

# Chromosome variations and diversity of *Epidendrum ibaguense* Lindl. (Orchidaceae) on the Tepequém's Tepuy, Roraima, Brazil

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**ABSTRACT.** Studies addressing chromosome variations have elucidated many points regarding the taxonomy of the Orchidaceae. *Epidendrum* L. besides being one the largest orchid genera, present remarkable morphological, and inter- and intraspecific chromosome variations. Thus, based on a previous report on flower color variation in individuals of *E. ibaguense* (magenta, pink, white, and red), our aim was to determine its chromosome number and test whether this trait is associated with flower color variation in natural populations on the Tepequém's Tepuy, Roraima. Root apices were pre-treated with 8-hydroxyquinoline at 4°C for 24 h and subsequently submitted to conventional cytogenetic procedures. Slides with the best spreading and contraction of chromosomes were photographed under light

microscopy. Chromosome number was determined by counting at least 10 mitotic metaphase cells per individual. The types of interphase nuclei were determined for 30 nuclei per individual. *E. ibaguense* presented intra- and interpopulation variation in chromosome number, with  $2n = 58, 72,$  and  $76$ . The chromosome number  $2n = 58$  was most commonly found in individuals with magenta, pink, and white flowers, while the remaining two chromosome numbers occurred mostly in red-flowered individuals. The types of interphase nuclei were associated with the chromosome number. Individuals with  $2n = 58$  presented a predominance of semi-reticulated nuclei, while in those with  $2n = 72$  and  $76$  the nuclei were predominantly non-reticulated. The dominance of dispolyploidy in *E. ibaguense* suggests that this cytotype provides this species with a territorial advantage and a higher reproductive success, possibly contradicting the polyploid hypothesis. Our results suggest that chromosome number may not represent a reproductive barrier in genus *Epidendrum*.

**Key words:** Chromosomes; Hybridization; Cytogenetics; Amazon; Orchids

## INTRODUCTION

Chromosome analyses provide strong support for plant biosystematics, especially when the variation is a key trait that helps to recognize the validity of a certain species (Stace, 1991). According to Guerra (1986) and Stace (1991), the divergence in chromosome number between morphologically close species can split distinct taxa, remarkably when the morphological variation is also present.

Thus, studies addressing chromosome variations have undoubtedly shed light into taxonomic statuses of the Orchidaceae, one of the largest Angiosperm families. Among the many genera composing this family, *Epidendrum* L. stands out with about 1500 described species (Chase et al., 2003). In *Epidendrum*, chromosome number can vary greatly, ranging from  $2n = 24$  in *E. fulgens* Brongn. to  $2n = 240$  in *E. cinnabarinum* Salzm. Despite such variation, chromosome number is currently known for only 3% of the *Epidendrum* species. Apart from these interspecific differences, chromosome number varies within a given species, and even between neighboring populations (e.g., *E. denticulatum* Barb.,  $2n = 38, 40$ ; *E. radicans* Pav. ex Lindl. Rodr.,  $2n = 40, 57, 60, 62, 64, 70$ ; and *E. xanthinum* Lindl.,  $2n = 28, 30, 40, 60,$  ca.  $80$ ) (Pinheiro et al., 2009; Felix and Guerra, 2010; Assis et al., 2013). The largest number of cytotypes is currently known for *E. secundum* Jacq., with  $2n = 28, 40, 48, 52, 68,$  and  $80$  (Pinheiro et al., 2009) or  $2n = 30, 42, 50, 54, 56, 58,$  and  $84$  (Assis et al., 2013). Additionally, in *E. secundum* the chromosomes also vary in size, revealing a strong karyotypic asymmetry and bimodality (Assis et al., 2013).

When compared to dysploidy, polyploidy is considered a major adaptive mechanism in plants, expanding their ecological and geographic distribution (Briggs and Walters, 1997). Along with hybridization, polyploidy is recognized as a key process in plant speciation (Rieseberg, 1997; Grant, 1981). Studies suggest that polyploid organisms, which have more than two genomes in the same nucleus, have increased adaptability to harsh environmental conditions, as well as being good colonizers (Ramsey and Schemske, 1998; Schifino-Wittmann, 2004; Fawcett and Van de Peer, 2010). Moreover, polyploidy is apparently related

to subspecific morphological variation (Pinheiro and Cozzolino, 2013). In *Epidendrum*, besides chromosome number variations, morphological traits of the species also vary greatly (Dressler, 1993, 2005; Pinheiro and Barros, 2007; Pansarin and Amaral, 2008).

