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## A natural intergeneric hybrid of Gesneriaceae from Brazil

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### Abstract

A natural hybrid between *Goyazia* and *Mandirola* (Gloxiniinae, Gesneriaceae) from Cerrado (Brazil) is here described, supported by pollen morphology, cytological data and morphological characters. The microsporogenesis of *Mandirola hirsuta* and that of the hybrid were analyzed in order to evaluate the cytogenetic characteristics. The haploid chromosome numbers observed were  $n = 12$  for *M. hirsuta* and  $n = 11, 13, 16$  and  $26$  for the hybrid. Structural abnormalities (monads, dyads, triads and micronuclei) were observed at the final of the hybrid's meiosis. High viability rates of the pollen were recorded for *Goyazia* and *Mandirola* (>90%) and low viability for the hybrid (34.7%). The pollen grains were acetolyzed, measured and photographed for pollen morphology analysis. Quantitative pollen data were analyzed through descriptive and multivariate statistics. The hybrid has intermediate pollen characteristics between *G. petraea* and *M. hirsuta*; it is more related to *G. petraea* by the measures of diameters and ectoapertures; it is more similar to *M. hirsuta* mainly regarding the microreticulum on the mesocolpium region. The hybrid and *Mandirola* share vegetative and flower size, while the colors of the hybrid are similar to *Goyazia*. Pollen morphology, cytological data and morphological characters brought clear evidence for the recognition of the intergeneric hybrid, which we named as *Goydirola x punctata*.

### Introduction

The importance of natural hybridization to evolution process and to origin of new species of plants has had distinct viewpoints: a) natural hybridization played a major role in the evolution of plant species (Lotsy 1916, Anderson 1949, Stebbins 1959, Raven 1976, Grant 1981, Whitham *et al.* 1991, Rieseberg & Ellstrand 1993, Rieseberg 1997, Mallet 2007, Frajman *et al.* 2009); b) natural hybridization is common but not universal, it is concentrated in a small portion of taxa (Ellstrand *et al.* 1996, Whitney *et al.* 2010).

The recognition of hybrid origin of a lineage is not always easy. The first evidence commonly used is the mosaic of both parental and intermediate morphological characters of the hybrid (Rieseberg 1995), but chemical, molecular and cytogenetic characters are also used (Rieseberg & Ellstrand 1993, Machado 2008, Pessoa *et al.* 2020). The establishment of a new species or hybrid swarm, resulted of interspecific crosses, is dependent of polyploidization, allopolyploidization, asexual reproduction, etc. (e.g. Rieseberg *et al.* 2003, Mallet 2007, Rieseberg & Willis 2007, Soltis & Soltis 2009).

The analysis of the meiotic process responsible for microsporogenesis in putative hybrids, as well as the verification of the pollen viability after microgametogenesis, can provide clues regarding the similarity between the genomes involved in the hybridization process, indicating in addition the reproductive viability of this hybrid (Fernandes *et*

al. 2018). Microsporogenesis in hybrids often presents problems both in the pairing of homologous chromosomes in prophase I and in the disjunction of chromosomes in anaphase I and II, resulting in anomalous structures such as monads, dyads, triads or polyads and micronuclei, or even in unbalanced gametes and sterile pollen grains, such as observed by Guo *et al.* (2010) in interspecific hybrids of *Solanum* Linnaeus (1753: 184).

There is an extensive list of natural hybrids between plant species or artificial hybrids (Knobloch 1972, Lexer *et al.* 2003, Machado 2008, Whitney *et al.* 2010, Baiakhmetov *et al.* 2020). However, spontaneous (natural) hybridization between genera of plants is a less common event (Skog 1976, Ellstrand *et al.* 1996, Machado 2008, Whitney *et al.* 2010, Schilling 2011, Mitchell *et al.* 2019, Zhou *et al.* 2020) due to longer time since divergence (Moyle *et al.* 2004, Moyle & Nakazato 2010). Of the 3437 hybrids recorded by Whitney *et al.* (2010), only 3.5% represented intergeneric hybrids.

Natural hybridization between species of Gesneriaceae in Brazil was reported by Chautems (1988), Chautems & Matsuoka (2003) and Araujo *et al.* (2005) in *Nematanthus* Schrader (1821: 718), by Ferreira *et al.* (2013) in *Sinningia* Nees (1825: 297) and by Chautems (2002) and SanMartin-Gajardo & Sazima (2005) in *Vanhouttea* Lemaire (1845: 346). Wiehler (1976a) described various artificial hybrids between genera of Gesneriaceae and there is a list of all artificial hybrids on the website of the Gesneriad Society ([http://www.gesneriadsociety.org/ir\\_ges/index.htm](http://www.gesneriadsociety.org/ir_ges/index.htm)). Gesneriaceae is the eighth of the 25 families most prone to hybridization in the data set of Whitney *et al.* (2010). Smith *et al.* (2016) reported three species of *Columnnea* Linnaeus (1753: 638) that may represent lineage from hybrid ancestor and one that probably is a more recent hybrid. Smith *et al.* (1995) showed that the natural interspecific hybrids in *Cyrtandra* Forster & Forster (1776: 5) more frequently occurred between different sections of this genera (Clark *et al.* 2009). They supposed that this occurs due to lack of sympatry for species within a section, but may also be attributed to the difficulty in recognizing hybrid individuals from two morphologically similar species. However, natural hybrids between genera in this family could be a rare phenomenon probably because the effective mechanisms of reproductive isolation are pollination syndromes, geographical or ecological isolation and time of flowering (Wiehler 1983).

