

# TWO CYTOTYPES AND A NEW HYBRID IN *Salvinia* SÉGUIER

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**ABSTRACT** - Two cytotypes ( $2n=4x=36$  and  $2n=6x=54$ ) found in *Salvinia minima* Bak. are discussed, the first from Brazil and the second from Argentina. The hexaploid cytotype, presumably a hybrid between *Salvinia minima* and *S. sprucei* Kuhn, was collected from the Solimões River near Manaus, Brazil and from Trinidad. Discussing its intermediate morphology, the authors attempt to explain the hybridization as a result of the seasonal and sporadic occurrence of *Salvinia sprucei* in the Amazonian basin, assuming that the still unknown chromosome number of the latter species would correspond to the diploid level ( $2n=2x=18$ ).

## Dois Citótipos e Um Novo Híbrido em *Salvinia* Séguier

**RESUMO** - Dois citótipos ( $2n=4x=36$  e  $2n=6x=54$ ) foram encontrados em *Salvinia minima* Bak., o primeiro deles proveniente do Brasil e o segundo da Argentina. O citótipo hexaplóide é, provavelmente, um híbrido entre *Salvinia minima* e *S. sprucei* Kuhn e foi coletado no Rio Solimões próximo a Manaus, Brasil e em Trinidad. Na discussão da morfologia intermediária, os autores tentam explicar a hibridização como resultado da ocorrência sazonal e esporádica de *Salvinia sprucei* na Bacia Amazônica, admitindo ainda que o número cromossômico não conhecido da última espécie, deveria corresponder ao nível diplóide ( $2n=2x=18$ ).

**Key-Words:** *Salvinia*-hybrids-Amazonia

While carrying on a comparative analysis of the neotropical species of *Salvinia*, we discovered some specimens showing morphological characteristics intermediate between *S. minima* and *S. sprucei* from Amazonian populations.

A hypothesis is advanced to explain the origin of the intermediate specimens.

## MATERIAL AND TECHNIQUES

Voucher specimens used for chromosome counts and also for clearing, staining and drawing leaf characters, are given below:

### *Salvinia minima*:

ARGENTINA: Provincia Buenos Aires, Isla Martín García, cantera,

08/01/1995, *Hurrellet al.* 2231 (LP) ( $2n=54$ ).

BRAZIL: Amazonas, near Manaus, Rio Solimões, Lago do Castanho, austral border (with *Salvinia auriculata*, *S. sprucei*, *S. minima* x *S. sprucei*?), 13/07/1976, (LP); idem, *Salvinia minima* Baker, *S. sprucei* Kuhn + specimens with intermediate morphology between both species, 13/07/1976, *de la Sota* 6352 *pro parte, b* (LP).

ENGLAND: Royal Botanic Gardens, Kew, Richmond, Surrey, Lower Nursery, *cult.* 10/11/1992, from plants collected in Brazil, Rio de Janeiro, Jacarepaguá, Parque Ecológico "Chico Mendes", 09/04/1992, *de la Sota* 7004 (LP, K) ( $2n=36$ ).

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### *Salvinia sprucei*:

BRAZIL: Amazonas, near Manaus, in Solimões River, Lago do Castanho, austral border (*Salvinia minima*, *S. sprucei*+ plants with intermediate morphology between both species), 13/07/1976, *de la Sota 6352, a & 2do. a* (LP), Amazonas, near Manaus, Paraná Xiborena, with *S. auriculata*, *S. minima* x *S. sprucei*?, 13/07/1976, *de la Sota 6349* (LP).

### *Salvinia minima* X *S. sprucei*?

BRAZIL: *de la Sota 6352, pro parte*(LP), *de la Sota 6353, pro parte*(LP); Amazonas, near Manaus, Paraná Xiborena, 13/07/1976, *de la Sota 6350*(LP); Amazonas, Amazon River, few km from the mouth of Rio Negro, 11/09/1974, *Conant 958, pro parte* (GH).

PERÚ: Dep. Loreto, Río Tepiche, Santa Elena, 16/10/1968. *Mc Daniel 11300 & Marcos, pro parte* (GH).

