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Short communication

Updating the list of chromosome numbers for *Philodendron* (Araceae)

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ABSTRACT

Aiming for a better understanding of karyotype evolution within *Philodendron*, we report chromosome counts for 23 species of the genus, of which 19 are being reported for the first time, thus increasing to 84 (*ca*. 17% of the genus) the total number of species with available chromosome counts. The diploid numbers $2n = 32$ and $2n = 34$ were the most common, with 10 and 11 species, respectively, whereas only two species presented different chromosome numbers (*P. giganteum* with $2n = 30$ and *P. adamantinum* with $2n = 36$). The results are discussed in the context of previous analyses of karyotypes of *Philodendron* spp., taking into account bidirectional dysploidy as the main mechanism of chromosome number evolution within the genus.

Keywords: aroids, diploid number, dysploidy, karyotype, *Philodendron*

Philodendron (Araceae) is one of the most prominent monocot groups in the humid Neotropical forests, being composed mostly of lianescent species (Grayum 1996; Croat 1997). The genus is the second largest of the aroid family, comprising almost 500 species (Boyce & Croat 2016), which have been traditionally subdivided into three major groups: *P.* subgenus *Meconostigma* (21 spp.), *P.* subgenus *Pteromischum* (82 spp.) and *P.* subgenus *Philodendron* (*ca*. 380 spp. subdivided into 10 sections) (Sakuragui *et al*. 2005; Barbosa & Sakuragui 2014; Calazans *et al*. 2014). There is a considerable ecological variation within *Philodendron*, mainly observed among the species of *P.* subgenus *Philodendron*, which also presents the widest geographic distribution, ranging from Mexico to Uruguay (Croat 1997; Mayo *et al*. 1997).

Considering the proportion of 19% of angiosperms with known chromosome numbers (Rice *et al*. 2015), the members of Araceae have been relatively well sampled in cytogenetic studies, with a coverage of 26% of the approximately 3400 species (Bogner & Petersen 2007; Cusimano *et al*. 2012; Boyce & Croat 2016). Recently, Correia-da-Silva *et al*. (2014) reviewed the list of chromosome numbers previously published for *Philodendron* species, besides reporting new chromosome counts for the group. According to these authors, the coverage of the genus is considerably lower than the observed in other genera of Araceae, with only 13% of the species with available chromosome counts (66 out of *ca*. 500). Although there is a certain degree of variation of chromosome numbers within the genus, ranging from $2n = 28$ to 40, most of the species present either $2n = 32$

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(45.4%; 30 spp.) or 2*n* = 34 (27.3%; 18 spp.) (Correia-da-Silva *et al*. 2014). Therefore, in order to increase the list of chromosome counts for *Philodendron*, as well as aiming for a better understanding of the karyotype evolution within the genus, we bring diploid numbers for 23 species, 19 of which are being reported for the first time.

All plant materials were obtained from the Araceae living collection held at the Royal Botanic Gardens, Kew, except for the accession of *P. mello-barretoanum* Burle-Marx ex G.M.Barroso, which is cultivated in the *Philodendron* living collection of the Department of Genetics of the Federal University of Pernambuco (Universidade Federal de Pernambuco – Recife, Brazil) (Tab. S1 in supplementary material).

Chromosome counts followed the procedures used by Correia-da-Silva *et al*. (2014), with some modifications. Root tips were collected, pre-treated with 2 mM 8-hydroxyquinoline at room temperature (*ca*. 25 °C) for 1 h and, and at 10 °C for 23 h, fixated in Carnoy (3:1 ethanol:acetic acid, v/v) at room temperature for 4-6 h and stored at –20 °C. Subsequently, root tips were digested for 24 h at 37 °C in an enzymatic solution containing 2% (w/v) cellulase from *Aspergillus niger* Tiegh. (Sigma-Aldrich) and 20% (v/v) pectinase from *A. niger* (Sigma-Aldrich) and squashed in a drop of 45% acetic acid. Chromosome preparations were stained and mounted with a DAPI-glycerol solution (2 µg/mL 4',6-diamidino-2-phenylindole and glycerol; 1:1, v/v) for 10-15 min. Cell images were acquired with a Leica DMLB epifluorescence microscope and a Leica DFC 340FX camera with the Leica CW4000 software.