*E. ibaguense* Kunth. is distributed from Mexico to Bolivia, and is commonly found in rocky outcrops, at elevations ranging from 200 - 1000 m, both in the savannas and Rain forests (Freitas, 2001; Luz and Franco, 2012; Flora do Brasil 2020 em construção, 2017). In Brazil, its distribution is limited to the North and Midwest regions, within the Amazon Domain (Tropicos.org domain, 2017; Flora do Brasil 2020 em construção, 2017). Individuals of *E. ibaguense* with different flower colors were reported on a “Tepuy” (Luz and Franco, 2012). This is a type of ancient, table-top Mountain with a particular flora (Alves et al., 2007). Based on this report, and given the great variation in chromosome number in genus *Epidendrum*, this study aimed 1) to determine the chromosome numbers, and 2) to test for an association between chromosome number and flower color variation in natural populations of *E. ibaguense* on the Tepequém’s Tepuy, municipality of Amajari municipality, Roraima.

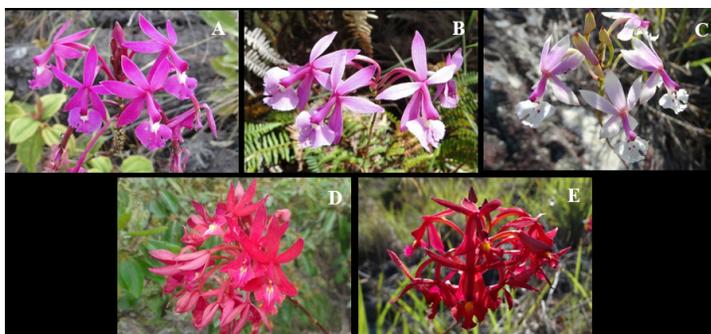
## MATERIAL AND METHODS

### Study site

We collected individuals of *E. ibaguense* from the Tepequém’s Tepuy, in the municipality of Amajari/RR. The Tepequém’s Tepuy is a remain of an eroded sandstone plateau, reaching up to 1100 m. Its flora is composed mainly of grasses, shrubs, and subshrubs (Silva, 1997). The studied populations of *E. ibaguense* are located at the following coordinates: 3°45'56.96750"N and 61°42'09.59398"W, at 831 m (population 1), and 3°45'55.34681"N and 61°41'18.97437"W, at 1056 m (population 2).

### Sample selection and collection

To determine the chromosome numbers of *E. ibaguense*, we selected individuals with different flower colors: magenta and pink (the most common individuals), and red and white (rare individuals) (Figure 1). The morphological characterization of vouchers used in the cytogenetic analysis is shown in Table 1.



**Figure 1.** Flower color variation of *Epidendrum ibaguense* on the Tepequém’s Tepuy/RR. **A.** magenta flowers; **B.** pink flowers; **C.** white flowers; **D.** terrestrial red flowers from population 1 (1); **E.** rupicolous red flowers from the population 2 (2).

## Chromosome analysis

We previously treated root apices of *E. ibaguense* with 8-hydroxyquinoline at 4°C for 24 h, and postfixed the samples in Carnoy (ethanol:acetic acid, 3:1) at 4°C. The root apices were hydrolyzed in 1 N HCl for 10 min at 60°C, smashed on glass slides, and stained with 2% Giemsa for 20 min (adapted from Guerra, 1988). We photographed those slides with the best spreading and contraction of chromosomes under a light microscope. We determined the chromosome number for at least 10 mitotic metaphases per individual, and the type of interphasic nuclei in 30 samples per individual.

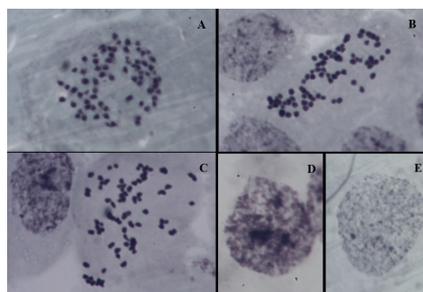
**Table 1.** Morphological traits of the vouchers used in the cytogenetic analysis.

