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Karyotypic studies in the Chilean genus *Placea* (Amaryllidaceae)

Estudios cariotípicos en el género chileno *Placea* (Amaryllidaceae)

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ABSTRACT

Chromosome numbers and karyotypes of seven specific taxa of the Chilean endemic genus *Placea* were determined. Chromosome numbers of *P. lutea*, *P. ornata*, *P. grandiflora*, *P. germainii* and *P.* aff. *davidii* are described for the first time. All taxa are diploid with $2n=2x=16$ and karyotypes are composed of four metacentric (4 m), ten submetacentric (10 sm), and two subtelocentric (2 st) chromosomes. The most symmetrical karyotype was observed in *P. lutea* (AI: 6.84) while the most asymmetrical karyotype was shown by *P. arzae* (AI: 9.72). The constancy in karyotype formulae and high similarity in asymmetry indexes suggest that some orthoselection mechanism might be involved in *Placea*'s chromosomal evolution. In spite that no significant karyotypic differences were observed, the species may be differentiated by their chromosomal sizes. Moreover, the tribal position of *Placea* and its likely relationships with other hippeastroid genera are discussed.

Keywords: Chile, endemic flora, Hippeastreae, karyology.

RESUMEN

Se determinaron números cromosómicos y cariotipos de siete especies del género endémico chileno *Placea*. Los números cromosómicos para *P. lutea*, *P. ornata*, *P. grandiflora, P. germainii* y *P*. aff. *davidii* son descritos por primera vez. Todas las taxa son diploides con $2n=2x=16$ y los cariotipos están compuestos por cuatro cromosomas metacéntricos (4 m), diez submetacéntricos (10 sm) y dos subtelocéntricos (2 st). El cariotipo más simétrico fue observado en *P. lutea* (AI: 6,84), mientras que el cariotipo más asimétrico fue encontrado en *P. arzae* (AI: 9,72). La constancia en las fórmulas cariotípicas y la alta similaridad en los índices de asimetría sugieren que algún mecanismo de ortoselección podría estar involucrado en la evolución cromosómica del género *Placea.* A pesar que no se observaron diferencias cariotípicas significativas, las especies pueden ser diferenciadas por los tamaños cromosómicos. Además, se discuten la posición tribal de *Placea* y su relación probable con otros géneros Hippeastroides.

Palabras clave: Chile, flora endémica, Hippeastreae, cariología.

INTRODUCTION

Placea Miers ex Lindl. (Hippeastreae, Amaryllidaceae) is an endemic genus from Central Chile (Traub & Moldenke 1949). It is of considerable interest due to its narrow geographical distribution between Illapel, region Coquimbo (ca. 31ºS) and Rancagua region (ca. 34ºS) covering coastal and Andean mountains, besides hilly areas of the Central valley (Muñoz 2000, Ravenna 2003). *Placea*'s inflorescence consists of (1) 3-8 (12) flowers, with mainly whitish (more rarely red-purple, yellow or carmine-pink) tepals with red or purple longitudinal stripes and they are characterized by a conspicuous paraperigone or corona (Traub & Moldenke 1949, Traub 1963, Ravenna 2003). Due to their beautiful showy flowers, these plants have been suggested to have a high floricultural potential (Arriagada & Zöllner 1996, Bridgen *et al.* 2001, Baeza & Schrader 2004).

Chromosome studies have been carried out in almost every genera of Amaryllidaceae tribe Hippeastreae, to which *Placea* belongs (Meerow & Snijman 1998, Meerow *et al*. 1999, Meerow *et al.* 2000). Chromosome numbers have been determined for several South American species of *Hippeastrum* Herb. (e.g. Naranjo & Andrada 1975), *Habranthus* Herb. (e.g. Naranjo 1974), *Rhodophiala* C. Presl (Grau & Bayer 1991, Naranjo & Poggio 2000, Baeza *et al*. 2006, also treated as *Myostemma* Salisb.), *Phycella* Lindl. (Palma-Rojas 2000, Grau & Bayer 1991, Baeza *et al.* 2007a, 2007b), *Rhodolirium* Phil. (Palma-Rojas 2000, Naranjo & Poggio 2000, Baeza *et al.* 2009), *Zephyranthes* Herb. (e.g. Naranjo 1974) and the monotypic genus *Traubia* Mold. (Grau & Bayer 1991). However, chromosome studies in *Placea* have been addressed only in two species: *P. arzae* Phil. (Naranjo 1985) and *P. amoena* Phil. (Baeza & Schrader 2004, Baeza *et al.* 2007b), both species being diploids ($2n = 2x = 16$). The chromosome number and karyotype of the remaining *Placea* species is unknown.

