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NATURAL HYBRIDIZATION IN *HYPOCHOERIS* (ASTERACEAE-CICHORIOIDEAE)*

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Summary: Two *Hypochoeris* L. species: *H. chillensis* (Kunth) Hieron., *H. microcephala* var. *albiflora* (Kuntze) Cabrera and their putative hybrids has been analyzed by numerical methods, using 21 morphometric or qualitative characters, in order to study the composition of a hybrid swarm growing in Ciudad Universitaria (Buenos Aires). Principal Component Analysis (PCA), Wells' Distance Diagram, and Wilson's Character Count Procedure were applied for predicting hybridity. Morphology of pollen and achene surface observed by SEM are reported. The significance of the hybridization is discussed in relation with the geographical distribution and the morphological variation observed in *H. chillensis*.

Key words: Hybridization, *Hypochoeris*, numerical analysis, pollen morphology, achene surface.

Resumen: Hibridación natural en *Hypochoeris* (Asteraceae-Cichorioideae). Dos especies de *Hypochoeris: H. microcephala* var. *albiflora* (Kunth) Hieron., *H. chillensis* (Kuntze) Cabrera y sus probables híbridos fueron caracterizados numéricamente usando 21 caracteres. Para diagnosticar esta hibridación se utilizó el Análisis de Componentes Principales (PCA), el diagrama de Wells y el índice de Wilson. El estudio incluyó el análisis de la superficie del aquenio y del polen por medio de microscopio electrónico de barrido (MEB). El significado de esta hibridación es discutido en relación al área de distribución geográfica y variación morfológica observada en *H. chillensis*.

Palabras clave: Hibridación, *Hypochoeris*, análisis numéricos, polen, superficie de aquenio.

INTRODUCTION

The genus *Hypochoeris* L. is distributed in Western Eurasia and South America. Different authors (Stebbins, 1971; Tomb, 1977) reported variable number of species for the genus (from 50 to 100). The species are classified into five sections and only one of them -*Achyrophorus* (Scop) Benth. & Hook.- is represented in America.

Some of the European species (i.e., *H. radicata* L.) show considerable variability originated by natural hybridization. This evolutionary process is responsible of variation in morphological characters such as leaf shape, root form, and capitula, arising a lot of subspecies, varieties, and forms (Aarssen, 1981).

The South American species, all belonging to the same section, occupy different habitats: high regions along the Andes, humid and arid plains in Argentina (including Patagonia) and tropical and subtropical regions of South Brazil.

At least, one of the most widely distributed South American species, *H. chillensis* (Kunth) Hieron. (the" achicoria" or "cerraja") shows, as *H. radicata,* a wide morphological variation, which has originated more than one confusion at taxonomic level. *Hypochoeris chillensis*, like *H. radicata* have a perennial root system.

Hypochoeris chillensis includes two taxa previously described as species: *H. tweedie* (Kunth) Cabrera and *H. brasiliensis* (Less) Benth. & Hook. ex Griseb. Both were considered by Cabrera (1976) as variations without systematic value.

Natural hybridization between *H. chillensis* and other two species has been previously reported (Wulff, 1992; Wulff & Burghardt, 1994; Burghardt & Wulff, 1996). The present work was carried out in order to analyze, by means of a numerical study based on 21 qualitative and quantitative morphological characters, the occurrence of natural hybridization between *H. chillensis* and *H. microcephala* var. *albiflora* (Kuntze) Cabrera. Some palynologic and fruit surface characters, useful for the analysis, are included as well.

* Dedicated to Prof. Dr. Juan H. Hunziker on the ocassion of his 75th anniversary.

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MATERIALS AND METHODS

Materials

Hypochoeris chillensis

ARGENTINA, Ciudad Autónoma de Buenos Aires. Ciudad Universitaria: 15-11-93 A.F.Wulff 602, 603, 605, 606, 608, 796, 797, 798, 799, 800, 801.

H. microcephala. var. *albiflora*

ARGENTINA, Ciudad Autónoma de Buenos Aires, Ciudad Universitaria: 15-11-93 A.F. Wulff 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812.

Putative hybrids

ARGENTINA, Ciudad Autónoma de Buenos Aires, Ciudad Universitaria: 15-11-93 A.F. Wulff 610, 611, 612, 613, 614, 615, 616, 617, 618.

