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Infraspecific characterization and chromosome numbers in *Anredera cordifolia* (Basellaceae).

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ABSTRACT: Two infraspecific taxa in *Anredera cordifolia* are cytotaxonomically studied: *Anredera cordifolia* (TEN.)STEEN. subsp. *cordifolia* (2n=36) and *A. cordifolia* subsp. *gracilis* (MIERS) XIFREDA & ARGIMÓN (2n=24). Valuable characters to recognize it comprise vegetative morphology, occurrence of fruit with seed production, pollen grain sizes and exine sculpture, and ploidy level.

KEYWORDS: Basellaceae, *Anredera cordifolia*, chromosome numbers, ploidy level, pollen grains, infraspecific characterization.

Introduction

Anredera cordifolia (TEN.)STEEN. was described by TENORE (1853) as *Boussingaultia cordifolia* from material collected in Buenos Aires, Argentina, that was growing at the Real Orto Botanico di Napoli, Italy. The plant has been introduced to Naples, possibly by aerial or basal tubers, several years before by AMADO BONPLAND (TENORE 1833) who was living in Buenos Aires at the house of PIETRO DE ANGELIS, editor and friend of MICHELE TENORE (GUARINO & al. 1992). There can be no doubt, however, about the origin of the plant referred to by Tenore, for it still grows in the area were it was introduced. Herbarium sheets

located at NAP were studied and the scientific name was also recently typified (GUARINO & al. 1992). Since TENORE described this species based upon cultivated collections from Argentina, the new name *B. gracilis* has been created for MIERS (1864) based on wild plants collected at Sao Paulo, Brazil.

The new species has been previously wrongly determined by HOOKER (1837), as the peruvian entity *Boussingaultia baselloides* HBK. It was described and drawn from materials introduced to England from Buenos Aires by Tweedie (HAUMAN 1925). That incorrect identification has involved into taxonomic confusion. Most earlier authors had applied the name *B. baselloides*, or the combination *Anredera baselloides* in HOOKER'S sense, in nearly all contributions to literature.

The taxonomic problems of the group were partially recognized by HAUMAN (1925), who in discussing the taxa, has ignored *B. cordifolia* but pointed out morphologic variability under the name *B. gracilis*. He described the new forma *pseudo-baselloides* on the base of cultivated plants, and cited HOOKER'S plate 3620 in synonymy. VAN STEENIS (1957) transferred the nomenclature to *Anredera cordifolia*, studying cultivated materials from Malaysia, and considered the differences on pistil morphology a rather variable character. He and subsequent writers of regional floras, failed to ignore the formation of fruits or considered the taxon as sterile, or pointed out "fruit unknown" (BAILEY 1949, HATSCHBACH 1974, REITZ 1968, TRONCOSO 1987, VAN STEENIS 1957, VILLA CARENZO 1966, ZAPATER 1991). A new attempt to clarify *A. cordifolia* was that of SPERLING (1987) in a world-wide revision. Sperling determined that fruits with mature seeds were rarely produced and that the plant seemed to spread about by tubers. He also ascribed himself the first description of the fruit. Indeed, MIERS (1864) was the author who first described fruits and seeds, frequently found in wild plants. Variations in floral morphology and fruiting specimens are common in the natural area of dispersion, the southern South America (Bolivia, Paraguay, Brazil, Uruguay and Argentina).The plant has been spread about from South America by Spanish and Portuguese traders at an early date. Ornamental and medicinal, it is extensively cultivated and spontaneous in subtropical gardens of the world.

With the aim of clarifying the systematics and to contribute to the knowledge of the relationships between both wild and cultivated strains in *A. cordifolia,* we performed morphological observations and karyological studies.

A new combination was established and the name *Anredera cordifolia* (TEN.)STEEN. subsp. *gracilis* (MIERS)XIFREDA & ARGIMÓN was recently published (XIFREDA 1999). The results that involve studies on the chromosome numbers and morphological recognition at the infraspecific level are here presented.

