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Karyological investigations on several species of genus *Rebutia* Sect. *Digitorebutia* (Cactaceae)

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Abstract — These are the first avaiable data about the karyology of the taxa belonging to *Rebutia* Sect. *Digitorebutia,* the section of genus *Rebutia* K. Schum. with more species. Eight species of Sect. *Digitorebutia* were investigated. Out of the species studied, seven (*R. pygmaea*, *R. raulii*, *R. steinmannii, R. major*, *R. diersiana*, *R. haagei* and *R. gavazzii*) are polyploid, with tetraploid chromosome number 2n=*4x*=44, and one (*R. leucanthema*) is diploid, with chromosome number 2n=*2x*=22; *x*=11. This is the first karyotype information of all the species investigated.

Key words: Cactaceae, chromosome number, *Rebutia* Sect. *Digitorebutia*, taxonomy.

INTRODUCTION

Digitorebutia (Fric and Kreuz. ex Buining) Buining and Donald is the section of genus *Rebutia* K. Schum. with more species as currently circumscribed, that is about fifty taxa. Most of them are recognized at the species level (Mosti 1999, 2000a, 2000b; Mosti and Papini 2005). This section is characterized by style and stamen filament partially fused with the internal side of the floral tube (Donald and Brederoo 1975). The filament and the style are instead completely cohalescent with the tube in *Rebutia* Sect. *Aylostera* (Speg.) Buining and Donald and completely free in *Rebutia* Sect. *Cylindrorebutia* Buining and Donald and in *Rebutia* sect. *Setirebutia* Buining and Donald. In all these four sections the floral tubes show externally a hairy epidermis (in *Aylostera* even bristles); in *Rebutia* sect. *Rebutia*, instead the external suface of the floral tube is naked and filament and style are completely free as in *Cylindrorebutia* and in *Setirebutia*. The species of *Digitorebutia* are often composed by few or very few populations located at medium-high or high altitudes (3000-4000 m) of the andean region of Bolivia and Argentina. Hence these species grow genetically isolated by physical and geographical barriers. After Mosti (1999; 2000a; 2000b) and Sida (1997), these species are characterized by evident characters such as color and shape of the floral parts, the number of ribs, the features of the areola and the spines equipment. Each taxon is often well characterized even by microcharacters such as the scupltures on the seed testa cells (Mosti 1999, 2000a, 2000b; Mosti and Raffaelli 2003) and the stigma morphology (Mosti *et al.* 2010). Hunt (2006), recognized as "good" species of section *Digitorebutia,* in his checklist only *R. pygmaea* (R. Fries) Britton and Rose, *R. ritteri* (Wessner) Buining and Donald and *R. steinmannii* (Solms-Laubach) Britton and Rose of all those reviewed by Mosti (1999, 2000a, 2000b), hence producing a long list of synonyms. However Hunt's (2006) restrictive treatment was not based on experimental data, neither morphological or biomolecular investigations. Hence we preferred to maintain Mosti's (1999; 2000a; 2000b) taxonomic view. Within *Digitorebutia* it is possible to recognize three large groups related to, respectively, *R. atrovirens* (Backeberg) Sida, *R. pygmaea* and *R. steinmannii*. In fact, Rausch (1986), the main collector and discoverer of these plants, related to these taxa most of the currently recognized taxa of

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Digitorebutia, mainly described at the infraspecific level. On the other hand RAUSCH (1986), after a first description of these taxa as belonging to genus *Rebutia*, preferred to insert *Digitorebutia* nested within genus *Lobivia*, currently synonymized with *Echinopsis* (HUNT 2006). The insertion of *Digitorebutia* in *Lobivia* was not accepted by other authors (SIDA 1997; MOSTI 1999, 2000a; 2000b; MOSTI and PAPINI 2004; HUNT 2006).

The taxa considered by RAUSCH (1986) closely related to *R. pygmaea* (i.e. *R. colorea*, *R. diersiana*, *R. friedrichiana*, *R. minor*, *R. polypethala*, *R. violaceostaminata*) and to *R. haagei* (i.e. *R. canacruzensis*, *R crassa*, *R. eos*; *R. nazarenoensis*, *R. pallida*, *R. pelzliana*, *R. orurensis*), all together here called the "pygmaea group", are generally characterized by well visible and straight ribs, and radial spines that are frequently short and running parallelly to the shoot surface (adpressed). The taxa belonging to the "steinmannii group" (i.e. *R. steinmannii*, *R. applanata*, *R. christinae*, *R. cincinnata*, *R. major*, *R. parvula*) own generally radial spines outwards oriented, on average quite long with respect to the rest of the section and often twisted. The taxa belonging to the "atrovirens group" (i.e. *R. atrovirens*, *R. haefneriana*, *R. huasiensis*, *R. yuquinensis*, *R. pauciareolata*, *R. pseudoritteri*, *R. raffaellii*, *R. raulii*, *R. ritteri*, *R. zecheri*) have an equipment of radial spines with features that are intermediate between those of the previous groups, while sometimes the ribs run spirally (a character common to many species of sect. *Aylostera*). The ribs are often formed by well distinguishable tubercles. Tepals color: in the "atrovirens group" the color tones changed from red-orang to a more or less intense red, to more or less violaceous, while in the "steinmannii group" the taxa have tepals changing from orange to red. In the "pygmaea group", including in our treatment both *R. pygmaea* and *R. haagei* (with taxa related to both), the color tones are more extended, comprising also pink and yellow, besides those already present in the other two groups. Moreover the filaments in "atrovirens group", are always red.