Among the analyzed karyotypes, the diploid numbers 2*n* = 32 and 2*n* = 34 were the most frequent, being observed for 10 (*P. annulatum*, *P. ernestii* (Fig. 1A), *P. glanduliferum* (Fig. 1B), *P. glaziovii*, *P. inconcinnum* (Fig. 1C), *P. jacquinii* (Fig. 1D), *P. lacerum* (Fig. 1E), *P. longilaminatum* (Fig. 1F), *P. schmidtiae* and *P. smithii* (Fig. 1G)) and 11 (*P. angustilobum*, *P. burle-marxii* (Fig. 1H), *P. cordatum*, *P. erubescens* (Fig. 1I), *P. krugii*, *P. maximum* (Fig. 1J), *P. mello-barretoanum* (Fig. 1K), *P. renauxii*, *P. tenue*, *P. tripartitum* and *P. uleanum* (Fig. 1L)) species, respectively (Tab. S1 in supplementary material). Only *P. giganteum* (2*n* = 30; Fig. 1M) and *P. adamantinum* (2*n* = 36) had different chromosome numbers. Therefore, we further confirm the numbers 2*n* = 32 and 2*n* = 34, particularly the first one, as the most important in the genus, although without any clear pattern of distribution among the different sections of *P.* subgenus *Philodendron* (Tab. S1 in supplementary material).

Considering the two diverging counts previously published for *P. giganteum*, 2*n* = 30 (Simmonds 1954) and 2*n* = 34 (Jones 1957), we confirmed the data from the first analysis (Tab. S1 in supplementary material). As previously pointed out by Correia-da-Silva *et al*. (2014) for several other *Philodendron* species, this seems to be the case of a miscount by Jones (1957), instead of the existence of a chromosome number polymorphism within the species. Such likely miscounts may be linked to the use paraffin sections by the author to obtain the chromosome preparations for materials with small chromosomes such as *Philodendron* spp. (Jones 1957), instead of the most usual flattening of macerated meristems by squashing between slide and coverslip. Similarly, the data previously published by Sharma & Mukhopadhyay (1965) for *P. lacerum* (2*n* = 36) and *P. erubescens* (2*n* = 32) and were divergent from the chromosome numbers observed here, which were 2*n* = 32 (Fig. 1E) and 2*n* = 34 (Fig. 1I), respectively (Tab. S1 in supplementary material). On the other hand, for *P. cordatum*, the number 2*n* = 34, which Jones (1957) had previously observed, was corroborated here (Tab. S1 in supplementary material).

Regarding the analyzed species of *P.* subgenus *Meconostigma*, *P. adamantinum*, showed a commonly observed number for eastern Brazilian species of the subgenus (2*n* = 36), as well as *P. corcovadense*, *P. saxicola* and *P. undulatum*, for instance (Correia-da-Silva *et al*. 2014), while *P. mello-barretoanum* showed 2*n* = 34, which is being reported for the first time for the subgenus. As our new results for *P. lacerum* associates the species with a quite common diploid number for *P.* subgenus *Philodendron* (2*n* = 32), *P. rugosum* is currently the only species of the mentioned group with a double-checked diploid number of 2*n* = 36 (Bogner & Bunting 1983; Petersen 1989). In addition, the number 2*n* = 36 has been observed almost only in the heliophytes of *P.* subgenus *Meconostigma*, which has been indicated as a derivative habit within the group (Calazans *et al*. 2014; Loss-Oliveira *et al*. 2016). Thus, *x* = 18 may not be the primitive basic number in *Philodendron*, not representing the whole genus, as previously discussed by Correia-da-Silva *et al*. (2014). Instead, we suggest either 2*n* = 32 or 2*n* =34 as the ancestral diploid number for the group, although further confirmation is necessary through an analysis of ancestral chromosome number reconstruction. Therefore, bidirectional dysploidy (starting from 2*n* = 32 or 2*n* = 34) could be regarded as the main cause of chromosome number variation among *Philodendron* species, as largely observed among Araceae genera, probably being the most significant events during the karyotype evolution within the family (see Sousa & Renner 2015), besides being frequently reported for other angiosperm groups, such as *Brachypodium* (Poaceae) (Idziak *et al*. 2014) and *Melampodium* (Asteraceae) (McCann *et al*. 2016), for instance.

Including the results presented here, chromosome counts are now available for 84 species of *Philodendron* (ca. 17% of the ca. 500 species), excluding the findings for the cultivated hybrids presented by Catalano *et al*. (1964), Catalano & Landi (1966) and Jones (1957) (Tab. S1 in supplementary material). As previously mentioned, assumptions regarding the association between taxa and diploid numbers cannot be easily defined, due to the still low coverage of some groups, such as *P.* subgenus *Pteromischum*, for which there are chromosome counts for only two species (Tab. S1 in