The aim of this work is to determine the chromosome number and the karyotype of the genus *Placea*, and their relationships with other Hippeastroid genera in order to increase the knowledge about the karyological diversity of this endemic genus.

MATERIALS AND METHODS

The study was conducted during March to September 2008. In order to disclose the karyotype of each *Placea* species germplasm from seeds and bulbs was obtained from different localities in Chile (Table I, Fig. 1). Seeds were sowed in Petri dishes with water-moistened filter paper and placed at 4ºC until roots reached one-centimeter of length. Bulbs were placed in glass containers with water-moistened absorbent paper at room temperature until roots sprouted. Root tips were pre-treated in colchicine (0.05%) for 18 h at room temperature, then fixed in a freshly prepared mixture of absolute ethanol-glacial acid acetic (3:1) for 24 h and stored in 70% ethanol. Treated root tips were hydrolyzed in HCl 1N for 10 minutes at 60ºC, macerated, stained with lacto-propionic orcein and squashed on a slide (Araneda *et al*. 2004). Slides were made permanent using liquid nitrogen and mounted in a glycerin drop. Plates were observed using a light microscope with incorporated digital camera. Images were analyzed with Micro Measure 3.3 (Reeves 2001). The length and shape of chromosomes were determined to construct the karyotypes by using Adobe Photoshop 7.0 (Seven metaphase plates from three to ten plants per species were selected). Chromosomes were classified according to Levan *et al.* (1964), the abbreviations being m, sm, and st which correspond to metacentric, submetacentric, and subtelocentric chromosomes, respectively.

The following parameters were calculated in each metaphase plate for the numerical characterization of the karyotypes: mean haploid chromosome length (CL), and total complement length (TCL). These parameters were compared by one-way ANOVA and Tukey's test was carried out to test differences between each pair of means. Statistical evaluation was carried out using SPSS 14.0.

Karyotype asymmetry was estimated using intrachromosomal asymmetry index (A_1); interchromosomal asymmetry index (A_2); coefficient of variation of chromosome length (CV_{cl}); coefficient variation of centromeric index (CV_{ci}) and a new asymmetry index (AI) proposed by Paszko (2006). All the species were identified using the key provided by Traub & Moldenke

Table I. Taxa and localities of *Placea* used in this chromosomal study.
Tabla I. Taxa y localidades de *Placea* usadas en este estudio cromosómico.

Taxa	Locality
Placea amoena Phil.	Coquimbo. Prov. Choapa, Illapel, Cuesta El Espino. 4-XI-2004. P. Guerrero *et al.* 215 (INIA)
Placea arzae Phil.	Santiago. Prov. Chacabuco. Camino a Farellones. 18-XI-2005. M. Rosas 47 (INIA).
Placea aff. *davidii* Ravenna	Santiago. Prov. Chacabuco. Cordillera de Quilapilún. 28-XII-2004. N. García 726 (SGO, CONC)
Placea germainii Phil.	Santiago. Prov. Chacabuco. Caleu. La Capilla.10-XII-2005. O. Fernández & P. Novoa 17 (JBN)
Placea grandiflora Lem.	Santiago Prov. Chacabuco. Caleu. Cerro El Roble. 10-XII-2004. O. Fernández 67 (JBN).
Placea lutea Phil.	Valparaíso. Prov Aconcagua. Catemu, Mine of Cerro Negro. 25-X-2004. P. Guerrero *et al.* 222 (INIA).
Placea ornata Miers ex Lindl.	Valparaíso. Prov Aconcagua. Panquehue. 12-IX-2004. O. Fernández 68 (JBN).