*Mandirola* Decaisne (1848: 468) and *Goyazia* Taubert (1896: 451) (Gesneriaceae-Gloxiniinae) are herbs, with scaly rhizomes and corolla lilac, white or pink, with varied patterns of shape and different patterns of spots on mouth. These genera are endemic from Brazil, occurring in rock outcrops in Cerrado Biome (Perret *et al.* 2013, Araujo 2020a, 2020b). They are closely related and are distinguished by the size of the plant, leaves and corolla and the stem stiff and thin in *Goyazia* (Weber 2004, Roalson *et al.* 2005, Perret *et al.* 2013; Araujo *et al.* 2012, Chautems *et al.* 2018, Araujo 2020a, Araujo 2020b). *Goyazia* include three species: *G. petraea* (Phillips 1970: 221) Wiehler (1976b: 395) (distributed in Mato Grosso do Sul and Mato Grosso States), *G. rupicola* Taubert (1896: 451) (Pará, Tocantins, Maranhão, Goiás and Minas Gerais States) and *G. villosa* (Gardner 1842: 469) Howard (1975: 367) (Tocantins State). The delimitation of *Mandirola* species is not clear (Roalson *et al.* 2005, Araujo *et al.* 2012, Perret *et al.* 2013), but Araujo (2020b) discriminated three species: *M. hirsuta* (Candolle 1838: 536) Araujo & Chautems (2012: 894) (distributed in Mato Grosso, Minas Gerais and São Paulo States), *M. multiflora* (Gardner 1842: 468) Decaisne (1848: 468) (Distrito Federal, Tocantins and Goiás States) and *M. rupestris* (Gardner 1842: 480) Roalson & Boggan (2005: 230) (Pará, Tocantins, Bahia and Maranhão States). Fiorini *et al.* (2019) recognized seven genetic groups in *Mandirola*, geographically well delimited, with low intrapopulation variability and high haplotype and nucleotide diversities. *Mandirola* and *Goyazia* are associated with humid microhabitats of rock outcrops (Araujo 2020a, 2020b). Probably the current distribution area of *Mandirola* was occupied before the establishment of the savanna matrix, whose rapid expansion fragmented and restricted the connectivity among geographic groups of this genus (Fiorini *et al.* 2019). This process was described by Fiorini *et al.* (2019) to *Mandirola*, but since *Goyazia* had its origin in these both same environmental conditions and time as *Mandirola* and is also restricted to the same micro-habitats of its sister genus, it may also have passed through the same fragmentation process.

Based on pollen morphology, cytological data, morphological studies of herbarium specimen, living material and field observation of almost all populations of *Mandirola* and *Goyazia* and one individual collected in Mato Grosso (Brazil), we aim to 1) test the hybrid origin of this individual, 2) find macro-and micromorphological characters to distinguish the putative hybrid from the parents.

## Material and methods

**Plant Material:**—During a field work in Chapada dos Guimarães (MT-Brazil), in February 2011, the authors found a specimen that presented mosaic and intermediate morphological characters between *Goyazia* and *Mandirola* which

occur in the same area. The rhizome of this specimen was collected and kept in cultivation. Specimen voucher was collected from cultivation in February 2015, preserved in 70% ethanol, herborized and deposited at SORO herbarium (Araujo 1170). Vouchers of *Goyazia* (Araujo 1061, 1062, 1067) and *Mandirola* (Araujo 1063, 1064, 1066, 1068, 1073) collected in the same area were deposited at HUFABC (Table 1). Some flowers were preserved in 70% ethanol and were used for observation and description of morphological data. Living specimens were photographed in the field and in cultivation.

**TABLE 1.** Locality of putative hybrid (*Goydirola*) and parental species (*Goyazia petraea* and *Mandirola hirsuta*) studied.

Species	Voucher	Geographical coordinates
<i>Goyazia petraea</i>	A.O. Araujo <i>et al.</i> 1061	15°22'44.8"S, 55°50'31.5"W
<i>Goyazia petraea</i>	A.O. Araujo <i>et al.</i> 1062	15°22'44.8"S, 55°50'31.5"W
<i>Goyazia petraea</i>	A.O. Araujo <i>et al.</i> 1065	15°25'44"S, 55°49'59.7"W
<i>Goyazia petraea</i>	A.O. Araujo <i>et al.</i> 1067	15°26'16.8"S, 55°50'16.3"W
<i>Goydirola</i>	A.O. Araujo <i>et al.</i> 1170	15°22'44.8"S, 55°50'31.5"W
<i>Mandirola hirsuta</i>	A.O. Araujo <i>et al.</i> 1063	15°22'44.8"S, 55°50'31.5"W
<i>Mandirola hirsuta</i>	A.O. Araujo <i>et al.</i> 1064	15°22'44.8"S, 55°50'31.5"W
<i>Mandirola hirsuta</i>	A.O. Araujo <i>et al.</i> 1073	15°24'24.8"S, 55°49'27.3"W
<i>Mandirola hirsuta</i>	A.O. Araujo <i>et al.</i> 1066	15°26'16.8"S, 55°50'16.3"W
<i>Mandirola hirsuta</i>	A.O. Araujo <i>et al.</i> 1068	15°26'16.8"S, 55°50'16.3"W

The flower buds of *Goyazia*, *Mandirola* and the putative hybrid were collected at different stages of development and promptly fixed in the field in Carnoy fixative solution (absolute ethanol / glacial acetic acid, 3: 1, v / v). After 24 hours at room temperature, the fixed materials were stored in a freezer at -20° C.

**Pollen Viability and Meiotic Analysis:**—In order to access pollen viability, anthers of five pre-anthesis flower buds were squashed following the protocol presented by Alexander (1980) and analyzed under light microscope. Pollen viability indexes (P.V.%) were calculated based on the proportion of viable pollen grains—those fully stained and with regular size and shape. At least 1,500 pollen grains per population were analyzed. For the meiotic studies and the calculation of the meiotic index (M.I%) anthers of immature flower buds were squashed with acetocarmine 1.2% following Guidini *et al.* (2017). The meiotic index was obtained based on the percentage of regular tetrads observed, with four similar cells and absent micronuclei. The images were captured using a Nikon 80i photomicroscope associated with the AR-NIS image analysis program.

**Chromosome count:**—Cytogenetic studies were carried out in two populations of *Goyazia petraea* (Araujo 1062 and 1065), a population of the putative hybrid (Araujo 1170) and a population of *Mandirola hirsuta* (Araujo 1068), using the squash of the fixed flower buds according to the procedure mentioned above, in order to observe haploid and diploid chromosome numbers. The haploid numbers were observed in meiotic cells in diakinesis and the diploid number was observed in metaphasic cells of the somatic tissues of the anthers.

