, O. (1989)	L-M7	Serra da Estrela (Barragem do Covão do Curral)
SANTOS, M. F.; CARVALHO, G. (1994)	L-M7	Coimbra (Mondego river)
CARVALHO, G. (1989)	L-Lc	Vacariça

Reference	Name of the taxon	Collector and date of isolation	Culture medium	Origin: (harvest place)
616	<i>P. boryanum</i> (Turp.) Menegh. v. longicorne Reinsch	PAIVA, J. (1993)	L-M7	Madeira
392	<i>P. duplex</i> Meyen v. gracillimum W. & G. S. West	CARVALHO, G.; LOURENÇO, O. (1989)	L-Lc	Weir of Coimbra (Mondego river)
391	<i>P. duplex</i> Meyen v. rugulosum Raciborski	CARVALHO, G.; LOURENÇO, O. (1988)	L-Lc	Quiaios (Lagoa das Braças)
716	<i>P. duplex</i> Meyen. v. rugulosum Raciborski	FERNANDES, J. (1994)	S2T2	Quiaios (Lagoa da Vela)
395	<i>P. duplex</i> Meyen v. subgranulatum Raciborski	MONTEZUMA DE CARVALHO (1990)	L-Lc	Açores
577	<i>P. integrum</i> Nägeli v. integrum	PAIVA, J. (1993)	L-M7	Madeira
575	<i>P. primum</i> (Printz) Hegewald	SANTOS, S. (1993)	L-M7	Coimbra (Mondego river)
PLANKTOSPHAERIA G. M. Smith				
530	<i>P. maxima</i> Bischoff & Bold	PAIVA, J. (1991)	L-M7	S. Tomé
611	<i>P. maxima</i> Bischoff & Bold	PAIVA, J. (1992)	L-M7	S. Tomé (between S. Luis and Chamilço)
612	<i>P. texensis</i> Bischoff & Bold	PAIVA, J. (1992)	L-M7	Príncipe (Fundão)
PLANOCHLORIS Komárek				
532	<i>P. pyrenoidifera</i> (Kors.) Komárek	LEITÃO, A. (1992)	L-M7	Évora
PSEUDODIDYMOCYSTIS Hegewald & Deason				
559	<i>P. inconspicua</i> (Korsikov) Hindák	SANTOS, M. F. (1979)	L-M7	Ançã
QUADRIGULA Printz				
396	<i>Q. closterioides</i> (Bohl.) Printz	LOURENÇO, O. (1988)	L-M7	Near Sortelha
RADIOCOCCUS Schmidle				
428	<i>R. planktonicus</i> Lund	MONTEZUMA DE CARVALHO (1990)	M7	Açores
SCENEDESMUS Meyen				
429	<i>S. aculeolatus</i> Reinsch	MONTEZUMA DE CARVALHO (1990)	L-M7	Açores
535	<i>S. aculeolatus</i> Reinsch	SANTOS, M. F. (1975)	L-M7	Tentugal (rice swamp)
555	<i>S. aculeolatus</i> Reinsch	LOURENÇO, O. (1992)	L-M7	Coimbra
442	<i>S. armatus</i> Chodat	MONTEZUMA DE CARVALHO (1990)	L-M7	Açores
562	<i>S. armatus</i> Chodat	SANTOS, M. F. (1974)	L-M7	Coimbra
563	<i>S. armatus</i> Chodat	SANTOS, M. F. (1975)	L-M7	Tentugal
		LEITÃO, A. (1992)	L-M7	Évora



564	<i>S. armatus</i> Chodat	LEITÃO, A. (1992)	L-M7	Evora
437	<i>S. armatus</i> Chodat v. <i>asymmetricus</i> Bour-rely	MONTEZUMA DE CARVALHO (1990)	L-M7	Açores
551	<i>S. armatus</i> Chodat v. <i>longispina</i> (Chod.) Hegewald & Hindák	LOURENÇO, O. (1992)	L-M7	Coimbra
548	<i>S. asymmetricus</i> (Schröd.) Chodat	SANTOS, M. F. (1981)	L-M7	Serra da Estrela (Lagoa Comprida)
754	<i>S. asymmetricus</i> (Schröd.) Chodat	SANTOS, M. F.; CARVALHO, G. (1994)	M7	Serra da Estrela (Covão do Boeiro)
565	<i>S. brasiliensis</i> Bohlin	PAIVA, J. (1992)	L-M7	S. Tomé (Diogo Vaz)
557	<i>C. communis</i> Hegewald	LOURENÇO, O. (1992)	L-M7	Coimbra
644	<i>S. communis</i> Hegewald	SANTOS, S. (1993)	L-M7	Coimbra (Mondego river)
431	<i>S. danubialis</i> Hortobágyi	MONTEZUMA DE CARVALHO (1990)	L-M7	Açores
527	<i>S. denticulatus</i> Lagerheim	MONTEZUMA DE CARVALHO (1990)	L-M7	Açores
584	<i>S. denticulatus</i> Legerheim	SANTOS, S. (1993)	L-M7	Coimbra (Mondego river)
542	<i>S. dispar</i> (Bréb.) Rabenhorst	SANTOS, M. F. (1975)	L-M7	Between Meãs and Montemor-o-Velho (rice swamp)
534	<i>S. granulatus</i> W. & G. S. West	SANTOS, M. F. (1975)	L-M7	Tentugal (rice swamp)
424	<i>S. obtusus</i> Meyen	MONTEZUMA DE CARVALHO (1990)	L-M7	Açores
619	<i>S. obtusus</i> Meyen	PAIVA, J. (1993)	L-M7	Madeira
479	<i>S. opoliensis</i> Richter	CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Ditch of Maiorca (Montemor-o-Velho)
560	<i>S. pannonicus</i> Hortobágyi	CARVALHO, G. (1992)	L-M7	Friumes (Alva river)
719	<i>S. pannonicus</i> Hortobágyi	FERNANDES, J. (1994)	S2T2	Quiaios (Lagoa da Vela)
561	<i>S. pleiomorphus</i> Hindák	LEITÃO, A. (1992)	L-M7	Evora
545	<i>S. quadrispina</i> Chodat	SANTOS, M. F. (1981)	L-M7	Serra da Estrela (Barragem do Covão do Curral)
618	<i>S. quadrispina</i> Chodat	PAIVA, J. (1993)	L-M7	Madeira
549	<i>S. raciborskii</i> Woloszynska	CARVALHO, G.; SANTOS, M. F. (1984)	L-M7	Mira
522	<i>S. serratus</i> (Corda) Bohlin	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)
547	<i>S. smithii</i> Chodat	SANTOS, M. F. (1975)	L-M7	Mira
212	<i>S. spinosus</i> Chodat	CARVALHO, G.; SANTOS, M. F. (1984)	L-M7	Mira
540	<i>S. tenuispina</i> Chodat	LEITÃO, A. (1992)	L-M7	Evora
<b>SCHROEDERIA</b> Lemmermann				
546	<i>S. setigera</i> (Schröd.) Lemmermann	LOURENÇO, O. (1992)	L-M7	Coimbra
<b>SCOTIELLOPSIS</b> Vinatzer				
589	<i>S. terrestris</i> (Reisigl) Puncoch & Kalina	PAIVA, J. (1992)	L-M7	Príncipe

Reference	Name of the taxon	Collector and date of isolation	Culture medium	Origin: (harvest place)
<b>SIDEROCELI</b> (Naumann) Fott				
416	<i>S. kolkwitzii</i> (Naum.) Fott	CARVALHO, G.; SANTOS, M. F. (1985)	S2T2	Tentugal
<b>TETRALANTOS</b> Telling				
480	<i>T. lagerheimii</i> Telling	CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Ditch of Maiorca (Montemor-o-Velho)
<b>TETRASTRUM</b> Chodat				
576	<i>T. komarekii</i> Hindák	SANTOS, S. (1993)	M7	Coimbra (Mondego river)
721	<i>T. komarekii</i> Hindák	FERNANDES, J. (1994)	M7	Quiatos (Lagoa da Vela)
<b>TREBOUXIA</b> De Puyrnaly				
413	<i>T. potteri</i> Ahmadjian ex Gärtner	SANTOS, M. F. (1981)	L-Lcol	Coimbra (Choupal)
412	<i>T. simplex</i> Tschermak-Woess	CARVALHO, G. (1988)	L-Lcol	Coimbra (Botanical Garden)
<b>Order Tetrasporales</b>				
<b>CHAETOPELTI</b> Berthold				
489	<i>C. orbicularis</i> Berthold	CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Ditch of Maiorca (Montemor-o-Velho)
<b>CHARACIOCHLORIS</b> Pascher				
386	<i>C. sasae</i> Nozaki	CARVALHO, G.; LOURENÇO, O. (1989)	M7	Weir of Coimbra (Mondego river)
<b>CHLAMYDOCAPSA</b> Fott				
526	<i>C. maxima</i> (Malnx) Ettl & Gärtner	LOURENÇO, O. (1991)	M7	Serra da Estrela (Barragem do Covão do Curral)
<b>CHLORANGIELLA</b> De Toni				
501	<i>C. basiannulata</i> (Skuja) Silva	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)
<b>CYANOPTYCHE</b> Pascher				
387	<i>C. gloeocystis</i> Pascher	LOURENÇO, O. (1989)	S2T2	Sintra (Monserrate Park)
<b>PLEOTILA</b> Mrozinska-Webb				
502	<i>P. ramosa</i> Mrozinska-Webb	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)

**SPHAERELLOCYSTIS** Ettl

- 581 *S. stellata* Ettl  
 582 *S. stellata* Ettl  
 613 *S. stellata* Ettl

**TETRASPORA** Link

- 468 *T. lemmermannii* Fott

**Order Volvocales****MESOSTIGMA** Lauterborn

- 660 *M. viride* Lauterborn

**EUDORINA** Ehrenberg

- 486 *E. elegans* Ehrenberg

**PLEODORINA** Shaw

- 485 *P. californica* Shaw

**PTEROMONAS** Seligo

- 347 *P. angulosa* Lemmermann

**STEPHANOSPHAERA** Cohn

- 477 *S. pluvialis* Cohn

**VOLVOX** Linnaeus

- 469 *V. aureus* Ehrenberg  
 470 *V. tertius* Meyer

**Order Chaetophorales****CHLOROKYBUS** Gettler

- 458 *C. atmophyticus* Gettler  
 491 *C. atmophyticus* Gettler

**CLONIOPHORA** Tiffany

- 475 *C. spicata* (Schmidle) Islam

- SANTOS, M. F. (1979)  
 SANTOS, S. (1993)  
 PAIVA, J. (1992)

- LOURENÇO, O. (1991)

- SANTOS, M. F.; CARVALHO, G. (1994)

- LOURENÇO, O. (1991)

- CARVALHO, G.; SANTOS, M. F. (1991)

- CARVALHO, G. (1988)

- CALADO, A.; RINO, J. (1991)

- CARVALHO, G.; SANTOS, M. F. (1991)

- CARVALHO, G.; SANTOS, M. F. (1991)

**CLASS ULOTHREICHOPHYCEAE**

- SANTOS, L. (1990)

- LOURENÇO, O. (1991)

- CARVALHO, G.; SANTOS, M. F. (1991)

M7

M7

M7

L-M7

L-M7

L-M7

L-M7

L-M7

L-M7

L-M7

L-M7

L-M7

Cantanhede  
 Coimbra (Mondego river)  
 Príncipe (Fundão)

Serra da Estrela (Lagoa Comprida)

Coimbra (Mondego river)

Quaios (Lagoa da Vela)

Ditch of Maiorca (Montemor-o-Velho)

Barrô

Vilar Dam (Tavora river)

Mondego river (old stream)

Ditch of Maiorca (Montemor-o-Velho)

Margarça

Quaios (Lagoa da Vela)

Near Maiorca (Mondego river)

Reference	Name of the taxon	Collector and date of isolation	Culture medium	Origin: (harvest place)
<b>LEPTOSIRA Borzi</b>				
569	<i>L. obovata</i> Vischer	PAIVA, J. (1992)	M7	S. Tomé (Diogo Vaz)
583	<i>L. obovata</i> Vischer	PAIVA, J. (1992)	M7	S. Tomé (between S. Luis and Chamigco)
<b>PSEUDENDOCOLONIUM Wille</b>				
494	<i>P. postratum</i> Tupa	SANTOS, L. (1990)	Lc	Margaraca
<b>PSEUDOPLEUROCOCCUS Snow</b>				
482	<i>P. printzii</i> Vischer	LOURENÇO, O. (1991)	M7	Coimbra (S. Sebastião)
<b>PSEUDOTETRACYSTIS Arneson</b>				
607	<i>P. terrestris</i> Arneson	PAIVA, J. (1992)	M7	S. Tomé (S. Nicolau)
<b>Order Oedogoniales</b>				
<b>BULBOCHAETE Agardh</b>				
552	<i>B. sp.</i>	CARVALHO, G. (1992)	L-M7	Friumes (Alva river)
<b>OEDOGONIUM Link</b>				
655	<i>O. pusillum</i> Kirchner	PAIVA, J. (1992)	S2T2	S. Tomé (Diogo Vaz)
649	<i>O. tapinosporum</i> Wittrock	PAIVA, J. (1992)	S2T2	Príncipe (Fundão)
<b>Order Siphonales</b>				
<b>PROTOSIPHON Klebs</b>				
418	<i>P. botryoides</i> (Kütz.) Klebs	CARVALHO, G.; LOURENÇO, O. (1989)	Lc	Marrocos (near Taza)
<b>Order Siphonocladales</b>				
<b>CLADOPHORELLA Fritsch</b>				
471	<i>C. calcicola</i> Fritsch	LOURENÇO, O. (1989)	S2T2	Lagoa de Obidos
<b>Order Ulothrichales</b>				
<b>CHAETOPHORA Schrank</b>				
457	<i>C. elegans</i> (Roth) Agardh	SANTOS, L. (1991)	L-M7	Alentejo (Oeiras stream)
447	<i>C. incrassata</i> (Hudson) Hazen	SANTOS, L. (1991)	L-M7	Alentejo (Oeiras stream)