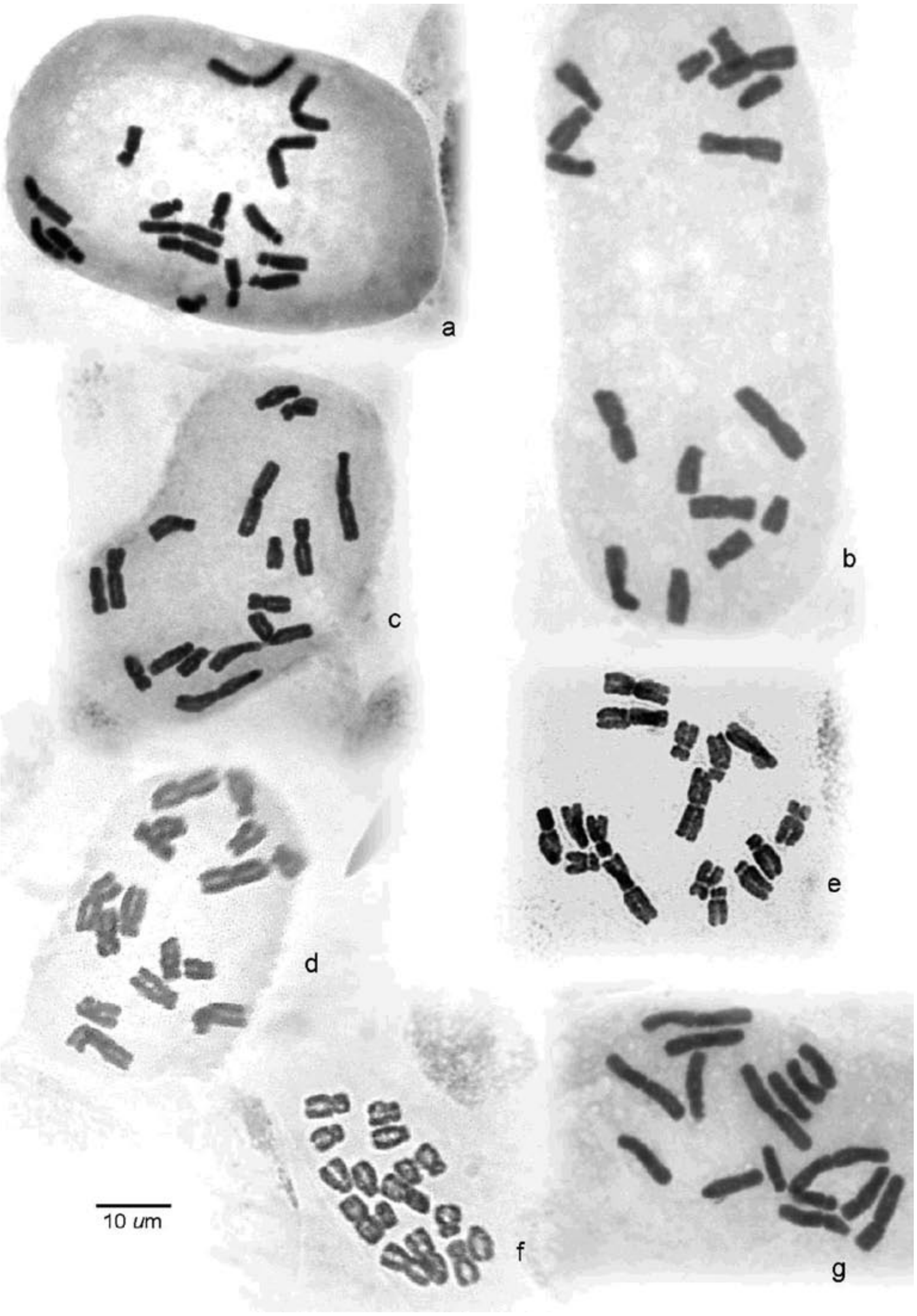

Figure 1. Somatic chromosomes of *Placea*. a) *P. grandiflora,* b) *P.* aff. *davidii,* c) *P. arzae,* d) *P. lutea,* e) *P. amoena,* f) *P. germaiini,* g) *P. ornata.* Scale bar = 10 µm. All figures to same scale.

Figura 1. Cromosomas somáticos de *Placea*. a) *P. grandiflora,* b) *P.* aff. *davidii,* c) *P. arzae,* d) *P. lutea,* e) *P. amoena,* f) *P. germaiini,* g) *P. ornata.* Escala =10 µm. Todas las figuras están en la misma escala.