Finally in *Digitorebutia* there is a group of species, i.e. *R. leucanthema* Rausch, *R. mixticolor* Ritter and *R. nigricans* (Wessner) Hunt, with distinctive features that do not allow to insert them neither in one of the above cited groups, nor in a fourth group that may comprise all of them.

The aim of this investigation was to analyse the chromosome number in diploid phase and the karyotype of eight species belonging to *Rebutia* Sect. *Digitorebutia*. Among these species four (*R. pygmaea*, *R. diersiana*, *R. gavazzii* and *R. haagei*) belong to the "pygmaea group"; two species [*R. steinmannii* and *R. major* (Rausch) Sida] to the "steinmannii group", one species (*R. raulii* Rausch) to the "atrovirens group", while one species (*R. leucanthema* Rausch) cannot be inserted in any of the above mentioned three groups.

Most chromosome counts in Cactaceae are available for genera *Echinocereus, Opuntia* and *Mammillaria.* In *Echinocereus*, chromosome counts are available for 23 (COTA and PHILBRICK 1994) of the 48 recognized species (TAYLOR 1985); 19 species are diploid (2n=22) and 4 tetraploid (2n=44). In a sample of ten species of *Melocactus*, five resulted diploid and five tetraploid (Das and Mohanty 2008). In *Mammillaria* (DAS *et al.* 1998; DEL ANGEL *et al.* 2006; BRIONES *et al.* 2004) the investigated species resulted all diploid (2n=22). Even studies in *Ferocactus* individuated only diploid species (DAS et al. 2000). In general about 30% of the investigated species of Cactaceae showed a polyploid karyotype (PINKAVA *et al.* 1985). Polyploidy was found in 20 genera of the family (COTA and PHILBRICK 1994). The occurrence of the basic number x=11 in most genera of the Cactaceae would indicate that polyploidy is probably a derived condition (COTA and PHILBRICK 1994). Triploids, tetraploids and hexaploids have been documented in the family (PINKAVA and PARFITT 1982; 1988; PINKAVA *et al.*

TABLE 1 — Nomenclature for centromeric position on cromosome (LEVAN *et al.* 1964) expressed as a ratio: long arm/short arm = **R**.

Term	R value	Centromeric location
M	1	median point
m	1.0 - 1.7	median region
sm	1.7 - 3.0	submedian region
st	3.0 - 7.0	subterminal region
t	7.0 - ∞	terminal region
T	∞	terminal point

Fig. 1 — *Rebutia pygmaea* (R. Fries) Britton and Rose (A). *Rebutia raulii* Rausch (B). *Rebutia steinmannii* (Solms-Laubach) Britton and Rose (C). *Rebutia major* (Rausch) Sida (D). *Rebutia diersiana* Rausch (E). *Rebutia haagei* Fric and Shelle (F). *Rebutia gavazzii* Mosti (G). *Rebutia leucanthema* Rausch (H).

1985). *Opuntia* is the genus of Cactaceae in which the highest frequency of polyploids has been reported, that is 20% of the investigated species (PINKAVA *et al.* 1985). Species of *Opuntia* resulted diploid, tetraploid and pentaploid (BANDYOPADHYAY and SHARMA 2000), three species resulted octoploid (PALOMINO and HERAS 2001), while *Opuntia rubescens* was the only species found to be decaploid (KATAGIRI 1953). Tetraploidy is however the most frequent form of polyploidy in the Cactaceae (PINKAVA *et al*. 1985).

Currently no data is available about the karyology of the taxa belonging to *Rebutia* Sect. *Digitorebutia.* ROSS (1981) published the chromosome number (both 2n = 44) of two species of Sect. *Aylostera: Rebutia kupperiana Boedeker* and *R. spegazziniana Backeberg.* The only other information in genus *Rebutia* (in our knowledge) is about two other species of Sect. *Rebutia*: *R. minuscula* K. Schum. and *R. violaciflora* Backeberg, both diploid (2n = 22).