**Pollen Morphology:**—The pollen material of the putative hybrid and parental species were collected (Table 1) and analyzed using: a) the light microscopy (LM) for description of overall pollen morphology; b) by scanning electron microscopy (SEM) for capturing ornamentation details, and c) by transmission electron microscopy (TEM) for detection of the ultrastructure of exine in the hybrid. For LM, the pollen grains were acetolyzed according to the acetolysis technique (Erdtman 1960), following modifications cited by Melhem *et al.* (2003). They were measured (pollen diameter  $n \leq 25$ ; aperture and exine thickness  $n \leq 10$ ) up to seven days after their preparation (Salgado-Labouriau *et al.* 1965). For SEM non-acetolysed pollen grains were analysed following Melhem *et al.* (2003), and for TEM the protocols of Haddad *et al.* (1998) and Sabatini *et al.* (1963) were followed. Permanent slides of light microscopy are deposited in the pollen reference collection of the Plant Morphology and Palynology Laboratory (LaMPali), Departamento de Biologia Aplicada à Agropecuária, Universidade Estadual Paulista, UNESP, Jaboticabal, Brazil.

Descriptive statistical analysis was conducted to obtain the means (x), standard deviation (sx), standard error (s), 95% confidence interval (CI), coefficient of variability (CV) and range (R) according to Vieira (2011) and Zar (2010). To compare the values of the pollen grain diameters we used the graphs of MINITAB 10.3 for Windows software in order to represent the means and the confidence interval values. The principal component analysis (PCA) and cluster analysis (CA-UPGMA based on Euclidean distance) were performed using the programs FITOPAC 1 (Shepherd 1996)

and PC-ORD version 5.15 (McCune & Mefford 2011) to verify if the pollen data allow the grouping of the analyzed species (PCA), and whether the pollen characteristics provide relation among the putative hybrid and parental species analyzed (CA). We used 13 metric variables for the PCA and CA (Table 6).

The pollen terminology followed Punt *et al.* (2007) and Halbritter *et al.* (2018). For pollen description was adopted Bellonzi *et al.* (2020); for the polar area index, Faegri & Iversen (1966); and for the width index colpi, Gasparino *et al.* (2013). Photomicrographs were performed with a light microscope LEICA DM1000LED for LM photos, and the electromicrographs with a JEOL JSM 5410 scanning electron microscope for the SEM images, and JEOL JEM 1010 transmission electron microscope for the TEM image.

## Results

### Taxonomy

*Goydirola* A.O.Araujo & M.Peixoto, *gen. hybr. nov.* (*Goyazia* Taubert (1896: 451) × *Mandirola* Decaisne (1848: 468)).

*Goydirola* × *punctata* A.O.Araujo & M.Peixoto, *nothosp. nov.* (*Goyazia petraea* (Phillips 1970: 221) Wiehler (1976b: 395) × *Mandirola hirsuta* (Candolle 1838: 536) Araujo & Chautems (2012: 894)).

Type:—Living plant in cultivation at Biritiba Ussu, near Mogi das Cruzes, São Paulo, Brazil (from field collected material in Mato Grosso, Chapada dos Guimarães, 551 m, 15°22'44.8"S, 55°50'31.5"W), when it bloomed, the branches were preserved in alcohol and later the present exsiccate was prepared, 27 October 2020, *A.O. Araujo 1170* (holotype SORO!).

Herb 15–20 cm tall, stems vinaceous, 0.2 cm diam., internodes 1.2–2.7 cm, with rhizome, saxicolous. Leaves opposite, petiole 0.07–0.2 cm, vinaceous, blade oval, 2.3–2.5 × 1.5–1.9 cm, apex acuminate, base cordate, margin serrate, above dark green, pubescent, beneath vinaceous, sparsely pubescent with pubescent veins. Inflorescence reduced to solitary flower, axillary. Flowers obliquely borne on pedicels, with 2 bracteole, 0.1–0.2 cm long, vinaceous, pubescent; calyx dialysepalous, sepals 0.5–0.6 cm, lanceolate, margin entire, vinaceous, pubescent; corolla salverform, 0.3–0.4 cm wide at the middle, tube 0.9–1.1 cm long, outside purple, beneath white with pale lilac dots, inside purple, beneath white with purple dots, mouth 0.4–0.5 cm in diam., white with purple dots reaching the lobes (dots randomly arranged), lobes lilac with purple dots, unequal, upper two 0.3–0.5 × 0.4–0.7 cm; 2 lateral 0.3–0.7 × 0.4–0.7 cm, lower one 0.4–0.6 × 0.5–0.7 cm; stamens 4, included, filaments 0.7–0.9 cm long, white, anthers coherent, staminode 1; nectary annular; ovary densely pubescent, stigma bilobed.

Distribution and Ecology:—The hybrid was collected at Chapada dos Guimarães—MT, Brazil, in February 2011, in shaded rock outcrop. This area is included in Cerrado Biome. Just one hybrid individual was found on the same rock outcrop on which the parental species were. *G. petraea* and *M. hirsuta* are common in this area and frequently are in sympatry on the rock outcrops (Fig. 1C). This location was visited several times by the authors (2004, 2013, 2015), but the hybrid was not observed again. Thus, we assume that it was generated in a rare hybridization event between parental species.

Phenology:—The hybrid was found in the field with flowers in February (Fig. 1A, B). In cultivation it also flowered in February.

Etymology:—*Goydirola* is the combination between *Goyazia* and *Mandirola*. The epithet *punctata* refers to the dots in the tube, mouth and lobes of corolla.

*Goydirola* × *punctata* is similar to *Mandirola hirsuta* in vegetative and flower sizes, but the colors of this hybrid are similar to *Goyazia petraea* (Table 2, Fig. 1).