SWITZERLAND: Zürich, Botanical Garden, collected by Hall from Trinidad in 1977 and cultivated at the Botanical Garden in Zürich since Nov. 1977, 20/01/1982, *Schneller s/n* (Z) (n=27);

VENEZUELA: Edo. Falcón, marches of El Caballo and neighbourhoods 11° 04-05'N-69°16'W, 18/02/1977, *Steyermark & González 113783* (GH).

Material from Brazil and from Kew (*de la Sota 7004*) were pretreated with saturated 8-hydroxyquinoline at noon and left overnight at room temperature in the dark, fixed in alcohol-

acetic acid (3:1), refrigerated 1-2 days, washed in distilled water, 2 hours in 2% solution of cellulase in 1% acetic acid at room temperature in the dark, washed, hydrolyzed in 1N hydrochloric acid, washed, fixed again 1 hour in 3:1, stained in Feulgen and squashed in hot acetocarmin.

Another and simpler technique was employed for the Argentinian material (*Hurrell et al. 2231*). It was pretreated at noon with saturated 8-hydroxyquinoline for 2 hours at room temperature, fixed in 3:1 24 hours at room temperature, squashed and stained with acetic hematoxyline (Núñez, 1968).

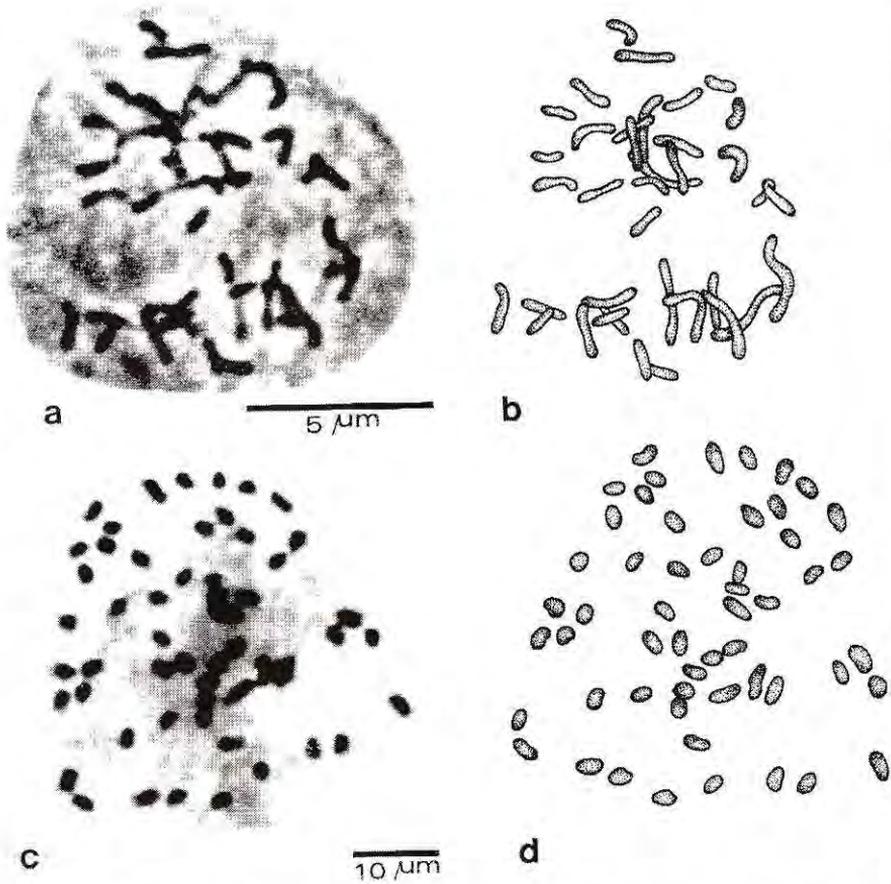
In the first case, the slides were photographed with a Zeiss phase contrast microscope. In the second one, a Nikon Labophot 2 photomicroscope was used.

