CHROMOSOME NUMBER AND SATELLITED CHROMOSOME MORPHOLOGY OF ELEVEN SPECIES OF *ARACHIS* (LEGUMINOSAE)

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Summary: Peñaloza, A.P.S. & J.F.M. Valls. 2005. Chromosome number and satellited chromosome morphology of eleven species of *Arachis* (Leguminosae). Bonplandia 14(1-2): 65-72. ISSN: 0524-0476.

The chromosome number was determined in eleven recently-described species of *Arachis* belonging to five different taxonomic sections. Two new species of section *Arachis, A. Gregoryi* and *A. Krapovickasii*, present 2n=20 chromosomes and lack the small 'A' pair, while *A. linearifolia* and *A. Schininii* have 2n=20, including the small 'A' pair of chromosomes, typical of the A genome of *A. hypogaea*. *Arachis Schininii*, previously listed under *A. Hoehnei*, a species lacking this pair of chromosomes, has many morphological differences, and is therefore considered to be a new species. *Arachis submarginata* (sect. *Extranervosae*), *A. Pflugeae* and *A. Hassleri* (sect. *Procumbentes*), and *A. seridoënsis* and *A. interrupta* (sect. *Heteranthae*) also have 2n=20. One species of section *Rhizomatosae*, *A. nitida*, is tetraploid (2n=40), but is morphologically distinct from the other tetraploids in this section. The new species of section *Erectoides*, *A. porphyrocalyx*, shows 2n=18. This is the first count of 2n=18 chromosomes for an *Arachis* species outside section *Arachis* and its origin is probably not associated with the origin of the 18 chromosomes occurring in three species of section *Arachis*.

Key words: wild peanut, chromosome morphology, satellited chromosome, 'A' chromosomes.

Resumen: Peñaloza, A.P.S. & J.F.M. Valls. 2005. Número cromosómico y morfología del cromosoma satelitado en once espécies de *Arachis* (Leguminosae). Bonplandia 14(1-2): 65-72. ISSN: 0524-0476.

Se determinó el número cromosómico de once especies recientemente descriptas del género *Arachis*, pertenecientes a cinco secciones taxonómicas. En la sección *Arachis, A. Gregoryi* y *A. Krapovickasii* presentan 2n=20 cromosomas sin el par "A", mientras *A. linearifolia* y *A. Schininii* presentan 2n=20, incluyendo el par "A", típico del genomio A de *A. hypogaea*. *Arachis Schininii* presenta características citológicas (presencia del par "A") y morfológicas distintas de *A. Hoehnei*, bajo la cual estuvo incluida. *Arachis submarginata* (sección *Extranervosae*), *A. Pflugeae* y *A. Hassleri* (sección *Procumbentes*), y *A. seridoënsis* y *A. interrupta* (sección *Heteranthae*) también presentan 2n=20. Una especie de la sección *Rhizomatosae*, *A. nitida*, es tetraploide, con 2n=40, pero morfológicamente distinta de las demás especies tetraploides de la sección. En la sección *Erectoides*, la nueva especie *A. porphyrocalyx* presenta 2n=18. Este es el primer recuento de 18 cromosomas en especies de *Arachis* que no pertenecen a la sección *Arachis* y su origen no está asociado al origen de los 18 cromosomas de las tres especies de la sección *Arachis*.

Palabras clave: maní silvestre, morfología cromosómica, cromosomas SAT, cromosomas 'A'.

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Introduction

The genus *Arachis* L. is a diverse taxon containing the cultivated peanut, *A. hypogaea* L. and its wild relatives. *Arachis* species are distinguished from those of other genera mainly by flowering above the ground but producing fruits and seeds below the soil surface. Wild *Arachis* species are native to a large region of South America, from the Brazilian Atlantic Coast to the foothills of the Andes Mountains in NW Argentina and from the mouth of the Amazon River in northern Brazil to approximately 34ºS, along the northern edge of the La Plata River in Uruguay. The native habitats of wild *Arachis* are highly varied, including savannas, semi-arid areas, edges of tropical rain forest and humid swamps.