	Magenta		Pink	White	Red (1)	
	1	2	2	2	1	2
Population						
Leaf length	59.41	55.38	60.05	54.2	71.36	28.65
Leaf width	2.63	2.4	1.87	1.81	2.77	3
Inflorescence length*	65	113.75	78.5	36	137	25
Flower /Inflorescence	5F 8B	5F 8B	5F 9B	3F 6B	8F 18B	2F 4B
Sepal length	11.75	13.85	16.22	14.05	15.98	13.47
Petal length	16.42	13.26	17.44	13.77	15.75	15.03
Labellum length	19.7	18.99	18.13	19.27	19.54	19.37
Labellum diameter	14.94	11.36	12.51	11.47	10.45	10.28
Column length	9.49	8.69	11.95	9.07	10.05	9.98
Habit	Terrestrial	Rupicolous	Terrestrial	Terrestrial	Terrestrial	Rupicolous

Values are reported in millimeters, \*except for inflorescence length, which is in centimeters. F- Flowers and B- Buds.

## RESULTS

*E. ibaguense* presented intra- and interpopulation variation in chromosome number, with  $2n = 58$ ,  $72$ , and  $76$  (Figure 2), and  $2n = 58$  being most common in four individuals from both studied populations. This finding represents a valuable contribution to the cytogenetic status of *E. ibaguense* since  $2n = 70$  has been the only chromosome number report up to now (Pinheiro et al., 2009). At population 1, the terrestrial, magenta-flowered individual presented  $2n = 58$ , while the terrestrial, red-flowered individual sheltered in the shade presented  $2n = 72$ . At population 2, the rupicolous individuals with magenta, pink, and white flowers presented  $2n = 58$ , while rupicolous, red-flowered individuals exposed to the sunlight presented  $2n = 76$ . Moreover, in individuals with  $2n = 58$ , interphasic nuclei were predominantly semi-reticulated, whereas in those with  $2n = 72$  and  $76$  nuclei were predominantly non-reticulated.



**Figure 2.** Metaphasic cells of *Epidendrum ibaguense* showing condensed chromosomes. **A.**  $2n = 58$ , magenta-flowered morphotype; **B.**  $2n = 72$ , terrestrial, red-flowered morphotype (population 1); **C.**  $2n = 76$ , rupicolous, red-flowered (population 2). Type of interphasic nuclei: **D.** semi-reticulated; **E.** non-reticulated.

## DISCUSSION

In *E. ibaguense*, the chromosome number was first reported by Pinheiro et al. (2009). These authors determined  $2n = 70$  for individuals from Serra de Pacaraima, located on the border between Venezuela and Roraima. In our study, in both studied populations,  $2n = 58$  was the most frequent chromosome number, while  $2n = 72$  and  $2n = 76$  were less frequent. Therefore, in both populations of the Tepequém's Tepuy, diploid individuals of *E. ibaguense* ( $2n = 58$ ) may have greater reproductive and environmental success, as suggested by their higher local abundance. Our results suggest that in *E. ibaguense*, descendant and ascendant dispyloid events may have caused the chromosome variation in this species on the Tepequém's Tepuy.

According to Felix and Guerra (2010), in the subfamily Epidendroideae, only five of the 128 genera present a stable number of chromosomes. *Epidendrum* is one of the few genera that present variation in chromosome number, including at the intraspecific level [e.g., *E. ciliare* L., *E. denticulatum*, *E. radicans*, *E. xanthinum*, and in the complex *E. secundum* (Tanaka and Kamemoto, 1984; Pinheiro et al., 2009; Felix and Guerra, 2010; Assis et al., 2013) and *E. ibaguense* (this study)].

Such variation in chromosome number in *Epidendrum* suggests that determining a basic karyotype for this genus is uncertain, bringing about the difficulty in estimating the level of polyploidy and the karyotypic evolution in this taxon (Felix and Guerra, 2000). However, dysploidy (loss or gain of chromosomes) has been considered the most important evolutionary events in Orchidaceae (Felix and Guerra, 2010), while in plants as a whole, as well as in many orchid genera, polyploidy plays such a role (Felix and Guerra, 2000, 2005; Conceição et al., 2006).

It has been observed that highly polymorphic species are adapted to environmental conditions that impose strong selective pressures. Some groups within Orchidaceae that grow on inselbergs show levels of polyploidy higher than those related to epiphytic species (Felix and Guerra, 2010). In *E. cinnabarinum*, for instance, the intraspecific polyploidy seems to be related to the acquisition of the rupicolous habit (Felix and Guerra, 2010). Interestingly, this and other polyploid species are found in disturbed environments (Assis, 2009; Pinheiro et al., 2009; Felix and Guerra, 2010). Duplicated or repetitive gene sequence may provide a local advantage for polyploid organisms (Fawcett and Van der Peer, 2010). On the other hand, such a variation in chromosome number may not represent an effective reproductive barrier neither for *Epidendrum* nor the remaining groups of plants (Cozzolino et al., 2004; Marques et al., 2010).