MATERIALS AND METHODS

Specimens - The employed living specimens originated directly, by vegetative propagation, from the original specimens collected by WALTER RAUSCH

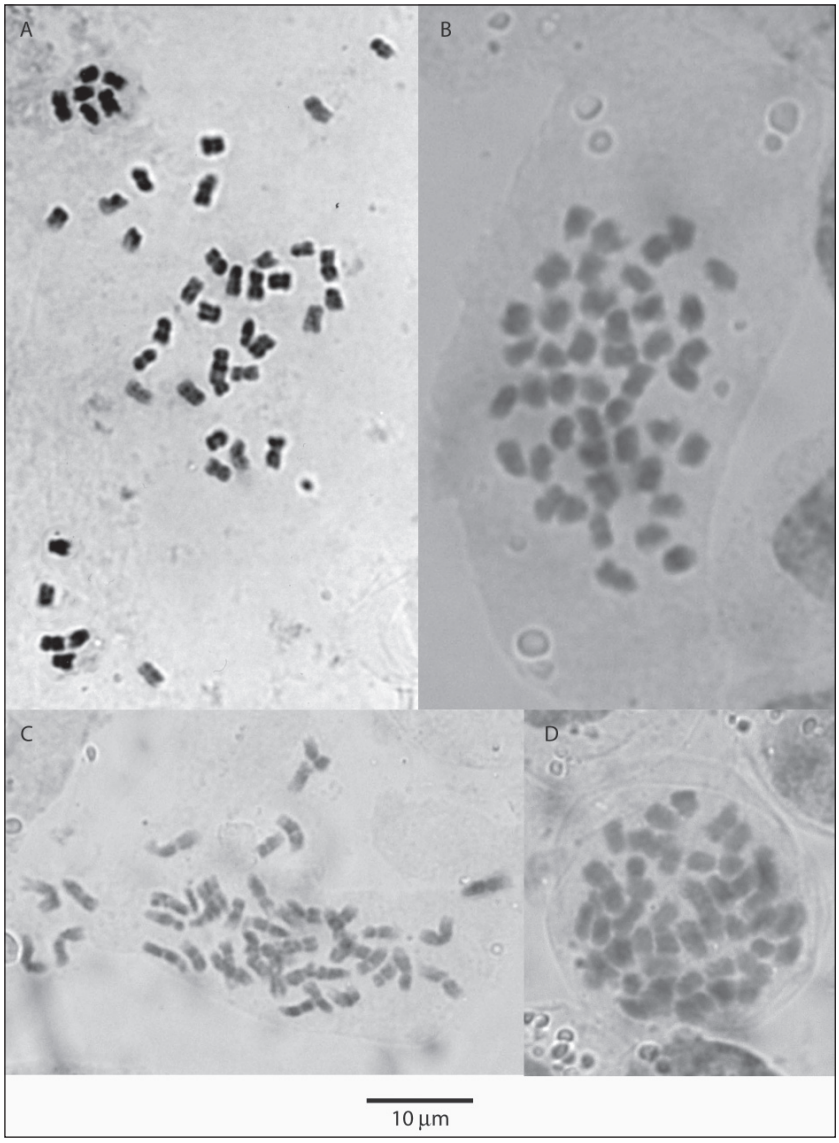


Fig. 2 — Photomicrographs of mitotic metaphase plates of: *Rebutia pygmaea* 2n=44 (A), *Rebutia raulii* 2n=44 (B), *Rebutia steinmannii* 2n=44 (C), *Rebutia major* 2n=44 (D).

(WR) and D.J. FERGUSON (DJF) in the years '70 and '80 of the last century and are cultivated by S. Mosti. The corresponding exsiccata are available by the Tropical Herbarium of Florence (FT):

Rebutia pygmaea (R. Fries) Britton and Rose; DJF 278, Argentina, Jujuy, Tres Cruces; cult. S. Mosti 2011, FT.

Rebutia gavazzii Mosti; WR 828, Bolivia, Chuquisaca, Sud Cinti; cult. S. Mosti 2011, FT.

Rebutia haagei Fric and Schelle; WR 35, Argentina, Jujuy, Humahuaca; cult. S. Mosti 2011, FT.

Rebutia diersiana Rausch; WR 631, Bolivia, Chuquisaca, Yuquina, near Culpina; cult. S. Mosti 2011, FT.

Rebutia steinmannii (Solms-Laubach) Britton and Rose; WR 208, Bolivia, Oruro, Eucaliptos; cult. S. Mosti 2011, FT.

R. major (Rausch) Sida; WR 334, Argentina, Jujuy, near Tafna; cult. S. Mosti 2011, FT.

Rebutia raulii Rausch; WR 485, Bolivia, Chuquisaca, Nor Cinti; cult. S. Mosti 2011, FT.

Rebutia leucanthema Rausch; WR 305, Bolivia, Chuquisaca, Nor Cinti, Cana Cruz; cult. S. Mosti 2011, FT.