For the hystological analysis of the hemiblades and their optical sections, dry material was rehydrated in boiling water plus a few drops of non-ionic detergent, cleared and bleached with OHK 5% and commercial NaOCl and stained with tannic acid and iron chloride (Foster, 1934). For observations and drawings, a Willd M5 stereomicroscope and a Willd M20 microscope were used.

## RESULTS

### - Two mitotic chromosome counts in *Salvinia minima*

*Salvinia minima* is a native species of America, distributed from Hermepin Co., Minnesota, USA, to Chascomús, Pcia. Buenos Aires, Ar-



**Figure 1.** *Salvinia minima* Bak. a-b., mitotic metaphase with 36 chromosomes (de la Sota 7004(LP,K)); c-d, mitotic metaphase with 54 chromosomes (Hurrell et al. 2231(LP)).

gentina, that is, from 47°NL to 35°SL. This species was introduced and subspontaneous? in Africa (Senegal: Dakar, sending v. *A. Sanokho* aus Dakar, (Z); Zaire, Boma, Pont sur la Lukunga vers son embouchure avec le Bas-Fleuve, *Lebrun 11190* (K); Léopoldville, Boma, Luki, *Wagemans 2463* (K); Boma, Rivière Lukunga au pont de la route Boma-Banana, *Wagemans 1830 & 1848* (K)).

We determined two mitotic chromosome counts for this species:

$2n=4x=36$  for the Brazilian plants and  $2n=6x=54$  for the Argentinian ones. The chromosome count of  $2n=5x=45$  for a probable pentaploid hybrid was also reported for *S. minima* (Schneller, 1980).

**- An intermediate morphology between *Salvinia minima* and *S. sprucei***

Analysing the leaf histology and the indument of a Brazilian population of *Salvinia minima* and specimens from Trinidad cultured in the Botanic

Garden at Zürich, one of us (the senior author) found a gradual intermediate location of the leaf papillae and also length of hairs (Tab. 1).

Since the material was sterile, special attention was given to leaf characters.

In the figure 2A, *Salvinia minima* exhibited a regular distribution of the papillae covering the whole surface of the blade, the larger ones being found along the midrib whereas the smaller ones near the margin. Its size, shape and hairs on the top of each papilla, free from one another in the same papilla but intermingled with the hairs from the neighbouring ones, confirmed data given previously (de la Sota, 1963; de la Sota & Jankowski, 1996).

In *Salvinia sprucei*, fig. 2B, its blade is hooded with small papillae, confined to the margin (1-3 rows covering ca. 1/5 of the leaf surface (Tab. 1) and with short free hairs, as already described by the senior author (de la Sota, 1964).

The populations from the Amazonian basin (*de la Sota 6352 p.p.*, *idem 6353 p.p.*, *ibidem 6350*) and from Trinidad (*Schneller, s/n*), in which concerns the size and distributional pattern of the papillae (from 20 to 50% of the leaf surface, *cf.* table 1) and the shape and size of their hairs, completely free (fig. 2AxB), show intermediate characters between *Salvinia minima* and *S. sprucei*. The plants (*Schneller, s/n*) from Trinidad are closer to *Salvinia*

*sprucei* than to *S. minima*, with regard to the shape and venation of their leaves.

As it can be seen in the figure 2, L1-L7, the leaves of the three *taxa* were comparatively analysed through optical sections. Only three significative differences were observed:

-The outline of the abaxial epidermis cells (L1): it appears as intermediate in the presumptive hybrid between *Salvinia minima* (A) and *S. sprucei* (B).

