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SHORT COMMUNICATION

Characterization of the karyotypic morphology of *Haplopappus foliosus* (Hook. & Arn.) (Asteraceae)

Caracterización de la morfología cariotípica de *Haplopappus foliosus* (Hook. & Arn.) (Asteraceae)

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ABSTRACT

The characterization of the chromosomal morphology of *Haplopappus foliosus* (Hook. & Arn.) determined a chromosomal number of 2n = 10, with a karyotypic formula of one subtelocentric pair (st) and four submetacentric pairs (sm). The intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) showed a moderate tendency towards telocentricity and low variability in the sizes of chromosome pairs.

Keywords: endemic shrub, karyotype, karyotypic morphology.

RESUMEN

La caracterización de la morfología cromosómica de *Haplopappus foliosus* (Hook. & Arn.) determinó un número cromosómico de 2n = 10, con una fórmula cariotípica de un par subtelocéntrico (st) y cuatro pares submetacéntricos (sm). Los índices de asimetría intracromosómica (A1) e intercromosómica (A2) mostraron una moderada tendencia a la telocentría y baja variabilidad en los tamaños de los pares cromosómicos.

Palabras claves: arbusto endémico, cariotipo, morfología cariotípica.

The taxonomic history of the genus *Haplopappus* Cass (Asteraceae) has been complex, beginning with Hall (1928), who described 149 species for the genus, evenly divided between North and South America. Subsequently, interpretations of generic limits have focused almost exclusively on the North American elements of the genus. Some authors support Hall's treatment (Jackson 1966, Moran 1969, Grau 1976), while others propose that at least the North American sections be treated as separate genera or combined with existing genera (Anderson *et al.* 1974, Clark *et al.* 1980). Thus, over the last eight decades, there has been almost complete disintegration of the genus, and all taxa recognized by Hall (1928) have been accommodated within previously named genera, leading to a formal redescription of the genus (Lane & Hartman 1996). Currently, 70 species are recognized, distributed exclusively in South America (Klingenberg 2007).

The main controversies in the taxonomic history of the genus stem from the existence of hybrids, classifications primarily made based on morphological characters, which do not always allow for accurate differentiation between species (Lane & Hartman 1996). Therefore, the incorporation of information from new characters is a significant contribution to the resolution of taxonomic problems (Siljak-Yakovlev & Peruzzi 2012). The inclusion of cytogenetic characters in such cases is of great assistance, as it allows discrimination between parentals and hybrids and is based on traits that are less affected by the environment. One species of the genus *Haplopappus* is *Haplopappus foliosus* (Hook. & Arn. 1983), which is an endemic shrub of Chile, distributed along the coastal zone from the Atacama region to the Maule region (Hoffmann 1998, Rodríguez *et al*. 2018). Despite being a common species in Chile, cytogenetic data is scarce. At the chromosomal level, only a haploid value of n = 5 has been determined (Brown & Clark 1981, Frías 2005) without a detailed description of its karyotype. Therefore, the aim of this study is to characterize the karyotypic morphology of *H. foliosus*.

Seeds of *H. foliosus* were collected during the fruiting stage (March/April) from 30 randomly chosen individuals belonging to the population located in the BioParque Puquén (32°14′16″S - 71°31′16″W), in Los Molles, Valparaíso region, Chile. The reference material is deposited in the herbarium of the Facultad de Ciencias Forestales y Conservación de la Naturaleza, Universidad de Chile.