ELAKATOTHRIX Wille

448 E. genevensis (Reverd.) Hindák

FOTTEA Hindák

646 F. stichococcoides Hindák

GEMINELLA Turpin

430 G. interrupta (Turpin) Lagerheim

GLOEOTILA Kützing

461 G. curta Skuja

INTERFILUM Chodat & Topali

590 I. paradoxum Chodat & Topali

591 I. paradoxum Chodat & Topali

JAAGIELLA Vischer

441 J. alpicola Vischer

KOLIELLA Hindák

574 K. spiculiformis (Vischer) Hindák

MICROSPORA Thuret

399 M. amoena (Kütz.) Rabenh. v. gracilis

(Wille) De-Toni

499 M. wittrockii (Wille) Lagerheim

Order Zygnematales

CLOSTERIUM Nitzsch

358 C. acerosum (Schrank) Ehrenberg

361-a C. acerosum (Schrank) Ehrenb. v. elonga-

tum Brébisson

SANTOS, M. F. (1980)

L-M7

Coimbra

LOURENÇO, O. (1991)

L-M7

Quiaios (Lagoa das Braças)

SANTOS, S. (1993)

L-S2T2

Coimbra (Mondego river)

LOURENÇO, O. (1990)

L-M7

Serra da Estrela (Lagoa Comprida)

SANTOS, L. (1990)

L-M7

Margarça

PAIVA, J. (1993)

L-S2T2

Madeira

SANTOS, M. F.; CARVALHO, G. (1993)

L-S2T2

Azeição (Quinta da Bacalhoa)

CARVALHO, G. (1989)

Lc

Coimbra (airborne alga)

SANTOS, S. (1993)

L-M7

Coimbra (Mondego river)

LOURENÇO, O. (1990)

L-M7

Manteigas

SIMÃO, I. (1991)

L-M7

Near Portela (Mondego river)

CLASS ZYGOPHYCEAE

LOURENÇO, O. (1988)

L-M7

Almofala de Baixo

CARVALHO, G.; LOURENÇO, O. (1989)

L-M7

Between Montemor-o-Velho and Maiorca  
(Mondego river)

Reference	Name of the taxon	Collector and date of isolation	Culture medium	Origin: (harvest place)
361-b	<i>C. acerosum</i> (Schrank) Ehrenb. v. minus Hantzsch	CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Between Montemor-o-Velho and Malorca (Mondego river)
459	<i>C. acutum</i> Bréb. v. variable (Lemm.) Krieger	LOURENÇO, O. (1991)	L-M7	Quiaios (Lagoa das Braças)
407	<i>C. cornu</i> Ehrenberg	LOURENÇO, O. (1989)	L-M7	Sintra (Monserrate Park)
504	<i>C. cornu</i> Ehrenberg	LOURENÇO, O. (1991)	L-M7	Quiaios (Lagoa das Braças)
366	<i>C. diana</i> Ehrenb. v. brevis (Petk.) Krieger	CARVALHO, G.; LOURENÇO, O. (1987)	L-M7	Mondego river (old stream)
384	<i>C. eboracense</i> Turner	LOURENÇO, O. (1990)	L-M7	Serra da Estrela (Manteigas)
498	<i>C. eboracense</i> Turner	PAIVA, J. (1991)	L-M7	Minho (Lindoso Dam)
362	<i>C. ehrenbergii</i> Meneghini	LOURENÇO, O. (1988)	L-M7	Unhais da Serra
476	<i>C. incurvum</i> Brébisson	LOURENÇO, O. (1991)	L-M7	Quiaios (Lagoa das Braças)
359	<i>C. intermedium</i> Ralfs	LOURENÇO, O. (1988)	L-M7	Sortelha
363	<i>C. moniliferum</i> (Bory) Ehrenberg	CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Portela de Formoselha (Mondego river)
364	<i>C. moniliferum</i> (Bory) Ehrenberg	CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Pateira de Fermentelos
365	<i>C. moniliferum</i> (Bory) Ehrenberg	SANTOS, M. F. (1975)	L-M7	Near Montemor-o-Velho
383	<i>C. moniliferum</i> (Bory) Ehrenberg	LOURENÇO, O. (1990)	L-M7	Manteigas
382	<i>C. moniliferum</i> (Bory) Ehrenb. v. submoniliferum (Woron.) Krieger	LOURENÇO, O. (1989)	L-M7	Turil
440	<i>C. navicula</i> (Bréb.) Lütkenmüller	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)
COSMARIUM Corda				
378	<i>C. angulosum</i> Brébisson	LOURENÇO, O. (1989)	L-M7	Between Covas and Vilar de Mouros
375	<i>C. binum</i> Nordstedt	CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Weir of Coimbra (Mondego river)
369	<i>C. biretum</i> (Bréb.) Ralfs	CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Ponte de Formoselha (Mondego river)
404	<i>C. brebissonii</i> (Menegh.) Ralfs	LOURENÇO, O. (1990)	L-M7	Serra da Estrela (Lagoa Comprida)
371	<i>C. caelatum</i> Ralfs	LOURENÇO, O. (1989)	L-M7	Between Covas and Vilar de Mouros
355	<i>C. crenatum</i> Ralfs	LOURENÇO, O. (1988)	L-M7	Between Góis and Pampilhosa da Serra
403	<i>C. difficile</i> Lütkenmüller	LOURENÇO, O. (1990)	L-M7	Serra da Estrela (Lagoa Comprida)
623	<i>C. difficile</i> Lütkenmüller	PAIVA, J. (1993)	L-M7	Madeira
490	<i>C. granatum</i> Brébisson	CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Ditch of Malorca (Montemor-o-Velho)
349	<i>C. hammeri</i> Reinsch	LOURENÇO, O. (1988)	L-M7	Manteigas
377	<i>C. hornsvandense</i> Gutw. v. dubovianum	LOURENÇO, O. (1988)	L-M7	Serra da Estrela (Baragem do Covão do

349	<i>C. hammertii</i> Kienboen					
377	<i>C. hornavanzense</i> Gutw. v. dubovianum (Lüttk.) Ruzicka					
379	<i>C. laeve</i> Rabenhorst					
566	<i>C. pachydermum</i> Lundell v. aethiopicum W. & G. S. West					
368	<i>C. quadrum</i> Lundell v. sublatum (Nordst.) W. & G.S. West f. dilatatum Scott & Grönblad					
370	<i>C. regnellii</i> Wille v. pseudoregnellii (Messik.) Krieger & Gerloff					
625	<i>C. regnellii</i> Wille v. pseudoregnellii (Messik.) Krieger & Gerloff					
406	<i>C. sinostegos</i> Schaarschm. v. obtusius Gutwinski					
376	<i>C. sportella</i> Brébisson					
405	<i>C. sportella</i> Bréb. v. subnudum W. & G. S. West					
622	<i>C. sportella</i> Bréb. v. subnudum W. & G. S. West					
380	<i>C. subgranatum</i> (Nordst.) Lütikemüller v. borgei Krieger					
COSMOCLADIUM Brébisson						
342	<i>C. perissum</i> Roy & Bisset					
DESMIDIUM Agardh						
554	<i>D. swartzii</i> Agardh					
EUASTRUM Ehrenberg						
488	<i>E. binale</i> (Turp.) Ehrenb. v. gutwinski (Schm.) Homfeld					
351	<i>E. humerosum</i> Ralfs					
409	<i>E. humerosum</i> Ralfs					
		LOURENÇO, O. (1988)	L-M7	Serra da Estrela (Barragem do Covão do Curral)		
		PEREIRA, L. (1990)	L-M7	Comeal da Torre		
		PAIVA, J. (1992)	L-M7	S. Tomé (Diogo Vaz)		
		CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Weir of Coimbra (Mondego river)		
		LOURENÇO, O. (1989)	L-M7	Sintra (Monserrate Park)		
		PAIVA, J. (1993)	L-M7	Madeira		
		LOURENÇO, O. (1990)	L-M7	Serra da Estrela (Lagoa Comprida)		
		LOURENÇO, O. (1989)	L-M7	Between Covas and Vilar de Mouros		
		LOURENÇO, O. (1990)	L-M7	Serra da Estrela (Lagoa Comprida)		
		PAIVA, J. (1993)	L-M7	Madeira		
		CARVALHO, G.; SANTOS, M. F. (1988)	L-M7	Between Fig. Campo and Pereira do Campo		
		LOURENÇO, O. (1988)	L-M7	Serra da Estrela (Barragem do Covão do Curral)		
		CARVALHO, G. (1992)	L-M7	Friumes (Alva river)		
		LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Barragem do Covão do Curral)		
		LOURENÇO, O. (1988)	L-M7	Serra da Estrela (Barragem do Covão do Curral)		
		LOURENÇO, O. (1990)	L-M7	Serra da Estrela (Lagoa Comprida)		

Reference	Name of the taxon	Collector and date of isolation	Culture medium	Origin: (harvest place)
410	<i>E. pectinatum</i> Brébisson	LOURENÇO, O. (1990)	L-M7	Serra da Estrela (Lagoa Comprida)
446	<i>E. verrucosum</i> Ehrenberg	LOURENÇO, O. (1991)	L-M7	Quialoes (Lagoa das Braças)
GONATOZYGON De Bary				
350	<i>G. kinahani</i> (Arch.) Rabenhorst	CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Mondego river (old stream)
558	<i>G. kinahani</i> (Arch.) Rabenhorst	CARVALHO, G. (1992)	L-M7	Friumes (Alva river)
MESOTAENIUM Nägeli				
451	<i>M. endlicherianum</i> Nägeli	LOURENÇO, O. (1991)	M7	Quialoes (Lagoa das Braças)
MICRASTERIAS Agardh				
353	<i>M. denticulata</i> Brébisson	LOURENÇO, O. (1988)	L-M7	Buçaco
NETRIUM (Nägeli) Itzigson & Rothe				
624	<i>N. digitus</i> (Ehrenb.) Itzigson & Rothe v. <i>naegeli</i> (Bréb.) Krieger	PAIVA, J. (1993)	L-M7	Madeira
PENIUM Brébisson				
445	<i>P. spirostriolatum</i> Barker	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)
PLEUROTAENIUM Nägeli				
374	<i>P. maximum</i> (Reinsch) Lundell	LOURENÇO, O. (1989)	L-M7	Sintra (Monserrate Park)
SPIROGYRA Link				
588	<i>S. submaxima</i> Transeau	SANTOS, M. F.; CARVALHO, G. (1993)	L-M7	Sintra (Monserrate Park)
SPIROTAENIA Brébisson				
463	<i>S. erythrocephala</i> Itzigsohn	LOURENÇO, O. (1989)	L-M7	Between Covas and Vilar de Mouros
STAURASTRUM Meyen				
356	<i>S. dispar</i> Brébisson	CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Weir of Coimbra (Mondego river)
628	<i>S. dispar</i> Brébisson	PAIVA, J. (1993)	L-M7	Madeira
450	<i>S. laeve</i> Ralfs	MONTEZUMA DE CARVALHO (1990)	L-M7	Açores
357	<i>S. manfeldtii</i> Delponste	CARVALHO, G.; LOURENÇO, O. (1989)	L-M7	Weir of Coimbra (Mondego river)
372	<i>S. meriant</i> Reisch	LOURENÇO, O. (1989)	L-M7	Between Covas and Vilar de Mouros
		LOURENÇO, O. (1988)	L-M7	Serra da Estrela (Baragem do Covão do



348	<i>S. proboscideum</i> (Bréb.) Archer	LOURENÇO, O. (1988)	L-M7	Serra da Estrela (Barragem do Covão do Curral)
495	<i>S. seibaldi</i> Reinsch v. <i>ornatum</i> Nordstedt	LOURENÇO, O. (1991)	L-M7	Quialos (Lagoa das Braças)
360	<i>S. senarium</i> (Ehrenb.) Ralfs	LOURENÇO, O. (1988)	L-M7	Serra da Estrela (Barragem do Covão do Curral)
373	<i>S. tetracerum</i> Ralfs	LOURENÇO, O. (1989)	L-M7	Sintra (Monserrate Park)
722	<i>S. tetracerum</i> Ralfs	FERNANDES, J. (1994)	M7	Quialos (Lagoa da Vela)
472	<i>S. tohopekaligense</i> Wolle v. <i>brevispinum</i> G. M. Smith	CARVALHO, G.; SANTOS, M. F. (1991)	L-M7	Near S. Martinho de Arvore (Mondego river)
<b>STAUROESMUS</b> Telling				
460	<i>S. incus</i> (Bréb.) Telling v. <i>ralfsii</i> (West) Telling	LOURENÇO, O. (1991)	L-M7	Quialos (Lagoa das Braças)
<b>TETMEMORUS</b> Ralfs				
444	<i>T. brébissonii</i> (Menegh.) Ralfs	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)
<b>XANTHIDIUM</b> Ehrenberg				
439	<i>X. brébissonii</i> Ralfs	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)

#### DIVISION CHROMOPHYTA

##### CLASS CHRYSOPHYCEAE

##### SUBCLASS HETEROCHRYSPHYCIDAE

##### Order Chromulinales

##### CHRYSONEBULA

- 657 *C. flava* Starmach  
647 *C. flava* Starmach

##### DERMATOCHRYSIS

- 514 *D. reticulata* (Meyer) Entwistle & Andersen

- Quialos (Lagoa das Braças)  
Madeira

Colmbra

Reference	Name of the taxon	Collector and date of isolation	Culture medium	Origin: (harvest place)
	<b>Order Micrococcales</b>			
	<b>CHARACIOPSIS Borzi</b>			
456	<i>C. acuta</i> (Braun) Borzi	LOURENÇO, O. (1991)	L-M7	Quialos (Lagoa das Braças)
481	<i>C. saccata</i> Carter	SANTOS, M. F. (1989)	L-M7	Colmbra
	<b>OPHIOCYTIUM Nägeli</b>			
510	<i>O. capitatum</i> Wolle	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)
	<b>PSEUDOSTAURASTRUM Chodat</b>			
568	<i>P. enorme</i> (Ralfs) Chodat	PAIVA, J. (1992)	L-M7	S. Tomé (Diogo Vaz)
	<b>Order Tribonematales</b>			
	<b>TRIBONEMA Derbès &amp; Solier</b>			
520	<i>T. aequale</i> Pascher	SANTOS, M. F. (1975)	Lcol	Tentugal (rice swamp)
	<b>Order Vaucheriales</b>			
	<b>BOTRYDIUM Wallroth</b>			
512	<i>B. cystosum</i> Vischer	CARVALHO, G. (1990)	M7	Barró
	<b>VAUCHERIA De Candolle</b>			
493	<i>V. aversa</i> Hassall	SANTOS, L. (1990)	M7	Margaraca
585	<i>V. racemosa</i> (Vauch.) De Candolle	FERRAND-ALMEIDA, F. (1992)	M7	Cernache
421	<i>V. repens</i> Hassall	LOURENÇO, O. (1988)	M7	Buçaco
422	<i>V. sessilis</i> (Vauch.) De Candolle	LOURENÇO, O. (1988)	M7	Tomar
	<b>Order Chroococcales</b>			
	<b>APHANOTHECE Nägeli</b>			
654	<i>A. naegeli</i> Wartm.	PAIVA, J. (1992)	M7	S. Tomé (between S. Luis and Chamigo)