**Cytogenetic Analysis:**—The results of the meiotic analysis and the observed chromosome numbers are presented at Table 3. For the investigated hybrid (*Araujo 1170*), haploid numbers  $n = 12, 13, 16$  and  $26$  were identified, with  $n = 13$  being the most common number (Fig. 2). In diakinesis, cells with univalent and multivalent chromosomes were observed. The diploid number  $2n = 26$  was counted (Fig. 2E). Anomalous structures were observed, such as triads (17.2%), dyads (7.8%) and monads (1.2%), with the occurrence of tetrads with micronuclei (Fig. 2F–H), at the end of microsporogenesis. In this access, pollen grains with different sizes were observed (Fig. 2I).

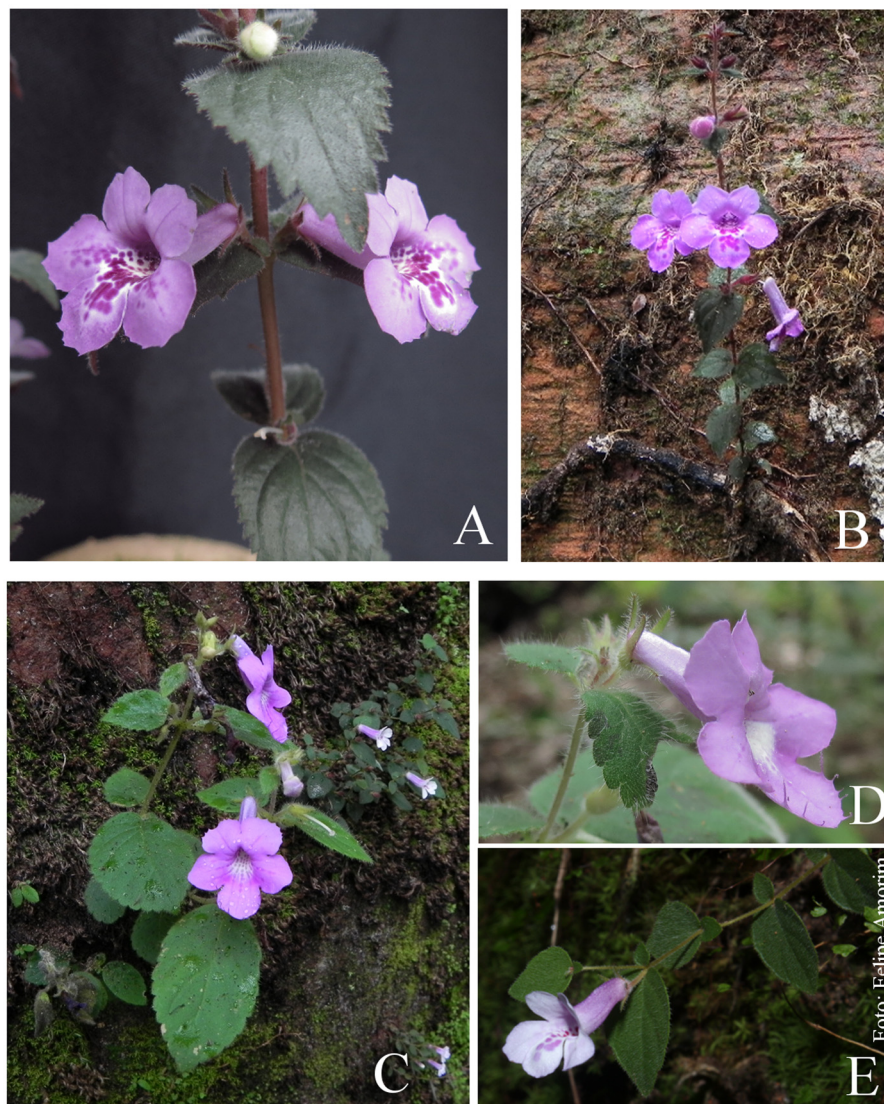
**Pollen Morphology, Pollen Description:**—The hybrid *Goydirola* and parental species have similar characteristics of pollen grains regarding the pollen unit, polarity, type and number of ectoapertures (3-colporate, Fig. 3D), type of endoaperture (olongate, Fig. 3K, Q) and microreticulate ornamentation. Nonetheless, some pollen characteristics varied among the specimens, such as *amb* and size of the microreticulum on the mesocolpium (Tables 4–5, Fig. 3).

**TABLE 2.** Morphological comparison between *Goydirola* and parental species (*Goyazia petraea* and *Mandirola hirsuta*: only parental populations found at the same location of the hybrid).

Character	<i>Goyazia petraea</i>	<i>Goydirola</i>	<i>Mandirola hirsuta</i>
Plant size (cm)	3.3–12.5	15–20	3.4–20
Stems diameter (cm)	0.03–0.1	0.2	0.1–0.2
Stems color	vinaceous to green	vinaceous	green to brownish
Petiole size (cm)	0.02–0.07	ca. 0.14	0.14–0.49
Leaf blade size (cm)	0.9–1.4 × 0.7–1.0	2.3–2.5 × 1.5–1.9	1.5–5.2 × 0.91–2.8
Anisophylly / isophylly	anisophylly to pronounced anisophylly	isophylly	isophylly to anisophylly
Leaf margin	serrate	serrate	crenate or serrate
Leaf color			
Beneath	vinaceous	vinaceous	bright green, rarely dark green
Above	dark green	dark green	bright green
Leaf texture	papyraceous	papyraceous	membranaceous
Calyx color	vinaceous or dark green	vinaceous	bright green
Corolla tube			
Color outside beneath	white with pale lilac dots	white with pale lilac dots	white with pale lilac dots
Color inside beneath	white with lilac or purple dots	white with purple dots	white with pale lilac dots
Size (cm)	0.5–0.8	0.9–1.1	0.8–1.0
Corolla mouth			
Color	white with lilac or purple dots reaching the lobes (dots randomly arranged)	white with purple dots reaching the lobes (dots randomly arranged)	white with pale lilac dots (dots arranged in rows)
Wide (cm)	0.3–0.4	0.5–0.7	0.36–0.49
Proportion: width and height	wider than high	wider than high	higher than wide
Corolla lower lobes			
Margin	dentate	dentate	fimbriate
Color	white to pale lilac with lilac or purple dots	lilac with purple dots	lilac without dots
Length ratio between the upper and lower lobes	upper 1.5–2 times smaller than the lower	upper 1.2–1.3 times smaller than the lower	upper 1.4–1.75 times smaller than the lower

