# CHROMOSOMAL OBSERVATIONS ON ELIEA (HYPERICACEAE), A MONOSPECIFIC MADAGASCAR ENDEMIC

# Frank Almeda and Ricardo Pacifico

California Academy of Sciences Institute for Biodiversity Science and Sustainability Department of Botany 55 Music Concourse Drive, Golden Gate Park San Francisco, California 94118-4503, U.S.A. falmeda@calacademy.org; ricardo\_b9@hotmail.com

## ABSTRACT

A meiotic chromosome number of n=9 is reported for *Eliea articulata*, a monospecific genus of Hypericaceae endemic to Madagascar. This first report for the genus is a new number for the small tribe Cratoxlyeae which includes only one other genus, *Cratoxylum*. A hypothetical scenario for the origin of n=9 is provided in the context of known chromosome numbers for *Cratoxylum* and the two other tribes in the Hypericaceae. Given that x=12 has been proposed as the basic chromosome number for the family with numbers of n=11 and n=10 also reported for related species and genera, n=9 appears to have arisen as a descending dysploid from these higher numbers. Field photos of *Eliea articulata* are provided along with a distribution map and camera lucida drawings of meiotic chromosome figures.

## RÉSUMÉ

Un nombre de chromosomes méiotiques de *n*=9 est rapporté pour *Eliea articulata*, un genre monospécifique d'Hypericaceae endémique à Madagascar. Ce premier rapport pour le genre est un nouveau nombre pour la petite tribu Cratoxlyeae qui ne comprend qu'un seul autre genre, *Cratoxylum*. Un scénario hypothétique pour l'origine de *n*=9 est fourni dans le contexte des nombres chromosomiques connus pour *Cratoxylum* et les deux autres tribus des Hypericaceae. Etant donné que x=12 a été proposé comme le nombre de chromosomes de base pour la famille avec des nombres de *n*=11 et *n*=10 également rapportés pour des espèces et des genres apparentés, *n*=9 semble être apparu comme un dysploîde descendant à partir de ces nombres plus élevés. Des photos de terrain d'*Eliea articulata* sont fournies, ainsi qu'une carte de répartition et des dessins en camera lucida de figures de chromosomiques méiotiques.

# INTRODUCTION

*Eliea* Cambess. is a monospecific genus of shrubs and small trees with showy white flowers (Fig. 1) that is endemic to the littoral forest that once formed a nearly continuous fringe in eastern Madagascar from Sambava in the north to the vicinity of Fort Dauphin in the south (Schatz 2001; Gouvenain & Silander 2003). It commonly grows on white coastal sands but also ranges to elevations of ca. 1000 m elevation (Fig. 2). It has been variously placed in Subfamily Hypericoideae of a broadly defined Clusiaceae, but recently it has been assigned to a more narrowly defined Hypericaceae that consists of three tribes (Hypericeae, Vismieae, and Cratoxyleae). Collectively, as many as 9–10 but as few as five genera have been attributed to the Hypericaceae based on morphological and molecular data (Stevens 2007; Nürk et al. 2012). A recent appraisal of generic limits in the Hypericaceae using molecular data recognizes a single broadly defined *Hypericum* L. in the tribe Hypericeae (Ruhfel et al. 2011) together with *Harungana* Lam., *Vismia* Vand., and *Psorospermum* Spach in the Vismieae (Ranarivelo 2017), and *Cratoxylum* Blume and *Eliea* in the Cratoxyleae (Stevens 2007). The generic composition of the Cratoxyleae, for the most part, has never been seriously challenged and molecular data now confirm the long-time placement of *Eliea* in the Cratoxyleae based on morphology (Ruhfel 2011).

Chromosome number diversity and evolution of Hypericaceae received considerable early attention (Robson & Adams 1968) focused primarily on *Hypericum*, the largest genus in the family with ca. 470–500 species (Christenhusz et al. 2017; Nürk et al. 2012). We here add the first chromosome number report for *Eliea* and summarize the little that is known about chromosome numbers for the Cratoxlyeae and Vismieae in the context of chromosome number data for the Hypericaceae generally.