**CLASS XANTHOPHYCEAE**

**DIVISION CYANOPHYTA**

**CLASS CYANOPHYCEAE**

**SUBCLASS COCCOGONOPHYCIDAE**

- 604 *G. rupestris* (Lyngb.) Bornet  
635 *G. rupestris* (Lyngb.) Bornet

SANTOS, M. F. (1993)  
PAIVA, J. (1992)

M7 Coimbra (Botanical garden)  
M7 S. Tomé (Santa Catarina)

## Order Nostocales

## ANABAENA Bory

- 466 *A. laxa* (Rabenh.) A. Braun  
505 *A. cylindrica* Lemmermann

CARVALHO, G. (1989)  
PAIVA, J. (1991)

Lc Near Vacariça  
Lc S. Tomé

## CALOTHRIX Agardh

- 571 *C. atricha* Frémy  
633 *C. braunii* (A. Br.) Bornet & Flahault

PAIVA, J. (1992)  
PAIVA, J. (1993)

M7 S. Tomé (S. Nicolau)  
M7 Madeira

## CYLINDROSPERMUM Kützinger

- 441 *C. alatosporum* F. E. Fritsch  
483 *C. maius* Kützinger  
570 *C. gorakhpurensis* Singh

LOURENÇO, O. (1991)  
LOURENÇO, O. (1989)  
PAIVA, J. (1992)

M7 Carril (Entelhais)  
M7 Sintra  
M7 S. Tomé (S. Nicolau)

## LYNGBYA Agardh

- 653 *L. maior* Meneghini

PAIVA, J. (1992)

M7 S. Tomé (Diogo Vaz)

## MICROCHAETE Thuret

- 630 *M. tenera* Thuret  
631 *M. tenera* Thuret  
632 *M. tenera* Thuret

PAIVA, J. (1993)  
SANTOS, M. F. (1979)  
LOURENÇO, O. (1991)

L-M7 Madeira  
L-M7 Amieiro (near Arazede)  
L-M7 Serra da Estrela (Lagoa Comprida)

## MICROCOLEUS Desmazières

- 656 *M. vaginatus* (Vauch.) Gomont

SANTOS, M. F. (1993)

S2T2 Coimbra (Botanical garden)

## NOSTOC Vaucher

- 650 *N. carneum* Agardh  
484 *N. ellipso sporum* Rabenhorst  
610 *N. ellipso sporum* Rabenhorst  
636 *N. ellipso sporum* Rabenhorst

PAIVA, J. (1992)  
CARVALHO, G. (1989)  
PAIVA, J. (1992)  
PAIVA, J. (1993)

M7 S. Tomé (between S. Luis and Chamifço)  
Lc Near Vacariça  
M7 S. Tomé (Santa Catarina)  
M7 Madeira

Reference	Name of the taxon	Collector and date of isolation	Culture medium	Origin: (harvest place)
609	<i>N. gelatinosum</i> Schousboe	PAIVA, J. (1992)	M7	S. Tomé (S. Nicolau)
651	<i>N. humifusum</i> Carmichael	PAIVA, J. (1992)	M7	S. Tomé (Monte Café)
<b>PHORMIDIUM</b> Kützling				
648	<i>P. favosum</i> (Bory) Gomont	PAIVA, J. (1993)	S2T2	Madeira
762	<i>P. favosum</i> (Bory) Gomont	LOURENÇO, O. (1987)	S2T2	Serra da Estrela (Lagoa Comprida)
763	<i>P. favosum</i> (Bory) Gomont	SANTOS, M. F.; CARVALHO, G. (1994)	M7	Serra da Estrela (Barragem do Covão do Curral)
<b>TOLYPOTHRIX</b> Kützling				
594	<i>T. byssoidea</i> (Berk.) Kirchner	SANTOS, M. F.; CARVALHO, G. (1993)	M7	Azeitão (Quinta da Bacalhoa)
662	<i>T. byssoidea</i> (Berk.) Kirchner	SANTOS, M. F. (1988)	M7	Coimbra (Botanical Garden)
<b>Order Stigonematales</b>				
<b>HAPALOSIPHON</b> Nägeli				
443	<i>H. hibernicus</i> W. & G. S. West	LOURENÇO, O. (1991)	L-M7	Serra da Estrela (Lagoa Comprida)
652	<i>H. intricatus</i> W. & G. S. West	PAIVA, J. (1992)	L-S2T2	Príncipe (Pico Papagalo)
<b>SCYTONEMA</b> Agardh				
572	<i>S. chiastrum</i> Gettler	PAIVA, J. (1992)	L-M7	S. Tomé (between S. Luis and Chamico)
579	<i>S. chiastrum</i> Gettler	PAIVA, J. (1992)	L-M7	S. Tomé (Monte Café)
580	<i>S. crispum</i> (Agardh) Bornet	PAIVA, J. (1992)	L-M7	S. Tomé (S. Nicolau)
573	<i>S. obscurum</i> Hansgirg	PAIVA, J. (1992)	L-M7	S. Tomé (Monte Café)
<b>STIGONEMA</b> Agardh				
427	<i>S. ocellatum</i> (Dillw.) Thuret	LOURENÇO, O. (1990)	L-M7	Serra da Estrela (Lagoa Comprida)
769	<i>S. ocellatum</i> (Dillw.) Thuret	SANTOS, M. F.; CARVALHO, G. (1994)	S2T2	Serra do Gerês (Malhadoura)

## 2.2. — LIST

Algae received by exchange and included in ACOI

Reference	Name of the taxon	Culture medium	Origin
<b>RHODOPHYCEAE</b>			
1351.1	<i>Bangia atropurpurea</i> (Roth) Agardh	7 + 5	SAG
<b>CHRYSTOPHYCEAE</b>			
7	<i>Chrysoaernella hieroglyphica</i> (Waern) Gayral et Lepailleur	ES/TII	CAEN
9	<i>Nematochryopsis roscoffensis</i> Chadefaud	ES/TII	CAEN
119.79	<i>Phaeothamnion</i> sp. Lagerheim	7	SAG
128.79	<i>Ruttnera spectabilis</i> Geitler	6	SAG
<b>PRYMNESIOPHYCEAE</b>			
17	<i>Chrysotila lamellosa</i> Anaud	ES/TII	CAEN
5.83	<i>Corcontochrysis noctivaga</i> Kalina	7	SAG
944.1	<i>Cricosphaera carterae</i> (Braarud & Fagerland) Braarud	6	SAG
2167	<i>Cricosphaera carterae</i> (Braarud & Fagerland) Braarud	ES	UTEX
50	<i>Platychrysis piennari</i> Gayral et Fresuel	ES/TII	CAEN
45	<i>Prymnesium parvum</i> Carter	ES/TII	CAEN
995	<i>Prymnesium parvum</i> Carter	ES	UTEX
<b>XANTHOPHYCEAE</b>			
805.2	<i>Botrydium cystosum</i> Vischer	1b	SAG
48.84	<i>Chloridella neglecta</i> Pascher et Geitler	1	SAG
B36.84	<i>Chlorobotrys regularis</i> (West) Bohlin	1	SAG
812.1	<i>Chlorocloster engadinensis</i> Vischer	1b	SAG
835.1	<i>Heterococcus brevicellularis</i> Vischer	1b	SAG
848.1	<i>Monodus subterraneus</i> Petersen	1b	SAG
8.83	<i>Monodus unipapilla</i> Reisinger	1	SAG
850.1	<i>Neprodiaella brevis</i> Vischer	1	SAG
11.85	<i>Pseudostaurastrum enorme</i> (Ralfs) Chodat	7	SAG
<b>CHLOROMONADOPHYCEAE</b>			
1195.1	<i>Vacuolaria virescens</i> Cienkowski	7	SAG
<b>EUGLENOPHYCEAE</b>			
1261.5	<i>Phacus pusillus</i> Lemmermann	3b	SAG

Reference	Name of the taxon	Culture medium	Origin
<b>CHLOROPHYCEAE</b>			
211	<i>Chlorella vulgaris</i> Beijerinck	Lc	C. C. A. P.
	<i>Chlorella vulgaris</i> Beijerinck	Lc	C. B. S. C.
16.85	<i>Chlorochytrium lemnae</i> Colm	1	SAG
110.80	<i>Coleochaete scutata</i> Brébisson	1	SAG
12.86	<i>Dictyochloropsis symbiontica</i> Tschermak-Woess var. <i>pauciautostopora</i> Tschermak-Woess	1	SAG
418.1	<i>Draparnaldia plumosa</i> (Vaucher) Agardh	1	SAG
13.86	<i>Dunaliella tertiolecta</i> Butcher	14	SAG
B1821	<i>Fritschiella tuberosa</i> Iyengar	Lcol	UTEX
	<i>Hyalotheca dissiliens</i> (Smith) Brébisson	Wc	U. F. S. Carlos (Brasil)
B515	<i>Hydrodictyon reticulatum</i> (L.) Lagerheim	S2T2	UTEX
462	<i>Klebsormidium subtilissimum</i> (Rabenh.) Silva, Mattox et Blackwell	Lcol	UTEX
575.1a	<i>Oedogonium cardiacum</i> Wittrock	1	SAG
53.80	<i>Scenedesmus subspicatus</i> Chodat	7	SAG
86.81	<i>Scenedesmus subspicatus</i> Chodat	7	SAG
	<i>Spondylosium pandoriforme</i> (Heim.) Teiling	Wc	U. F. S. Carlos (Brasil)
41.85	<i>Tetraselmis striata</i> Butcher	6	SAG

### 3 — AMENDMENTS TO THE PREVIOUS LISTS

#### 3.1. — Corrections to some determinations

- 176 — Instead of *Closterium gracile* Breb. must be *Cl. limneticum* Lemn.
- 199 — Instead of *Staurastrum apiculatum* Bréb. must be *Staurodesmus dejectus* (Bréb.) Teiling var. *apiculatum* (Bréb.) Teiling.
- 178 — Instead of *Scenedesmus arcuatus* Lemm. must be *Sc. arcuatus* Lemn. var. *platydiscus* G. M. Smith.
- 318 — Instead of *Scenedesmus opoliensis* P. Richt. var. *mononensis* Chodat. must be *Sc. maximus* (W. & G. S. West) Chodat.
- 10 — Instead of *Scenedesmus quadrispina* Chodat must be *Sc. armatus* (Chodat) Chodat.
- 179 — Instead of *Scenedesmus spinosus* Chodat. must be *Sc. tenuispina* Chodat.

## 3.2. — Nomenclatural modification (according to Hindák, 1990)

- 118 — *Scenedesmus acutiformis* Schröder is transferred to *Enallax acutiformis* (Schröder) Hindák.  
 207, 260, 317 — *Scenedesmus acutus* Meyen is transferred to *Sc. obliquus* (Turpin) Kützing.  
 259 — *Scenedesmus costatus* Schmidle is transferred to *Enallax costatus* (Schmidle) Pascher.  
 1, 232 — *Scenedesmus oahuensis* (Lemm.) G. S. Smith is transferred to *Sc. Nagellii* Brébisson.  
 235 — *Enallax coelastroides* (Bohl.) Skuja is transferred to *Enallax costatus* (Schmidle) Pascher.

## 3.3. — Recovering of culture extinct Algae

- 489 — *Chaetopeltis orbicularis* (will replace the culture with the number 100).  
 354 — *Cosmarinum subcrenatum* (will replace the culture with the number 116).

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## APONTAMENTOS SOBRE A FLORA GALEGA — XIV

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### RESUMO

São citados 22 taxões de diverso interesse corológico para a Galiza dos quais 5 taxões são citados pela primeira vez para este território: *Cistus* × *cyprius* Lam., *Escallonia macrantha* Hooker & Arnott in Hooker, *Oxalis articulata* Savignay in Lam., *Senecio petasites* (Sims) DC. e *Silene muscipula* L.

### ABSTRACT

22 taxa of varied corologic interest are mentioned here, among them 5 species not previously cited from this territory: *Cistus* × *cyprius* Lam., *Escallonia macrantha* Hooker & Arnott in Hooker, *Oxalis articulata* Savignay in Lam., *Senecio petasites* (Sims) DC. and *Silene muscipula* L.

### *Alopecurus myosuroides* Hudson

(sin. *Alopecurus agrestis* L.)

A CORUNHA: As Júbias, na beira de uma estrada, 7/14/1989, 29TNH5099, leg. Luz Fdez. Prada & Jesús Rei Gómez.

Esta planta foi encontrada por LAÍNZ no herbário de Planelas, procedente de Ferrol, depois de ter passado inadvertida a PAU (1924) quando fez a revisão do mesmo herbário; já fora citada aliás do mesmo lugar por LÓPEZ ALONSO (LAÍNZ, 1965: 326, 1967: 48). Não existe confirmação da referência de LANGE (1861: 25) para a província de Ourense, onde é muito provável



a sua presença pois aparece nos limites da província. ORTIZ & RODRÍGUEZ-OUBIÑA (1987: 99) deram a segunda localidade certa da Galiza, do Porrinho (Ponte-vedra).

Esta é uma nova localidade, relativamente próxima da de Planellas, onde por outra parte não se tornou a encontrar.

### **Amaranthus albus L.**

LUGO: Becerreá, Ponte sobre o rio Cruzul, 15/09/1992, 29TPH 5245. Em comunidades nitrófilas na beira da estrada nacional VI.

Primeira citação provincial para esta planta ruderal originária da América do Norte que se encontra naturalizada na maior parte da Europa. CARRETERO (1987: 439) recolheu esta planta em Carril (Ponte-vedra), GÓMEZ VIGIDE & *al.* (1989: 111) deram-na da Serra de Outes (A Corunha), posteriormente CARRETERO (1990: 565) assinalou-a para toda a Península Ibérica, com excepção das províncias de Lugo, Ourense e Oviedo.

### **Campanula rotundifolia L. s. l.**

A CORUNHA: Paradela, 20/07/1989, 29TNH8659.

Primeira localidade para a província da Corunha e segundo galega já que anteriormente fora encontrada em Trevinca (GUTIÁN & *al.*, 1985: 199).