Considering the microreticulate exine ornamentation of *Goydirola* and parental species pollen grains, in some specimens the microreticulum occurs in different sizes (Table 5, Fig. 3). In the apocolpium region, the microreticulum are homobrochate (Fig. 3B–C, H–I, O–P), however the mesocolpium region presents microreticulum heterobrochate in *Goydirola* and *Mandirola hirsuta* (Fig. 3J–SEM, L–M, R–S, T). Furthermore, *Goydirola* and *M. hirsuta* have pollen grains with circular or triangular amb (Fig. 3G, N), while *G. petraea* has only circular amb in its pollen grains (Fig. 3A). In TEM, the pollen grains of *Goydirola* have tectum thick, discontinuous and slightly wavy, columellae long with similar diameters, sometimes incomplete; the foot layer is thinner than the nexine; intine is thick (Fig. 3J).

**Analysis of Quantitative Data:**—Through the measurements made and comparing the means and the interval of the pollen grains diameters of hybrid and parental species studied (Fig. 4A, B), we observed that the specimen *Goyazia petraea* 1062 has the smallest diameter values, while the specimen *Mandirola hirsuta* 1066 has the highest diameter values in polar diameter in equatorial view-PDEV (only this specimen and *G. petraea* 1067 have the size of the pollen grains from small to medium, Tables 4–5, Fig. 4A). In addition, regarding this diameter (PDEV), the hybrid has measurements of its pollen grains closest to specimens *G. petraea* 1062, *M. hirsuta* 1073 and *G. petraea* 1061, respectively, forming a continuous group of the smallest values for this dimension (Fig. 4A). However, concerning the equatorial diameter in equatorial view-EDEV (Fig. 4B), it is possible to notice that the hybrid has intermediate values between *G. petraea* and *M. hirsuta*.



**FIGURE 1.** Morphological comparison between hybrid (*Goydirola*) and parental species (*Goyazia petraea* and *Mandirola hirsuta*). (A–B) *Goydirola*. (A) plant with flowers and bud, (B) plant in the field, (C) *M. hirsuta* and *G. petraea* in the same rocky outcrop, (D) *M. hirsuta*, (E) *G. petraea*. Photography: A and D (A.O.Araujo), B and C (M.Peixoto), E (Felipe Amorim).

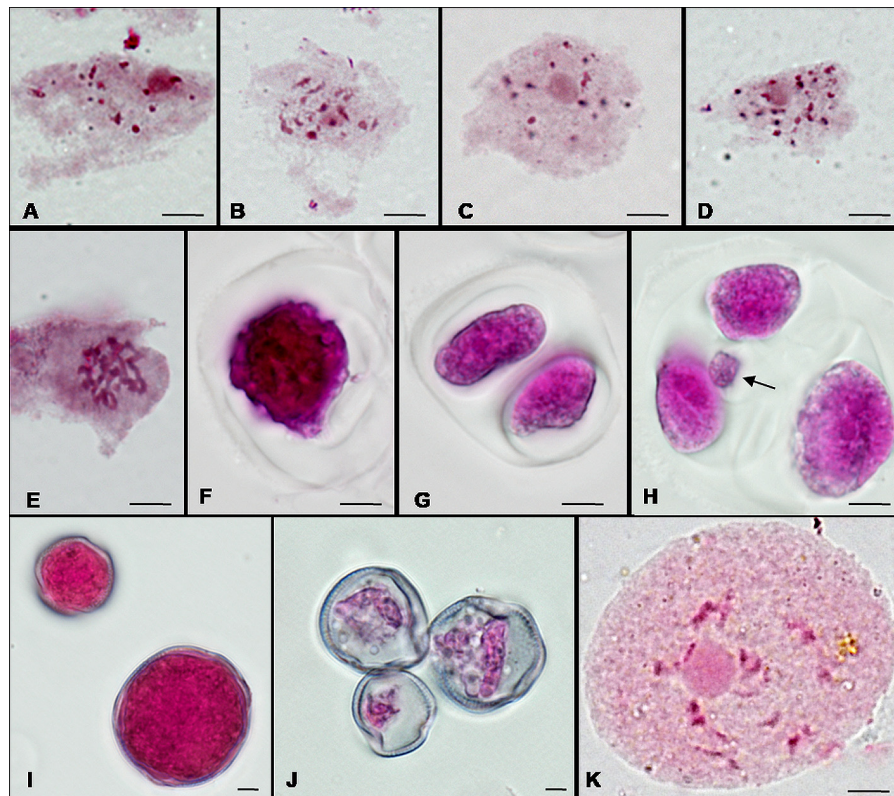
**TABLE 3.** Data of meiotic analysis in two populations of *Goyazia petraea*, one population of *Mandirola hirsuta* and the putative hybrid (*Goydirola*), with chromosome haploid ( $n$ ) and diploid number ( $2n$ ), index of pollen viability (PV%) and meiotic index (MI%).

Species	Access	$n$	$2n$	PV%	MI%
<i>Goyazia petraea</i>	AOA1062	-	-	99.6	94.2
<i>Goyazia petraea</i>	AOA 1065	-	-	98.6	87.5
<i>Goydirola</i>	AOA 1170	12,13,16,26	26	34.7	73.0
<i>Mandirola hirsuta</i>	AOA 1068	12	-	97.3	-