Krapovickas & Gregory (1994) published a detailed treatise on the morphology and taxonomy of 69 species of *Arachis*. They proposed the classification of these species into nine sections, according to their morphological similarities, cross-compatibility, pollen viability of hybrids, and chromosome morphology. A few, rare specimens somewhat doubtful concerning the classification into sections, were not included in the monograph. Since 1994, new expeditions have been conducted in Brazil, Bolivia and Paraguay, resulting in the collection of additional new species. These accessions, including some of the rare specimens temporarilly set aside or published as possibly belonging to some of the 69 species considered by Krapovickas & Gregory (1994), have been described as eleven new species of *Arachis* (Valls & Simpson, 2005).

Chromosome numbers and morphology are well-known sources of information used to clarify relationships at several taxonomic levels in plants, including the species of genus *Arachis*. Husted (1933, 1936) reported the karyotypic morphology in *A. hypogaea* and pointed out that this species has one pair of small chromosomes, designated as 'A', and one pair of chromosomes with large satellites, designated as 'B'. Most of the species of this genus were reported to be diploids with 20 chromosomes. Some diploid species of section *Arachis* also have the small 'A' pair. Only four species are tetraploid, based on x=10, and belong to sections *Arachis* (*A. hypogaea* and *A. monticola* Krapov. & Rigoni) and *Rhizomatosae* Krapov. & W.C. Greg. [*A. glabrata* Benth. and *A. pseudovillosa* (Chodat & Hassl.) Krapov. & W.C. Greg.]. The tetraploid species of section *Arachis* are believed to have evolved independently from those of *Rhizomatosae* (Smartt & Stalker, 1982).

Lavia (1996, 1998) reported 2n=18 chromosomes for *Arachis palustris* Krapov., W.C. Greg. & Valls and *A. praecox* Krapov., W.C. Greg. & Valls, diploid species classified by Krapovickas & Gregory (1994) as members of section *Arachis*. Peñaloza *et al.* (1996) and Peñaloza & Valls (1997) reported 2n=18 for *A. decora* Krapov., W.C. Greg. & Valls, also a species of section *Arachis*. The diploid condition is widely believed to be the more ancient characteristic and the tetraploid level more advanced. Lavia (1999) has suggested that the diploids with 2n=18 of section *Arachis* have evolved from a diploid with 2n=20 of the same section.

The presence or absence of a small chromosome pair, with late condensation and poor staining at prometaphase (the 'A' chromosome of Husted, 1933, 1936), as well as the type of satellited chromosome (SAT), according to the types classified by Fernández & Krapovickas (1994), are essential attributes to the accurate comprehension of *Arachis* taxonomy, allowing for the classification of the species into one of the nine sections in most cases.

In the present work, the chromosome number and the type of satellited chromosomes of eleven new species, described elsewhere by Valls & Simpson (2005) were determined in order to characterize the cytogenetic aspects of these new species and to contribute to their taxonomic classification into one of the nine pre-existing sections.

Material and methods

Seeds and plants were obtained from the Wild *Arachis* Genebank at Embrapa Genetic

Resources and Biotechnology (Cenargen), in Brasília, Brazil. The work was carried out at the Laboratory of Cytogenetics of Cenargen and voucher specimens are deposited at the Cenargen Herbarium (CEN). The list of species analyzed in this work, their collector numbers and source can be seen in Table 1.

Seeds were put to germinate on filter paper, moistened with 0.3% Ethephon solution, and maintained at 20-30ºC.

Mitotic preparations were obtained from young root tips, ca. 1 cm long, obtained from germinating seeds or from plants maintained in pots at the greenhouse. After pre-treatment in 0.002N 8-hidroxiquinoline at 17ºC for 4 hours, root tips were fixed in absolute ethanol:glacial acetic acid (3:1; v/v) solution for 24 hours and then were hydrolized in 5N HCl for 20 minutes, both at room temperature. The root meristems were stained with Schiff's solution, and squashed on a slide with a drop of acetic carmine 2%. Metaphase cells of each accession were photographed with Ilford Pan F Plus 50 ASA film.

The satellited chromosomes were classified according to the terminology suggested by Fernández & Krapovickas (1994) and Lavia (1999, 2001).