Karyological analyses - Karyological analyses were obtained from somatic mitoses taken directly from the first phases of the developing embryo root tips after seed germination. After a pretreatment of about 3 hours in 8-hydroxichinoline saturated solution at room temperature, the material was fixed in Farmer's fixative solution (Ethanol and Acetic Acid - 3:1) (LÖVE and LÖVE 1975) for many hours, also used as a preservative solution in the test tubes stocked at 5°C temperature.

Afterwards, the material was washed in distilled water and then hydrolyzed in HCl 1N at 60°C for 5 minutes. Finally, after HCl removing

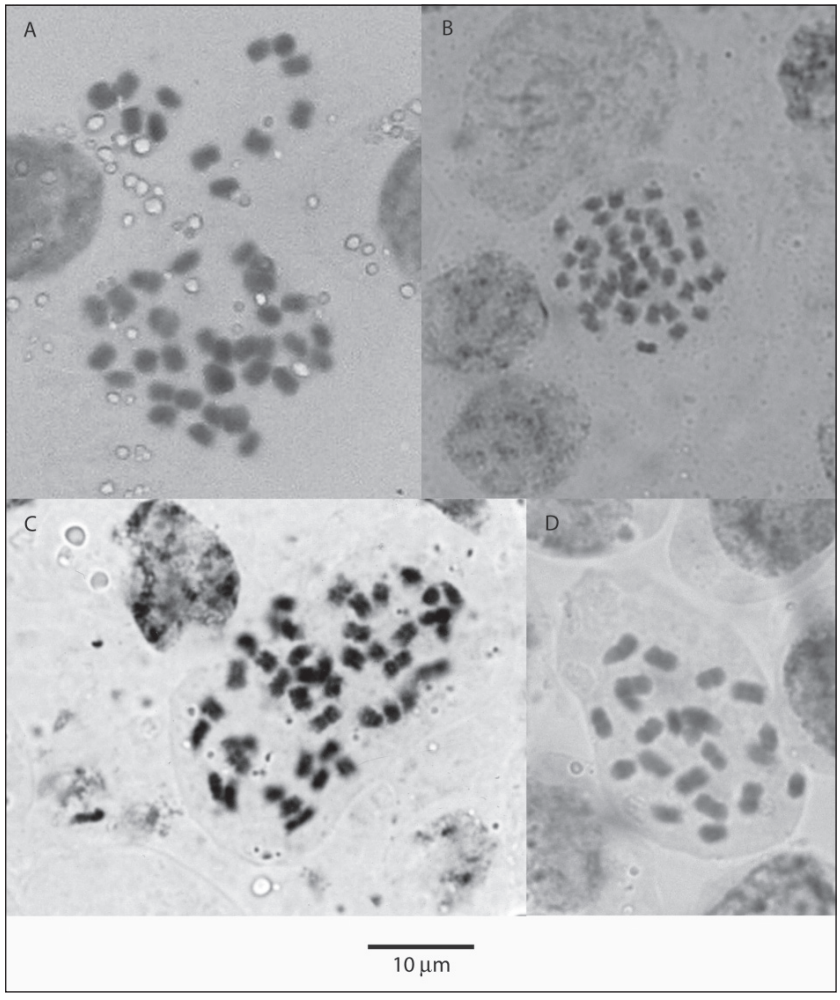


Fig. 3 — Photomicrographs of mitotic metaphase plates of: *Rebutia diersiana* 2n=44 (A), *Rebutia haagei* 2n=44 (B), *Rebutia gavazzii* 2n=44 (C), *Rebutia leucanthema* Rausch 2n=22 (D).

and after briefly washing in distilled water, the tips, were stained with 40% water solution of Lacto-propionic-orceine (DYER 1979) for 18-48 hours at room temperature.

Karyological investigations were carried out on mitotic metaphase plates of meristematic cells, with the technique of fresh squashes of root tips (DYER 1979): a very small piece of stained meristematic tissue was placed on a slide in a drop of 45% Acetic Acid, a clean coverslip was lowered over the drop and then gently pressured with the tip of a mounted needle until all the cells are dispersed in a large squash. Chromosome counts were made both during direct observations with the microscope, and on enlarged images of the original micrographs. Images were recorded with a microscope ZEISS Axiophot and digital photocamera Canon G5 connected to a personal computer. Measurements and values were processed in order to obtain chromosome ordering and homologue recognition, idiogram, karyotype formula and cromosome nomenclature proposed by LEVAN *et al.* (1964) (TABLE 1), centromeric index [As.k %] (ARANO and SAITO 1980) and the intra-[A1] and inter-chromosomic [A2] asymmetry indices proposed by ROMERO ZARCO (1986).

RESULTS

Rebutia pygmaea (R. Fries) Britton and Rose (Figs. 1A, 2A, 4a) – 2n = 44.