Vouchers are deposited at the herbarium of the Facultad de Ciencias Exactas y Naturales (BAFC).

Methods

Pollen morphology

Pollen grains from herbarium specimens for light microscopy (LM) studies were mounted in glycerine jelly and sealed with paraffin wax. Samples for scanning electron microscopy (SEM) were mounted on aluminum stubs using double-sided self-adhesive tape. The SEM preparations were sputter coated with a thin gold layer before viewing and photographing.

Description was made following El-Ghazaly (1980) and Punt *et al*. (1994). Measurements of the equatorial diameter of pollen grains were made on light microscope samples; averages from three sets of 30 pollen grains of each taxon were calculated.

Pollen fertility

Pollen stainability was studied with Alexander´s differential staining (1969). For each individual, 500 pollen grains were observed.

SEM morphological traits of achene surface

Achenes from specimens of the three taxa for SEM were mounted in the same way above described.

Numerical Analysis of Hybridization

For numerical analysis the following characters were used (codification of the stages of qualitative characters are included):

1) Shape of the involucre (0= bell-shaped, 1=intermediate, 2= cylindrical)
2) Apical diameter of the involucre (mm)
3) Lateral diameter of the involucre (mm)
4) Long of the involucre at the flowering (mm)
5) Long of the involucre at the fructification (mm)
6) Ligules sticking out of the bracts involucre (0=absent, 1=present)
7) Number of flowers per capitulum
8) Flower color (0=white, 1=pale yellow, 2=brillant yellow)
9) Palea length without cirrus (mm)
10) Cirrus length (mm)
11) Corolla-tube length (mm)
12) Ligule length (mm)
13) Achene color (0=redish brown; 1=intermediate; 2=olivaceous brown)
14) Achene ornamentation (*H. chillensis* like=0, intermediate=1, *H. microcephala* like=2)
15) Number of branching points
16) Number of basal stems
17) First internode length (cm)
18) Basal diameter of stems (cm)
19) Plant height (cm)
20) Trichome arrangement of the achene beak apex (sparse=0, fasciculated and dispersed=1, fasciculated=2)
21) Achene scales boundary (thin=0, irregular thickening=1, thick=2)

Measurements of characters 2, 3, 4, 5, 7, 9, 10, 11, and 12 were taken with a stereomicroscope Leitz and a micrometric ocular. When it was possible, ten measures were made for each character and the mean was calculated.

Principal Component Analysis

Individuals of the two taxa and the natural hybrids were the operative taxonomical units (OTU). A Principal Component Analysis (PCA) was applied using all the characters above cited and performed using NT-Sys Program, version 1.8 (Rohlf, 1993).

Wells Distance Diagram (Distance Diagram)

The hybridization hypothesis was analyzed by means of the method proposed by Wells (1980). In order to perform the Distance Diagram, each specimen was considered as an operational taxonomic unity (OTU) . The same characters used

for PCA were registered and codified. As reference points, individuals of *H. chillensis* and *H. microcephala* var. *albiflora* with morphological characteristics falling within the described range by Cabrera (1963) were utilized.

Character Count Procedure

A Character Count Procedure (Wilson, 1992) on the same individuals was carried out, but only considering the quantitative characteristics, as this method indicates. The analysis was as follows: 1) Each individual was assigned to one of the three groups (*H. chillensis*, *H. microcephala* var. *albiflora*, and the putative hybrids), 2) Characters for separating parents were selected, 3) For each character, was determined and tabulated whether or not the values for the hybrids were intermediate, 4) The number of intermediate characters was divided by the total number of considered characters.

RESULTS

Pollen morphology

All pollen grains are equinolophate and tricolporate. Fig. 1 shows pollen grains of the three taxa in polar and equatorial views.

Hypochoeris chillensis: Equatorial diameter 32 µm-38µm (mean=35 µm). Triangular amb with right supratectal spines (Fig. 1 A). Equatorial ridges with two series of spines (Fig. 1 B). Spinose paraporal lacunae (Fig. 1 C). Punctitegilate tectum (Fig. 1 C). Circular elliptic os.