Fig. 1A & B. Field photographs of Eliea articulata. (A. by N. Rakotonirina, B. by G. Schatz. Vouchers: A. Rakotonirina 643 (MO, P, TAN); B. Schatz 4367 (MO).



Fig. 2. Distribution of *Eliea articulata* in Madagascar. The white circles are vouchered collection coordinates from the GBIF database and the red triangle represents the vouchered collection for the chromosome number reported here.

### MATERIALS AND METHODS

Chromosome numbers for species discussed here are based on the summary by Federov (1974), the online IPCN Chromosome Reports (legacy.tropicos.org/Project/IPCN), and the CCDB–Chromosome Counts Database (ccdb.tau.ac.il/about/). Since these online databases are incomplete we also surveyed the literature for other published sources of chromosome number information.

The map was prepared using QGIS 3.4.6 (QGIS Development Team 2023) based on coordinates of *E. articulata* downloaded from the Global Biodiversity Information Facility (GBIF 2023). These were cleaned using the package CoordinateCleaner (Zizka et al. 2019), implemented in R (R Core Team 2018).

The photos in Figure 1 were obtained from Tropicos, botanical information system at the Missouri Botanical Garden (www.tropicos.org).

Flower buds of *Eliea articulata* (Lam.) Cambess. were collected in the field from a natural population at the southern limit of its range in Madagascar (Fig. 2). Buds were fixed in modified Carnoy's solution (4 chloroform, 3 ethanol, 1 glacial acetic acid, v/v/v) for 24 hours, then transferred to 70% ethanol for refrigerated storage. In the laboratory anthers were teased open and the contents squashed in 1% ferric aceto-carmine. Counts were made from pollen mother cells using a Zeiss light microscope with phase contrast and a 100× oil immersion objective. Drawings of meiotic configurations were made with camera lucida at a magnification of 1500×. The original drawings were scanned and minor edits to the images were made using GIMP 2.10 (https://www.gimp.org/). The voucher collection for the chromosome count reported here is as follows:

*Eliea articulata.* n=9. Madagascar: Toliara Province, ca. 6 km W of Fort Dauphin airport in the vicinity of Ambilinia along road from Fort Dauphin to Ambovombe, 25°0'19"S, 46°18'10"E, white sandy soil adjacent to large stand of *Nepenthes madagascariensis* Poir. at sea level, 16 Oct 1998, *F. Almeda 7891* (CAS).



Fig. 3. A–C. Camera lucida drawings of meiotic chromosome figures of *Eliea articulata*. A & B. Metaphase I (*n*=9). C. Telophase I (*n*=9).

#### OBSERVATIONS AND DISCUSSION

A count of n=9 is the first chromosome report for the monospecific Malagasy endemic *Eliea*. This count is based on metaphase I and telophase I figures (Fig. 3). Meiosis was regular in all cells examined showing clear and consistent counts of n=9.

*Cratoxylum*, the only other genus assigned to the tribe Cratoxlyeae consists of six species that range from India east to southern China and throughout Malesia (Gogelein 1967; Robson 1974). Chromosome counts are known for two species of *Cratoxylum*. A count of n=11 has been reported for *C. cochinchinense* (Lour.) Blume under the synonym *C. polyanthum* Korth. (Gill et al. 1979; Singhal et al. 1985). A count of n=7 has been reported for *C. formosum* (Jacq.) Benth. & Hook. ex Dyer (Tixier 1953). Although more counts will be needed to better understand the two disparate numbers for *Cratoxylum*, the collective numbers of n=7, 9, and 11 now known for the tribe are suggestive of a descending dysploid series based on n=11 leading to the lower numbers. This hypothesis is strengthened by the reports of n=10 for both *Harungana* and *Vismia* of the sister tribe Vismieae. A single count of n=10 has been reported for each of two unspecified Costa Rican species of *Vismia* (Robson & Adams 1968). There are no chromosome number reports for *Psorospermum*. We had hoped to locate voucher specimens for the two species of *Vismia* since only three species occur in Costa Rica (Hammel 2007). Unfortunately, our efforts were unsuccessful. Voucher specimens could not be located in the herbaria at FSU and DPU (which is now at NY) where they were reportedly deposited.