For obtaining mitotic plates, the seeds were germinated in the laboratory in Petri dishes. The apical root zone of approximately 5-8 mm in length was cut from 60 specimens and pre-fixed with a 0.002 M 8-Hydroxyquinoline solution for five hours at room temperature. Subsequently, the material was washed twice with distilled water. The material was then fixed in an Ethanol-acetic acid solution (3:1 v/v) at room temperature for 20 hours. The material was washed again twice, followed by acid hydrolysis with 5 N HCl for 10 minutes at room temperature. It was then washed twice with distilled water, and finally, the root apex was stained with 2% orcein lactic-acetic to proceed with the squashing of the root meristems. Images of the mitotic plates were captured using an OLYMPUS BX61 video microscope connected to a MS DOS computer (in the Laboratorio de Neurobiología y Biología del Conocer, Universidad de Chile), using the Spot diagnostic instruments program (version 4.5). Fifteen metaphase plates were measured using the MicroMeasure 3.3 program (Reeves 2001). The images of the mitotic plates were later edited to generate the karyotype using Photoshop. Based on the position of the centromere, the length of the long and short arms of each chromosomal pair was determined. The measurements were expressed as a percentage of the total length of the haploid chromosomal set. With these values, the karyotypic morphology was obtained, and karyotypes were prepared according to the chromosomal categories proposed by *Levan et al*. (1964), visualized in a karyo-ideogram, with the aim of graphically representing the size and morphology of each chromosomal pair. Additionally, intrachromosomal and interchromosomal asymmetry was determined using two numerical parameters, A1 and A2, proposed by Romero-Zarco (1986). The intrachromosomal asymmetry index varies from 0 to 1 and tends to zero when the chromosomes are metacentric (Cheenaveralah 1973). The interchromosomal asymmetry index measures the variation among chromosomes within the karyotype of a species. When the sizes of the chromosomes within a species are more or less uniform or show little variability, the interchromosomal asymmetry tends to exhibit very low values (Romero-Zarco 1986). Additionally, the total relative chromosome length (LR%) was calculated.

Haplopappus foliosus exhibits a karyotype of 2n = 10 with a haploid karyotypic formula of 4sm + 1st, meaning the first pair is subtelocentric and the remaining four pairs are submetacentric (Figs. 1a-1c, Table 1). The values of the intrachromosomal asymmetry index (A1) are 0.63 ± 0.04, indicating a moderate tendency towards telocentricity, and the interchromosomal asymmetry index (A2) is 0.17 ± 0.04, revealing uniformity or little variability in the sizes of chromosomal pairs relative to each other (Table 1). The LR% of the species is 20.00 ± 3.33 (Table 1).

The diploid chromosome number of 2n = 10 obtained in *H. foliosus* confirms the chromosomal number previously described by Brown & Clark (1981) and Frías (2005), which is equivalent to that documented for 32 species of the genus in South America (Brown & Clark 1981). Therefore, it is inferred that the ancestral basic number is n = 5, and it is conserved in South America despite the distinctive morphological characteristics of the species. This situation contrasts with what is found in North American species, where the chromosome number is characterized by high heterogeneity (n= 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, and 45), which correlates with a wide spectrum of morphological diversity observed among the species (Brown & Clark 1981).

Although Grau (1976) conducted a karyogram of the species, this is the first study to present the karyotypic morphology of *H. foliosus*, highlighting the structural characteristics of the chromosomes (Table 1). Furthermore, Grau (1976) reported a chromosomal pair with a satellite in position one. These karyotypic differences in the first chromosomal pair may be attributed to measurement challenges of the satellites or genuine differences between the analyzed populations. Therefore, it is essential to conduct

studies on different populations to assess the intraspecific genomic variability that may be occurring at the chromosomal level. On the other hand, the results show similarity in karyotypic morphology for many species within the genus, where taxa exhibit at least four pairs of submetacentric chromosomes. However, this similarity cannot be directly correlated with morphological differences within the genus. Grau (1976) cytologically investigated eight species of the genus in Chile and detected a chromosomal number of 2n = 10. The karyotype of these species was very similar, consisting of a large submetacentric chromosome pair (chromosome pair with satellite) and four smaller submetacentric pairs (Grau 1981). More recent studies, based on species of *Haplopappus* (*H. grindelioides*, *H. glutinosus*, *H. macrocephalus*, *H. stolpii*, and *H. donianus*), have also found a chromosomal number of 2n = 10 (Baeza *et al.* 2007, Baeza *et al.* 2016). Other studies have exclusively recorded pairs of submetacentric chromosomes, with satellites in pair 3 for some species and in pair 2 for others (Baeza & Schrader 2005). The continuity in chromosomal similarity among species within the genus is likely related to the group's evolutionary history and the fact that distinguishable chromosomal pairs have originated through rearrangements in shape, size, and specificity within this genus.