Putative hybrids: Equatorial diameter ranged from 30 µm to 38 µm (mean=33.14 µm). Circular amb with slightly curved supratectal spines (Fig. 1 D). Equatorial ridges with a single series of spines (Fig. 1 E). Paraporal lacunae with or without spines (Fig. 1 F). Punctitegilate tectum (Fig. 1 F). Circular-elliptic os.

Hypochoeris microcephala var. *albiflora*: Equatorial diameter rather constant: 30 mm - 34 µm (mean=33.2 µm). Circular amb with slightly curved supratectal spines (Fig. 1 G). Equatorial ridges with a single series of spines (Fig. 1 H). Paraporal lacunae always without spines (Fig. 1 I). Solidum tectum (Fig. 1 I). Oblongus os.

Pollen fertility was 92-98% for both parent species, while the putative hybrids pollen fertility varied from 34 to 60%.

SEM morphological characteristics of achene

Hypochoeris chillensis showed achene beak apex with sparse, adpressed trichotomata of 16.7 µm mean length (13-21µm) (Fig. 2 A). Scales with thin ciliate margin; cilia straight to recurvate of 19 µm (13-24 µm) (Fig. 2 B, C).

Putative hybrids beak apex with fasciculate and dispersed trichotomata, most of them adpressed, 18.8 µm length (8.7-26 µm) (Fig. 2 D). Scales with irregular boundary, irregular thickening, ciliate, cilia incurvate, 11.9 µm length (8.7-17.4 µm) (Fig. 2 E, F).

Hypochoeris microcephala var. *albiflora* showed beak apex with fasciculate trichotomata, not adpressed, 31.7 µm length (26.1-39.1 µm) (Fig. 2 G). Scales with thick boundary, ciliate, cilia incurvate, 7.2 µm length (4.3-13 µm) (Fig. 2 H, I).

Numerical Study of Hybridization

Principal Component Analysis

The position of the OTUs in a space delimited by the three first principal components revealed three groups well defined without any continuity among them. The hybrid group was in an intermediate position between individuals of both species that could be its putative parents (Fig. 3).

Distance Diagram

The Distance Diagram is showed in Fig. 4. The individuals from both species were assembled in two different groups and those that show intermediacy were located in an intermediate area between them. These specimens were located into the semicircle that includes all the samples of *H. chillensis* and *H. microcephala* var. *albiflora*.

Character Count Procedure

Means of nine quantitative characters in the putative hybrids showed intermediacy while only one was not intermediate (Table 1).