### **Carex humilis Leyss.**

PONTE-VEDRA: Basadre, 5/06/1988, 29TNH5835. Silheda, Bazar, 26/03/1991, 29TNH5835.

Citada nos ervaçais e landas de natureza calcária da cornija cantábrica (ALLORGE, 1941: 234). LAÍNZ (1967: 47) faz a primeira referência concreta para a Galiza situada em Palas de Rei (Lugo), nos terreiros serpentínicos próximos desta localidade, mas em diferente província. Posteriormente o mesmo LAÍNZ (1968: 36) dá uma segunda citação galega, de Baldaio, no Concelho de Tor-doia (A Corunha). Nova para a província de Ponte-vedra.

**Centaurea corcubionensis** Laínz

A CORUNHA: Póvoa do Caraminhal, monte A Curota, 15/06/1985, 29TNH0009.

Planta descrita originariamente do Pindo, Concelho de Carnota, província da Corunha (LAÍNIZ, 1967: 40); posteriormente o mesmo autor acrescentou várias localidades novas da mesma província corunhesa também: Camarinhas, Malpica e Zás (LAÍNIZ, 1971: 27).

Esta nova localidade está situada mais para o sul, afastada da restrita área donde era conhecido anteriormente este endemismo do subsector Compostelano (sector Galaico-Português).

**Cistus × cyprius** Lam. (*Cistus ladanifer* × *laurifolius*)

OURENSE: Rubiá, Covas, 7/07/1988, 29TPH7705.

Primeira citação galega. BOLAÑOS & GUINEA (1949: 193-199) citam-no do centro e sul da Península Ibérica.

**Deschampsia setacea** (Hudson) Hackel

[sin. *Deschampsia discolor* (Thuill.) Beauv.]

A CORUNHA: Zas, Brandonhas, barragem da Fervença, 28/07/1987, 29TNH0361.

Planta palustre invulgar na Galiza, citada por várias vezes na província de Lugo: LANGE (1861: 38), MERINO (1909: 295) e ALLORGE (1927: 949). Esta é pois a primeira citação para a província da Corunha.

**Dittrichia viscosa** (L.) W. Greuter

[sin. *Inula viscosa* (L.) Aiton]

A CORUNHA: Fene, beiras da estrada das Pias, frente à entrada de ASTANO, 5/10/1988, 29TNJ6713.

OURENSE: Verim, Rasela, 17/11/1991, 29TPG3145.

Citação nova para a província da Corunha e ampliação de área para a província de Ourense. Conhecíamos duas citações da província de Ponte-vedra: uma, do vale do Minho, de Planellas

(MERINO, 1906: 336), confirmada posteriormente por PAU (1924: 44); outra, de GÓMEZ VIGIDE & al. (1989: 108), do Porrinho. GARCÍA MARTÍNEZ & al. (1991: 48) acarretaram a primeira citação para a província de Ourense, perto da barragem de Velhe.

**Escallonia macrantha** Hooker & Arnott in Hooker

A CORUNHA: Val-do-vinho, praia de Vila-Rube, 03/06/1991, 29TNJ7631.

Primeira citação para a Galiza desta espécie alóctona nativa do Chile e em estado silvestre em diversos lugares da Europa Ocidental. Utilizada frequentemente como sebe na comarca das Marinhas, aparece algumas vezes subespontânea, embora uma separação clara dos indivíduos espontâneos e os procedentes de cultivos abandonados não resulte fácil.

**Euphorbia maculata** L.

A CORUNHA: Santiago de Compostela, Campus Universitário, 26/06/1989, 29TNH3847.

Primeira citação provincial desta planta de origem americana que já fora citada por RODRÍGUEZ GRACIA (1986: 33) e GÓMEZ VIGIDE & al. (1989: 109) da província de Ponte-vedra. PINTO DA SILVA & DA SILVA (1956: 24) citam-na como subespontânea em Portugal, desde Barcelos até Monção, Faro e Alfarelos. Posteriormente ORTIZ & RODRÍGUEZ-OUBIÑA (1990: 76) fizeram umas novas referências desta planta também em Portugal, de Coimbra e Parede.

**Euphorbia segetalis** L.

PONTE-VEDRA: Redondela, Estação dos caminhos de ferro, 10/02/1990, 29TNG3281.

Citada por PLANELLAS (1852: 355) para a Galiza, PAU (1924: 68) confirmou posteriormente a sua presença. É citada por MERINO (1906: 534) de vários pontos de Ourense: A Rua, O Bolo, As Ermidas e Cudeiro, e também de Lugo: S. Clódio, Monte-furado e Sequeiros. GUITIÁN & GUITIÁN (1986: 155) assinalaram-na como

pouco frequente nas áreas de Corrubedo (A Corunha). Esta é primeira citação provincial.

**Hainardia cylindrica** (Willd.) W. Greuter

[sin. *Monerma cylindrica* (Willd.) Cosson & Durieu]

A CORUNHA: Ferrol, Doninhos, Outeiro, na beira da estrada, 5/06/1992, 29TNJ5616. Ferrol, Covas, postduna da praia de S. Jurjo, 23/07/1922, 29TNJ5720.

Duas novas citações para a Galiza e primeiras para a província da Corunha desta gramínea assinalada apenas na província de Ponte-vedra segundo o listado de RODRÍGUEZ GRACIA & al. (1989: 81).

**Lactuca viminea** Presl. subsp. *viminea*

PONTE-VEDRA: Merça, 14/07/1992, 29TNH5935.

Medra nos lugares áridos do interior de Lugo e Ourense (MERINO, 1906: 469). PAU (1924: 50) encontrou-a também no herbário de Planellas, de Ourense. Esta é a primeira referência para Ponte-vedra.

**Melilotus officinalis** (L.) Pallas

A CORUNHA: Santiago de Compostela, 7/07/1989, 29TNH3747.

Planta nitrófila, das beiras dos caminhos. LAÍNZ (1968: 8) assinalou esta planta de Bóveda e Cervantes (Lugo) e COSTA TENORICO (1984: 265) citou-a da comarca de Trives (Ourense). Primeira citação provincial.

**Origanum vulgare**

A CORUNHA: Ares, Redes, beira de caminho, 9/09/1989, 29TNJ 6508. Leg. *Nuria Caruncho Conde*.

MERINO (1906: 195) aludiu às referências de PLANELLAS (1852: 338) que indicava a existência desta espécie em Santiago (A Corunha) e na ria de Ponte-vedra; no entanto PAU (1924: 63) não a encontrou na revisão do seu herbário. Mais tarde MERINO

achou esta espécie em Sequeiros, na província de Lugo (LAÍNZ, 1955: 131). A nossa citação é a confirmação da presença desta planta na província da Corunha.

**Oxalis articulata** Savignay in Lam.

A CORUNHA: Manhom, praia do Esteiro, nas areias nitrificadas da postduna, 27/10/1991, 29TPJ0345.

Primeira citação para a Galiza desta planta procedente das zonas tépidas da América do Sul e já conhecida de diversas localidades da Península Ibérica.

**Senecio petasites** (Sims) DC.

A CORUNHA: Ferrol, praia da Granha, 18/08/1991, 29TNJ5914. De origem mexicana, esta é a primeira citação para a Galiza.

**Silene muscipula** L.

(sin. *Silene arvensis* Loscos nom. illeg.)

A CORUNHA: Aranga, A Castelá, 6/06/1987, 29TNH7983.

TALAVERA (1990: 361), não a dá nem para a Galiza nem para o Noroeste da Península, sendo esta a primeira citação galega.

**Solanum laciniatum** Aiton

A CORUNHA: A Corunha, A Zapateira, proximidades do Colégio Universitário, 21/11/1988, 29TNH9980. Ferrol, Ponta Arnela, 29TNJ6215. Ferrol, A Granha, alcantilado na parte posterior da praia artificial, 18/08/1991. 29TNJ6014.

Primeiras citações para a província da Corunha desta solanácea arbustiva de origem australiana e neozelandesa, citada recentemente como novidade galega para a província de Pontevedra (BUJÁN & *al.*, 1989: 571).

**Symphytum tuberosum** L.

A CORUNHA: Ferrol, Serantes, nos prados ensopados da beira do rio da Sardinha, 02/03/1983, 29TNJ6217. Cedeira, Peneiro, na beira do rio das Mestas, 28/03/1988, 29TNJ7931.

OURENSE: A Veiga, Jares, 14/07/1987, 29TPG7079.

As citações conhecidas da Galiza são raras e a maioria, do interior do país. É dada por MERINO (1906: 149) como espécie rara na Galiza e a cita de Galdo (segundo RODRÍGUEZ FRANCO), arredores de Lugo (segundo CASTRO PITA) e BER. CARREIRA ALVAREZ (1955: 523) dá a referida espécie como subespontânea no Vale de Burom. GÓMEZ VIGIDE (1985: 372) citou-a de Rubiá (Ourense). Estas são primeiras citações provinciais para A Corunha.

### **Verbascum sinuatum L.**

A CORUNHA: Ferrol, Esteiro, 02/07/1988, 29TNJ6315. Ferrol, descampados do porto comercial, 02/07/1988, 29TNJ6214.

MERINO (1906: 127) citou apenas dois exemplares da ilha da Toja. Esta é pois a confirmação da existência desta planta na Galiza para além de ser primeira citação provincial.

### **Zantedeschia aethiopica (L.) Sprengel**

A CORUNHA: Ferrol, Doninhos, na ribeira oeste da lagoa, 06/06/1991, 29TNJ5515. Ferrol, A Granha, canteira próxima da estrada a S. Filipe, 13/04/1992, 29TNJ5914. Vale-do-vinho, Meirás, 22/05/1992, 29TNJ6525. Vale-do-vinho, Lago, 22/05/1992, 29TNJ 6870.

Primeiras citações para a província da Corunha desta planta ornamental, procedente de África do Sul que já fora citada anteriormente na Galiza, de Baiona, na província de Ponte-vedra (RODRÍGUEZ GRACIA, 1983: 21).

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\* These results were presented in the Triel 23, 2-5 in June 1993, Helsinki, Finland.



## STUDIES ON THE $\beta$ -GLUCOSIDASE FROM *DICHOMITUS SQUALENS* (KARST.) REID INDUCED BY CARBOXYMETHYLCELLULOSE

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### SUMMARY

The strain of *Dichomitus squalens* being studied was cultured in a liquid medium having as a sole carbon source 1% soluble cellulose-carboxymethylcellulose (CMC). Some kinetics parameters of the  $\beta$ -D-glucosidase component from *D. squalens* have been determined.

The Michaelis constant (km) for ONPG (o-nitrophenyl  $\beta$ -D-glucopyranoside) is 1.41 mM, the  $V_{max}$  is 9 nmoles  $min^{-1}$ , at pH 4.0 and 50° C.

The  $\beta$ -glucosidase exhibits a rapid thermal inactivation at 70° C although it exhibited 100% activity at 50° C, that is more or less constant until 64° C, temperature in which the residual activity was still 80%.

The profiles of pH effect on the activity and on the stability enzyme are similar which show the denaturation of enzymes on values lower than 2.76 and higher than 6.35. D-glucose is shown to be a non competitive inhibitor ( $K_i = 3.35$  mM) when the ONPG is the substrate.

### INTRODUCTION

THE breakdown of native cellulose to soluble sugars is a process which involves the action of a multi-enzymic system, one component of this system is the  $\beta$ -glucosidase which catalysis the hydrolysis of cellobiose on D-glucose.

In the present work some properties of the  $\beta$ -D-glucosidase from *D. squalens* were investigated using 1% of carboxymethyl-

\* These results were presented in the Tricel 93, 2-5 th June 1993, Helsinki, Finland.

cellulose (one artificial soluble cellulose) as the sole carbon source persuing other work (RESENDE *et al.*, 1992) where we also reported some kinetic parameters of one  $\beta$ -glucosidase from *D. squalens* induced by 1% of Whatmann cellulose.

## MATERIAL AND METHODS

### Material

The mycelium of a white-rot *D. squalens* (Karst.) Reid strain was isolated from a fruity-body collected from *Pinus* sp. and has been subcultured on potato dextrose agar.

Erlenmeyers of 250 ml containing 100 ml of a basal liquid medium, NORKRANS & HAMMARSTRÖM (1963) enriched with biotin 5  $\mu\text{g l}^{-1}$  and thiamine 100  $\mu\text{g l}^{-1}$  and supplemented with carboxymethylcellulose as the sole carbon source, were inoculated with mycelium discs which grew without agitation and were incubated at 28° C.

### Methods

#### a) Extracellular extracts preparation

The culture with 14 days of incubation were centrifuged and the extracellular extracts were added by sodium azide (in a final concentration of 0.01%) to prevent contamination and maintained at 4° C until be assayed.

#### b) Enzyme assays

$\beta$ -D-glucosidase activity was determined by incubating at 50° C, for 30 min, a reaction mixture containing 1 ml 0.2M of sodium acetate buffer, pH 4.0, 1 ml 5mM of ortho-nitro-phenyl- $\beta$ -D-glucopyranoside, 1 ml of enzyme solution (a suitable diluted extracellular extract) and 1 ml of water. The reaction was stopped by the addition of 4 ml 0.4M glycine-NaOH solution, pH 10.8 (Wood, 1968). The absorbance of the mixture resulting was measured at 430 nm, and the appropriate enzyme and substrate blanks were included.

One unit of activity (I. U.) is defined as the amount of enzyme, which under the reaction conditions, catalyses the release of 1  $\mu\text{mole}$  of ortho-nitrophenol per min (PARR, 1983).

c) *Kinetic properties*

The kinetic properties of a  $\beta$ -D-glucosidase were investigated, using the assay methods of LINEAWEAVER & BURK, 1934 and of DIXON & WEBB, 1964, but with different concentrations (ONPG) or in the presence of one inhibitor (various glucose concentrations).

d) *Temperature stability.*

Thermal deactivation experiments were carried out by pipetting 1 ml quantities of extracellular extracts (enzyme solution) into test tubes preheated to the incubating temperatures (between 50° C and 75° C) in a water bath. At noted times samples were removed and diluted directly into cold buffer (0.2M sodium acetate) and the diluted samples assayed for  $\beta$ -D-glucosidase activity as described in assay methods.

e) *pH activity*

pH activity experiments were carried out by pipetting 1 ml quantities of 0.2M sodium acetate buffer solutions of different pH values between 2.76 and 9.76; 1 ml of 5 mM ONPG, 1 ml of the extracellular extract (enzyme solution) and 1 ml of water.  $\beta$ -D-glucosidase activity was determined as described above in enzyme assays.

f) *pH stability*

A reaction mixture containing 1 ml 0.2M sodium acetate in different pH from 2.76 up to 9.76 and 1 ml of the enzyme solution was incubated at room temperature during 18 hours. Then we applied to all test tubes the same method previously described to determine the  $\beta$ -D-glucosidase activity.