Results

The results obtained are reported in Table 2. They concern the chromosome number, the presence or absence of the small 'A' pair of chromosomes and the type of satellited chromosomes for the eleven recently-described species of *Arachis*. Prometaphase and metaphase cells can be seen in Figure 1.

Table 1. Herbarium specimens and accessions of *Arachis*.

Section/ Species/Collector number	Accession code(BRA-)	Source	Lat. (S)	Long. (W)	Alt. (m)
Section *Arachis*					
A. linearifolia VPoBi 9401	022608	Santo Antônio do Leverger- MT-Brazil	15º 54'	56º 20'	110
A. Schininii VSW 9923	022926	Bella Vista - Paraguay	22º 23'	56º 24'	200
A. Gregoryi VSGr 6389	012696	Vila Bela - MT - Brazil	15º 22'	60º 14'	210
A. Krapovickasii WiSVg 1291	036901	San Jose de Chiquitos - Bolivia	18º 14'	60º 51'	-
Section *Erectoides*					
A. porphyrocalyx VMSv 7303	016039	Uberaba - MG - Brazil	19º 58'	47º 46'	490
Section *Extranervosae*					
A. submarginata VGaRoSv 12525	018147	Ribeirão Cascalheira - MT - Brazil	13º 12'	51º 54'	390
Section *Heteranthae*					
A. interrupta VFaPzSv 13082	030121	Monte Azul - MG - Brazil	14º 55'	43º 29'	470
A. seridosnsis VRSv 10969	025623	Acari - RN - Brazil	6º 21'	36º 36'	210
Section *Procumbentes*					
A. Pflugeae VRcSgSv 13589	032875	Porto Murtinho - MS -Brazil	21º 44'	57º 25'	190
A. Hassleri SvPzHn 3818	035971	Concepción - Paraguay	23º 23'	57º 25'	100
Section *Rhizomatosae*					
A. nitida VMPzW 14040	034983	Porto Murtinho - MS - Brazil	21º 52'	57º 34'	200

Collectors: **Bi**: L.B. Bianchetti; **ca**: L.F. Freitas; **da**: L. Galgaro; **dr**: A. Gripp; **en**: R. Heyn; **M**: J.P. Moss; **mo**: A. Pott; **mz**: E.A. Pizarro ; **o** : V.R. Rao; **oc**: R.C. Oliveira; **oo**: D.M.S. Rocha; **p**: C.E. Simpson; **pg**: A.K. Singh; **pv**: G.P. Silva; **s** : J.F.M. Valls; **sg**: I.G. Vargas ; **t** : W.L. Werneck; **ti**: D.E. Williams.
Brazilian States: **Md** : Minas Gerais; **Mp**: Mato Grosso do Sul; **MT**: Mato Grosso; **oN**: Rio Grande do Norte.

Table 2W Chromosome number, presence or absence of 'A' chromosome pair and type of satellited chromosome of eleven species of *Arachis*.

Section/ Species	Collector number	2n	'A' pair	Type of SAT chromosome [a]
Arachis				
A. linearifolia	V 9401	20	+	not analyzed
A. Schininii	V 9923	20	+	not analyzed
A. Gregoryi	V 6389	20	-	6
A. Krapovickasii	Wi 1291	20	-	5
Erectoides				
A. porphyrocalyx	V 7303	18	-	8
Extranervosae				
A. submarginata	V 12525	20	-	3A
Heteranthae				
A. interrupta	V 13082	20	-	10
A. seridosnsis	V 10969	20	-	10
Procumbentes				
A. Pflugeae	V 13589	20	-	9
A. Hassleri	Sv 3818	20	-	9
Rhizomatosae				
A. nitida	V 14040	40	-	3A

[a] According to Fernández & Krapovickas (1994) and Lavia (1999, 2001) classification.

Discussion

Section *Arachis*

The species of section *Arachis* show great variation with regard to cytogenetic aspects. Some species have the small 'A' pair of chromosomes, similar to those observed in *A. hypogaea* by Husted (1933, 1936). Most species of section *Arachis* with the 'A' pair are perennials (Lavia, 1999).