The chromosomal asymmetries reported for the species under study (Table 1) align with Romero-Zarco's (1986) assertion regarding A1, as when the indices deviate from zero, chromosomes tend towards telocentricity. The values of A2 revealed little variability in chromosomal sizes within the species. This value is consistent with previous studies on species such as *H. glutinosus* (A2 = 0.19), *H. grindelioides* (A2 = 0.15), and *H. stolpii* (A2 = 0.16), which also exhibit low values (Baeza & Schrader 2005). Therefore, it appears that the karyotypes of this genus are rather symmetrical.

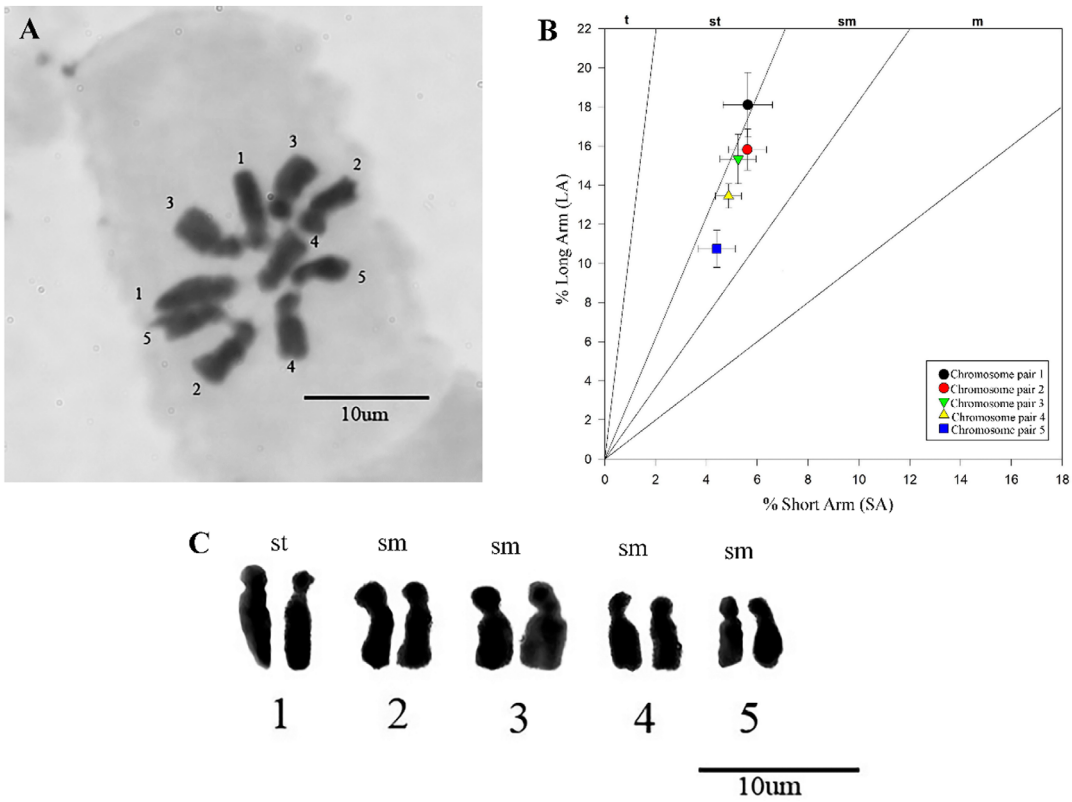

Figure 1. A: Metaphase plate of *H. foliosus* (2n = 10) 40x. Homologous chromosomes have been numbered in decreasing order according to their size. B: Karyo-ideogram of *H. foliosus*. Chromosomes with centromere in the region: t: terminal, st: subterminal, sm: submedian, and m: median. C: Karyotype of *H. foliosus*. Chromosome pairs have been numbered in decreasing order. / Placa metafásica de *H. foliosus* (2n = 10) 40x. Los cromosomas homólogos se han enumerado en orden decreciente en cuanto a su tamaño. B: Cario – ideograma de *H. foliosus*. Cromosomas con el centrómero en la región: t: terminal, st: subterminal, sm: submedia y m: media. C: Cariotipo de *H. foliosus*. Los pares de cromosomas se han enumerado en orden decreciente.

Table 1. Chromosomal characteristics of *H. foliosus*. Cp: Chromosomal pair, CP: Centromere position, LLA%: Relative length of long arm, LSA%: Relative length of short arm, and ICr%: Relative centromeric index. LR%: Relative total chromosomal length, A1: Intra-chromosomal asymmetry index, A2: Inter-chromosomal asymmetry index, LSA/LLA: Ratio of relative length of short arm/long arm. (SD: Standard deviation), CI% average centromeric index. Species with haploid chromosomal set n = 5. / Características cromosómicas de *H. foliosus*. Cp: par