(1949) and later contributions by Ravenna (1981) and Muñoz (2000). The reference materials are deposited in the herbarium of the Universidad de Concepción (CONC), Instituto Nacional de Investigaciones Agropecuarias (INIA), Jardín Botánico Nacional (JBN) and Museo Nacional de Historia Natural (SGO).

RESULTS

The analyzed parameters are summarized in Table II. Mitotic metaphases and diploid karyotypes of the species are shown in Figures 1 and 2, respectively.

All seven accessions analyzed showed a common karyotype constituted by eight chromosome pairs of different sizes and the common karyotype formula was 4m + 10sm + 2st. In most of the taxa, the longest pair corresponded to the first metacentric pair and the shortest pair was the sixth pair (submetacentric) (Table II). The shortest chromosomes were observed in *P.* aff. *davidii* and *P. lutea,* while the longest chromosomes were found in *P. amoena*. Chromosomes of *P. amoena* were 30% longer than chromosomes of *P.* aff. *davidii*.

For TCL, ANOVA discriminated among taxa (F= 18.225, $P < 0.001$). Considering the CL of each chromosomal pair, all the chromosomal pairs were different among taxa (Table II). In all the assessments, with exception of the seventh and eighth chromosome pairs, *P. amoena* was different from each other species. In general, the examined karyotypes were asymmetrical with respect to chromosome length and heterogeneous regarding chromosome uniformity. Chromosomes with low length variability were found in *P. ornata, P.* aff. *davidii* and *P. lutea* (A_2=0.27 to 0.28). All analyzed taxa showed high degrees of karyotype asymmetry indicated by their high A_1 values (0.92 to 0.93). However, according to the asymmetry index (AI), the most symmetrical karyotype was observed in *P. lutea* (AI=6.84), while the most asymmetrical karyotype was exhibited by *P. arzae* (AI=9.72). The scatter diagram of CV_{ci} and CV_{cl} (Fig. 3) showed two groups of species: one composed by the most asymmetrical karyotypes (AI=8.72-9.72) and the other with the most symmetrical karyotypes (AI=6.84-7.42). *Placea amoena* fell apart, with the lowest relative variation in centromere position, although still variable in chromosome length.

DISCUSSION

The chromosome number and karyotype structure, but not the total chromosome length, were the same for every *Placea* species. Chromosome numbers in *P.* aff. *davidii*, *P. ornata*, *P. grandiflora* and *P. germainii* are reported for the first time, establishing a diploid number ($2n$=$2x$=16) for the genus *Placea*. Chromosome numbers of the remaining taxa (*P. arzae* and *P. amoena*) agree with previous reports (Naranjo 1985, Baeza & Schrader 2004). The basic chromosome number x = 8, probably was derived by a reduction process from x = 11 (Naranjo & Poggio 2000). The basic number x = 8 has been reported in related hippeastroid genera from South America, such as *Phycella*, *Rhodophiala*, *Rhodolirium* and *Traubia* (Grau & Bayer 1991, Naranjo & Poggio 2000, Palma-Rojas 2000, Baeza *et al*. 2007a, 2007b, 2009).

The stable chromosome number in genus *Placea* suggests that neither polyploidy nor aneuploidy nor dysploidy have played a significant role in its diversification. This fact strongly contrast with studies that have stressed how chromosomal numeric alteration processes have played an important role in the evolution of other hippeastroid genera, such as *Hippeastrum*, *Rhodophiala*, *Habranthus* and *Zephyranthes* (Naranjo 1969, Naranjo 1974, Naranjo & Andrada 1975, Flory 1977, Naranjo & Poggio 2000).

