POLLEN GRAINS FROM UMBELLIFERAE OF RIO GRANDE DO SUL. GENERA *ERYNGIUM* L. SECTIO *PANNICULATA* WOLFF *

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SUMARIO

En el presente trabajo, fueron estudiados los granos de polen de 15 especies del género *Eryngium* L. Sectio Panniculata Wolff de Rio Grande do Sul (Brasil).

Estos granos de polen no son muy diferentes en aspectos morfológicos, pero algunas especies pueden ser apartadas por los estudios estadísticos de los ejes.

Los granos polen de *Eryngium* L. son prolados o perprolados con una débil exina.

Within the Umbelliferae family, the genera *Eryngium* L. is the most representative of Rio Grande do Sul, not only by the number of species (59 % of the total) but by the number of individuals too. Sometimes they charaterize some fields.

The Sectio *Panniculata* Wolff (54 % of the Eryngium from Rio Grande do Sul, Rambo 1957), was characterized by Wolff (1913) with vegetative characters (principally leaf morphology) which are confusing to be used.

Because of the difficulties mentioned it was thus resolved the pollen grains should be used as an attempt to get a more definite separation of them, although Erdtman (1952) put the Umbelliferae as a stenopalynous family. (Families whose pollen grains have small differences in shape, apertures and membranes).

The following species belong to the Section:

Sectio XXXI - Panniculata Wolff (in Engler, 1913)

Subsectio Ensiformia (Lancifolia) Wolff (Not present in RGS)

Subsectio Eupanniculata Wolff

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** Reseacher of "Universidade Federal do Rio Grande do Sul" and fellows of "Conselho Nacional de Pesquisas".

Serie PSEUDOAREATA Wolff (Not present in RGS)
Serie PLATYPHYLLA (Latifolia) Wolff
E. schwakeanum Urb. [1]
E. lassauxii Decne [1]
E. chamissonis Urb.
E. decaisneanum Urb.
E. pandanifolium Cham. et Schl.
E. horridum Malme
E. eburneum Decne
E. balansae Wolff
E. stenophyllum Urb. [1]
E. regnellii Malme [1]
E. megapotamicum Malme
Serie SPARGANIFOLIA Wolff (Not present in RGS)
Series ANGUSTIFOLIA Wolff
Subseries PRISTIS Wolff
E. paraguariense Urb.
E. canaliculatum Cham. et Schl.
E. scirpinum Cham.
E. pristis Cham. et Schl.
Subseries JUNCEA Wolff
E. junceum Cham. et Schl.
E. eriophorum Cham. et Schl.
Subseries ZOSTERIFOLIUM Wolff
E. zosterifolium Wolff
Subseries LUZULIFOLIA Wolff
E. luzulifolium Cham. et Schl.

MATERIAL AND METHODS

Drying plants from ICN and PACA Herbaria were used. The following fifty species were studied: *E. chamissonis* Urb. (PACA 49781 - Loc. RS. Osório-Lagoa dos Quadros in I/18/1951; ICN 4695 - Loc. RS. Barra do Ribeiro, Morro da Formiga in XII/6/1937). *E. pandanifolium* Cham. et Schl. (ICN 7312 - Loc. RS. Soledade in I/2/1970). *E. decaisneanum* Urb. (ICN 7491 - Loc. RS. São Lourenço in II/23/1970). *E. horridum* Malme (ICN 4165 - Loc. RS. Viamão, Itapoan in I/5/1967; ICN 4689 - Loc. RS. Barra do Ribeiro, Ponta de Formiga in XII/6/1967). *E. eburneum* Decne (ICN 4172 - Loc. RS. Torres in V/6/1967; ICN 4139 - Loc. RS. Panambi in III/2/1967). *E. balansae* Wolff (ICN 4692 - Loc. RS. Barra do Ribeiro in XII/8/1967; ICN 4693 - Loc. RS. Barra do Ribeiro in XII/8/1967). *E. megapotamicum* Malme (ICN 4725 - Loc. RS. Viamão, Itapoan in

[1] Species citad by other authors, which weren't studied.