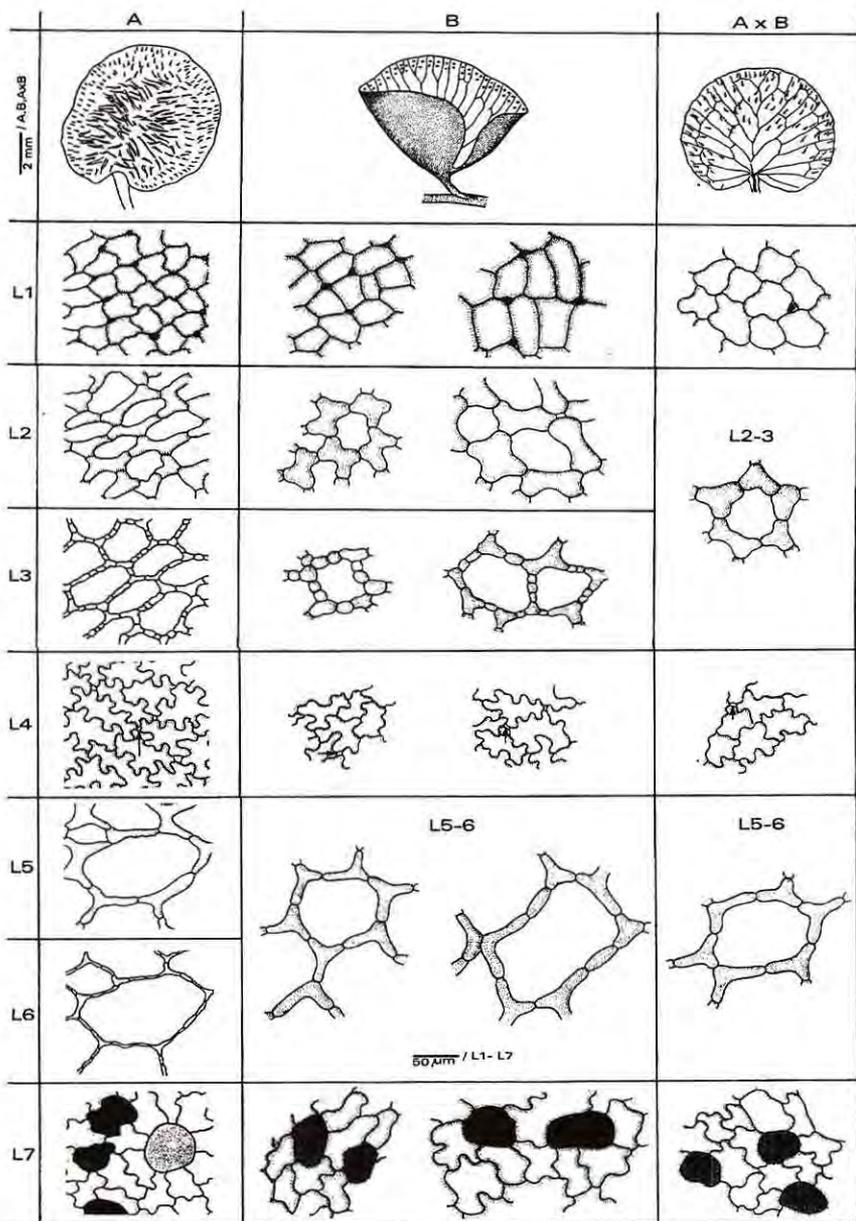
-The wall of the upper air chambers: there is only one cellular level in the presumptive hybrid (L2), the parents having two levels (L2 and L3).

-The walls of the lower air chambers: only in *Salvinia minima* it was possible to differentiate 2 levels or sublevels of construction (L5 and L6).

The two examples illustrated for *Salvinia sprucei* under B of figure 2, show a little difference in size and only in size and shape in the case of upper epidermis (L1). However, both cellular outlines belong to the same type, neither waved nor amoeboid, as in *Salvinia minima*.