Materials and Methods

Mitotic studies were performed on root-tips from germinating seeds or aerial bulbils and basal tubers. Both kinds of materials were semi-submerged in water. The root-tips obtained were pretreated at 20-22° C for 2 hours with a saturated

solution of alfa-bromonaphtalene and fixed in 3:1 (absolute alcohol: glacial acetic acid) for more than 12 hours at 4° C. After hydrolysis in ClH 5N for 10 minutes, staining and squashing were made in 45 % propionic acid haematoxylin (2 %), ferric citrate (1%) was used as mordant (NUÑEZ 1968). Mounting was made in DPX (Fluka) after removal of the coverslip. Meiotic studies were undertaken in young inflorescences previously fixed in Newcomer's modified fixative (HUNZIKER 1966). The squash was made as in mitosis. Chromosome morphology was described according to the nomenclature of LEVAN & al. (l964). Consequently the chromosomes were divided into two groups: m chromosomes (centric index: i = small arm length x 100 / total chromosome length = 50-37.5) and sm chromosomes (i = 37.5-25).

Pollen fertility was determined by ALEXANDER stain (1969). Scanning electron microscopy of pollen grains and fruits were carried out at the SEM Service of La Plata Museum, Argentina. Samples were mounted onto SEM stubs using double-side adhesive tape, coated with gold using Jeol JFC-1100 ion sputter coater and subsequent examination was with a Jeol JSM-T100 SEM.

The localities where fresh materials were collected, the collectors and the herbaria where the voucher specimens were deposited are indicated in Table 1.

Results and Discussion

Wild forms and cultivated forms have been recognized taxonomically. Documented names and synonymy are as below indicated.

1. ***Anredera cordifolia*** (TEN.) STEEN. subsp. ***cordifolia,*** Flora Malesiana ser. 1, 5 (3): 303-304. 1957.

≡ *Boussingaultia cordifolia* TEN., Ann. Sc. Nat. 3, 19: 355. 1853. Lectotype: Culta Hort. Naples, Coll. Tenore (NAP!).

= *Boussingaultia gracilis* f. *pseudobaselloides* HAUMAN Anales Mus. Nac. Hist. Nat.. Buenos Aires 33: 355. 1925. Lectotype (**here designated**): Buenos Aires, environs de la capitale. Tweedie ex Herb. Hooker (K).

≡ *Boussingaultia gracilis* var. *pseudobaselloides* (HAUMAN) BAILEY, Man Cult. Pl.: 368. 1949. LAWRENCE, Gentes Herb. 8 (1): 28. 1949.

– *Boussingaultia baselloides* auct. non HUMB., BONPL. & KUNTH: HOOKER, Bot. Mag. 64, tab. 3620. 1837.

– *Anredera baselloides* auct. non (HUMB., BONPL. & KUNTH) BAILLON: BAILLON, Hist. Pl. 9: 147, 208-210. 1887.

2. ***Anredera cordifolia*** (TEN.) STEEN. susbsp. ***gracilis*** (MIERS) XIFREDA & ARGIMÓN, Cat. Plant. Vasc. Rep. Argentina. II Dicotiledóneas, Apen. 1: 1245. 1999.

≡ *Boussingaultia gracilis* MIERS, J. Bot. 2:161, tab 18. 1864. Type: Brasil, Prov. S. Paulo, Weir 486 (Holotype BM, isotypes K, F).

Table 1. List of the *Anredera cordifolia* materials studied, indicating probably ploidy level, number of cells analyzed, meiotic configurations and pollen fertility, procedence and voucher number. Collectors: FTOM: Federico Teodoro Otto Mollard, AMS: Andrea Mariel Sanso, AFW: Arturo Federico Wulff, CCX: Cecilia Carmen Xifreda.