I/10/1968). *E. paraguariense* Urb. (PACA 35254 - Loc. RS. Bom Jesus, Serra da Rocinha in II/14/1947). *E. canaliculatum* Cham. et Schl. (PACA 64330 - Loc. RS. São Joaquim in II/18/1958). *E. scirpinum* Cham. (PACA 36765 - Loc. RS. Cambará in I/5/1948). *E. pristis* Cham et Schl.(ICN 4097 - Loc. RS. Viamão, Parque Saint Hilaire in V/5/1966; ICN 4143 - Loc. RS. Viamão, Parque Saint Hilaire in I/5/1967). *E. junceum* Cham. et Schl. (PACA 3032 - Loc. RS. Pelotas, Aeroposto in XII/16/1938). *E. eriophorum* Cham. et Schl. (ICN 1633 - Loc. RS. Porto Alegre, Morro Santana in III/24/1958; ICN 4911 - Loc. RS. Camaquan in VI/17/1968). *E. zosterifolium* Wolff (PACA 53913 - Loc RS. Bom Jesus, Serra da Rocinha in II/3/1953). *E. luzulifolium* Cham. et Schl. (ICN 7618 - Loc. RS. Viamão, Viamópolis in V/9/1970).

The pollen grains were treated by the acetolytic method (Erdtman, 1952). The tubes with acetolyze mixture were boiled for one minute in waterbath.

The acetolyzed grains were mounted in glycerin-jelly, and slides were sealed with paraffin (Salgado-Labouriau, 1966). At first, the jelly with much water caused trouble, because the pollen grains were caused to swell one or two days after they were acetolyzed. This problems was solved, when the jelly glass was put in a drier. The swelling became nonexistent and besides, it became easier to work with the small portions of gelatine, When the ambient temperature is high it is wise to place both the jelly glass and the tube with the acetolysed grains in ice.

The measurements of pollen dimensions were made until one week the pollen materials were mounted (Salgado-Labouriau, 1966).

Differences between central and lateral capitula were seen in flowering and fructification (some abnormal anthers and fruits on the lateral capitula, Viana, F.M.S., personal communication). Due to this fact, it was established the supposition of existing differences between pollen grains from one or other capitulum (lateral and central). Twenty five measurements were made from each sample (Ferreira et alia, 1966; Salgado-Labouriau, 1966), polar or equatorial axis (equatorial view), taking central or lateral capitula separately (25 measuremants each one).

Later 50 new measurements were made to obtain more samples (Ting et al., 1964). Other acetolyzis were made without separating the pollen grains from lateral or central capitula.

The average of the sample, standard deviation and standard error were calculated. The significance of differences was tested by the use of 99 % confidence intervals (table III), from polar and equatorial diameters in equatorial view, because differences between two axes were large, therefore, only grains in equatorial view were found.

For the critical species comparative graphics on rectangular coordinate system were introduced, which have the advantage of immediate visualization and establish an area of dispersion which wasn't found by others

authors when the diameters were compared isolatedly (Salgado-Labouriau, 1967; Melhem, 1968).

The maximum of 10 measurements of membrane and apertures was made when the visibility of the microscope (WILD-M20) made it possible and the average can be found on table IV.

The terminology adopted in this paper is based on Erdtman glossary (1952).

All drawings were made with a camera lucida of WILD M20 microscope.

For the *t* test (Student) the following formulas were used:

$$s = \sum_{i=1}^{n} (x_i - \bar{x})^2/n - 1 \qquad \text{standard deviation (sample)}$$

$$\sigma^2 = \frac{n(s_1^2 + s_2^2)}{2n - 2} \qquad \text{standard deviation (population)}$$

$$t = \frac{\bar{x} - \bar{x}_1}{\sigma} \left(\frac{n^2}{2n}\right)^{1/2}$$

$$\mu = \bar{x} \pm t_{n-1} \cdot s_{\bar{x}} \qquad t_{48 \text{ or } 49} = 2.68 \quad t_{98} = 2.63$$

$$x = \frac{\sum x_i}{n} \qquad \text{arithmetic mean}$$

$$s_{\bar{x}} = \frac{s}{\sqrt{n}} \qquad \text{standard error of the mean}$$

$$x = \bar{x} \pm t \cdot s \qquad \text{confidence intervals}$$

RESULTS

Comparative study between the pollen grains from lateral and central capitula

In using the *t* test (Student) for the comparison of 2 averages of the measurements of the central and lateral capitula (Fischer, in Goodman, 1963) it was seen that with a few exceptions there was no separation in the level of 1 % (table I).