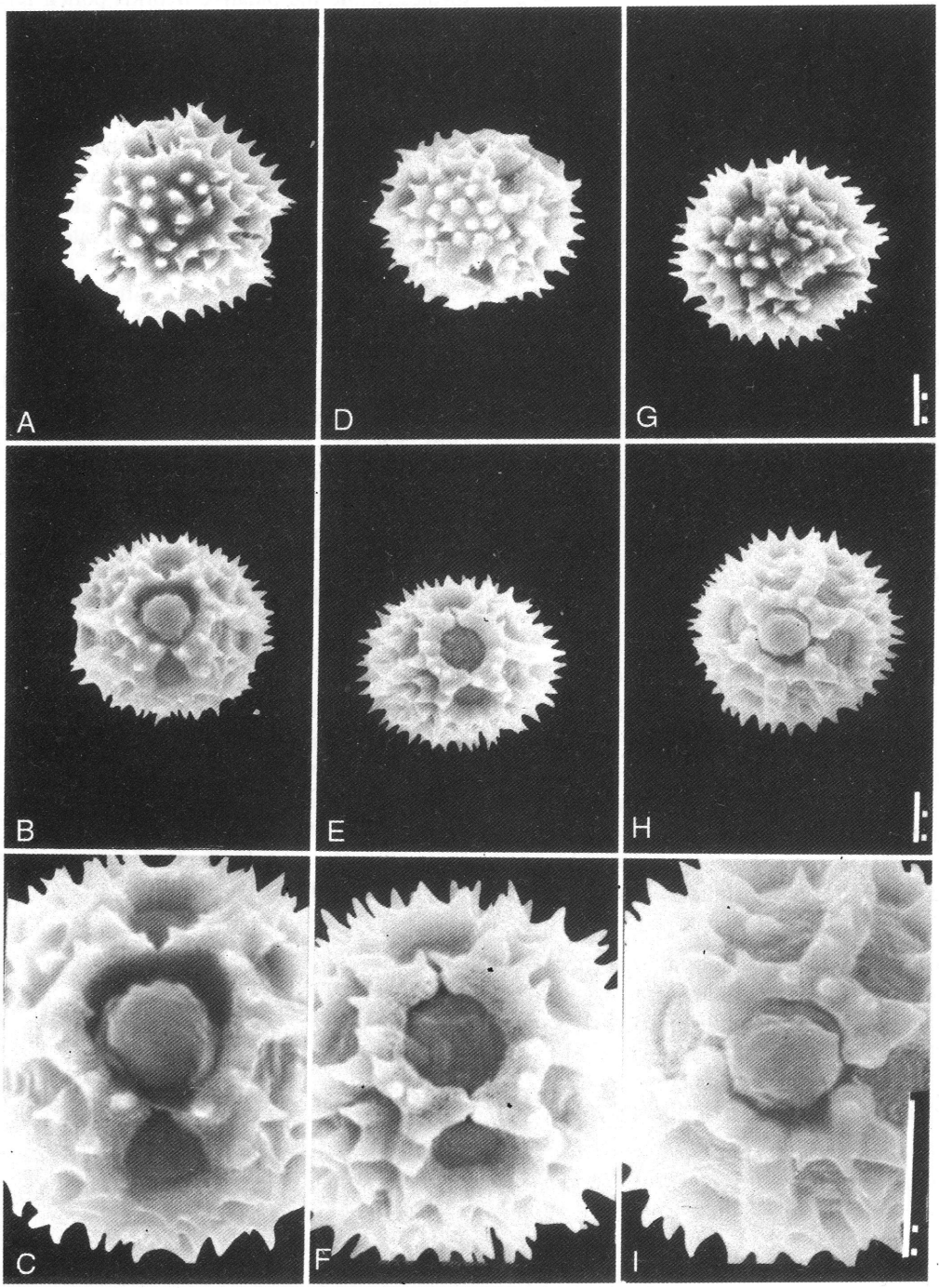

Fig. 1. Pollen morphology with SEM. **A-C**: *H. chillensis*. **D-F**: Hybrid. **G-I**: *H. microcephala*. **A**, **D** and **G** = polar view; **C**,**D**,**E**,**F**,**H** and **I**= equatorial view. Bar indicates 10 μm.