The count corresponding to the tetraploid number 2n = 44 (x = 11) was made on 11 mitotic metaphase plates, but the karyotype is related to the selected micrograph for Fig. 2A. It is the first report for this taxon. The formula is 2n = 4x = 44 = 4M + 32m + 8sm. The total chromosome length varied from 3.15 μm to 1.8 μm. The centromeric index was: As.k = 0.57, A1 = 0.24 and A2 = 0.14. The karyotype therefore was the most symmetric in the studied group, because of a large number of centromeric chromosomes and a some submedians. Chromosomes were generally of small size and showed a very low degree of polymorphism in their shapes.

Rebutia raulii Rausch (Figs. 1B, 2B, 4b) – 2n = 44.

The count corresponding to the tetraploid number 2n = 44 (x = 11) was made on 10 mitotic metaphase plates, but the karyotype is related to the selected micrograph for Fig. 2B. It is the first report for this taxon. The formula is 2n = 4x = 44 = 28m + 4sm + 4st + 8t. The total chromosome length varied from 4.2 μm to 2.45 μm. The centromeric index was: As.k = 0.65, A1 = 0.41 and A2 = 0.11. The karyotype therefore was quite asymmetric, because of a large number of subtelocentric and telocentric chromosomes. The chromosomes were generally of small size and showed a very low degree of size variation.

Rebutia steinmannii (Solms-Laubach) Britton and Rose (Figs. 1C, 2C, 4c) – 2n = 44.

The count corresponding to the tetraploid number 2n = 44 (x = 11) was made on 14 mitotic metaphase plates, but the karyotype is related to the selected micrograph for Fig. 2C. It is the first report for this taxon. The formula is 2n = 4x = 44 = 2M + 32m + 10sm. The total chromosome length varied from 5.7 μm to 2.45 μm. The centromeric index was: As.k = 0.57, A1 = 0.28 and A2 = 0.22. The karyotype therefore was quite symmetric, because of a large number of centromeric chromosomes and a some submedians. Chromosomes were generally of small size and showed a low degree of shape polymorphism.

Rebutia major (Rausch) Sida (Figs. 1D, 2D, 4d) – 2n = 44.

The count corresponding to the tetraploid number 2n = 44 (x = 11) was made on 9 mitotic

TABLE 2 — Synoptic data table of caryological investigation on *Rebutia*.

Taxa	Ploidy level	Formula	Total lenght (mm)	As.K %	A1	A2
R. pygmaea	2n=4x=44	4 M + 32 m + 8 sm	3.15 – 1.8	0.57	0.24	0.14
R. raulii	2n=4x=44	28 m + 4 sm + 4 st + 8 t	4.2 – 2.45	0.65	0.41	0.11
R. steinmannii	2n=4x=44	2 M + 32 m + 10 sm	5.7 – 2.45	0.57	0.28	0.22
R. major	2n=4x=44	8 M + 20 m + 8 sm + 8 t	6.75 – 1.9	0.63	0.38	0.30
R. diersiana	2n=4x=44	30 m + 6 sm + 8 T	3.65 – 1.8	0.63	0.38	0.13
R. haagei	2n=4x=44	4 M + 32 m + 4 sm + 4 t	2.44 – 0.9	0.59	0.30	0.20
R. gavazii	2n=4x=44	34 m + 4 sm + 2 st + 4 t	3.25 – 1.4	0.55	0.29	0.22
R. leucanthema	2n=2x=22	2 M + 16 m + 4 t	4.10 – 2.15	0.61	0.32	0.14

metaphase plates, but the karyotype is related to the selected micrograph for Fig. 2D. It is the first report for this taxon. The formula is 2n = 4x = 44 = 8M + 20m + 8sm + 8t. The total chromosome length varied from 6.75 μm to 1.9 μm. The centromeric index was: As.k = 0.63, A1 = 0.38 and A2 = 0.30. The karyotype was therefore quite asymmetric, because of a large number of centromeric chromosomes, a small number of submedians and several telocentric chromosomes. Chromosomes were generally of small size and showed the highest degree of size variation in the studied group.

Rebutia diersiana Rausch (Figs. 1E, 3A, 4e) – 2n = 44.

The count corresponding to the tetraploid number 2n = 44 (*x* = 11) was made on 7 mitotic metaphase plates, but the karyotype is related to the selected micrograph for Fig. 3A. It is the first report for this taxon. The formula is 2n = 4x = 44 = 30m + 6sm + 8T. The total chromosome length varied from 3.65 μm to 1.8 μm. The centromeric index was: As.k = 0.63, A1 = 0.38 and A2 = 0.13. The karyotype therefore was quite asymmetric, because of a large number of centromeric chromosomes, a small number of submedians and several telocentric chromosomes. Chromosomes were generally of small size and showed a low degree of size variation.

Rebutia haagei Fric and Shelle (Figs. 1F, 3B, 4f) – 2n = 44.