## RESULTS AND DISCUSSION

The results presented here let us to consider that the  $\beta$ -glucosidase induced whether by 1% Whatmann cellulose (crystalline cellulose) or by 1% of carboxymethylcellulose (soluble and substituted cellulose) have identical kinetic characteristics (when we compare these results — Figs. 1, 2, 3 and 4 with the results obtained and referred in RESENDE *et al.*, 1992).

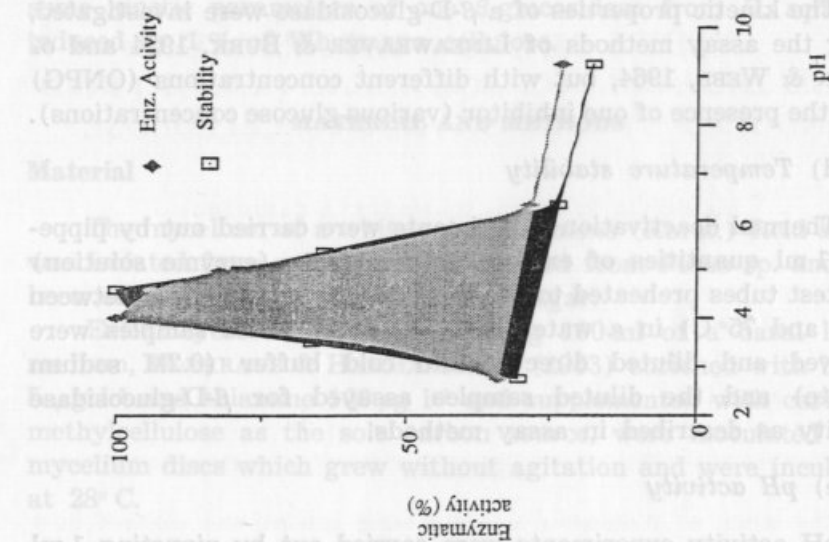


Fig. 2. — Effect of pH on *D. squalens*  $\beta$ -glucosidase activity and on the stability. Relative activity (%).

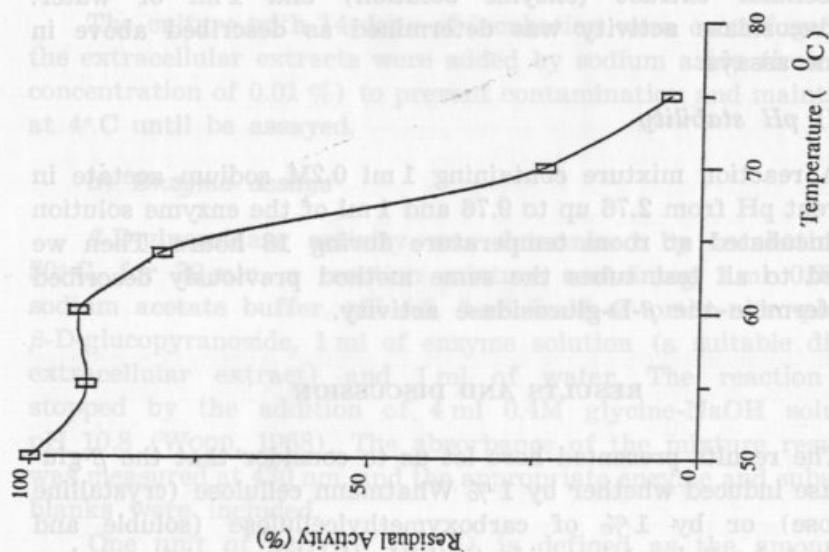


Fig. 1. — Thermal stability of *D. squalens*.

We verified that at 50° C the enzyme presents 100 % activity, a value that remains more or less constant only until 64° C when there is 80 % residual activity, as can be seen in Fig. 1, that shows the thermal stability of *Dichomitus squalens*  $\beta$ -D-glucosi-

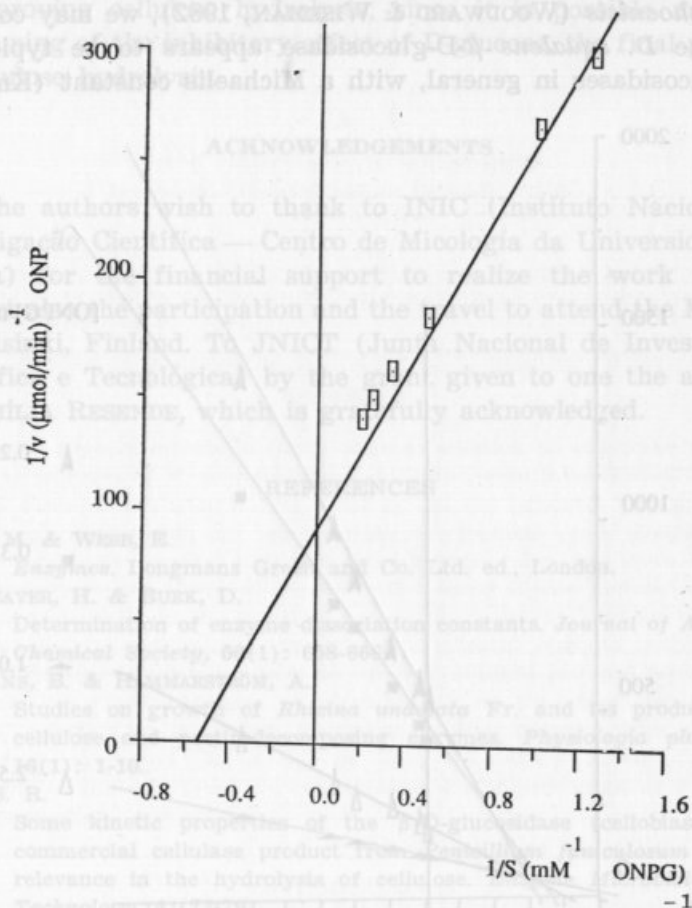


Fig. 3. — LINEWEAVER-BURK plot:  $V_{\text{max}} = 9$  nmoles min<sup>-1</sup>  
 $K_m = 1.41$  mM.

dase. However at 70° C it presents a rapid thermal inactivation (the value is only 23 %).

The profiles of the pH effect on the activity and on the stability (Fig. 2) of the  $\beta$ -glucosidase, were similar, and they seemed to indicate that the enzyme suffered denaturation in the

pH under 2.76 and over 6.35. The best results occurred in pH between 4.0 and 5.0 with 100% relative activity.

Once the Michaelis constant for ONPG for the  $\beta$ -D-glucosidase shows wide variations, from 0.05 mM for *Saccharomyces cerevisiae* (WOODWARD & WISEMAN, 1982) up to 44 mM for *Aspergillus phoenicis* (WOODWARD & WISEMAN, 1982), we may consider that the *D. squalens*  $\beta$ -D-glucosidase appears to be typical of  $\beta$ -D-glucosidases in general, with a Michaelis constant ( $K_m$ ) for

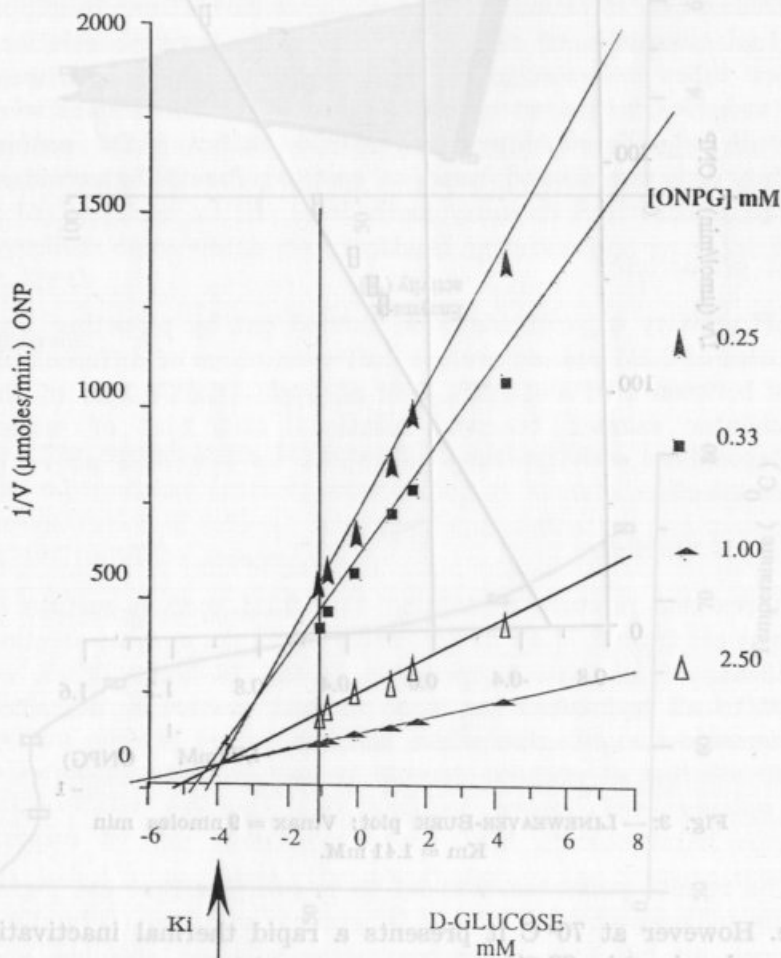


Fig. 4.— Constant of inhibition (DIXON & WEBB method)  
 $K_i = 3.35$  mM.



CNPG of 1.41 mM and the  $V_{max}$  value of 9 nmoles  $\text{min}^{-1}$ , at pH 4.0 and 50°C (Fig. 3).

When the substrate is ONPG, D-glucose is shown to be a non competitive inhibitor,  $K_i = 3.35$  mM (maybe a mixt competition — Fig. 4). This may be a significant advantage in terms of improving cellulose hydrolysis, since it is possible means a overcoming of the inhibitory effect of D-glucose, the final product of cellulose hydrolysis.

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6.4% of 1.41 mM and the  $V_{max}$  values of 8 units/min at pH 4.0 and 50°C (Fig. 3). The  $K_m$  values were 0.0011 and 0.0012 M, respectively. When the substrate concentration was shown to be a non-competitive inhibitor,  $K_i$  was 0.0035 M (Fig. 4). This may be a significant advantage of using cellulose hydrolysis as a substrate since it is possible to measure the inhibitory effect of  $D$ -glucose, the final product of cellulose hydrolysis, with a change in  $V_{max}$  in a double-reciprocal plot.

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## MUTAGENIC EFFECTS OF COLCHICINE ON *VIGNA UNGUICULATA* (L.) WALPERS (PAPILIONACEAE) IN NIGERIA

by

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### ABSTRACT

The mutagenic effects of 0.2% aqueous solution of colchicine on morphological features of *V. unguiculata* (L.) Walp., were investigated. Soaking sprouted seeds in the solution for 3 hours did not produce tetraploid cells but induced suppression of lateral root development and elongation of shoot-root axis. The treated materials later became taller and more vigorous than the controls and produced longer pods bearing higher number of seeds. Differences observed in the mean values of plant height, number of leaves per plant, length and breadth dimensions of abaxial stomata, pollen grain diameter, number of pods as well as number of seeds per pod were highly significant ( $P < 0.05$ ). Manner of pigmentation on the stem and perianth was not affected by treatment. Exposure to the mutagen was lethal to some of the cultivars. However, the need to further investigate these effects and to find utility of the treatment in the improvement of *V. unguiculata* production is suggested.

### INTRODUCTION

Cultivated *Vigna unguiculata* (L.) Walp., called beans in Nigeria, is a widely distributed annual herb within tropical and subtropical regions (COBLEY, 1962). As a cheap source of protein, its importance in the dietary needs of humans and livestock on a global scale (LELEJI, 1975; SINGH & RACHIE, 1985; UGBOROGHO & AGOMO, 1989b, UGBOROGHO & OBUTE, 1990, 1993) has been acknowledged. Consequently, considerable measures of

human and financial resources have been channelled towards improving desirable qualities of the plant aimed at increased yield and utilization. However, genetic variability available to researchers derives, in the main, from extant spontaneous mutations that are conserved by natural selection.

The process of natural selection can only act on ready made mutations. Since spontaneous mutations are both slow and random processes (STEBBINS, 1950; SWANSON, 1957; ALLARD, 1960; SCHULZ-SCHAEFFER, 1985) the need of wider genetic variability required for better improvement would not be satisfied by spontaneous mutations alone. Mutans of *V. unguiculata* have been produced with ionising radiations (APPA RAO & JANA, 1976; GHOSH *et al.*, 1979). The deployment of chemical mutagens to induce mutations has not been adequately attempted for *V. unguiculata*.

Colchicine which is a highly potent C-mitotic agent for several plants does not appear to effect changes in the chromosome complement of *V. unguiculata*. Several genic mutations, however, have been reported in several plants through the action of colchicine. FRANZKE & ROSS (1952) reported colchicine induced mutations that affected agronomic characters in sorghum. Similarly, this mutagen has induced formation of chimeral sectors and different floral colour variations on the same branch of flax, soybean, corn and wheat (DIRK *et al.*, 1956). According to reports of UGBOROGHO & SODIPO (1985) features such as retarded seedling emergence, enhanced mitotic indices, chromosomal aberrations in addition to polyploidisation have been effected in tomato by colchicine treatment.

It is against this background that we undertook to investigate the effects of colchicine treatment on the life history and genetic make up of *V. unguiculata* cultivars. We hope that desired effects, if produced and sustained would be an advantage to be utilized in crop improvement.

#### MATERIALS AND METHODS

Dry seeds of *V. unguiculata* (L.) bought from various markets in Nigeria were sorted into homogenous groups based on the criteria published elsewhere (UGBOROGHO & AGOMO, 1989a, b; UGBOROGHO & OBUTE, 1990, 1993) and referred to as cultivars. The cultivar groups were designated with letters of the English

alphabet A.....T. Cultivars A, B, C, were chosen for this investigation because of their white seed coat and different types of hilum ring colour while cultivars H, J and P were chosen for their different coat colours.