## DISCUSSION AND CONCLUSIONS

The intermediate leaf morphology (shape of the blade, distribution and development of the papillae and hairs and the outline of the upper epidermal cells, as it has been shown in



**Figure 2.** A-AxB, shape of the leaf and distribution of the papillae. A, *Salvinia minima* Bak., *Salvinia sprucei* Kuhn, AxB, *Salvinia minima* x *Salvinia sprucei*. L1-L7, optic sections of the leaf; L1, upper epidermis; L2, upper level of the dorsal air chambers walls; L3, lower level of the dorsal air chambers walls; L4, floor between dorsal and ventral air chambers, with an arrow showing intercellular space; L5-L6, upper and lower levels of the ventral air chambers walls; L7, lower epidermis with hydroptic cells (dotted). A and its row L1-L7, from de la Sota 6353(LP); B and both rows L1-L7, from de la Sota 6352(LP); AxB and its six rows, from Hall s/n(Z). L.A.Cassà & V.h.Calvetti del.

several plants from Brazil, Perú, Trinidad and Venezuela) are indications of a hybrid condition. The fact notwithstanding that the viewpoint advanced by Cassá de Pazos (1994), introduces some doubt about the taxonomic value of histological characters. It would be interesting to apply in the near future clonal methods to prove her point. Anyway, the straight or waved patterns in the outline of the upper epidermic cells in *Salvinia* are diagnostic characters.

The hybridization between both taxa would be in part supported by the chromosome numbers known at present: *Salvinia minima*,  $2n=36$  and  $54$ ; *Salvinia* sp.,  $n=27$  showing normal meiosis in the microsporangia, and fertile spores forming gametophytes (cf. Schneller, *s/n*, determined by him as *Salvinia minima*).

It seems to us that the specimens from the NW of South America would be hexaploid, originated as a result of a crossing between *Salvinia minima* ( $4x$ ) and *S. sprucei* ( $2x$ ), followed by chromosome doubling. It depends, however, on the supposition that the last taxon is really a diploid.

The specimens of the hybrid collected in the Solimões River near Manaus, and the connection between this river and Rio Negro, (showing intermediate morphology in their leaf characters, distribution and size of the papillae-hairs) make it feasible the possible hybridization between *Salvinia minima* (which is very frequent in the Solimões) and the sporadic and seasonal occurrence of *S. sprucei*. Moreover, it could explain

why Spruce found during the summer of 1851 the type specimen of this taxon (Brazil: *ad oram merid.flum Amazonum at vet. fl. Solimões*, June 1851, *Spruce 1636* (BM,K,P)). The presence of *Salvinia sprucei* in the Amazonian basin is rare and seasonal because only during the rainy season (winter) some plants can travel, through the Casequiare channel, from the Orinoco to the Amazonian basin.

Surely, only few plants can reach alive the "whitewater" of the Solimões, after their long journey along the Rio Negro ("blackwater") which is extremely poor in nutrients (Sioli, 1950) and is characterized by an acid pH of around 5 (Junk & Howard-Williams 1984:277). It is likely that the few individuals reaching the Solimões-Amazonian, where *Salvinia minima* is found covering the water surface, could hybridize with the latter and the hybrids then repeatedly backcross. *Salvinia sprucei* is really little frequent in the Amazon River and eventually disappears from there. So, when the senior author was looking for this species in January of 1960, just in the type locality, he could not find any plant. But several years later, in July 1976 and exactly in the same place, he found this taxon mixed in a low percentage with *Salvinia minima* and also with intermediate forms between both species.

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Data Number	A	B	C	D	E	det.
6352 2 <sup>a</sup> a	3	1	20	1	NC	s
6352 a	9	2	20	3-20	15	s
6349	7	3	20	2-5	5	s
6352 b	7	NC	100	13-25	13-35	m
6352 1 <sup>a</sup> a	3	3	20	6-10	NC	m x s
6350	4	NC	50   100	20-25	15-40	m x s
6352 b	8	NC	100	18-30	15	m
6353	7	NC	100	40-45	14	m

**Figure 3.** Ramdon samples from the Amazonian population where the hybrid was detected. References: Number:herbarium specimens collected by *de la Sota*. Data: measured characteristics, A, number of papillae; B, rows of papillae; C, cover percentage of the hemiblade by papillae(20-100%); D, height of the papillae; E, length of the hairs; det.(determinations) s, *S. sprucei*, M, *S. minima*, m x s, *S. minima* x *S. sprucei*.NC: not counted. These lectures have been taking from squares of 3250 mm<sup>2</sup>, observed 50x. The sizes given in D and E relatives and taken from the original drawings.

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