cromosómico, CP: posición del centrómero, LLA%: longitud relativa brazo largo, LSA%: longitud relativa brazo corto y ICr%: índice relativo centromérico. LR%: longitud total cromosómica relativa, A1: índice de asimetría intracromosomal, A2: índice de asimetría intercromosomal, LSA/LLA: relación de longitud relativa de brazo corto/brazo largo. (SD: desviación estándar), CI% promedio índice centromérico. Especie con juego cromosómico haploide n = 5.

Cp	CP	LLA%	LSA%	ICr%
1	st	18.24 ± 1.65	5.75 ± 0.98	0.24 ± 0.04
2	sm	15.79 ± 1.05	5.8 ± 0.75	0.27 ± 0.04
3	sm	15.19 ± 1.27	5.43 ± 0.71	0.26 ± 0.04
4	sm	13.35 ± 0.62	4.96 ± 0.51	0.27 ± 0.03
5	sm	10.88 ± 0.96	4.61 ± 0.74	0.30 ± 0.04

Characteristics	*H. foliosus*
Karyotypic formula	1 st + 4 sm
Number of metaphase plates	15
LR (%) ± SD	20.00 ± 3.33
Asymmetry index A1	0.63 ± 0.04
Asymmetry index A2	0.17 ± 0.04
LSA/LLA (%)	0.37 ± 0.04
CI%	0.27 ± 0.04

The cytogenetic evidence presented, based on quantitative analysis of karyotypic morphology, suggests that *H. foliosus* shares the same chromosomal number (2n=10) and considerable similarity in chromosomal morphology with species within the genus *Haplopappus* that have been studied. Additionally, for an adequate assessment of biodiversity in particular regions and to understand the current structure and dynamics of vegetation, the analysis of chromosomal data is of paramount importance. This is because the number of chromosomes and homology largely determine mating behavior and fertility, and thus reproductive behavior and patterns of variation (Stace 2000), the understanding of which is crucial for achieving a classification that reflects the process of evolution.

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REFERENCES

Anderson, L.C., Kyhos, D.W., Mosquin, T., Powell, A.M., Raven, P.H. 1974. Chromosome numbers in Compositae. IX. *Haplopappus* and other Astereae. American Journal of Botany 61(6): 665-671.