### **Treatment with colchicine**

20 seeds randomly picked from each cultivar group were soaked in Petri dishes containing tap water to trigger sprouting. After a period of 12 hours, 10 of the sprouted seeds from each group were selected and transferred to 0.2% aqueous solution of colchicine from 1400-1700 hours GMT in an air conditioned room. The rest seeds served as control samples; while the treated samples were washed and placed in Petri dishes containing moistened absorbent paper to grow. Both control and treated materials were transplanted into soil in black polyethylene bags and after about 3 weeks removed to soil in the biological garden of University of Lagos. These were monitored till they reached maturity, when all the measurements and observations were made.

### **Epidermal strips**

Rectangular cuttings were obtained from the equatorial regions of mature terminal leaflets. These were either mechanically stripped to get the abaxial epidermal strip; or treated with concentrated nitric acid until air bubbles appeared indicating separation of the epidermes. Forceps and mounted needles were used to remove the strips which were placed with the near-mesophyll surface on a drop of 50% glycerine on a glass slide. Such preparations were left to stand overnight at room temperature to ensure full turgidity. The number of epidermal cells and stomata were scored at  $\times 400$  field view on a Wild Heerbrugg phase contrast microscope. Stomatal indices were computed with the technique of STACE (1965) while a  $\times 10$  eye piece fitted with micrometer was used to measure the stomatal lengths and breadths.

### **Vegetative features**

Height of plants and number of leaves per plant, were measured with a metre rule; while growth habit and pattern of pigment deposition on the stems were visually scored.

### Reproductive features

Perianth colour was visually assessed to ascertain variation in the control and treated materials.

### Pollen grains

Pollen was teased out from mature anthers and stained with 1:1 acetocarmine-glycerine and the preparation was covered with coverslip and kept overnight at 60° C in an oven. Stained pollen grains were enumerated at  $\times 400$  magnification and the percentage of this to the total number of pollen grains counted represented the percentage stainability. Pollen diameter was also measured with an eye piece graticule for the triporate and monoporate types of pollen grains.

### Cytological studies

Root tips were obtained at 1400 hours GMT from plants growing on moistened sawdust in Petri dishes. These were pre-treated with saturated aqueous solution of paradichlorobenzene for 1.5 hours at room temperature. The root tips were fixed in 1:3 acetic acid alcohol solution for 12-24 hours and hydrolysed in 1N hydrochloric acid for 15 minutes at 60° C in an oven. Squash preparations were made by staining the meristematic region of the root tips with 2% acetocarmine on a slide and covering it with coverslip. By gently tapping with the wooden end of a mounted needle the cells were separated. Chromosome counts were obtained from plates with the best separated chromosomes at  $\times 400$  magnification.

### RESULTS

Treatment with 0.2% aqueous colchicine solution had a pronounced growth retarding effect on seedlings of all the cultivars. The effect was so much drastic for treated seedlings of cultivars B, C and P that growth was not sustained in these after a few days. Retardation in growth led to development of tap roots that were thicker and more bulbous at the tips in treated cultivars (Fig. 1). Evidently lateral root development was suppressed and this led to stouter and shorter lateral roots in the treated cultivars (Fig. 1).

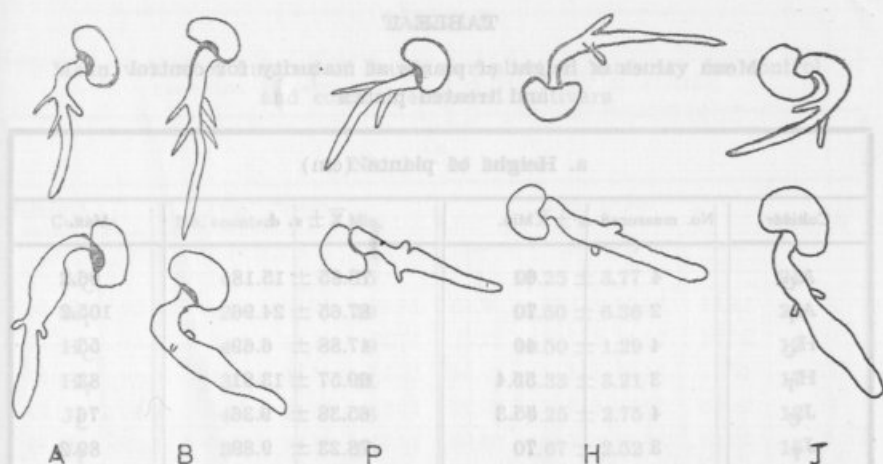


Fig. 1. — Normal seedlings of control and enlarged seedlings of colchicine treated material after two days sprouting  $\times 5$ .

Also suppressed by colchicine treatment was shoot development as evident in Fig. 2. However, after transplanting to soil in the garden the treated plants of cultivars A, H and J took to more vigorous growth and produced more luxuriant foliage than the controls.

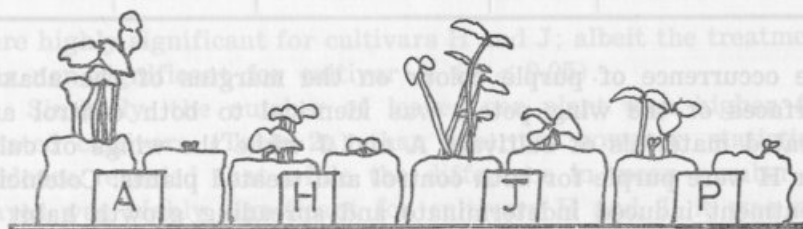


Fig. 2. — Shoot suppression by colchicine treatment. The alternate polyethylene bags contain treated cultivars.

### Qualitative morphological characters

Treatment with colchicine produced no noticeable effect on the pattern of pigmentation of the stem and perianth. Bases of lateral leaflets, bases of branches and those of petioles were pigmented in both the control and treated materials. Similarly,

TABLE 1

Mean values of height of plants at maturity for control and treated plants

a. Height of plants (cm)				
Cultivar	No. measured	Min.	$\bar{X} \pm s. d.$	Max.
A <sub>C</sub>	4	60	78.85 $\pm$ 15.18	96.2
A <sub>T</sub>	2	70	87.65 $\pm$ 24.96	105.2
H <sub>C</sub>	4	40	47.88 $\pm$ 6.69	55
H <sub>T</sub>	3	55.4	69.57 $\pm$ 13.81	83
J <sub>C</sub>	4	55.3	65.38 $\pm$ 9.36	76
J <sub>T</sub>	3	70	78.23 $\pm$ 9.89	89.2

\* The subscript C refers to control; while T refers to colchicine-treated.

b. Summary table of comparison of means between control and colchicine-treated plants using the t-test ( $\alpha = 0.05$ )

Cultivars	d. f.	Tabulated t-value	Cal t-value	Comment
A <sub>C</sub> vs. A <sub>T</sub>	4	2.132	2.11	n. s.
H <sub>C</sub> vs. H <sub>T</sub>	5	2.015	8.01	**
J <sub>C</sub> vs. J <sub>T</sub>	4	2.132	5.08	**

the occurrence of purple colour on the margins of the abaxial surfaces of the wing petals was identical to both control and treated materials of cultivars A and J while the wings of cultivar H were purple for both control and treated plants. Colchicine treatment induced indeterminate and spreading growth habit in cultivar A while the determinate and erect habit observed in cultivars H and J were not affected by the treatment.

### Quantitative morphological characters

Despite the initial retardation of growth, colchicine treatment had stimulatory influences on later stages of growth in all the cultivars. As shown in Table 2a, the treated cultivars altogether were taller than the control materials at maturity. Moreso, statistical evidence (Table 2b) revealed that the treatment effects



TABLE 2

Mean values of number of leaves per plant at maturity for control and colchicine-treated cultivars

a. Number of leaves				
Cultivar	No. counted	Min.	$\bar{X} \pm s. d.$	Max.
A <sub>C</sub>	4	15	19.25 $\pm$ 3.77	24
A <sub>T</sub>	2	19	23.50 $\pm$ 6.36	28
H <sub>C</sub>	4	9	10.50 $\pm$ 1.29	12
H <sub>T</sub>	3	11	13.33 $\pm$ 3.21	17
J <sub>C</sub>	4	6	9.25 $\pm$ 2.75	12
J <sub>T</sub>	3	10	12.67 $\pm$ 2.52	15

b. Summary table of comparison of means between control and treated cultivars				
Cultivars	d. f.	Tab. t-value	Cal. t-value	Comment
A <sub>C</sub> vs. A <sub>T</sub>	4	2.132	2.09	n. s
H <sub>C</sub> vs. H <sub>T</sub>	5	2.015	2.39	**
J <sub>C</sub> vs. J <sub>T</sub>	5	2.015	2.70	**

were highly significant for cultivars H and J; albeit the treatment was non significant for cultivar A ( $P < 0.05$ ).

Similarly, the number of leaves per plant was higher for treated cultivars (Table 2a) than controls; however, statistical evidence revealed that while the difference in mean number of leaves was highly significant for cultivars H and J, it was not significant for cultivar A (Table 2b;  $P < 0.05$ ).

#### Stomata on terminal leaflets

Colchicine treatment led to reduction in both the number of epidermal cells and stomata per  $\times 400$  field view. This was further corroborated by the stomatal indices computed for the control and treated cultivars (Table 3a). Furthermore, stomatal length and breadth were longer and wider in treated materials than in controls. The exception, though, was in the breadth of treated

TABLE 3

Abaxial stomatal dimensions (length and breadth) and indices of terminal leaflets of colchicine-treated and control cultivars

a.							
Cultivar	Length ( $\mu\text{m}$ )			Breadth ( $\mu\text{m}$ )			Stomatal indices %
	Min.	$\bar{X} \pm \text{s. d.}$	Max.	Min.	$\bar{X} \pm \text{s. d.}$	Max.	
A <sub>C</sub>	18.13	26.02 $\pm$ 1.71	29.40	13.46	17.13 $\pm$ 2.01	24.52	22.34
A <sub>T</sub>	26.04	23.11 $\pm$ 2.83	32.31	17.28	21.54 $\pm$ 2.65	28.60	15.47
H <sub>C</sub>	19.11	24.25 $\pm$ 3.15	32.34	16.17	17.99 $\pm$ 1.79	20.58	24.69
H <sub>T</sub>	26.00	30.56 $\pm$ 2.11	35.28	19.11	20.63 $\pm$ 2.16	29.40	19.53
J <sub>C</sub>	20.58	24.99 $\pm$ 3.15	29.40	14.70	18.54 $\pm$ 2.43	23.52	23.38
J <sub>T</sub>	26.46	30.02 $\pm$ 2.65	32.28	16.17	18.49 $\pm$ 1.54	20.58	16.67

b. Summary table of comparison of means of stomatal dimensions for colchicine-treated and control materials

	Cultivar	d. f.	Tabulated t-value	Calculated t-value	Comment
Length of stomata	A <sub>C</sub> vs. A <sub>T</sub>	74	1.980	6.15	**
	H <sub>C</sub> vs. H <sub>T</sub>	74	1.980	17.05	**
	J <sub>C</sub> vs. J <sub>T</sub>	74	1.980	12.89	**
Breadth of stomata	A <sub>C</sub> vs. A <sub>T</sub>	74	1.980	12.60	**
	H <sub>C</sub> vs. H <sub>T</sub>	74	1.980	8.0	**
	J <sub>C</sub> vs. J <sub>T</sub>	74	1.980	0.16	n. s.

cultivar J which showed almost the same dimension as the control. Statistical evidence showed that the observed differences in lengths and breadths of stomata for control and treated materials were highly significant ( $P < 0.05$ ) for all the cultivars except for the breadth dimensions of cultivar J which was not significant (Table 3b).

#### Pollen grains

Pollen from both control and treated materials were highly fertile with stainability figures greater than 95%. Two types of pollen grains were observed in both control and treated

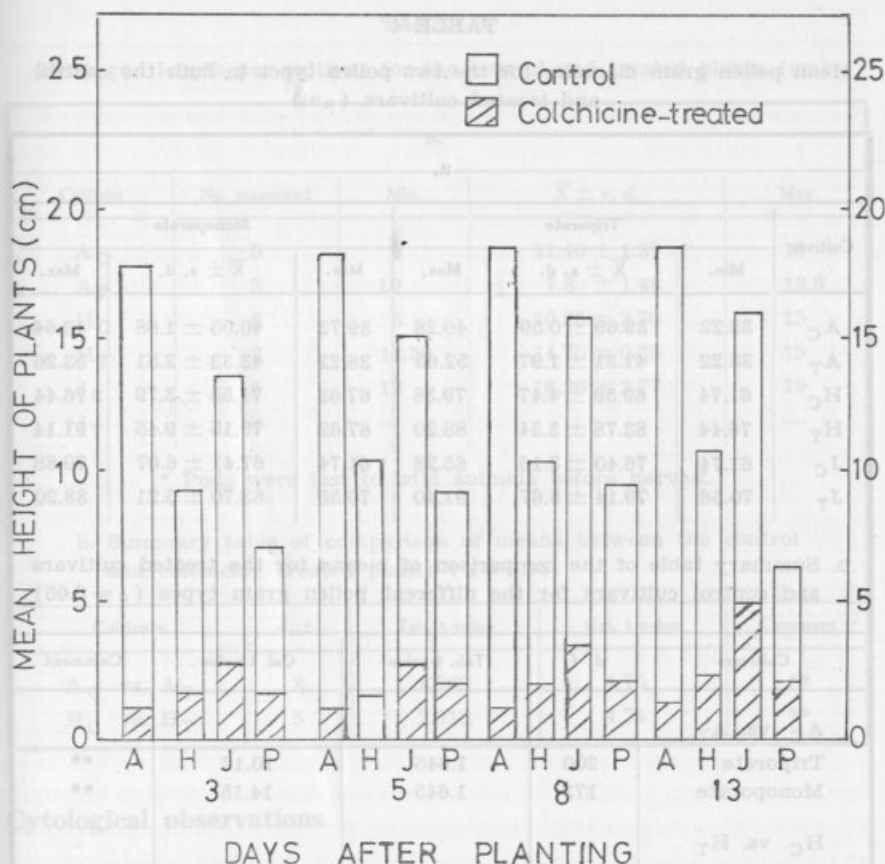


Fig. 3.—Histogram showing the heights of control and colchicine treated materials 13 days after planting.

materials; and the reticulate pattern of sculpturing was not noticeably affected by treatment with colchicine. However, the diameters of the two types of pollen grains were dissimilar (Table 4a). The variation in the mean diameter of monoporate and triporate pollen grains was highly significant ( $P < 0.05$ ; Table 4b) for all the cultivars.