The count corresponding to the tetraploid number 2n = 44 (*x* = 11) was made on 19 mitotic metaphase plates, but the karyotype is related to the selected micrograph for Fig. 3B. It is the first report for this taxon. The formula is 2n = 4x = 4 4 = 4M + 32m + 4sm + 4t. The total chromosome length varied from 2.44 μm to 0.9 μm. The centromeric index was: As.k = 0.59, A1 = 0.30 and A2 = 0.20. The karyotype therefore was quite symmetric, because of a large number of centromeric chromosomes, a small number of submedians and a very small number of telocentric chromosomes. Chromosomes were generally of small size and showed a low degree of polymorphism in their shape.

Rebutia gavazzii Mosti (Figs. 1G, 3C, 4g) – 2n = 44.

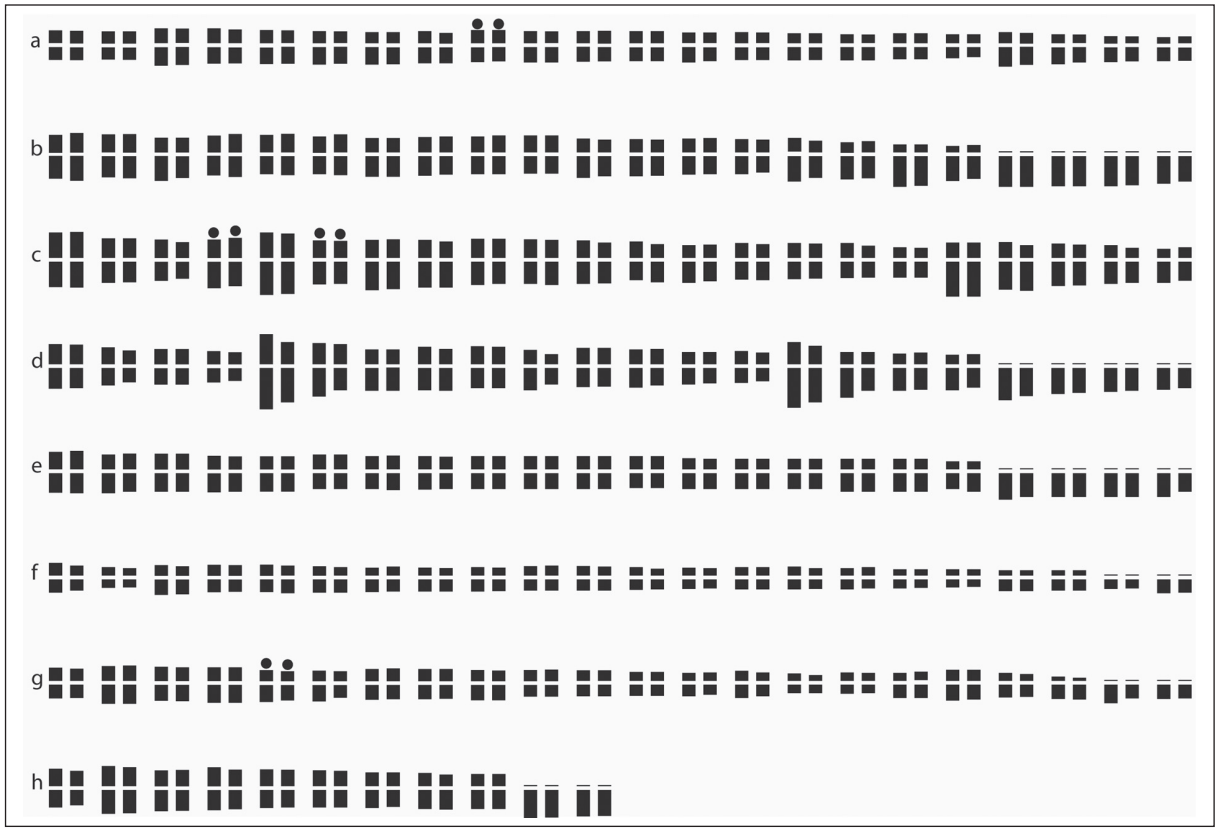
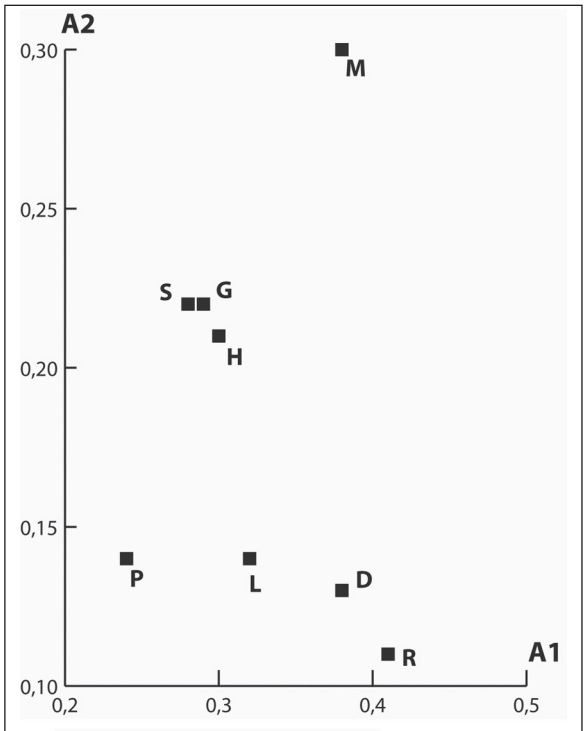