*E. ibaguense* is phylogenetically close to the Andean clade, mainly distributed between the Andean Mountains and Guyana (Pinheiro et al., 2009; Pinheiro and Cozzolino, 2013). In Brazil, it is found exclusively in the Amazon, particularly on the studied Tepuy. Little is known about these types of mountains, and biological information concerning this orchid group is important to understand its evolution.

Flower color variation in *E. ibaguense* may be associated with the attraction of the different local groups of pollinators (Proctor and Yeo, 1973; Richards, 1996). In the Amazonian orchids, the most common flower colors are yellow, white, and lilac, which lie within the visible spectrum of bees (Ribeiro et al., 1999). Red flowers are invisible to bees and are uncommon in orchids (Faegri and Van Der Pijl, 1979). According to Weiss (1995), flower color change evolved in response to a pollinator-driven selection, which may represent a functional convergence in the angiosperms. Red-flowered individuals of *E. ibaguense* may be coevolving with a specific group of pollinators.

There are still knowledge gaps concerning Amazonian Orchidaceae, and, thus, much

to be discovered about this family (Krahl et al., 2015). Moreover, studies addressing the origins of the tremendous diversity of *Epidendrum* are often constrained by the lack of model organisms capable of answering many fundamental questions about plant evolution (Pinheiro and Cozzolino, 2013). Therefore, punctual studies may fill such a gap about this orchid genus.

### Conflict of interest

The authors declare no conflicts of interest.

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### REFERENCES

- Alves RJV, Cardin L and Kropf MS (2007). Angiosperm disjunction “Campos rupestres - restingas”: a re-evaluation. *Acta Bot. Bras.* 21: 675-685. <https://doi.org/10.1590/S0102-33062007000300014>
- Assis FNM (2009). Variação numérica e evolução cariotípica em *Epidendrum* L. (Orchidaceae: Epidendroideae). Available at [<http://www.cca.ufpb.br/ppga/pdf/mestrado/Felipe%20Nollet-ms09.pdf>].
- Assis FNM, Souza BCQ, Medeiros-Neto E, Pinheiro F, et al. (2013). Karyology of the genus *Epidendrum* (Orchidaceae: Laeliinae) with emphasis on subgenus *Amphiglottium* and chromosome number variability in *Epidendrum secundum*. *Bot. J. Linn. Soc.* 172: 329-344. <https://doi.org/10.1111/boj.12045>
- Briggs D and Walters SM (1997). Plant variation and evolution. Cambridge University Press, Cambridge.
- Chase MW, Cameron KM, Barrett RL and Freudenstein JV (2003). DNA data and Orchidaceae systematics: a new phylogenetic classification. In: Orchid conservation. Natural History Publications (Dixon KW, Kell SP, Barrett RL, Cribb PJ, eds.). Sabah, 69-89.
- Conceição LP, Oliveira ALPC and Barbosa LV (2006). Characterization of the species *Epidendrum cinnabarinum* Salzm. (Epidendroideae: Orchidaceae) occurring in dunas do Abaeté-Salvador, BA-Brazil. *Cytologia (Tokyo)* 71: 125-129. <https://doi.org/10.1508/cytologia.71.125>
- Cozzolino S, D'Emérico S and Widmer A (2004). Evidence for reproductive isolate selection in Mediterranean orchids: karyotype differences compensate for the lack of pollinator specificity. *Proc. Biol. Sci.* 271 (Suppl 5): S259-S262. <https://doi.org/10.1098/rsbl.2004.0166>
- Dressler R (1993). Phylogeny and classification of the orchid family. Dioscorides Press, Portland.
- Dressler R (2005). How many orchid species? *Selbyana* 26: 155-158.
- Faegri K and Van Der Pijl L (1979). Principles of pollination ecology. 3rd edn. Pergamon Press, London.
- Fawcett JA and Van der Peer Y (2010). Angiosperm polyploids and their road to evolutionary success. *Trends Evol. Biol.* 1: 17-21.
- Felix LP and Guerra M (2000). Cytogenetics and cytotaxonomy of some Brazilian species of Cymbidioid orchids. *Genet. Mol. Biol.* 23: 957-978. <https://doi.org/10.1590/S1415-47572000000400041>
- Felix LP and Guerra M (2005). Basic chromosome numbers of terrestrial orchids. *Plant Syst Evol.* 254: 131-148.
- Felix LP and Guerra M (2010). Variation in chromosome number and the basic number of subfamily Epidendroideae (Orchidaceae). *Bot. J. Linn. Soc.* 163: 234-278. <https://doi.org/10.1111/j.1095-8339.2010.01059.x>
- Flora do Brasil 2020 em construção (2017). *Epidendrum*. Jardim Botânico do Rio de Janeiro. Available at [<http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB37569>]. Accessed April 18, 2017.
- Freitas FL (2001). Orquídeas na Amazônia. Roraima: Boa Vista.
- Grant V (1981). Plant speciation. Columbia University Press, New York.
- Guerra M (1986). Reviewing the chromosome nomenclature of Levan et al. *Rev. Bras. Genet.* 9: 21-40.
- Guerra MS (1988). Introdução à Citogenética Geral. Guanabara, Rio de Janeiro.