Baeza, C., Schrader, O. 2005. Comparative karyotype analysis in *Haplopappus* Cass. and *Grindelia* Willd. (Asteraceae) by double FISH with rRNA specific genes. Plant Systematics

and Evolution 251: 161-172. https://doi.org/10.1007/s00606-004-0231-2

Baeza, C., Schrader, O., Terrab, A., Stuessy, T., Rosas, M., Ruiz, E., Negritto, M. 2007. Recuentos cromosómicos en plantas que crecen en Chile III. Gayana Botánica 64(2): 175-183. http://dx.doi.org/10.4067/S0717-66432007000200002

Baeza, C.M., Peñailillo, P., Novoa, P., Rosas, M., Finot, V.L., Ruiz, E. 2016. Recuentos cromosómicos en plantas que crecen en Chile. IV. Gayana Botánica 73(2): 183-190. http://dx.doi.org/10.4067/S0717-66432016000200183

Brown, G., Clark, W. 1981. Chromosome numbers in South American *Haplopappus* Cass. (Compositae). American Journal of Botany 68: 1218-1221. https://doi.org/10.1002/j.1537-2197.1981.tb07828.x

Chennaveeraiah, M.S., Patil, B.C. 1973. Chromosome number and karyotype study in eight species of Crotalaria. Cytologia, 38(1): 73-79. https://doi.org/10.1508/cytologia.38.73

Clark, W.D., Brown, G.K., Mayes, R.A. 1980. Pollen morphology of *Haplopappus* and related genera (Compositae–Astereae). American Journal of Botany 67(9): 1391-1393. https://doi.org/10.1002/j.1537-2197.1980.tb07771.x

Frías, D. 2005. *Trupanea simpatrica* a new species of Tephritinae (Diptera:Tephritidae) infesting an endemic *Haplopappus* hybrid (Asteraceae) in Chile. Acta Entomológica de Chile 29(1): 13-45.

Grau, J. 1976. Chromosomenzahlen von südamerikanischen *Haplopappus*-Arten. Mitteilungen der Botanischen Staatssammlung, München 12: 403-410.

Grau, J. 1981. Zur Zytologie und systematischen Stellung von *Haplopappus gracilis* Phil. Mitteilungen der Botanischen Staatssammlung. München 17: 527-536.

Hall, H.M. 1928. The genus *Haplopappus*: a phylogenetic study in the Compositae. Carnegie Institution of Washington Publication. 389 pp.

Hoffmann, A. 1998. Flora Silvestre de Chile, Zona Central. Cuarta edición. Ediciones Fundación Claudio Gay. 254 pp.

Jackson, R.C. 1966. Some intersectional hybrids and relationships in *Haplopappus*. University of Kansas. Science Bulletin 46: 475-485.

Klingenberg, L. 2007. Monographie der südamerikanischen Gattungen *Haplopappus Cass.* und *Notopappus* L. Klingenberg (Asteracea–Astereae). Bibliotheca Botanica 157, E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart. 331 pp.

Lane, M.A., Hartman, R.L. 1996. Reclassification of North American *Haplopappus* (Compositae: Astereae) completed: *Rayjacksonia* gen. nov. American Journal of Botany 83(3): 356-370. https://doi.org/10.2307/2446170

Levan, A., Fredga, K., Sandberg, A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Moran, R. 1969. Five new taxa of *Haplopappus* (Compositae) from Baja California, Mexico. Transactions of the San Diego Society of Natural History 15: 150-164.

Reeves, A. 2001. MicroMeasure: a new computer program for the collection and analysis of cytogenetic data. Genome 44: 239-443.

Rodríguez, R., Marticorena, C., Alarcón, D., Baeza, C., Cavieres, L., Finot, V.L., Fuentes, N., Kiessling, A., Mihoc, M., Pauchard, A., Ruiz, E., Sanchez, P., Marticorena, A. 2018. Catálogo de las plantas vasculares de Chile. Gayana Botánica 75(1): 1-430. http://dx.doi.org/10.4067/S0717-66432018000100001

Romero-Zarco, C. 1986. A new method for estimating karyotype asymmetry. Taxon 36: 526-530. https://doi.org/10.2307/1221906

Siljak-Yakovlev, S., Peruzzi, L. 2012. Cytogenetic characterization of endemics: past and future. Plant Biosystems 146: 694-702. https://doi.org/10.1080/11263504.2012.716796

Stace, C.A. 2000. Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. Taxon 49(3): 451-477. https://doi.org/10.2307/1224344

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