Using rectangular coordinate systems (fig. 1), the visualization of it is better in the species *E. eburneum* Decne. Because of these results, it was settled not to make any saparation in the other acetolyzis of the same specimen (same inflorescence).

COMPARATIVE STUDY BETWEEN TWO DIFFERENT SPECIMENS, SAME SPECIES

It was thought, too, of existing differences between one plant and another of the same species. If so, it could not be considered as an only population of pollen grains. Using *t* test, it was verified this hypothesis of a significant difference in the level of 1 % can not be sustained only for the species *E. horridum* Malme.

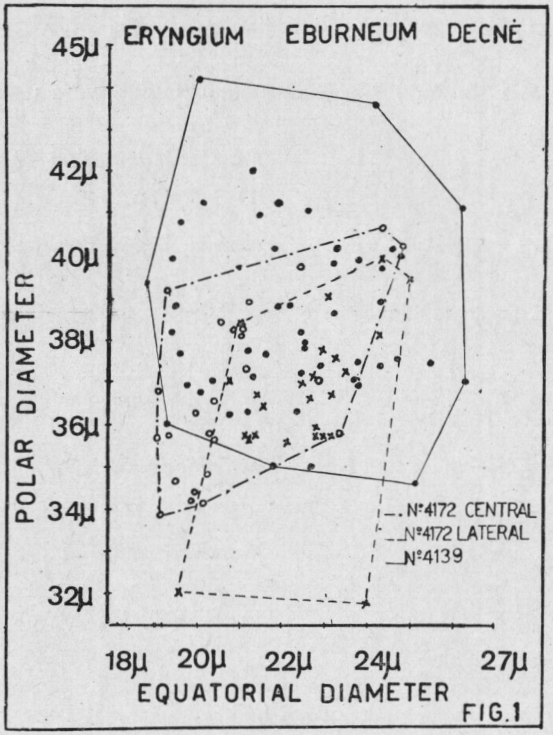

Fig. 1. — Comparative study throug rectangular coordinate system. Samples taken from lateral and central capitula

COMPARATIVE STUDY AMONG SPECIES

Axes: the results are on table III. All species showed P/E superior 1.5, thus becoming very difficult grains in polar view. This way all measurements were made in equatorial view.

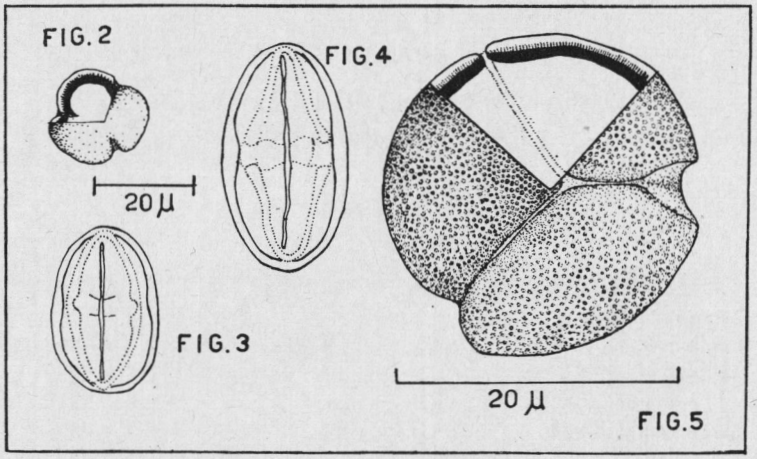

Fig. 2-5. — Pollen grains from *E. horridum* Malme, polar view. — Fig. 3, from *E. paraguarensis* Urb., equatorial view ; Fig. 4, from *E. chamissonis* Urb., equatorial view. — Fig. 5, pollen grains from *E. chamissonis* Urb., polar view, showing the colpi anastomosing in polar region.