Arachis linearifolia Valls, Krapov. & C.E. Simpson (V 9401) is one of the new species analyzed here belonging to this group. It is a perennial herb, with leaflets almost linear, otherwise very similar to *A. Diogoi* Hoehne and has 2n=20 and the small 'A' pair (Figure 1 A).

The other species of this group, *A. Schininii* Krapov., Valls & C.E. Simpson (V 9923), is quite peculiar, since it has some morphological characteristics in common with *A. Hoehnei* Krapov. & W.C. Greg., a species without the 'A' pair. However, *A. Schininii* has the small pair of chromosomes, as can be seen in Figure 1 B. The collection V 9923 has been previously identified by Krapovickas & Gregory (1994) as *A. Hoehnei* on morphological grounds, but this cytological difference alone is sufficient for its classification as a distinct species.

The other group of this same section, with species lacking the small 'A' pair of chromosomes, includes *A. Gregoryi* C.E. Simpson, Krapov. & Valls (V 6389) and *A. Krapovickasii* C.E. Simpson, D.E. Williams, Valls & I.G. Vargas (Wi 1291), both with 2n=20 chromosomes (Figs. 1C, 1E) and morphologically very similar to *A. magna* Krapov., W.C. Greg. & C.E. Simpson and *A. cruziana* Krapov., W.C. Greg. & C.E. Simpson, respectively. Polysomatic cells (2n=40) have been observed in *A. Gregoryi* (Fig. 1 D).

Section *Erectoides* Krapov. & W.C. Greg.