Fig. 4 — Karyograms of *Rebutia pygmaea* 2n=44 (a), *Rebutia raulii* 2n=44 (b), *Rebutia steinmannii* 2n=44 (c), *Rebutia major* 2n=44 (d), *Rebutia diersiana* 2n=44 (e), *Rebutia haagei* 2n=44 (f), *Rebutia gavazzii* 2n=44 (g), *Rebutia leucanthema* Rausch 2n=22 (h). (x 2000).

The count corresponding to the tetraploid number 2n = 44 (*x* = 11) was made on 15 mitotic metaphase plates, but the karyotype is related to the selected micrograph for Fig. 3C. It is the first report for this taxon. The formula is 2n = 4x = 44 = 34m + 4sm + 2st + 4t. The total chromosome length varied from 3.25 μm to 1.4 μm. The centromeric index was: As.k = 0.55, A1 = 0.29 and A2 = 0.22.

The karyotype therefore was quite symmetric, because of a large number of centromeric chromosomes a small number of submedians and several subtelocentric and telocentric chromosomes. Chromosomes were generally of small size and showed a low degree of polymorphism in their shapes.



taxon	A1	A2	
R. pygmaea	0,24	0,14	P
R. raulii	0,41	0,11	R
R. steinmannii	0,28	0,22	S
R. major	0,38	0,30	M
R. diersiana	0,38	0,13	D
R. haagei	0,30	0,21	H
R. gavazzii	0,29	0,22	G
R. leucanthema	0,32	0,14	L

Fig. 5 — Scatter diagram showing karyotype intrachromosomal asymmetry index (A1), due to ratio between arm length, against to interchromosomal asymmetry index (A2), due to variation between total length. The relative positions of the investigated species is explained in the text.

Rebutia leucanthema Rausch (Figs. 1H, 3D, 4h) – 2n = 22.

The counts corresponding to the diploid number 2n = 22 (*x* = 11) was made on 11 mitotic metaphase plates, but the karyotype is related to the selected micrograph for Fig. 3D. It is the first report for this taxon. The formula was 2n = 2x = 22 = 2M + 16m + 4t. The total chromosome length varied from 4.15 μm to 2.5 μm. The centromeric index was: As.k = 0.61, A1 = 0.32 and A2 = 0.14. The karyotype therefore was slightly asymmetric, because of a large number of centromeric chromosomes and a small number of telocentric chromosomes. Chromosomes were generally of small size and showed a low degree of shape polymorphism.

DISCUSSION

Of the eight investigated species of *Rebutia* sect. *Digitorebutia*, seven resulted to be polyploid, with a tetraploid chromosome number 2n = 44. This is the first information about the karyotype of all the species investigated in this study.

A wider sample would be necessary to suppose that polyploidy is the most frequent condition in section *Digitorebutia*. In any case tetraploidy was the only polyploid condition found in our sampling. Tetraploidy is considered to be the most successful condition among polyploids (De Wet 1980, Cota and Philbrick 1994).

The asymmetric karyotype depending on the chromosomes morphology can be linked to the evolutionary history of the taxa (Romero Zarco 1986). Generally the high intrachromosomal asymmetry index (A1) is considered as a specialized adaptation (Romero Zarco 1986). On the other hand, the interchromosomal asymmetry index (A2) may be directly related to the relative taxonomic distance between different taxa (Romero Zarco 1986).

The A1-A2 plot (Fig. 5) allow some considerations. The A1 index (intrachromosomal asymmetry) is variable between 0 (all metacentric chromosomes) and 1 (all telocentric chromosomes). The A2 index indicates the interchromosomal asymmetry and can vary between 0 and infinite. It is related to the diversity in dimension of the chromosomes complement.