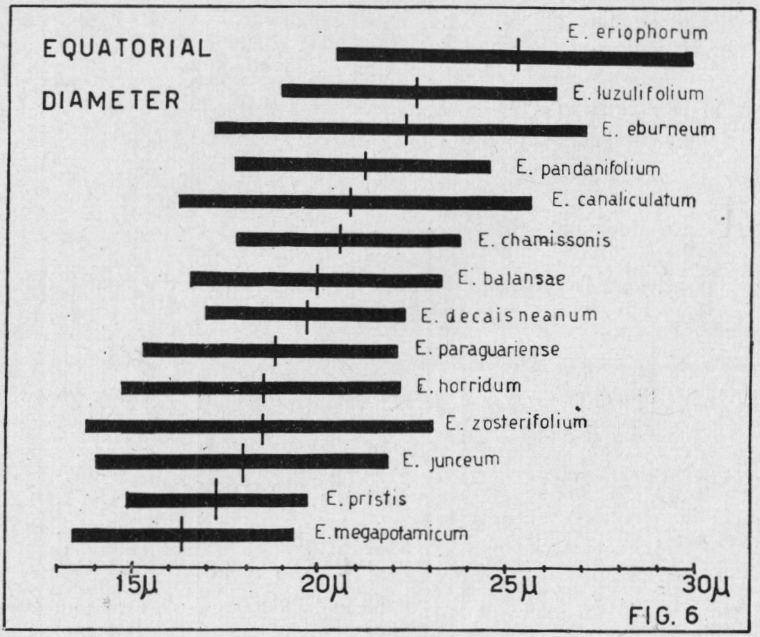

Fig. 6. — Comparative study among the species. Equatorial diameter, equatorial view Confidence intervals 99 %

TABLE I

Comparative study between lateral and central capitula (Ferreira and Irgang, 1970) *

Species	Equatorial diameter	Polar diameter
E. chamissonis	2.48	4.80
E. pandanifolium	**	**
E. horridum	1.71	9.12
E. eburneum	4.70	0.32
E. balansae	**	**
E. megapotamicum	1.62	2.31
E. pristis	0.31	6.46
E. paraguariense	2.34	0.03
E. canaliculatum	0.57	0.71
E. scirpinum	**	**
E. junceum	**	**
E. eriophorum	4.91	2.36
E. zosterifolium	0.89	2.54
E. decaisneanum	**	**

* $t_{.01\,(49)} = 2.68$.

** In these species the study was not realized.

TABLE II

Comparative study between different especimens, within the same species (Ferreira and Irgang, 1970)

Species	Equatorial diameter	Polar diameter
E. chamissonis	4695 and 49781 t = 1.61	4695 and 49781 t = 1.10
E. eburneum	4172 and 35354 t = 1.76	4172 and 35254 t = 0.42
E. horridum	4165 and 4689 t = 2.95	4165 and 4689 t = 2.69

$t_{.01\,(48)} = 2.68$.

TABLE III

Comparative study among the axes of different species studied. Ratio between polar and equatorial diameters (P/E from the means) and their $\log_{10}$. A 99 % confidence interval. (Ferreira and Irgang, 1970)

Species	Equatorial diameter micra			polar diameter micra			P/E
	x−t.s	x±t.$s_{\bar{x}}$	x+t.s	x−t.s	x±t.$s_{\bar{x}}$	x+t.s	$\log_{10}$
E. chamissonis	17.8	20.7 ±0.5	23.7	37.0	41.0 ±0 5	45.0	1.98 0.2967
E. pandanifolium	17.6	21.1 ±0.5	24.6	31.1	36.7 ±0.8	42.3	1.74 0.2405
E. horridum	14.6	18.4 ±0.5	22.2	24.7	33.0 ±1.3	41.5	1.79 0.2529
E. eburneum	17.1	22.2 ±0.8	27.3	33.0	38.6 ±0.8	44.2	1.74 0.2405
E. balansae	16.4	19.9 ±0.5	23.4	33.7	39.3 ±0.8	44.9	1.97 0.2945
E. megapotamicum	13.3	16.3 ±0.5	19.3	25.5	30.3 ±0.8	35.1	1.86 0.2695
E. decaisneanum	16.9	19.6 ±0.5	22.3	32.5	37.1 ±0.8	41.7	1.89 0.2765
E. pristis	14.7	17.2 ±0.3	19.7	29.0	33.0 ±0.5	37.0	1.92 0.2833
E. paraguariense	15.1	18.6 ±0.5	22.1	23.4	28.2 ±0.8	33.0	1.52 0.1818
E. canaliculatum	16.0	20.8 ±0.8	25.6	34.1	37.6 ±0.5	41.1	1.81 0.2577
E. scirpinum [1]	16.7 [2]	19.0	20.7	29.7	33.2	34.2	1.69 0.2279
E. junceum	13.9	17.9 ±0.5	21.9	30.9	36.8 ±0.8	42.9	2.06 0.3139
E. eriophorum	20.3	25.1 ±0.8	29.9	43.4	47.5 ±0.5	51.6	1.89 0.2765
E. zosterifolium	13.5	18.3 ±0.8	23.0	29.0	35.7 ±1.1	42.4	1.95 0.2900
E. luzulifolium	18.7	22.5 ±0.5	26.3	40.6	46.5 ±0.8	52.4	2.07 0.3160