Ploidy Level	N° of analyzed cells (mitosis)	N° of analyzed cells (meiosis)	Meiotic configurations (II, III, I)	Fertility	Procedence
	Anredera	*cordifolia*	subsp. *gracilis*		
2x	8	22	II	84-97%	Prov.Salta,Depto. Capital, Co. San Bernardo, CCX 1714 (SI)
	5	20	II	"	Prov. Buenos Aires, Pdo. Monte, San Miguel del Monte, FTOM s/n (BAFC)
	4	18	II	"	Prov. Salta, Depto. Capital, Co. San Bernardo, CCX 1713 (SI)
	10	28	II	"	Prov. Buenos Aires, Cap.Fed. Villa del Parque, CCX 732, 331 (SI)
	8	20	II	"	Prov. Jujuy, Depto. Santa Bárbara, a 5 km del Fuerte de Santa Bárbara, CCX 922 (SI)
	25	-	-	"	Prov.Tucumán,Depto.Capital, Avda. Mate de Luna, CCX & AMS 1053 (SI)
	-	25	II	"	Prov. Buenos Aires, Pdo. Avellaneda, Dominico, AFW 802 (BAFC)
	7	-	-	"	Prov. Jujuy, Depto. Capital, entrada a Mina 9 de Octubre, antes del río Zapla. CCX & AMS 899 (SI)
	20	25	II	"	Prov. Buenos Aires, Pdo. Lomas de Zamora, Lavallol, AFW 800 (BAFC)
	-	22	II	"	Prov. Buenos Aires, Pdo. Lanús, Lanús, AFW 801 (BAFC)
	Anredera	*cordifolia*	subsp. *cordifolia*		
3x	7	-	-	15-24%	Prov. Tucumán, Yerba Buena. CCX 1710, (SI)
	12	-	-	"	Prov. Tucumán, Yerba Buena. CCX 1711 (SI)
	14	28	III (9,34 ± 1.85) II (2,64 ± 2.22) I (1,31 ± 1.04)	"	Prov. Buenos Aires, Cap. Fed., Palermo Viejo, CCX 2006 (SI)
	-	29	III (9,11 ± 1.65) II (3,33 ± 2.25) I (2,77 ± 2.01)	"	Prov. Buenos Aires, Pdo. Gral. Sarmiento, Del Viso, AFW 804 (BAFC)
	10	-	-	"	Prov. Buenos Aires, Pdo. Avellaneda, Villa Dominico, AFW 805 (BAFC)
	10	-	-	-	Prov. Entre Ríos, Depto. Villaguay, Villaguay, FTOM s/n (BAFC)

Observations on *Anredera cordifolia* subsp. *cordifolia*

It is an extensively cultivated and spontaneous plant in tropical gardens of the world, recognized as a perennial herb with notably vegetative reproduction, propagated by the abundantly produced tubers at the base of the stems or in large clusters on leaf axils of old stems. The success of the invasive dispersal is the bulbilifery. The plants are also distinguished by the long leafy stems, succulent mucilaginous leaves, large and thickened, denses inflorescences, large flowers 4.5 - 6 mm diameter, grown on long pedicels.

Pollen grains are spheroidal (Fig. 1 B), 6-pantocolpate. Some pollen grains are irregularly pantocolpate, tectum spinulose and annular perforate or punctate (Fig. 1 D). The collection studied from Niue Island shown in NOWICKE (1996, Figs.9-10) with many irregularly pantocolpate grains and diameter size (43) 39-49 µm, belongs to this subspecies.

This cultivated bulbiliferous subspecies shows 2n=36 (Fig. 2 C) with a karyotype formulae of **30 m + 6 sm** (Fig. 3 B). The majority of the cells show at metaphase I only trivalents (12 III). The remaining cells show not only trivalents but also bivalents (II) and univalents (I) (Fig. 2 B, Table 1). The irregularity observed leads predominantly to aneuploid gametes and to virtual sterility reflected in the production of abnormal pollen grains (Table 1) and absence of seeds. It should be cytological recognized as an autotriploid. A similar behaviour is found in another representative of the family, *Ullucus tuberosus* CALDAS (PIETILA & al. 1990).

Observations on *A. cordifolia* subsp. *gracilis*

Embryological studies by ROCÉN (1927, sub *A. baselloides*) and POZNER & MALDONADO (1989), in an analysis of the sporogenesis and the gametophytic structure, did not reveal irregularities that might prevent the formation of fruits. In fact, an exhaustive analysis of alive plants and herbarium sheets, give evidence of the presence of fruit. The fruit is a small indehiscent globose capsule (Fig. 1 F, G) that encloses a unique seed with farinaceous perisperm and two annular cotyledons. The dispersal unit is the small utricle with the marcescent floral envelopes. Similarly, fruit presence in *Ullucus tuberosus* was appropriately pointed out by ROUSI & al. (1988).