The species with the lowest intrachromosomal asymmetry (A1) is *R. pygmaea*. It can be suggested that this low asymmetry index, might be linked to the high capability of this species

to adapt to habitat condition and hence not particularly specialized. As a matter of fact, *R. pygmaea* is the species in section *Digitorebutia*, of which more populations are known (RAUSCH 1986; PILBEAM 1997; MOSTI 2000a). *R. gavazzii* and *R. haagei* are more morphologically similar to *R. pygmaea* and showed higher intrachromosomal asymmetry index (Fig. 5). This data could be related to a more restricted geographical development of these two species. At least 3-4 different populations (RAUSCH 1986) of *R. haagei* are known (it is not a particularly low number, due to the difficutly of finding these rare species in the wild) and they grow only in northern Argentina and one in Bolivia. *R. gavazzii* is currently known in Bolivia and Argentina for 2 populations. Both *R. gavazzii* and *R. haagei* are considered as separate entities from *R. pygmaea* on the basis of morphological and micromorphological data, even if they are surely taxonomically close to this last (MOSTI 1999; 2000a; 2000b). *R. haagei* and *R. gavazzii*, are closer for the intrachromosomal and the interchromosomal asymmetry, however more distant for the flower morphology and spine apparata (MOSTI 1999; 2000a) as well as with respect to the more intermediate *R. pygmea.*

R. major (Fig. 5) is the species resulting to have the highest interchromosomal asymmetry (A2) and shows even a quite high intrachromosomal asymmetry (A1). In this case the higher asymmetry with respect to *R. steinmannii* can be attributed to the fact that *R. major* is the only argentinian species of the "steinmannii group" (MOSTI 2000a). Even *R. christinae* Rausch, a taxon considered closely related to *R. steinmannii* by RAUSCH (1986), grows in northern Argentina, but the insertion of this species within the "steinmanni group" is debatable because of its morphological characters (MOSTI 1999). The geographical isolation of *R. major* with respect to the other species of the "steinmanni group" (almost all endemic of Bolivia) and its following adaptation to a different environment, together with a high morphological specialization, may be related to the higher intrachromosomal asymmetry (A1) with respect to *R. steinmannii.* This last shows a low intrachromosomal asymmetry index (A1) (Fig. 5). Moreover the high nonhomogeneity of the chromosomes (A2) of *R. major* migth be related to a probable allopolyploid origin. Both *R. steinmannii* and *R. major* showed the largest chromosome size. This data may be interpreted as an indication of their common phylogenetic origin.

Examining those species that, together with *R. pygmaea,* showed a low interchromosomal (A2) asymmetry index (Fig. 9), we observed that *R. diersiana*, even if to be inserted in the "pygmaea group" (RAUSCH 1986, MOSTI 1999), appears to be quite different in its A1 with respect to *R. pygmea* itself. Such A1 index of *R. diersiana* (known only for two Bolivian populations) quite higher with respect to *R. pygmea*, might probably mean a higher degree of specialization (ROMERO ZARCO 1986). This observation would confirm that these two species are to be considered separate as stated by MOSTI (1999), thanks to some important macro and micromorphological characters, such as the morphology of the perianth segments and of the areola and the seed testa microsculpturing.

R. raulii, with its lowest A2 index, justifies, somehow, also its higher taxonomic distance with respect to the rest of the investigated species. This distance is supported also by morphological features that would suggest its insertion within the "atrovirens group". This last group comprises the taxonomic entities, among *Digitorebutia*, closer related to Sect. *Aylostera* of *Rebutia*, that is those that generally show intermediate macrocharacters between *Digitorebutia* and *Aylostera.* Unconspicuous ribs are more an *Aylostera* character, but the style only partially fused to the floral tube is a key *Digitorebutia* character. The quite high intrachromosomal asymmetry index (A1) of *R. raulii* is instead a data that may be linked to a high specialization degree and ecological adaptations (ROMERO ZARCO 1986). The interchromosomal asymmetry index (A2), quite similar between *R. pygmaea, R. raulii*, *R. diersiana* (Fig. 5), appears to be in this case more accidental, since these species are notably different under a morphological point of view. The low A2 index of this species may probably mean that these species would have originated through autopolyploid speciation.

Finally, *R. leucanthema* is the only species among those here investigated whose chromosome set resulted diploid, 2n=22. In genus *Rebutia* the diploid condition has been found only in the two investigated species of Sect. *Rebutia* (ROSS 1981). The diploidy, with respect to polyploidy, represents as such a sign of lower evolutionary degree within family Cactaceae (COTA and PHILBRICK 1994). This condition is remarked also by a quite low chromosome asymmetry indexes (Fig. 5) and would indicate a situation of primitivity of *R. leucanthema* karyotype within Sect. *Digitorebutia* and possibly a more basal

phylogenetic position. This species is morphologically characterized by ribs that are difficult to count, since they run spirally along the stem, and protruding tubercles. The white color of the flowers that characterizes this species is a feature that is not particularly frequent in genus *Rebutia* in general, and particularly in sect. *Digitorebutia*. The white flower is also related to a different pollinators choice. The diploid condition in this species, together with important differences in macro and micromorphological characters, is however a clue indicating an isolated taxonomic position (MOSTI 2000a), rather than a synonym of *R. pygmaea* (HUNT 2009).

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