$t_{.01\,(49)}$ t=2.68. [1] Only measurements × from 25. [2] Extremities in a sample of 25.

TABLE IV

Measurements of apertures and membranes of different species studied (Ferreira and Irgang, 1970)

Species	Exine	Nexine	Sexine	Colpus		Os	
				lenght	width	length	width
	µ	µ	µ	µ	µ	µ	µ
E. chamissonis	1.8	0.9	0.9	36.1	0.6	5.5	3.8
E. pandanifolium	2.0	0.8	1.2	35.4	0.8	7.1	3.9
E. horridum	1.7	0.9	0.8	24.9	0.3	5.8	2.4
E. eburneum	2.0	0.9	1.2	22.0	0.7	11.1[1]	2.7[1]
E. balansae	1.3	0.6	0.6	24.4	0.8	6.0[1]	2.9[1]
E. megapotamicum	1.9	0.8	1.1	20.0	1.1	12.0	2.1
E. paraguariense	1.6	0.8	0.8	27.7	0.6	7.0[1]	3.5[1]
E. canaliculatum	2.0	0.9	1.2	22.0	0.7	6.6	4.4
E. scirpinum	1.7	0.8	0.9	27.1	0.9	[2]	[2]
E. pristis	1.9	0.9	1.0	25.8	0.6	6.2	3.0
E. junceum	1.7	0.7	1.0	29.3	0.7	[2]	[2]
E. eriophorum	1.2	0.6	0.5	32.2	0.4	8.5	3.6
E. zosterifolium	1.9	0.9	1.0	33.7	0.6	5.0	3.4
E. decaisneanum	1.7	0.9	0.9	31.6	0.8	4.4	5.6
E. luzulifolium	1.5	0.7	0.7	38.9	0.9	11.1	4.6

[1] Rather inconspicuous - only 5 measurements were made.

[2] Rather inconspicuous - measurements were not made.

Apertures and membranes: Ten measurements were made of apertures and membranes, with the exception of a few cases where it was impossible because they were more inconspicuous.

Other results: The grains were medium size (ME) (Erdtman, 1952), tricolporate (fig. 2). Os lalongate (figs. 3 and 4).

All pollen grains showed thin exine. The measurements were made in polar region (equatorial view), the thickness of the grain membranes changing only close to the Os region where its visualization was very difficult (whole width).

Sexine finely tegillate. Over 500X, a punctuation can be observed on the surface of the grains which is more or less uniform in all species studied (Fig. 5).