As far as karyotypes are concerned, our results agree with previous reports for *Placea amoena* (4m + 10sm + 2st) (Baeza & Schrader 2004, Baeza *et al*. 2007b). However, the karyotype of *P. arzae* observed in this study disagrees with the 4m + 6sm + 6st with a satellite on the short arm of chromosome 6 (st) reported by Naranjo (1985). His observations were made in specimens obtained in Cautín, Villarrica (Chile, ca. 39º S), where *Placea* does not occur in nature. It seems likely that the material used by Naranjo (1985) was misidentified and might correspond to a species of the related genus *Rhodolirium* that occurs naturally in this area. *Placea* and *Rhodolirium* share the diploid number $2n$=16. However, the presence of a satellite on the short arm of the st chromosome pair has been reported in two species of the latter genus (*i.e. Rhodolirium montanum* Phil., cited as *Phycella* sp. in Palma-Rojas 2000 and cited as *Rhodophiala rhodolirion* (Baker) Traub in Naranjo & Poggio 2000, and *Rhodolirium andicola* (Poepp.) Ravenna, cited as *Rhodophiala andicola* (Poepp.) Traub in Naranjo & Poggio 2000). However, it would be necessary to check the herbarium voucher used by Naranjo (1985) in order to corroborate this hypothesis.

Karyotypes of all taxa are quite similar, being mostly comprised by submetacentric chromosomes. These results suggest the existence of interspecific stability in the karyotypes of *Placea*. The karyotypes were considered bimodal because of the presence of two outstanding groups of different mean sizes. The total complement is occupied by two large chromosome pairs and six medium-sized pairs. This process can be explained by karyotype orthoselection, where structural chromosome mutation occurs in a certain way, or by karyotype conservation, where the lack of structural mutations preserves the chromosome morphology. In our case, the constancy of the karyotype formula and similarity in asymmetry index (AI, A_1 and A_2), suggest that some orthoselection mechanism might be involved (White

TABLE II. Average length of total complement (TCL), chromosome length (CL), asymmetry index (AI), intrachromosomal and Interchromosomal asymmetry indexes (A_1 and A_2, respectively) of the place´s species studied.

TABLA II. Longitudes promedio del complemento cromosómico, longitudes de cromosomas e índices de asimetría (AI, A_1 y A_2) de las especies de *Placea* estudiadas.

Taxa	Mean of chromosome pairs length (CL)								AI±SD		A_1±SD		A_2±SD		TCL±SD	
	1	2	3	4	5	6	7	8								
P. amoena	14.95 c	13.30 d	10.62	8.56 c	7.68 b	7.43 c	8.13 b	6.85 bc	7.30	±1.43	0.92	±0.002	0.31	±0.01	155.03 c	±17.01
P. arzae	11.71 b	10.36 bc	8.53a	6.79 b	5.92 a	5.07 ab	5.57 a	6.60 bc	9.72	±0.63	0.93	±0.002	0.33	±0.03	121.09 b	±6.00
P. grandiflora	11.5 b	10.82 c	8.37a	6.03 ab	5.47 a	5.89 b	7.10 ab	6.94 c	8.72	±1.52	0.93	±0.001	0.31	±0.03	124.27 b	±13.89
P. germaiini	11.37 b	10.04 bc	8.07a	5.70 ab	5.30 a	4.46 a	6.41 ab	6.44 bc	9.56	±1.27	0.93	±0.001	0.32	±0.04	115.59 b	±24.38
P. lutea	9.60 ab	8.78 ab	7.04a	5.36 ab	5.38 a	4.14 a	5.30 a	6.25 ab	6.84	±0.63	0.93	±0.002	0.28	±0.02	104.59 ab	±24.63
P. ornata	9.69 ab	8.61 ab	7.29a	5.36 ab	4.92 a	4.43 a	5.94 ab	5.85 ab	7.41	±0.72	0.93	±0.002	0.28	±0.01	104.17 ab	±18.61
P. aff. *davidii*	8.33 a	7.92 a	6.84a	4.85 a	4.67 a	4.03 a	4.94 a	5.36 a	7.42	±0.88	0.93	±0.005	0.27	±0.01	93.89 a	±6.36

Values followed by the same letter are not significantly different ($p \leq 0.05$) / Valores seguidos de la misma letra no son significativamente diferentes ($p \leq 0.05$).

FIGURE 2. Diploid karyotypes. a) *P. grandiflora,* b) *P.* aff. *davidii,* c) *P. arzae,* d) *P. lutea,* e) *P. amoena,* f) *P. germaiini,* g) *P. ornata*. Scale bar = 10 µm. All figures to same scale.