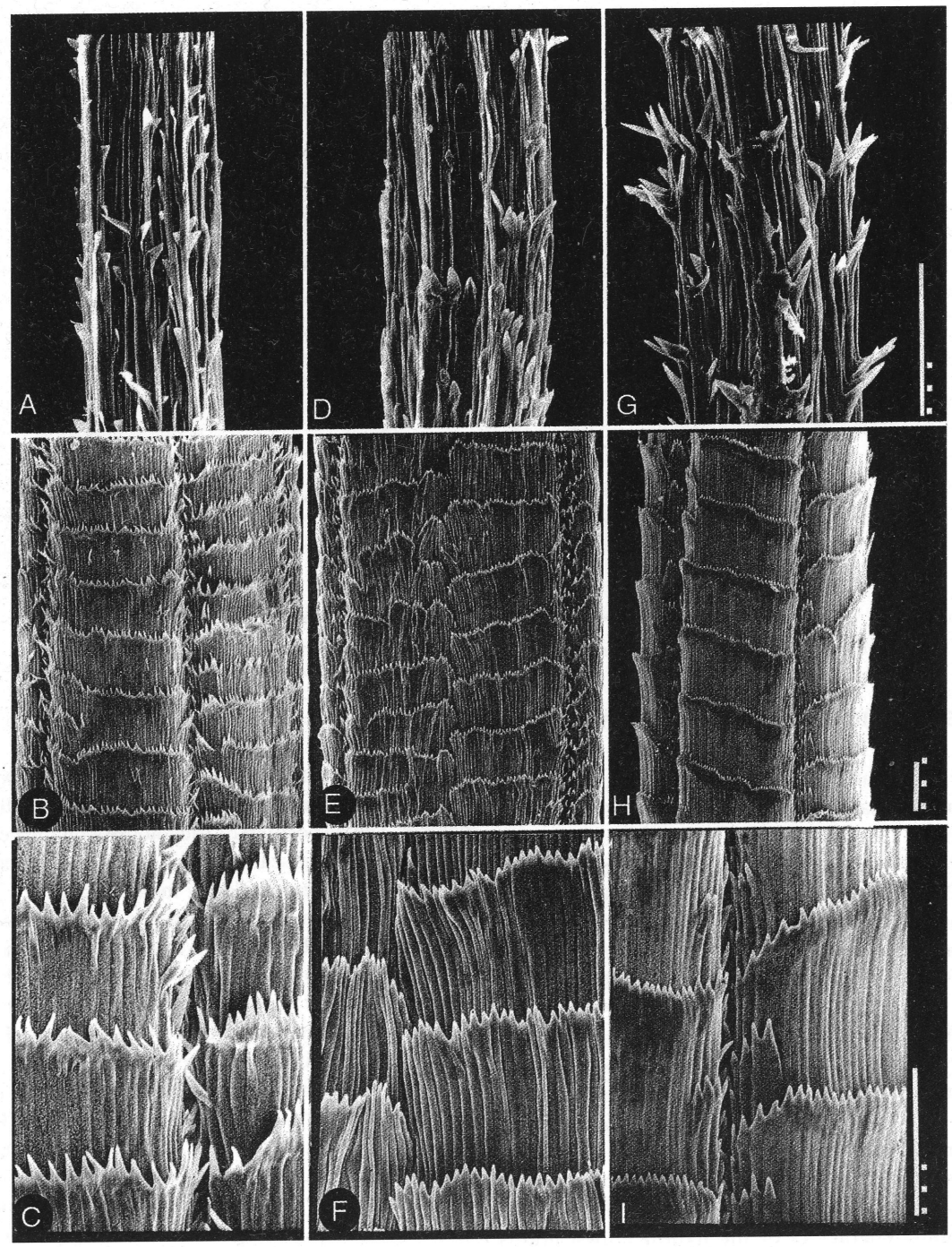

Fig. 2. Achene surface with SEM. **A-C**: *H. chillensis*. **D-F**: Hybrid. **G-I**: *H. microcephala*. **A**, **D**, **G**: Apex of the beak. **B**, **C**, **E**, **F**, **H**, **I**: Scales. Bar indicates 100 μm.

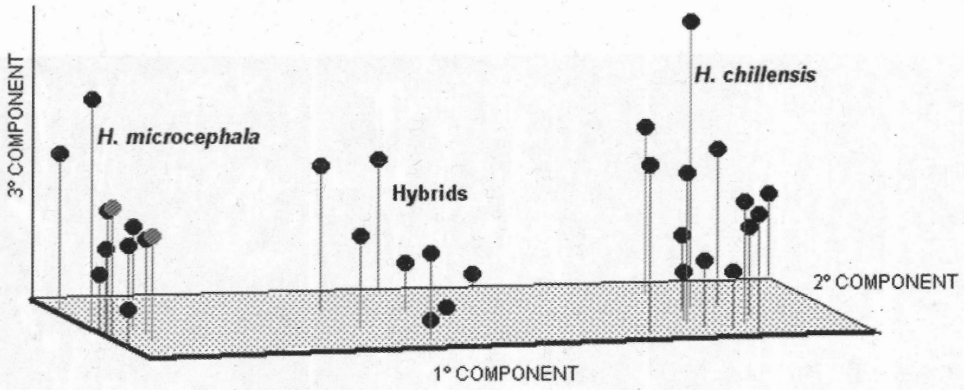

Fig. 3. First Principal Component Analysis (PCA) projection of the two *Hypochoeris* species and their putative hybrids.

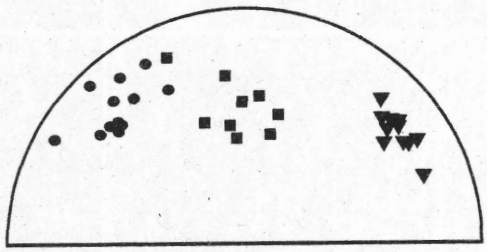

Fig. 4. Wells distance diagram of *H. chillensis* (●), *H. microcephala* var. *albiflora* (▼) and their putative hybrids (■).