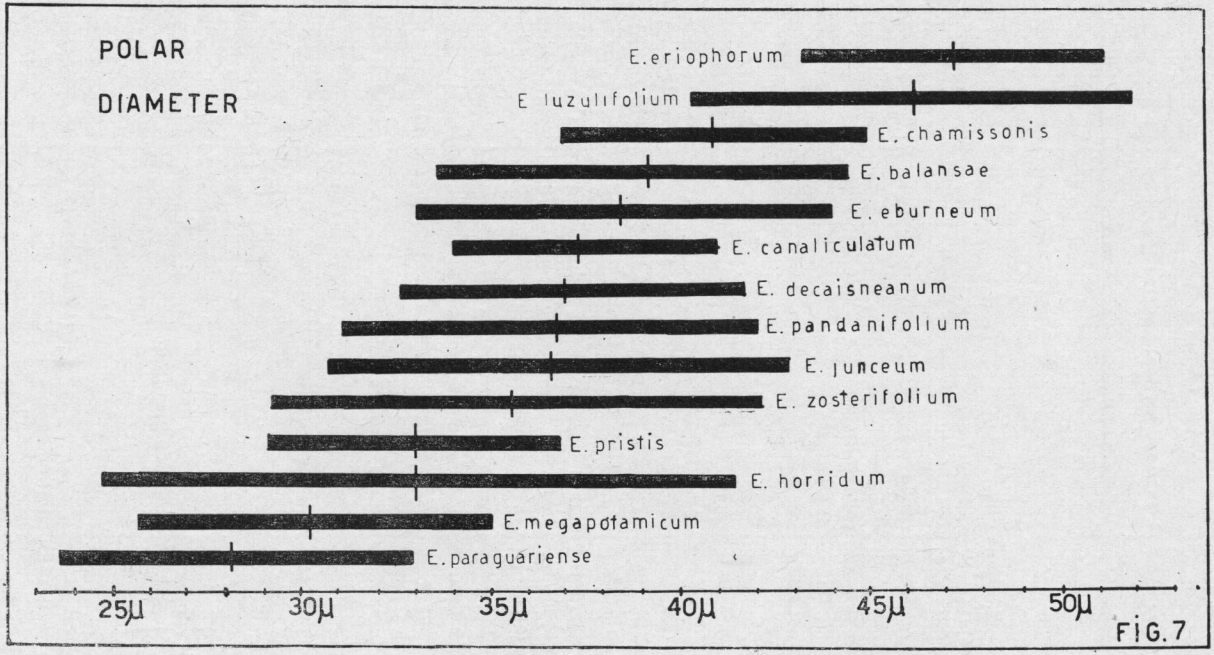

Fig. 7. — Comparative study among the species. Polar diameter, equatorial view. Confidence interval 99 %

TABLE V

Comparative studied between critical species (Ferreira and Irgang, 1970)

Species comparing	Equatorial diameter	Polar diameter
E. junceum-E. eriophorum	6.04	26.4
E. canaliculatum-E. paraguariense	5.22	9.07
E. canaliculatum-E. pristis	6.78	17.8
E. pandanifolium-E. chamissonis	1.45	14.2
E. chamissonis-E. decaisneanum	6.83	14.8
E. decaisneanum-E. pandanifolium	4.74	2.57

$t_{0.1\,(98)} = 2.63$.

DISCUSSION

Although Erdtman considers Umbelliferae as a stenopalynous family, several very interesting aspects have been found in the study of this family, principally in genera *Eryngium* L.

In this genera there is a quite perfect homogenity of the pollen grains on optic microscope. Therefore, some species showed different range among polar or equatorial axes in equatorial view.

On table III and figures 6 and 7, the 15 species of Sectio Panniculata Wolff can be compared, where there were slight differences among many species. However in the study of polar diameter three groups and 4 sub groups can be settled.

- Sectio Panniculata
  - 1. Minor pollen grains
    - Inferior (A)
      - *E. megapotamicum*
      - *E. paraguariense*
    - Superior (B)
      - *E. pristis*
      - *E. horridum*
  - 2. Medium pollen grains (C)
    - *E. pandanifolium, E. balansae, E. junceum, E. zosterifolium, E. decaisneanum, E. eburneum, E. canaliculatum.*
  - 3. Major pollen grains
    - Inferior (D)
      - *E. chamissonis*
    - Superior (E)
      - *E. luzulifolium*
      - *E. eriophorum*

The pollen grains from group A can be separated from those of group D and E. B can be separated from E but not from D. C is among the others, and cannot be distinguished from either of them.