- Krahl AH, Krahl DRP, Valsko JJ, Holanda ASS, et al. (2015). Biologia reprodutiva e polinização em orquídeas: com ênfase em espécies brasileiras e da região amazônica - uma revisão de literatura. *Natureza on-line* 13: 128-133.
- Luz FJF and Franco J (2012). Orquídeas de Roraima. Embrapa, Brasília.
- Marques I, Feliner GN, Draper Munt D, Martins-Loução MA, et al. (2010). Unraveling cryptic reticulate relationships and the origin of orphan hybrid disjunct populations in *Narcissus*. *Evolution* 64: 2353-2368.
- Pansarin ER and Amaral MCE (2008). Reproductive biology and pollination mechanisms of *Epidendrum secundum* (Orchidaceae). Floral variation: a consequence of natural hybridization? *Plant Biol (Stuttg)* 10: 211-219. <https://doi.org/10.1111/j.1438-8677.2007.00025.x>
- Pinheiro F and Barros F (2007). Morphometric analysis of *Epidendrum secundum* (Orchidaceae) in southeastern Brazil. *J. Nord. J. Bot.* 25: 129-136. <https://doi.org/10.1111/j.0107-055X.2007.00010.x>
- Pinheiro F and Cozzolino S (2013). *Epidendrum* (Orchidaceae) as a model system for ecological and evolutionary studies in the Neotropics. *Taxon* 62: 77-88.
- Pinheiro F, Koehler S, Corrêa AM, Salatino MLF, et al. (2009). Phylogenetic relationships and infrageneric classification of *Epidendrum* subgenus *Amphiglottium* (Laeliinae, Orchidaceae). *Plant Syst. Evol.* 283: 165-177. <https://doi.org/10.1007/s00606-009-0224-2>
- Proctor M and Yeo P (1973). The pollination of flowers. In: Handbook of experimental pollination biology (Jones CE and Little RJ, eds.). Scientific and Academic Editions, New York.
- Ramsey J and Schemske DW (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Syst.* 29: 467-501. <https://doi.org/10.1146/annurev.ecolsys.29.1.467>
- Ribeiro JE, Hopkins MJG, Vincentini A, Sothers C, et al. (1999). Flora da Reserva Ducke: Guia de Identificação das Plantas Vasculares de uma Floresta de Terra-Firme na Amazônia Central. INPA, Manaus.
- Richards PW (1996). Trees and shrubs: II. Reproductive biology. In: The tropical rain forest: an ecological study (Richards PW, ed.). Cambridge University Press, Cambridge, 101-120.
- Rieseberg LH (1997). Hybrid origins of plant species. *Annu. Rev. Ecol. Evol. Syst.* 28: 359-389. <https://doi.org/10.1146/annurev.ecolsys.28.1.359>
- Schifino-Wittmann MT (2004). Poliploidia e seu impacto na origem e evolução das plantas silvestres e cultivadas. *Rev. Bras. Agrociên.* 10: 151-157.
- Silva ELS (1997). A vegetação de Roraima. In: Homem, ambiente e ecologia no estado de Roraima (Barbosa RI, Ferreira EJJ and Castellón EG, eds.). INPA, Manaus.
- Stace CA (1991). Plant taxonomy and biosystematics. 2nd edn. Cambridge University Press, Cambridge.
- Tanaka R and Kamemoto H (1984). Chromosomes in orchids: counting and numbers. In: Orchid biology: reviews and perspectives III (Arditti J, eds.). Cornell University Press, Ithaca.
- Tropicos.org. (2017). Missouri Botanical Garden Available at: [<http://www.tropicos.org/Name/23504348>]. Accessed April 18, 2017.
- Weiss MR (1995). Floral color change: a widespread functional convergence. *Am. J. Bot.* 82: 167-185. <https://doi.org/10.2307/2445525>