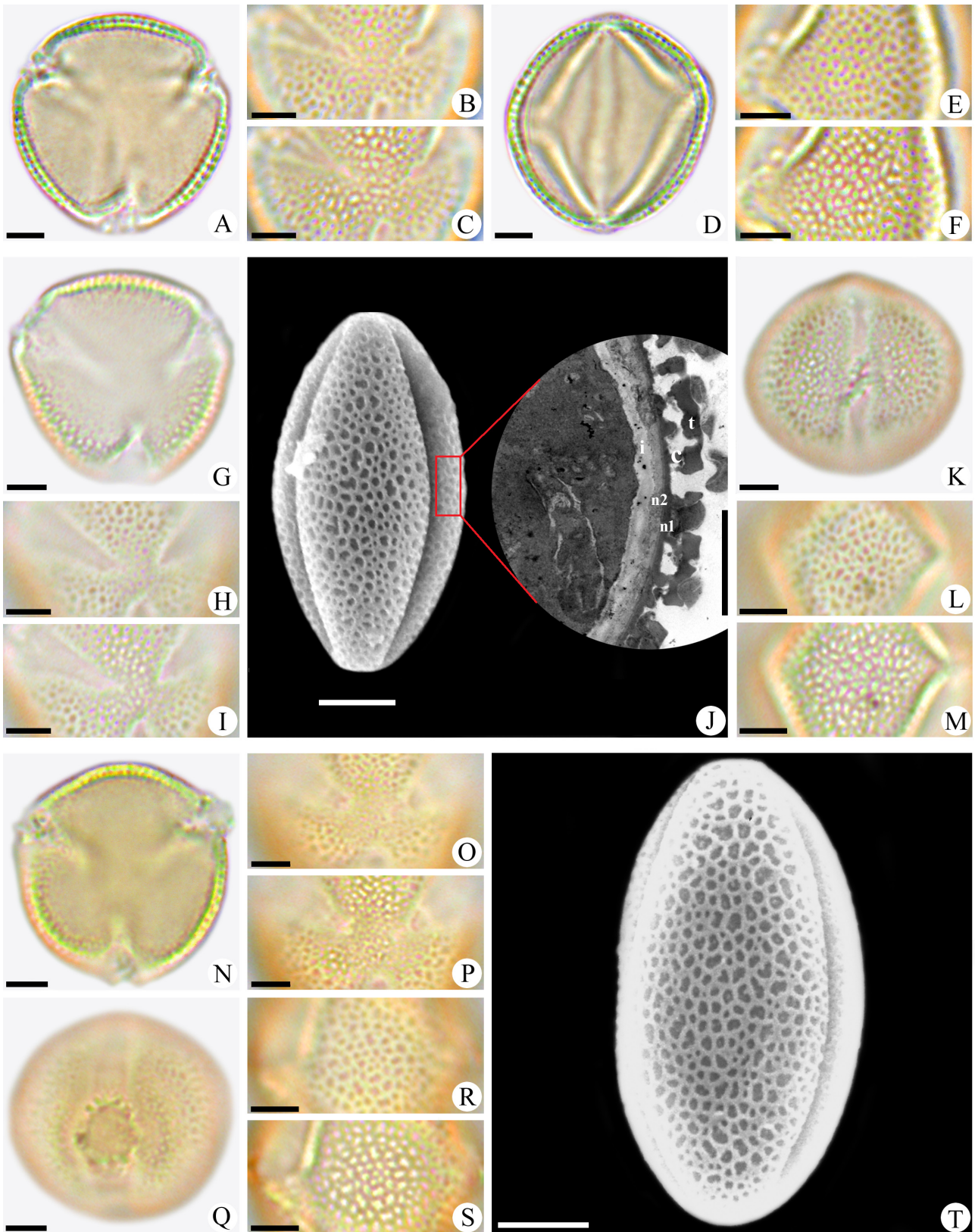
**TABLE 4.** Quantitative data of pollen grains from hybrid (*Goydirola*) and parental species (*Goyazia petraea* and *Mandirola hirsuta*) in  $\mu\text{m}$ .

Species	Voucher	(Range) $\bar{x} \pm s_x$	s	CI	CV
Polar diameter in equatorial view					
<i>G. petraea</i>	A.O. Araujo 1061*	(17.95–20.51) $19.23 \pm 0.57$	1.40	17.76–20.70	7.30
<i>G. petraea</i>	A.O. Araujo 1062	(15.38–17.95) $16.92 \pm 0.26$	1.28	16.39–17.45	7.58
<i>G. petraea</i>	A.O. Araujo 1067*	(23.08–25.64) $24.17 \pm 0.52$	1.37	22.91–25.44	5.67
<i>Goydirola</i>	A.O. Araujo 1170	(15.38–20.51) $18.46 \pm 0.26$	1.28	17.93–18.99	6.94
<i>M. hirsuta</i>	A.O. Araujo 1063	(17.95–25.64) $23.38 \pm 0.48$	2.38	22.40–24.36	10.17
<i>M. hirsuta</i>	A.O. Araujo 1064*	(20.51–25.64) $23.76 \pm 0.47$	1.80	22.76–24.76	7.59
<i>M. hirsuta</i>	A.O. Araujo 1073*	(17.95–20.51) $18.80 \pm 0.54$	1.32	17.41–20.19	7.04
<i>M. hirsuta</i>	A.O. Araujo 1066*	(22.50–27.50) $25.74 \pm 0.36$	1.47	24.98–26.49	5.71
<i>M. hirsuta</i>	A.O. Araujo 1068	(22.50–25.00) $23.10 \pm 0.22$	1.09	22.65–23.55	4.72
Equatorial diameter in equatorial view					
<i>G. petraea</i>	A.O. Araujo 1061*	(20.51–23.08) $21.79 \pm 0.57$	1.40	20.32–23.27	6.44
<i>G. petraea</i>	A.O. Araujo 1062	(15.38–20.51) $18.97 \pm 0.30$	1.48	18.36–19.58	7.80
<i>G. petraea</i>	A.O. Araujo 1067*	(20.51–25.64) $21.24 \pm 0.73$	1.94	19.46–23.03	9.12
<i>Goydirola</i>	A.O. Araujo 1170	(17.95–23.08) $21.23 \pm 0.28$	1.39	20.66–21.80	6.54
<i>M. hirsuta</i>	A.O. Araujo 1063	(15.38–23.08) $18.05 \pm 0.40$	2.02	17.22–18.88	11.21
<i>M. hirsuta</i>	A.O. Araujo 1064*	(12.82–20.51) $17.09 \pm 0.54$	2.09	15.94–18.25	12.25
<i>M. hirsuta</i>	A.O. Araujo 1073*	(15.38–20.51) $18.80 \pm 0.85$	2.09	16.61–21.00	11.13
<i>M. hirsuta</i>	A.O. Araujo 1066*	(17.50–27.50) $23.09 \pm 0.59$	2.43	21.84–24.34	10.50
<i>M. hirsuta</i>	A.O. Araujo 1068	(20.00–27.50) $23.40 \pm 0.32$	1.59	22.74–24.06	6.81

NOTE.  $\bar{x}$  = mean,  $s_x$  = standard deviation, s = standard error ( $\mu\text{m}$ ), CI = confidential interval at 95% ( $\mu\text{m}$ ), CV = coefficient of variability (%), \* $n < 25$ .