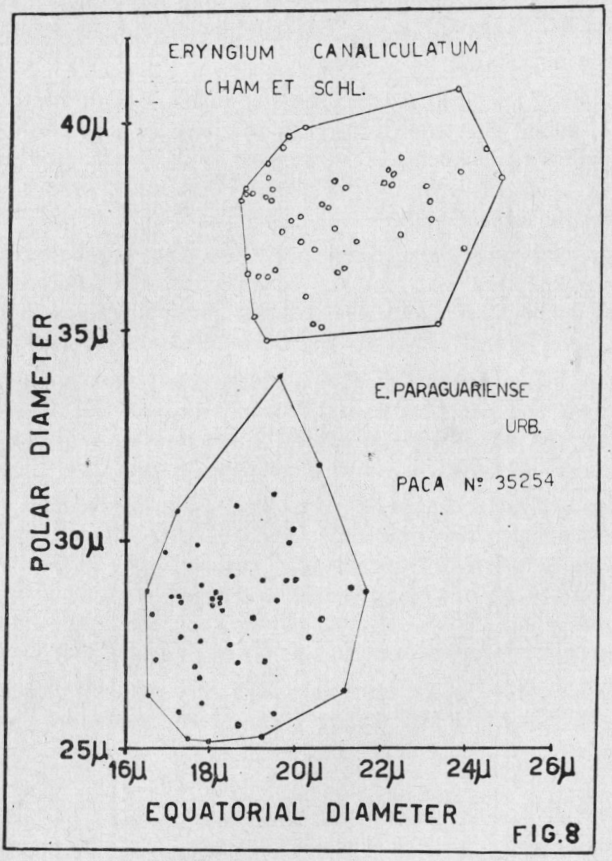

Fig. 8. — Comparative study between critical species: *E. canaliculatum* Cham. et Schl. and *E. paraguariensis* Urb.

Significance of differences was tested by the use od 99 % confidence interval (fig. 7). Considering the range of mean, only, it is very clear this difference (Table III).

The most interesting is in the critical species as shown on table V, when the t test was used. By morphological characters many of them are very hardly distinguishable but the pollen grains can be separated in this case. The ratios are highly significative for one to consider all as from the

same population. Sometimes, this can be visualized as in the critical species *E. canaliculatum* and *E. paraguariense* through projection in rectangular axis systems (fig. 8).

As there are not significant differences among especimes from different places (table II), this study considered all individuals from the same species as one population.

Some authors (Ferreira and Salgado-Labouriau, 1966; Matos and Melhem, 1966), found that the differences in shape or size could be due to different degrees of maturity of flowers or buds. Here, in this case, the authors tried to work always with flowers, not buds, because this could be too, an origin of variation.

For polar and equatorial diameters ratio the numbers outlined by Erdtman (1952) were used and the relation through common logarithm, which divided the class aroun the spheric grain alikely was done. This problem was very much discussed by Salgado-Labouriau (1966).

The size index presented by Ting et al. (1964) was not used because this ratio gives the size of the pollen grains only, while P/E shows the relations between polar and equatorial diameters thus giving the shape of the pollen grains which is more interesting in this case.

The pollen grains from this Sectio of Eryngium, sometimes, showed the colpi anastomosing at one pole only (fig. 5), with a small apolcopium in the other pole, which was not mesured because of the difficulty presented by grains that stayed in polar view even when were turned into glycerin (Salgado-Labouriau, 1966). The occurrence of colpi linked by one of the extremes had already been found in Leguminosae (Melhem, 1968).

The visualization of exine structures was very difficult and its interpretation was possible only after many grains were observed.

CONCLUSIONS

1. There were few differences in morphological view in optic microscope of the 15 species studied.
2. All species are tricolporate, showed thin exine with slight structure.
3. All species showed ratio P/E superior to 1.5.
4. There were not differences between pollen grains from central and lateral capitula.
5. In general, in the same species there were not differences in pollen grains from specimes from different places, so it was possible to consider as all of them being from the same population.

6. The species *E. megapotamicum* and *E. paraguariense* in equatorial view polar diameter, can be distinguished from *E. chamissonis*, *E. luzulifolium* and *E. eriophorum*. The first two have the pollen grains much smaller than the last three.

7. The species *E. pristis* and *E. horridum* can be distinguished from species *E. luzulifolium* and *E. eriophorum*, because the last two have the pollen grains larger than the first two.

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