#### Pod length and number of seeds per pod

The length of pods was observed to increase under the influence of colchicine as depicted in Table 5a. Such increase reflected on the higher values of the mean pod lengths for

TABLE 4

Mean pollen grain diameter for the two pollen types in both the control and treated cultivars ( $\mu\text{m}$ )

a.						
Cultivar	Triporate			Monoporate		
	Min.	$\bar{X} \pm \text{s. d.}$	Max.	Min.	$\bar{X} \pm \text{s. d.}$	Max.
A <sub>C</sub>	38.22	39.69 $\pm$ 0.59	40.28	39.72	40.05 $\pm$ 1.88	43.64
A <sub>T</sub>	38.22	41.31 $\pm$ 1.97	52.63	38.22	43.33 $\pm$ 2.51	53.26
H <sub>C</sub>	61.74	69.59 $\pm$ 4.47	79.38	67.62	71.55 $\pm$ 3.79	76.44
H <sub>T</sub>	76.44	83.78 $\pm$ 3.34	88.20	67.62	79.15 $\pm$ 9.45	91.14
J <sub>C</sub>	61.74	76.40 $\pm$ 3.16	85.28	61.74	67.41 $\pm$ 6.07	79.88
J <sub>T</sub>	70.56	79.14 $\pm$ 5.67	91.40	70.56	83.70 $\pm$ 5.21	88.20

b. Summary table of the comparison of means for the treated cultivars and control cultivars for the different pollen grain types ( $\alpha = 0.05$ )

Cultivars	d. f.	Tab. t-value	Cal. t-value	Comment
A <sub>C</sub> vs. A <sub>T</sub>				
Triporate	200	1.645	10.13	**
Monoporate	173	1.645	14.15	**
H <sub>C</sub> vs. H <sub>T</sub>				
Triporate	208	1.645	54.58	**
Monoporate	216	1.645	19.49	**
J <sub>C</sub> vs. J <sub>T</sub>				
Triporate	215	1.645	9.45	**
Monoporate	196	1.645	46.2	**

treated cultivars. Test for difference in means showed that such differences were highly significant ( $P < 0.05$ ; Table 5b).

The longer pods of the treated materials expectedly bore more seeds than the controls for cultivars A and H. The only pod on the treated material of cultivar J was lost to wild animals before it matured. The mean values of seeds per pod as well as the statistical evidence confirming treatment effects are depicted on Table 5a, b.

TABLE 5

Mean pod length per cultivar for the control and treated plants (cm)

a.				
Cultivar	No. measured	Min.	$\bar{X} \pm s. d.$	Max.
A <sub>C</sub>	5	6*	11.40 $\pm$ 1.37	10
A <sub>T</sub>	5	10	7.8 $\pm$ 1.48	13.5
H <sub>C</sub>	5	6	10.20 $\pm$ 3.70	15
H <sub>T</sub>	2	14.50	14.75 $\pm$ 0.35	15
J <sub>C</sub>	5	12	16.20 $\pm$ 2.77	19
*J <sub>T</sub>	—	—	—	—

\* Pods were lost to wild animals before harvest.

b. Summary table of comparison of means between the control and colchicine treated plants ( $\alpha = 0.05$ )

Cultivars	d. f.	Tab. t-value	Cal. t-value	Comment
A <sub>C</sub> vs. A <sub>T</sub>	8	1.860	4.75	**
H <sub>C</sub> vs. H <sub>T</sub>	5	2.015	4.74	**

### Cytological observations

Chromosome doubling was not effected with colchicine treatment contrary to expectation. Somatic chromosome counts of  $2n = 22$  for both treated cultivar H and the control while  $2n = 20$  was recorded for the control and treated material of cultivar A. Gross chromosome morphology was also not affected by colchicine treatment.

### DISCUSSION

In their paper, UGBOROGHO & SODIPO (1985) observed that the growth rate of *Lycopersicon esculentum* Miller was retarded by colchicine treatment. Although our observation in the present work is in line with this, it is equally at variance with these authors' findings in that the treated materials later overcame

TABLE 6

Mean number of seeds per pod for colchicine treated and control material

a.				
Cultivar	No. measured	Min.	$\bar{X} \pm s. d.$	Max.
A <sub>C</sub>	5	4	6.6 ± 2.4	10
A <sub>T</sub>	5	9	10.4 ± 1.14	12
H <sub>C</sub>	5	7	9.6 ± 3.97	15
H <sub>T</sub>	2	13	15.0 ± 3.34	14
J <sub>C</sub>	5	10	13.5 ± 0.71	19

b. Summary table of comparison of means between the control and colchicine treated material

Cultivars	d. f.	Tab. t-value	Cal. t-value	Comment
A <sub>C</sub> vs. A <sub>T</sub>	8	1.860	4.52	**
H <sub>C</sub> vs. H <sub>T</sub>	5	2.015	3.64	**

the induced suppression and even grew taller than the controls at maturity. A plausible explanation is that the «physiological shock» due to colchicine treatment may not have been strong enough to have sustained the initial effects. Contrary to this was the observation that cultivars B, C and P could not withstand colchicine stress suggesting that different physiological tolerance levels exist among these cultivars. However, both cases are in total agreement with the operations of natural selection whereby the non adapted mutants are selected against while adapted ones are selected for perpetuation. The reason adduced by UGBOROGHO & SODIPO (1985) for the death of seedlings was clumping of chromosomes in the meristems of *L. esculentum*. The same phenomenon most likely, operated in these *V. unguiculata* cultivars. It seems then that apart from the classical antimutagenic effect of colchicine, one may find both inhibitory and stimulatory effects of this mutagen on the growth and development of different strains of *V. unguiculata*.

The observed reduced stomatal indices in treated materials is an extension of larger and fewer stomata observed per field view. Such features are usual in autopolyploid cases according to SCHULZ-SCHAEFFER (1985). In fact, the works of other authors (ALLARD, 1960; STEBBINS, 1950; DAVIS & HEYWOOD, 1967; SWANSON, 1967; UGBOROGHO & SODIPO, 1985) are in agreement with our results. However, the breadth dimension of stomata for the treated material of cultivar J did not differ from that of the control. Environmental condition alone cannot be used to adequately explain this discrepancy considering the fact that treatment with colchicine increased the length of the stomata in the same material. However, it could be explained by low expressivity in mutant genes responsible for concurrent increase in length and breadth. Apparently, these genes have been modified to express increase in stomatal length alone for cultivar J.

Our observation of no phenotypic change in pigment distribution on treated plants is at variance with those of DIRK *et al.* (1956) on flax, soybean, corn and wheat. These authors obtained different colours of floral parts and fruits on the same branch, under the influence of colchicine. It might be that the *V. unguiculata* physiology is not amenable to colchicine effects in this regard. However, as earlier intimated, changes in genes responsible for pigment deposition may not have been enough to effect any phenotypic variation. HERSKOWITZ (1962) indicated that characters which appear phenotypically are penetrant although there could be various levels of expressivity. Our suggestion here is that whatever effect colchicine had on the genes that code for length and breadth of stomata, increase in stomatal breadth in cultivar J, had low expressivity.

The taller plants with higher number of leaves and more seeds per pod of the treated plants are trends in line with colchicine effects (ALLARD, 1960; SWANSON, 1957). High pollen stainability in both the control and treated material is a reflection of regular meiosis in both. The treatment further increased pollen diameter in both monoporate and triporate types. According to ALLARD (1960); UGBOROGHO & SODIPO (1985) colchicine treatment leads to increased pollen diameter as our results bear out.

Contrary to expectations the somatic number of chromosomes was not doubled by treatment with colchicine. However, other workers have obtained similar results (FRANZKE & ROSS, 1952;

DIRK *et al.*, 1956) with other plants. It follows, therefore, that despite the high potency of colchicine as a C-mitotic agent, its effects in inducing polyploidy may not be universal among angiosperm families. We are suggesting here that techniques other than the traditional treatment with colchicine should be developed for polyploidisation of *V. unguiculata*. Until this is achieved we may not have the wider genetic variability spectrum provided by polyploid and aneuploid levels for better crop improvement. However, the enhanced morphological characters induced by colchicine as noted in the present work need to be further investigated for its utility in *V. unguiculata* production.

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found in the order *B. nigra* > *B. pubescens* > *B. arvensis*. The pattern is not present in *B. alba*.

The morphological characters of seed coat confirmed the systematic interpretation of FIORI & PAOLETTI (1896-1898) and represent a useful diagnostic feature in mustard seed adulteration.

Key words: Seed, *Sisymbrium*, *Brassica*, officinalis.

#### RIASSUNTO

Gli A.A. hanno esaminato con lo stereomicroscopio ed il microscopio elettronico a scansione i semi di *Sisymbrium alba* ssp. *alba*, *B. arvensis*, *B. pubescens* e *Brassica nigra*.

Tre specie hanno evidenziato un modello tegumentale reticolato più pronunciato nella direzione *B. nigra* > *B. pubescens* > *B. arvensis*. Il modello non è presente in *B. alba*.

I caratteri morfologici del tegumento hanno confermato l'interpretazione sistematica di FIORI & PAOLETTI (1896-1898) e rappresentano utili elementi di diagnosi nella sofisticazione dei semi delle senapi.

Parole chiave: Seme, *Sisymbrium*, *Brassica*, officinalis.

\* Study subsidized by M. U. R. S. T. (60%)



## DIAGNOSTIC VALUE OF SEED COAT IN ITALIAN TAXA OF THE GENUS *SINAPIS* AND *BRASSICA NIGRA*\*

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### ABSTRACT

The Authors examined with the stereomicroscope and scanning electron microscope the seeds of *Sinapis alba* ssp. *alba*, *S. arvensis*, *S. pubescens* and *Brassica nigra*.

Three species exhibited a reticulate seed coat which is more pronounced in the order *B. nigra* > *S. pubescens* > *S. arvensis*. The pattern is not present in *S. alba*.

The morphological characters of seed coat confirmed the systematic interpretation of FIORI & PAOLETTI (1896-1898) and represent a useful diagnostic feature in mustard seed adulteration.

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Parole chiave: Seme, *Sinapis*, *Brassica*, officinale.

\* Study subsidized by M. U. R. S. T. (60 %).

## INTRODUCTION

THE officinal use of seeds of *S. alba* ssp. *alba*, *S. arvensis* and *B. nigra* was known at the time of Theophrastus (IV century BC) and has been the subject of numerous works (ANZALONE, 1989; BELJERINK, 1947; BIAGI & SPERONI, 1988; DE CAPITE, 1984; GASTALDO, 1987; GARNIER, 1961; LECLERC, 1966; MAUGINI, 1973; TOMASELLI, 1974; TONZIG, 1941). There is no evidence suggesting the officinal use of *S. pubescens* seeds.

The seeds of *S. alba*, *S. pubescens* and *B. nigra* are widely used not only in the food industry but also for medicinal purposes due to their antiscorbutic, antineuralgic and diuretic properties. Adulteration between the three species is frequent, and since the effects differ, botanical identification of these seeds is essential.

SCHULZ (1936) stated that the systematic placement of *Cruciferae* is highly complex and that the earliest classifications appear unrealistic in that they were based on few, often macroscopic characters while other features of considerable taxonomic value were neglected. CAUDA (1914), MUSIL (1948), BERGGREN (1960), VAUGHAN (1970), VAUGHAN & WHITEHOUSE (1971), CORNER (1976), MULLIGAN & BAILEY (1976), MATARESE PALMIERI (1990-1991) and BROCHMANN (1992) successfully use variations in seed coat to identify and classify taxa belonging to the *Brassica* tribe.

DE CANDOLLE (1824) attributed the four taxa examined in this study to the genus *Sinapis*. FIORI & PAOLETTI (1896-1898) placed *S. alba* in the genus *Sinapis* and *B. pubescens*, *B. nigra*, *B. sinapistrum* (= *S. arvensis*) in the genus *Brassica*, while SCHULZ (1959) attributed *S. arvensis*, *S. pubescens* and *S. alba* to the genus *Sinapis* and *B. nigra* to the genus *Brassica* (Table I).

The aim of this study was the characterization of the seeds of the four taxa examined in order to provide a useful diagnostic means in mustard seed adulteration as well as an evaluation of the taxonomic placement of each species.

## MATERIALS AND METHODS

The material consisted of seeds collected from wild species and seeds collected from herbarium specimens. The specimens are deposited in the Catania herbarium (CAT).

Morphobiometric analysis of seeds was carried out using a Wild M8 stereomicroscope. For the SEM study, seeds and seed

TABLE I  
Systematic interpretations of DE CANDOLLE (1824), FIORI & PAOLETTI (1896-1898)  
and SCHULZ (1959) in *Sinapis* sp. and *Brassica nigra* in Italy

	Genus	Sect.	Species
DE CANDOLLE	<i>Sinapis</i> L.	<i>Melanosinapis</i> DC. <i>Ceratosinapis</i> DC. <i>Leucosinapis</i> DC.	<i>S. nigra</i> L. <i>S. pubescens</i> L. <i>S. arvensis</i> L. <i>S. alba</i> L.
FIORI & PAOLETTI	<i>Sinapis</i> (Tourn.) L. <i>Brassica</i> (Tourn.) L.	— <i>Melanosinapis</i> (Schimp. et Spenn.) <i>Ceratosinapis</i> (DC. sect. <i>Sinapis</i> )	<i>S. alba</i> L. <i>B. pubescens</i> (L.) Ard. <i>B. nigra</i> (L.) Koch <i>B. sinapistrum</i> Boiss.
SCHULZ	<i>Sinapis</i> L. <i>Brassica</i> L.	<i>Ceratosinapis</i> DC. <i>Eriosinapis</i> Coss. <i>Leucosinapis</i> DC. <i>Melanosinapis</i> (DC.) Boiss.	<i>S. arvensis</i> L. <i>S. pubescens</i> L. <i>S. alba</i> L. <i>B. nigra</i> (L.) Koch

sections obtained by crushing, were dehydrated in the alcohol series (50°, 90°, 100°) and gold coated for 90 sec. before being observed with a Philips 501 scanning electron microscope.

The terminology of seed morphological characters follows that of BERGGREN (1981), while nomenclature for the species follows the recent work of PIGNATTI (1982).

#### *Specimens examined:*

*Sinapis alba* L. ssp. *alba*: SA 1s Padova; SA 2s Casola Valsenio (FO); SA 3s Modena; SA 4s Palermo; SA 5s Genova; SA 6s Numana (AN); SA 7s Pisa; SA 8s Milano; SA 9s Lucca; SA 10s Catania.