When chromosome numbers for the Cratoxyleae and Vismieae are compared with the better known and extensively sampled Hypericeae, a similar pattern of descending dysploidy is evident. The basic number for the Hypericeae (i.e. Hypericum s.l.) appears to be x=12 with an extensive descending series down to n=7 $(12\rightarrow11\rightarrow10\rightarrow9\rightarrow8\rightarrow7)$  and added tetraploidy based on all descending numbers from  $12\rightarrow7$ , together with some limited dysploidy on some of the polyploid increases (Robson 1974; Robson 1981; Robson and Adams 1968). If, as Robson and Adams hypothesize, the base number in Hypericum (and the family) is x=12, then n=11 in one species of *Cratoxylum* is likely a descending dysploid and the other species with n=7 may be a further dysploid reduction from other descending numbers in the series. Harungana and Vismia, both with n=10, also appear to be dysploid reductions from x=12 and *Eliea* with n=9, a new base number for the tribe, could have been derived as a further dysploid reduction from a base of 10. If n=11 is ultimately found to be a prevalent base number in Cratoxylum, then a competing scenario may come into play to account for the evolution of chromosome numbers in the family since the Cratoxyleae is basal and sister to the other two tribes of Hypericaceae (Ruhfel 2011). This alternative scenario would invoke x=11 as the base number with ascending dysploid cycles to generate n=12 and multiple cycles of descending dysploidy to derive all other lower numbers  $(7 \leftarrow 8 \leftarrow 9 \leftarrow 10 \leftarrow 11 \rightarrow 12)$ . Since ascending dysploidy is believed to be four times less common in flowering plants than descending dysploidy (Jones 1970; Goldblatt & Poston 1988), this scenario may be less likely, but plausible. Nevertheless, a base number of x=9 underscores the uniqueness of *Eliea* from a chromosomal perspective coupled with its phenetic distinctiveness (Gogelein 1967; Robson 1974; Stevens 2007). In the context of known chromosome numbers in the family, descending dysploidy from x=12 or x=11 appears to be the

most parsimonious scenario that could account for the origin of *Eliea's* unique chromosome number in the tribe Cratoxyleae.

# ACKNOWLEDGMENTS

We thank Seth Cotterell and Stephanie Keil of the libraries at CAS and the Missouri Botanical Garden respectively for assistance in obtaining a scanned copy of a literature reference, Nivo Rakotonirina and George Schatz for use of their field photographs in Figure 1, and the Madagascar Parks Association (MNP) and Parc Tsimbazaza for granting collecting permits. Field work was supported by the Elise and Lewis Rose Memorial Fund of the California Academy of Sciences. Gordon McPherson kindly reviewed the paper.