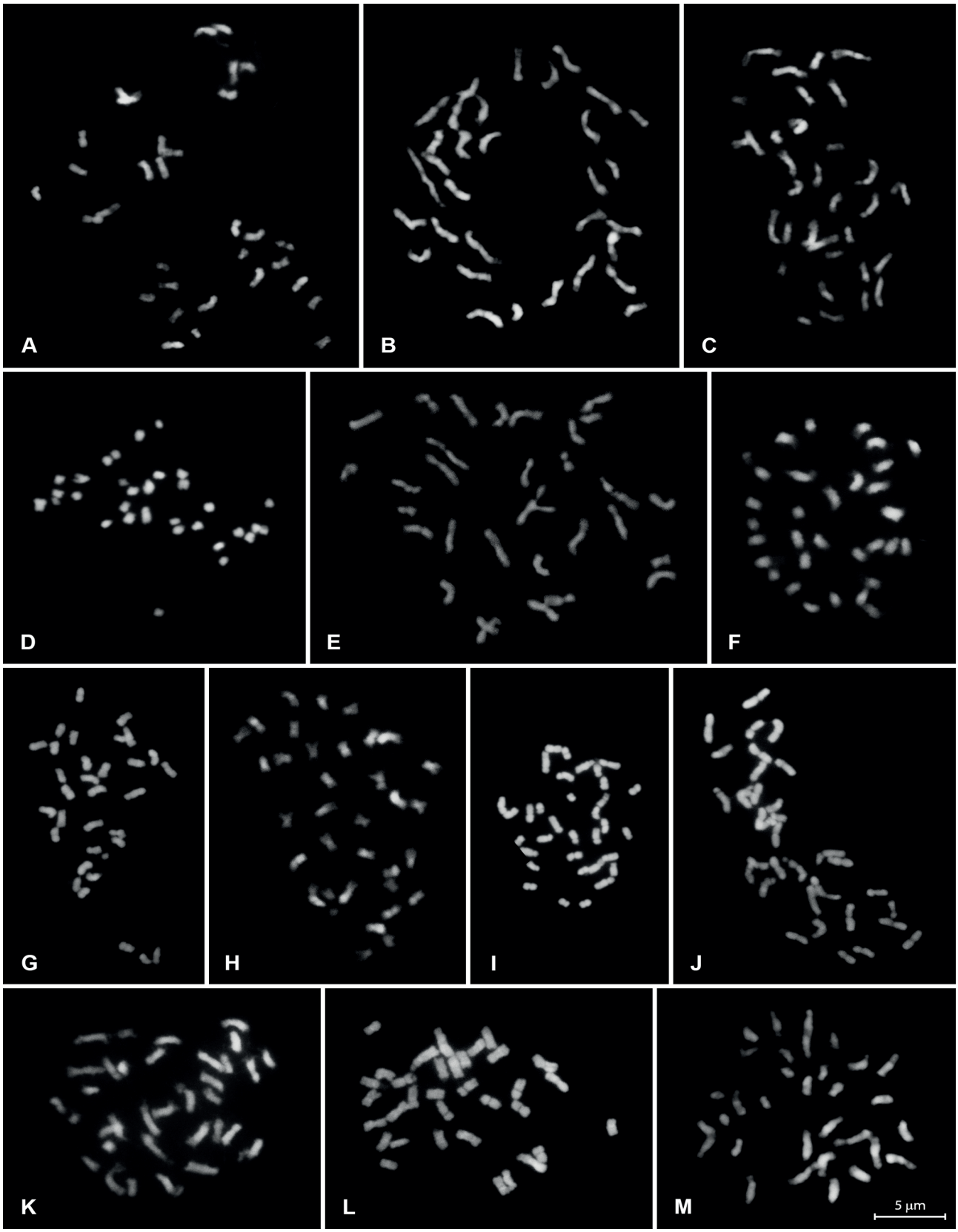

Figure 1. Mitotic chromosomes of *Philodendron* species stained with DAPI (4',6-diamidino-2-phenylindole). *Philodendron ernestii* (2*n* = 32; A); *P. glanduliferum* (2*n* = 32; B); *P. inconcinnum* (2*n* = 32; C); *P. jacquinii* (2*n* = 32; D); *P. lacerum* (2*n* = 32; E); *P. longilaminatum* (2*n* = 32; F); *P. smithii* (2*n* = 32; G); *P. burle-marxii* (2*n* = 34; H); *P. erubescens* (2*n* = 34; I); *P. maximum* (2*n* = 34; J); *P. mello-barretoanum* (2*n* = 34; K); *P. uleanum* (2*n* = 34; L); ; and *P. giganteum* (2*n* = 30; M).

supplementary material; see Correia-da-Silva *et al*. 2014). On the other hand, only by increasing the knowledge on the cytogenetic features of the *Philodendron* species, one may understand the evolutionary pathways of the karyotypes within the genus, as reported for *Typhonium* (Sousa *et al*. 2014), another aroid genus from the subfamily Aroideae.

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