Eighteen chromosomes were observed for

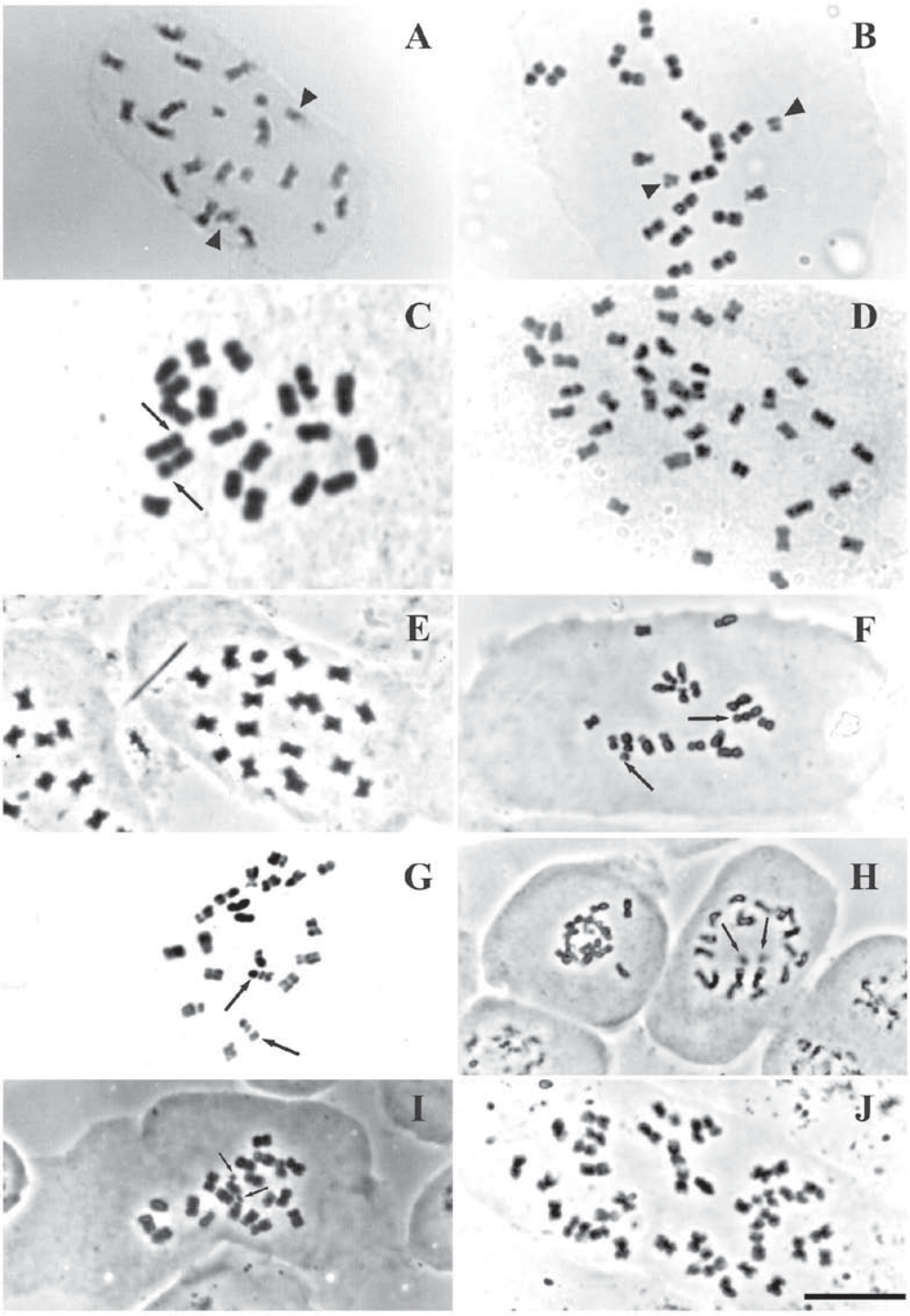

Fig. 1. Somatic prometaphases and metaphases in species of *Arachis* belonging to sections *Arachis* (A-E), *brectoides* (F), *bxtranervosae* (G), *mrocumbentes* (H-I) and *ohizomatosae* (J). A: *Arachis linearifolia* (V 9401), 2n=20. B: *AK pchininii* (V 9923), 2n=20. C: *AKd regoryi* (V 6389), 2n=20. D: *AKd regoryi* (V 6389), polysomatic cell, 2n=40. E: *AK hrapovickasii* (Wi 1291), 2n=20. F: *AKporphyrocalyx* (V 7303), 2n=18. G: *AKsubmarginata* (V 12525), 2n=20. H: *AK mflugeae* (V 13589), 2n=20. I: *AKe assleri* (Sv 3818), 2n=20. J: *AKnitida* (V 14040), 2n=40. Arrows indicate satellites and arrowheads indicate 'A' chromosomes. Bar in C=5 µm, in A-B and D-J=10 µm.

A. porphyrocalyx Valls & C.E. Simpson (V 7303) (Fig. 1 F). According to Lavia (1996), the basic chromosome number x=9 in *Arachis* species of section *Arachis* is probably derived from an ancestral x=10 by aneuploidy, supporting the hypothesis that x=9 is an advanced trait within the genus. Until now, this number has been reported for only three species of section *Arachis: A. palustris*, *A. praecox* (Lavia, 1996, 1998) and *A. decora* (Peñaloza *& al*., 1996; Peñaloza & Valls, 1997). The only known population of *A. porphyrocalyx* was collected at Uberaba, Minas Gerais State, and this location extends southward the area of distribution of *Arachis* species with 18 chromosomes. Although this species is found outside the area of distribution previously reported for species of section *Erectoides*, the root morphology, the presence of flowers concentrated at the base of the plant and the presence of anthocyanin in the flower calyx suggest its classification in this section (Valls & Simpson, 2005).

Arachis porphyrocalyx has SAT chromosome type 8 (Fig. 1 F), not reported previously for a species of section *Erectoides*, but the same type of satellite was observed by Lavia (1999) in one accession of *A. tuberosa* Bong. ex Benth., of the closely related and more primitive section *Trierectoides* Krapov. & W.C. Greg. Furthermore, *A. porphyrocalyx* has four pairs of submetacentric chromosomes, the same number as that reported for *A. stenophylla* Krapov. & W.C. Greg., which has been considered the most advanced species of section *Erectoides*, from the cytological standpoint (Fernández & Krapovickas, 1994).

Section *Extranervosae* Krapov. & W.C. Greg.