These susbspecies´s plants show distinguished small flowers. SPERLING (1987: 193) cites that these small flowered plants from natural areas have been taxonomically recognized as *Boussingaultia gracilis* MIERS.

Pollen size, exine sculpture (Fig.1, A, C. E) and fertility (Table 1) differ from the cultivated plants (Fig. 1 B, D). Here belongs the results obtained by NOWICKE´S (1975, Fig. 61) studying pollen materials from Brazil.

Somatic chromosome number was 2n=24 with a karyotype formulae of **20 m + 4 sm** (Fig. 2 D, Fig. 3 A). Both ploidy levels have similar chromosome sets. Meiosis was regular, (Fig.2 A) showing twelve bivalents at metaphase I.

Other species of the family show a probable basic number of twelve or six (x=12 or x=6) (BRÜCHER 1977, LARKA & al. 1992). Studies of DNA content on representatives of each taxon are in progress for clarification of the pattern of infraspecific variation established in this work (WULFF & al. in prep.).

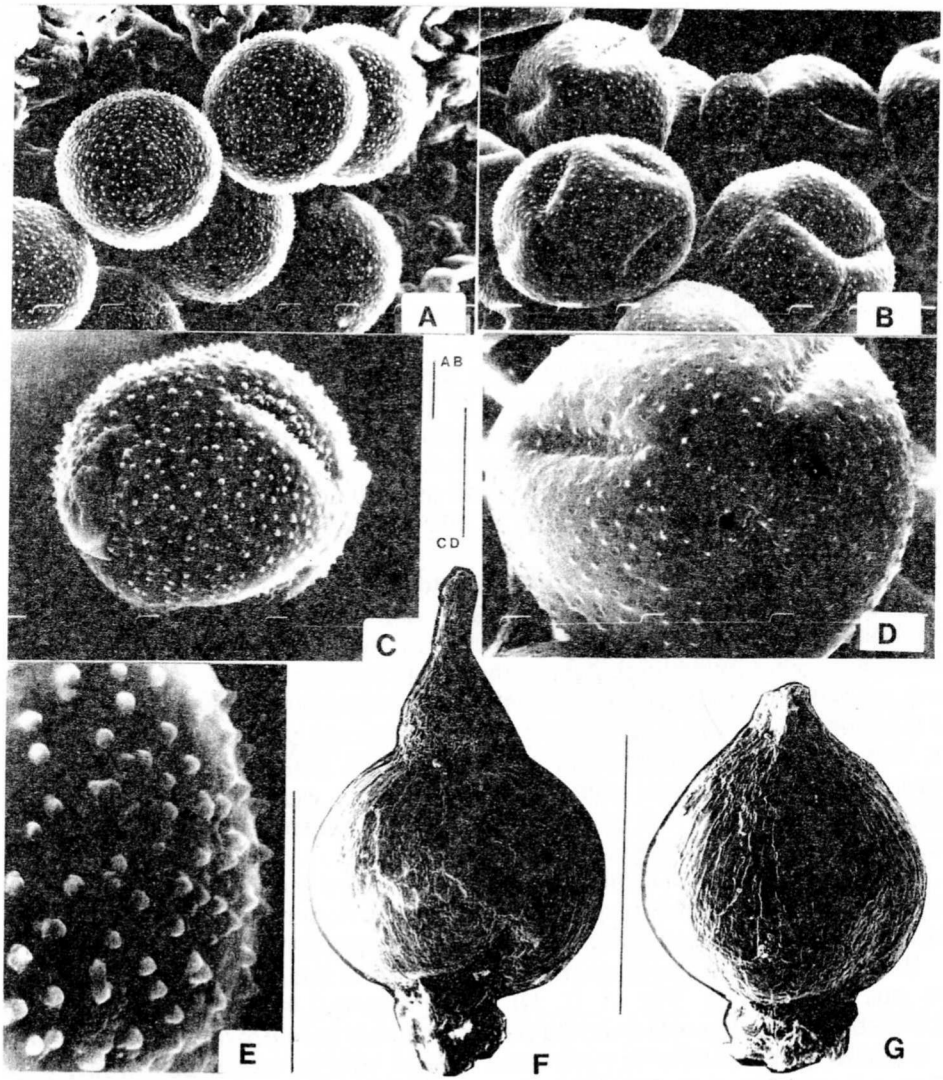

Figure 1. Pollen grains and fruits. *Anredera cordifolia* subsp. *gracilis*, CCX 732 (A, C, E, F, G). *A. cordifolia* subsp. *cordifolia*, CCX 2006 (B, D). A, B: pollen grains. C, D, E: pollen grain exine. F: inmature fruit. G: fruit. Scale A-E 10 μm; F-G 1 mm