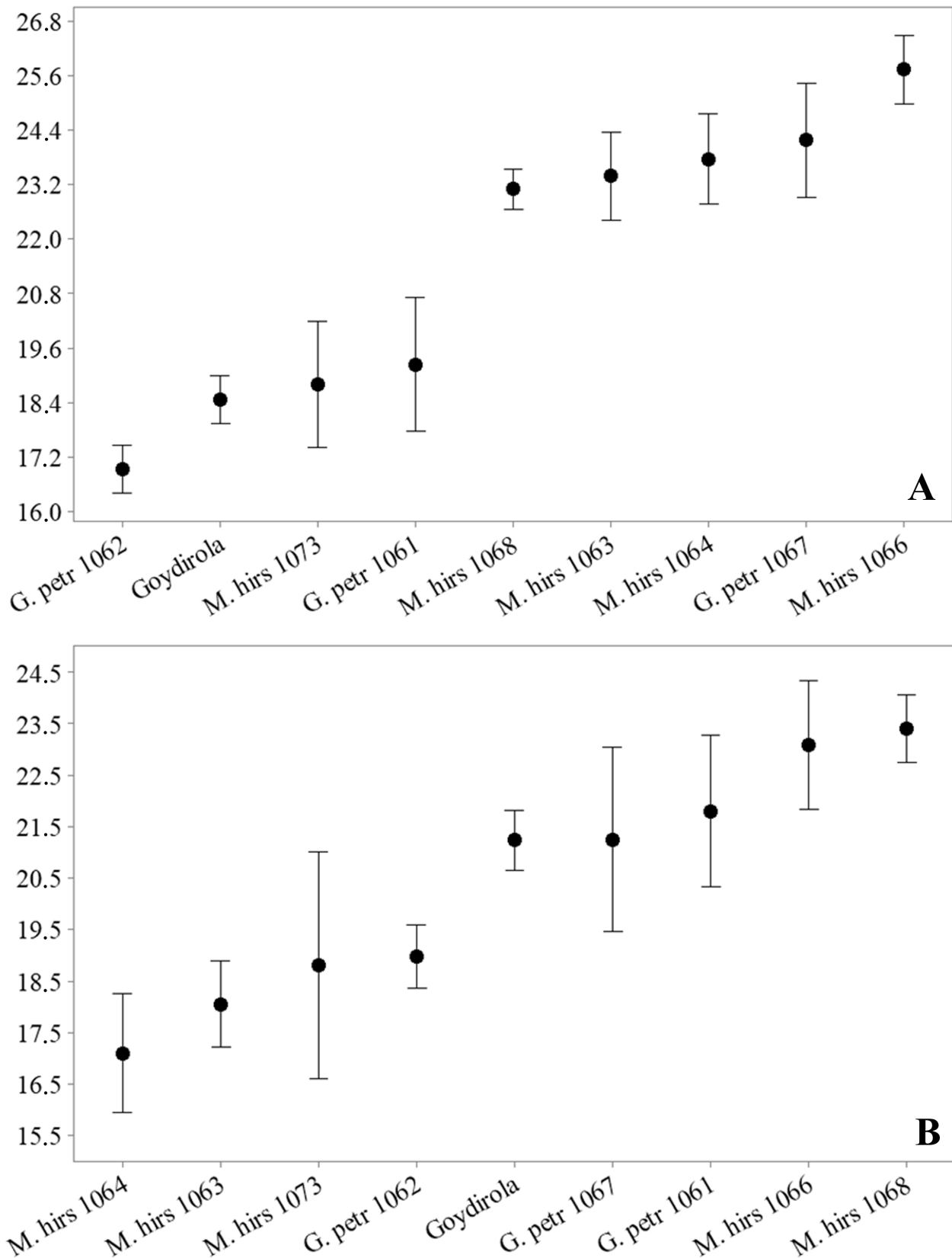


**FIGURE 2.** Phases of the meiotic process in the putative hybrid (A–J) and in *Mandirola hirsuta* (K), showing in: (A) diakinesis with  $n=12$ ; (B) diakinesis with  $n=13$ ; (C) diakinesis with  $n=16$ ; (D) diakinesis with  $n=26$ ; (E) mitotic metaphase cell in the anther somatic tissue, with  $2n=26$ ; (F) monad; (G) dyad; (H) tetrad with micronuclei (arrow); (I) pollen grains with different sizes; (J) unviable pollen; (K) diakinesis with  $n=12$ . Bar =  $5\mu\text{m}$ .



**FIGURE 3.** Photomicrographs and electron micrographs (SEM, TEM) of the pollen grains of hybrid (*Goydirola*) and parental species (*Goyazia petraea* and *Mandirola hirsuta*) studied. (A–F) *Goyazia petraea* [A–F = 1061], (G–M) *Goydirola*, (N–T) *Mandirola hirsuta* [N = 1064; O–P, R–S and T = 1068; Q = 1066]. (A, G and N) polar view with emphasis on the amb, (D, K and Q) equatorial view with emphasis on the apertures, (B–C, H–I and O–P) ornamentation in high and low focus on the apocolpium, (E–F, L–M and R–S) ornamentation in high and low focus on the mesocolpium, (J) ornamentation on the mesocolpium (SEM) with emphasis on the exine ultrastructure (TEM), (T) ornamentation on the mesocolpium (SEM). Scale bars: 2  $\mu\text{m}$  = J (TEM); 5  $\mu\text{m}$  = other images. Legends: c = columella, i = intine, n1 = foot layer, n2 = endexine, t = tectum.