Arachis submarginata Valls, Krapov. & C.E. Simpson (V 12525) has 2n=20 chromosomes, as occurs in *A. Pietrarellii* Krapov. & W.C. Greg., *A. Burchellii* Krapov. & W.C. Greg., *A. prostata* Benth. and *A. retusa* Krapov., W.C. Greg. & Valls, to which it is morphologically (Valls & Simpson, 2005) and/or molecularly associated (Galgaro & al., 1998). The satellited chromosome corresponds to 3A (Fig. 1 G), the most common type in the section (Fernández & Krapovickas, 1994; Lavia, 1999).

Section *Heteranthae* Krapov. & W.C. Greg.

In spite of their morphological and molecular differences (Veiga, 1994; Veiga *& al.*, 1999; Valls & Simpson, 1997, 2005), the two new species of section *Heteranthae*, *A. interrupta* Valls & C.E. Simpson (V 13082) and *A. seridoënsis* Valls, C.E. Simpson, Krapov. & R. Veiga (V 10969), have 2n=20 chromosomes. Both species have SAT chromosomes of type 10, as does *A. sylvestris* (A. Chev.) A. Chev., another species of this section (Fernández & Krapovickas, 1994; Lavia, 1996). Only one population of each of these species has been collected, so the presence of any cytogenetic variation cannot be determined. Chromosomes of both species are short in average, similar to the other species of this section.

Section *Procumbentes* Krapov. & W.C. Greg.

Arachis Pflugeae C.E. Simpson, Krapov. & Valls (V 13589) has 2n=20 chromosomes (Fig. 1 H), and polysomatic cells with 40 and 80 chromosomes were also observed.

Polyploidy in root cells of nodulating legumes is not a rare event, and was reported in *Arachis* (Fernández & Krapovickas, 1994; Lavia, 1996), *Vicia sativa* L., *Medicago sativa* L. and *Trifolium pratense* L. (Wipf, 1939; Wipf & Cooper, 1940), but it has not been well established whether nodules originate in polyploid cells, or whether abnormal cell division after infection causes polyploid cells to proliferate, as often happens in gall formation (Stalker *et al.*, 1994).

The other new species of section *Procumbentes*, *Arachis Hassleri* Krapov., Valls & C.E. Simpson (Sv 3818), also has 2n=20 chromosomes (Fig. 1 I). The two new species of section *Procumbentes* show the type 9 satellited chromosome that is typical of this section (Fernández & Krapovickas, 1994).

Section *Rhizomatosae* Krapov. & W.C. Greg.

Arachis nitida Valls, Krapov. & C.E. Simpson (V 14040) (Figure 1 J), the only recently-described species of section *Rhizomatosae*, has 2n=40 chromosomes, like

A. glabrata and *A. pseudovillosa*, the other two known species from series *Rhizomatosae* of the same taxonomic section. These two previously-described species, in spite of having 2n=40 chromosomes, produced hybrids with diploid annual species from section *Arachis* and with perennial species from sections *Procumbentes* and *Erectoides* (Krapovickas & Gregory, 1994). However, only one of such hybrids, from a section *Rhizomatosae* X section *Arachis* cross, produced flowers, and the *Rhizomatosae* parent (*Hammons & al. 569*) in fact belongs to the new *A. nitida* (Valls & Simpson, 2005), not to *A. glabrata*, which might indicate a better intersectional crossability of the new species.

Arachis nitida showed type 3A satellited chromosomes, as in *A. glabrata.* However, *A. nitida* has morphological similarity to *A. Burkartii* Handro, a species from series *Prorhizomatosae* Krapov. & W.C. Greg. (of section *Rhizomatosae*), which is diploid and produces no hybrids when crossed with other diploid species of the genus, therefore showing strong reproductive isolation, according to Krapovickas & Gregory (1994).

Conclusions

Most of the results obtained in the present work are compatible with the assignment of the new species into one of the nine sections proposed by Krapovickas & Gregory (1994). The chromosome number 2n=18 in *A. porphyrocalyx* shows that the process of loss of a pair of chromosomes might have occurred more than once in the genus, and probably in different ways. Improved comprehension of the evolution of the genus *Arachis* requires further cytogenetic studies of aditional accessions of each of the new species, since many species of *Arachis* have already shown intraspecific variation.

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