Figure 2. Chromosome numbers. *Anredera cordifolia* subsp. *gracilis*: 2n=24 (A, D). *A. cordifolia* subsp. *cordifolia*: 2n=36 (B, C). Meiosis: A, B. Mitosis: C, D. Empty triangles: trivalents (III), arrows: bivalents (II), full triangles: univalents (I). Scale 10 μm.

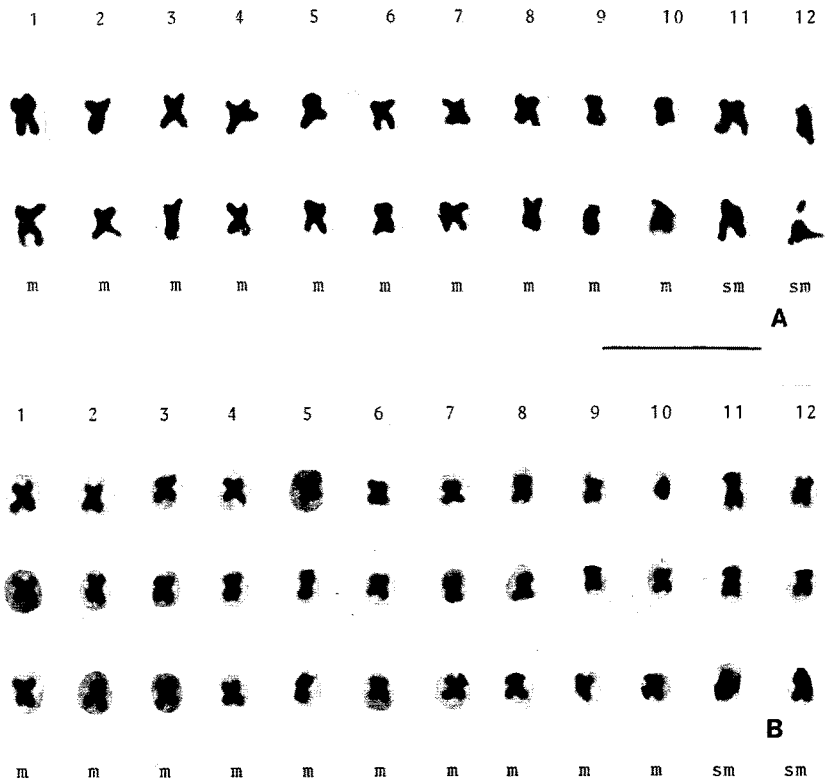

Figure 3. Karyograms. *Anredera cordifolia* subsp. *gracilis* (A). *A. cordifolia* subsp. *cordifolia* (B). Scale 10 μm.

Conclusions

This contribution points out two ploidy levels in *Anredera cordifolia:* 2n=36 in world wide vegetatively dispersed plants of *A. cordifolia* subsp. *cordifolia* and 2n=24 in *A. cordifolia* subsp. *gracilis*, wild plants with fruits and sexual reproduction. We are of the opinion that an useful purpose was served by maintaining taxonomic recognition to observed variation, and we believe that should be maintained as a species with two infraspecific taxa. Chromosome number is in correspondence with the sizes of vegetative organs and flowers, the pollen grains size and sculpture of the exine in dimension of the spicules and

density of them. Basellaceae family shows a similar correlation between chromosome number and morphology in *Ullucus tuberosus*. It were recognized two infraspecific taxa, at subspecies level for both wild and cultivated plants of *U. tuberosus*. The research on *Anredera* may also contribute to improve the breeding work on edible ulluco.

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