Discussion and Conclusions

Hybridization results in intermediacy between parents in many characters. Most of the numerical methods are based in this feature to demonstrate hybridity (Anderson, 1953; Adams, 1982; Wilson, 1992). All of the analyses here performed coincided in showing the hybrid swarm as composed by hybrids *H. microcephala* var. *albiflora* x *H. chillensis*.

Pollen grain size was rather constant in *H. microcephala* var. *albiflora* while *H. chillensis* exhibits a wider range of size pollen grains. Range in hybrids includes all pollen grain sizes of both putative parents, the mean of the hybrids is smaller than the *H. microcephala* one, because most of hybrid grains were small. This feature may be related probably to deficiencies in their genomic composition derived of cryptic structural hybridity (Wulff, 1992).

Some characters of the hybrid pollen grains (punctitegilate tectum, presence of spines in paraporal lacunae, circular elliptic os) were shared with *H. chillensis*. Circular amb, curvature of supratectal spines, and equatorial ridges with single series of spines were traits shared with *H. microcephala*.

The surface of the achene in the hybrids showed intermediate features between those of the putative parents. The disposition of trichotomata on the beak apex, thickness of scales margins, and also average of cilia length were evidently intermediate.

Character Count Procedure was successfully used for inferring hybridity in Asteraceae (Bartoli & Tortosa, 1998a, b). Our results using this procedure indicated that the hypothesis of divergence must be rejected. The deviation was very significant ($P < 0.05\%$) and confirmed the hypothesis of hybridity.

Principal Component Analysis was used for inferring hybridity or to show swarm structure (Palacios *et al.*, 1991; Mollard *et al.*, 2000). The results here presented showed three groups of points without continuity between them and the intermediate individuals were located in intermediate positions into the 3-dimensional space delimited by the three first principal components. Accordingly, the intermediate individuals could be considered as F_1 hybrids.

Table 1. Character Count Procedure (Willson´s Method)

Character	x̄ *H.chillensis*	δ	x̄ Hybrid	δ	x̄ *H. microcephala*	δ	Intermediate
3	7.85	0.988	7.844	1.574	7.127	0.998	+
7	170.75	20.356	93.444	19.635	41.818	6.047	+
10	1.951	0.24	2.531	0.413	2.538	0.258	+
11	5.42	1.077	5.153	0.702	4.412	0.29	+
12	2.208	0.148	3.163	0.373	3.481	0.361	+
15	6.917	1.73	4.778	1.922	3.182	0.751	+
16	3.25	1.422	2.556	1.74	9.091	1.446	-
17	17.792	7.074	9.761	2.863	5.451	0.847	+
18	0.386	0.12	0.233	0.048	0.146	0.027	+
19	52.625	5.693	42.489	8.244	23.845	2.25	+
							9:1

In the Diagram Distance, individuals from each species constituted a different group and the putative hybrids were located in an intermediate area between them. The intermediate specimens were located into the semicircle that includes the specimens of *H. chillensis* and *H. microcephala* var. *albiflora*, another evidence confirming hybridization between both species.

All the numerical methods applied in this paper were coincident in showing unquestionable evidences of hybridization between *H. chillensis* and *H. microcephala* var. *albiflora.* Although the structure of the hybrid swarm is not clear yet, the hybrids seem to be F1, but Distance Diagram evidenced morphological continuity among the individuals of *H. chillensis* and the hybrids. This feature would be evidence of introgression.

Karyotypes of these species were first described by Saez (1949, 1951) and later by Siljak-Yakovlev *et al.* (1994), Ruas *et al.* (1995), and Cerbah *et al.* (1995, 1999). The karyotypes of these species have shown similarities and these have been reflected on a quasi-regular meiosis in their hybrids (Wulff, 1992). However, Siljak-Yakovlev *et al.* (1994), Ruas *et al.*, (1995), and Cerbah *et al.* (1995, 1999) have shown some chromosomal variations on *H. chillensis* karyotypes of different provenances that were never discussed.

On the basis of evidences here described, we are able to propose that these differences could be arisen, in part due to hybridization or/and introgression which could be promoted, like in *H. radicata*, for the presence of a perennial root system.

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