FIGURA 2. Cariotipos diploides. a) *P. grandiflora,* b) *P.* aff. *davidii,* c) *P. arzae,* d) *P. lutea,* e) *P. amoena,* f) *P. germaiini,* g) *P. ornata*. Escala de la barra =10 µm. Todas las figuras están en la misma escala.

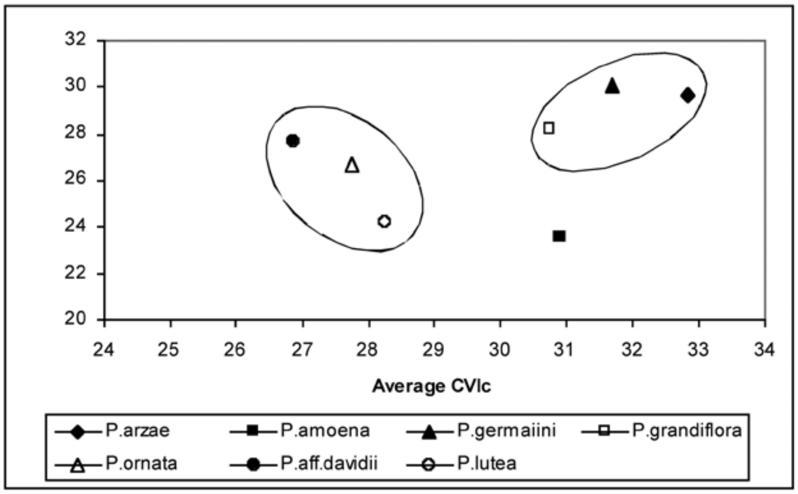

FIGURE 3. Scatter diagram for species of *Placea*. CV_{ci} versus CV_{cl}.

FIGURA 3. Diagrama de flujo de las especies de *Placea*, considerando CV_{ci} versus CV_{cl}.

1973, Sanso 2002). Similar results have been found in other South American monocots, such as *Rhodophiala* (Naranjo & Poggio 2000), *Hippeastrum* (Naranjo 1969, Naranjo & Andrada 1975) and *Alstroemeria* (Sanso 2002).

The chromosome size is also subject to evolutionary change. Frequently, the total mass of chromosomes in a nucleus has been found to be closely related to its DNA content. Thus, a factor responsible of the range of variation of chromosome size among species of the same genus may be polyploidy, repeated DNA content, or increase in the basic number (Sharma & Sen 2002, Schubert 2007). The variation of chromosome size found in this study ranges between 14.95-6.85 μm in *P. amoena* to 8.33-4.03 μm in *P.* aff. *davidii*. However, neither polyploidy nor an increase in the basic number were recorded in *Placea*. Therefore, differences in DNA content would be the most plausible explanation for chromosome size variation in this genus. However, this suggestion must be confirmed with DNA content measurements (e.g. flow cytometry). In spite of the similarity in the coefficients of variation assessed in all the species, the scatter diagram of CV_{ci} and CV_{cl} showed two groups of species and *Placea amoena* as an isolated species. This singular position of *Placea amoena* is correlated with both karyotype difference and its current taxonomic segregation. This result supports subgenus *Geissea* (Traub & Moldenke 1949), defined to distinguish the systematic placement of *P. amoena* from the rest of the genus, although the present phenetic analysis has no implications on whether it corresponds to a distinct evolutionary lineage within *Placea*.

The systematic and phylogenetic relationships of *Placea* within the tribe Hippeastreae is unknown, because previous phylogenetic studies in Amaryllidaceae have included only few samples of Chilean genera and none of *Placea* (Meerow *et al*. 1999, 2000). However, *Placea, Phycella*, *Rhodolirium* and *Traubia* share the same basic chromosome number *x*=8 (Naranjo 1985, Palma-Rojas 2000, Grau & Bayer 1991, Naranjo & Poggio 2000, Baeza *et al.* 2009) and the presence of a capitate stigma (Ravenna 2003). These shared traits suggest that these genera might be closely related within tribe Hippeastreae.

Finally, these results should be considered as a first insight into the karyotypic evolution of *Placea,* however, several other aspects must be considered and explored in order to achieve a better understanding of this phenomenon. It would be very useful to carry out an extensive survey across the geographical distribution range of *Placea* to assess intra- and interspecific variability, and perform a phylogenetic analysis to obtain a framework of its evolutionary history.

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