*S. arvensis* L.: SAR 1s Mt. Corsaro (AN); SAR 2s Camporosso (IM); SAR 3s Siena; SAR 4s Modena; SAR 5s Palermo.

*S. pubescens* L.: SP 1s Palermo; SP 2s Catania; SP 3s Cagliari.

*Brassica nigra* (L.) Koch: BN 1s Modena; BN 2s Parma; BN 3s Lucca; BN 4s Montesicuro (AN); BN 5s Genova; BN 6s Firenze; BN 7s Palermo; BN 8s Molina (PI).

#### *Abbreviations adopted:*

SA: *S. alba* ssp. *alba*; SAR: *S. arvensis*; SP: *S. pubescens*; BN: *B. nigra*; 1s, 2s, etc.: seed specimens; dM: longest diameter; Wi: seed width; Le: seed length; Th: seed thickness.

### RESULTS

Observation of the features given in Table II reveals characteristic tendencies at genus level. The symmetry of seeds in the *Sinapis* taxa is predominantly bilateral (89%). Bilateral seeds are less frequent (65%) in *B. nigra*, with an increase in radiosymmetrical seeds (35%).

The prevailing shape of seeds in *B. nigra* is elongate ellipsoidal (84%). This percentage diminishes in the *Sinapis* taxa (63%), while the number of compact ellipsoidal and spheroidal seeds increases.

The colour of seeds in the *Sinapis* taxa may vary from egg-yellow to various shades of brown and black, while seed colour in *B. nigra* is brown.

The size of seeds is smallest in *S. pubescens*, with average values increasing in the order *S. arvensis*, *B. nigra*, *S. alba* ssp. *alba* (Fig. 1).

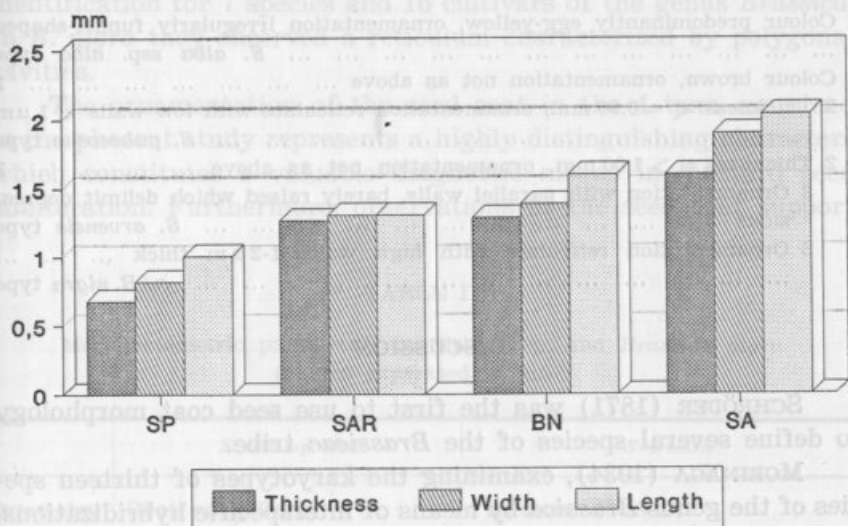


Fig. 1. — Size of seeds in *S. alba* ssp. *alba* (SA), *S. arvensis* (SAR), *S. pubescens* (SP) and *B. nigra* (BN).

The seeds coat exhibits four distinct morphotypes: *S. alba* ssp. *alba* type (Pl. I, figs. 1-4): cells delimit irregular funnel-shaped cavities with non-perforate base. Cross-sections show irregular layers which are indistinct and 70-100  $\mu\text{m}$  wide.

*S. arvensis* type (Pl. II, figs. 1-4): cells tend to incline forming folds which are arranged in parallel rows along the longitudinal axis of the seed and delimit barely distinguishable depressions. Cross-sections show distinct uniform layers 36-40  $\mu\text{m}$  thick.

*S. pubescens* type (Pl. III, figs. 1-5): reticulum with pits (dM 40-80  $\mu\text{m}$ ) having perforate base and provided with slightly raised thin walls formed of slightly longer cells. Cross-sections show irregular, indistinct layers.

*B. nigra* (Pl. IV, figs. 1-4): reticulum has finely perforate base with outline from circular to polygonal (dM 60-120  $\mu\text{m}$ ). Pits are delimited by prominent walls 1-2  $\mu\text{m}$  thick. Cross-sections show distinct layers, 23-50  $\mu\text{m}$  thick.

The colour, thickness and type of ornamentation of seeds of the four taxa represent distinguishing characters upon which a key to the morphotypes can be constructed:

- 1 Colour predominantly egg-yellow, ornamentation irregularly funnel-shaped ... .. *S. alba* ssp. *alba* type
- 1 Colour brown, ornamentation not as above ... .. 2
- 2 Thickness  $< = 0.90$  mm, ornamentation reticulate with low walls  $< 1 \mu\text{m}$  ... .. *S. pubescens* type
- 2 Thickness  $= > 1.00$  mm, ornamentation not as above ... .. 3
- 3 Ornamentation with parallel walls, barely raised which delimit depressions ... .. *S. arvensis* type
- 3 Ornamentation reticulate with high walls  $1-2 \mu\text{m}$  thick ... .. *B. nigra* type

#### DISCUSSION

SCHRÖDER (1871) was the first to use seed coat morphology to define several species of the *Brassicae* tribe.

MORINAGA (1934), examining the karyotypes of thirteen species of the genus *Brassica* by means of interspecific hybridizations, distinguished six groups: three comprised the parent species (among which *B. nigra*) having low chromosome number (8-10 n) and three the amphidiploid species with high chromosome number (17-19 n).

BERGGREN (1962), following the plan proposed by MORINAGA (1934), observed the seed coat of the parental and amphidiploid species. For *B. nigra* she reported low palisade cells in which height variations ranged from intermediate to marked, with consequent high reticulum. The lumina of the reticulum can measure  $10 \mu\text{m}$  while the angular pits, not usually elongate, measure  $70-150 \mu\text{m}$ . In a more recent study, BERGGREN (1981) described the cavities in *B. nigra* as having a concave base while the low reticulum in *S. alba* and *S. arvensis* varied from indistinct to almost indistinguishable.

VAUGHAN & WHITEHOUSE (1971), observing the external morphology and anatomy of seeds in 90 genera and 200 species belonging to the *Cruciferae*, reported that few genera or species exhibited exclusive distinguishing characters; consequently the different structural types observed in the seeds did not justify the division of this family into tribes. They described a reticulate surface and spherical seed shape in seeds of *B. nigra*. Similarly,



they observed spherical seed shape in *S. alba* and *S. arvensis* whereas the seed surface was smooth in the former and finely-reticulate in the latter. BUTH & ARA (1981) proposed a key of identification for 7 species and 16 cultivars of the genus *Brassica*. In *B. nigra* they observed a reticulum characterized by polygonal cavities.

The ornamentation of the seed coat in the 4 taxa examined in the present study represents a highly distinguishing characters which constitutes a valuable diagnostic means in mustard seed adulteration. Furthermore, observations of the seed coat support

TABLE II

Morphobiometric parameters in *Sinapis* sp. and *Brassica nigra*  
(values expressed in mm)

	<i>S. alba</i> ssp. <i>alba</i>	<i>S. arvensis</i>
Symmetry	Bilateral (92 %) radiosymmetric (6 %) asymmetric (2 %)	Bilateral (93,8 %) radiosymmetric (6,2 %)
Shape	Elongate ellipsoidal (89 %) spheroidal (9 %) irregular (2 %)	Spheroidal (37,2 %) compact ellipsoidal (32,8 %) elongate ellipsoidal (30 %)
Colour	Egg-yellow	Brown, black
Seed coat	Cavities funnel-shaped	Depressions barely defined
Size	Le 1,60(2,04)2,90 Wi 1,10(1,90)2,60 Th 1,00(1,60)2,60	Le 1,00(1,31)1,60 Wi 1,00(1,31)1,60 Th 1,00(1,27)1,60
	<i>S. pubescens</i>	<i>B. nigra</i>
Symmetry	Bilateral (80 %) radiosymmetric (20 %)	Bilateral (65 %) radiosymmetric (35 %)
Shape	Elongate ellipsoidal (87,8 %) spheroidal (11,8 %) compact ellipsoidal (1,1 %)	Largely elongate ellipsoidal (83 %) largely compact ellipsoidal (2 %) elongate ellipsoidal (1 %) spheroidal (14 %)
Colour	Brown	Brown
Seed coat	Reticulum with thin, slightly prominent walls	Reticulum with thick, prominent walls
Size	Le 0,70(1,01)1,20 Wi 0,70(0,82)1,10 Th 0,50(0,68)0,90	Le 0,80(1,55)2,60 Wi 0,50(1,24)2,10 Th 1,00(1,24)2,10

the systematic interpretation of FIORI & PAOLETTI (1896-1898) who placed *S. alba* in the genus *Sinapis* and within the genus *Brassica*: *B. nigra*, *B. pubescens* in sect. *Melanosinàpis*, *B. sinapistrum* (= *S. arvensis*) in sect. *Ceratosinàpis* (Table I).

In fact, the seeds of *B. nigra*, *S. pubescens* and *S. arvensis* exhibit a reticulate ornamental pattern. In *B. nigra* (Pl. IV, figs. 3 and 4), ornamentation is reticulate with wide pits (dM 60-120  $\mu\text{m}$ ) provided with densely perforate base due to the spacing of the cell testa. Pits are delimited by prominent thick walls (1-2  $\mu\text{m}$ ) formed by the grouping of longer cells. Ornamentation in *S. pubescens* (Pl. III, figs. 3 and 5) closely resembles that of *B. nigra* (FIORI & PAOLETTI place both species in the same section) and differs only for the narrower pits (dM 40-80  $\mu\text{m}$ ) and less prominent, thinner walls (< 1  $\mu\text{m}$ ) of the former. The sculpture pattern observed in *S. arvensis* (Pl. II, figs. 2 and 3) confirms the description of VAUGHAN & WHITEHOUSE (1971) of this species. It is characterized by cells which tend to incline forming folds arranged in parallel rows along the longitudinal axis of the seed. The cells delimit barely distinguishable depressions.

Finally, *S. alba* ssp. *alba* (Pl. I, figs. 2 and 3) totally lacks a reticulate pattern since cells delimit irregular funnel-shaped cavities with non-perforate base. Moreover, the predominant seed colour in this taxon is egg-yellow while seed colour in the remaining species varies from brown to black.

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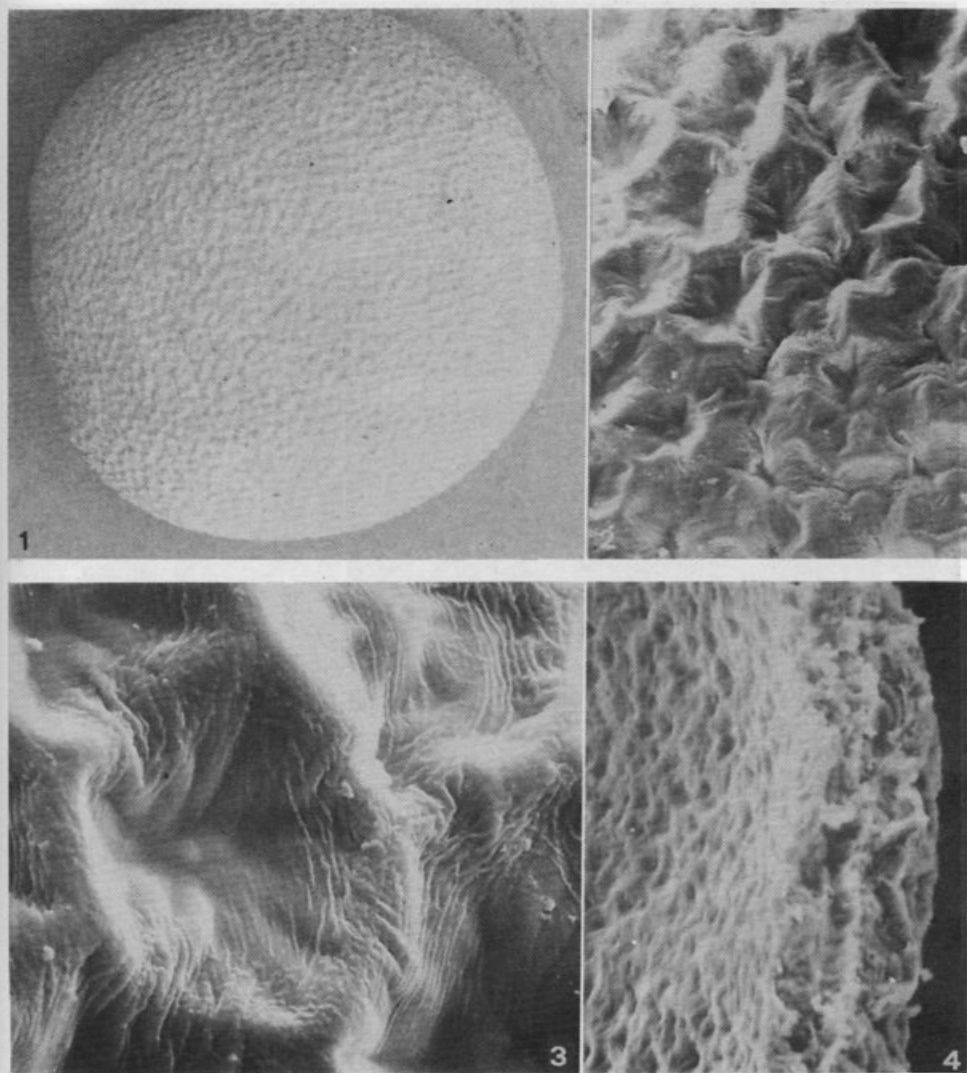


Fig. 1. — *S. alba* ssp. *alba*: whole seed ( $\times 35$ ). Figs. 2-3. — Ornamentation funnel-shaped ( $\times 250$ ;  $\times 500$ ). Fig. 4. — Section ( $\times 600$ ).

Figs. 1-2. — *S. pubescens*: whole seed ( $\times 75$ ;  $\times 35$ ). Figs. 3, 5. — Ornamentation reticulate with pits delimited by slightly raised walls  $< 1 \mu m$  thick ( $\times 250$ ;  $\times 500$ ). Fig. 4. — Section ( $\times 1200$ ).