# REFERENCES

- CHRISTENHUSZ, M.J.M., M.F. FAY, & M.W. CHASE. 2017. Plants of the world: An illustrated encyclopedia of vascular plants. Royal Botanic Gardens, Kew, UK and University of Chicago Press, Illinois, U.S.A.
- FEDEROV, A.A., ED. 1974. Chromosome numbers in flowering plants. Academy of Sciences of the U.S.S.R., Komarov Botanical Institute, Leningrad.
- GBIF.org 2023. GBIF Occurrence Download. https://doi.org/10.15468/dl.2x4cbv
- GILL, B.S., S.S. BIR, & V.K. SINGHAL. 1979. Chromosome number reports LXV. Taxon 28:630.
- GOGELEIN, A.J.F. 1967. A revision of the genus Cratoxylum Bl. (Guttiferae). Blumea 15:453–475.
- GOLDBLATT, P. & M.E. POSTON. 1988. Observations on the chromosome cytology of Velloziaceae. Ann. Missouri Bot. Gard. 75:192–195.
- GOUVENAIN, R.C. DE & J.A. SILANDER, JR. 2003. Littoral forest. In: S.M. Goodman and J.P. Benstead, eds. The natural history of Madagascar. University of Chicago Press, Chicago, Illinois, U.S.A. Pp. 103–111.
- HAMMEL, B. 2007. Hypericaceae. In: B. Hammel, M.H. Grayum, C. Herrera, & N. Zamora, eds. Manual de Plantas de Costa Rica. Vol. VI. Dicotiledóneas (Haloragaceae-Phytolaccaceae). Missouri Botanical Garden, St. Louis. Pp. 26–31.
- JONES, K. 1970. Chromosome changes in plant evolution. Taxon 19:172–179.
- NÜRK, N.M., S. MADRIÑÁN, M.A. CARINE, M.W. CHASE, & F.R. BLATTNER. 2012. Molecular phylogenetics and morphological evolution of St. John's wort (*Hypericum*: Hypericaceae). Molec. Phylogen. Evol. 66:1–16.
- QGIS DEVELOPMENT TEAM. 2023. QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available from: http://qgis.org (accessed 17 February 2023)
- R Core TEAM. 2018. R: A language and environment for statistical computing. Vienna, Austria. Available from: https:// www.r.project.org/ (accessed 20 February 2023)
- RABAKONANDRIANINA, E. & G.D. CARR. 1987. Chromosome numbers of Madagascar plants. Ann. Missouri Bot. Gard. 74:123–125.
- RANARIVELO, H.S. 2017. Systematics, biogeography, and species delimitation of the Malagasy *Psorospermum* (Hypericaceae). Dissertations. 690. https://irl.umsl.edu/dissertation/690
- ROBSON, N.K.B. 1974. Hypericaceae. In: C.G.G.J. van Steenis, ed. Flora Malesiana I, 8:1–29. Noordhoff, Leiden, The Netherlands.
- ROBSON, N.K.B. 1981. Studies in the genus *Hypericum* L. (Guttiferae). 2. Characters of the genus. Bull. British Mus. Nat. Hist. Bot. 8:55–226.
- ROBSON, N.K.B. 2012 Studies in the genus *Hypericum* L. (Hypericaceae) 9. Addenda, corrigenda, keys, lists, and general discussion. Phytotaxa 72:1–111.
- ROBSON, N.K.B. & P. ADAMS. 1968. Chromosome numbers in Hypericum and related genera. Brittonia 20:95–106.
- RUHFEL, B.R. 2011. Systematics and biogeography of the Clusioid clade (Malpighiales). Biological Sciences Faculty and Staff Research 3. https://encompass.eku.edu/bio\_fsresearch/3
- RUHFEL, B.R., V. BITTRICH, C.P. BOVE, M.H.G. GUSTAFSSON, C.T. PHILBRICK, R. RUTISHAUSER, Z. XI, & C.C. DAVIS. 2011. Phylogeny of the clusioid clade (Malpighiales): Evidence from plastid and mitochondrial genomes. Amer. J. Bot. 98:306–325.
- SCHATZ, G.E. 2001. Generic tree flora of Madagascar. Royal Botanic Gardens, Kew, UK and Missouri Botanical Garden. St. Louis, U.S.A.
- SINGHAL, V.K., B.S. GILL, & S.S. BIR. 1985. Cytology of woody species. Proc. Indian Acad. Sci. (sect. B) 94:607–617.
- STEVENS, P.F. 2007. Hypericaceae. In: Kubitski, K., ed. The families and genera of vascular plants. Springer Verlag, Berlin, Germany. Pp. 194–201.

TIXIER, P. 1953. Données cytologiques sur quelques Guttiférales du Viet-Nam. Rev. Cytol. et Biol. Vég. 14:12.

ZIZKA, A., D. SILVESTRO, T. ANDERMANN, J. AZEVEDO, C. DUARTE RITTER, D. EDLER, H. FAROOQ, A. HERDEAN, M. ARIZA, R. SCHARN, S. SVANTESSON, N. WENGSTRÖM, V. ZIZKA, & A. ANTONELLI. 2019. CoordinateCleaner: Standardized cleaning of occurrence records from biological collection databases. Methods Ecol. Evol. 10:744–751.