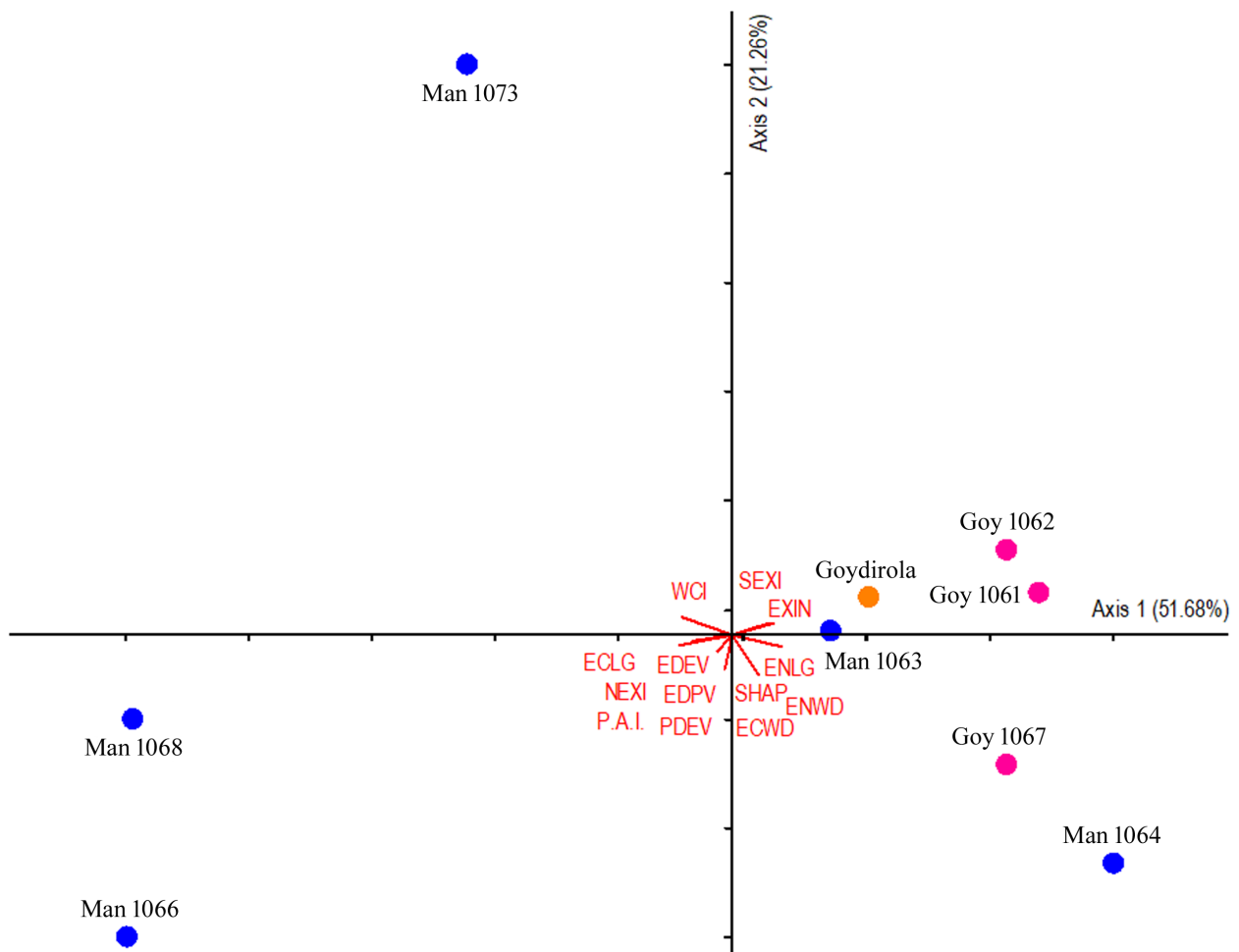


**FIGURE 4.** Representation of confidence interval of mean in 95% of the pollen grains of hybrid (*Goydirola*) and parental species (*Goyazia petraea* and *Mandirola hirsuta*) studied. (A) Polar diameter in equatorial view, (B) Equatorial diameter in equatorial view. The higher and lower boundaries showing the confidence interval; the average circle showing the arithmetic mean. The values are in  $\mu\text{m}$ . G. petr = *Goyazia petraea*; M. hirs = *Mandirola hirsuta*.

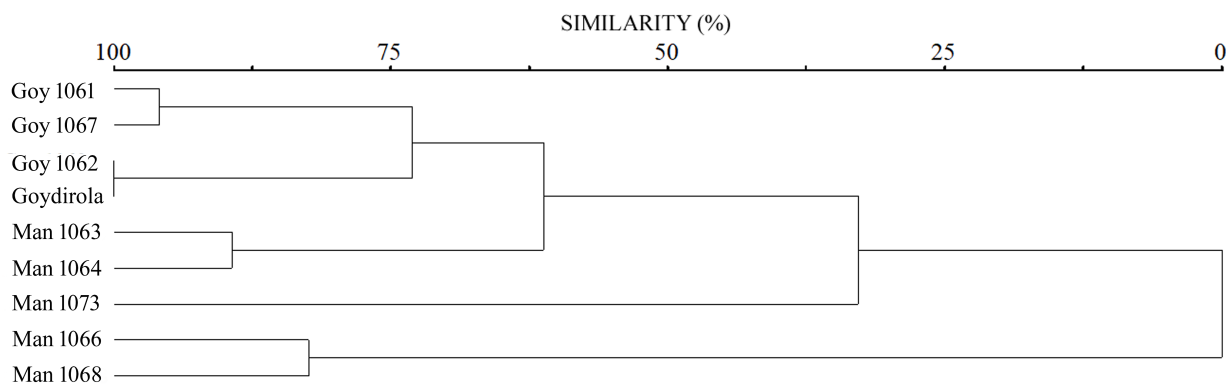
The first two axes of the multivariate analysis (principal component analysis–PCA) summarized 72.94% of the total of total data variance (Fig. 5). The first axis of the analysis explained 51.68% of the total variability of data, presenting as most significant variables the length of ectoaperture (ECLG) and width index of colpi (WCI), respectively (Table 6). Thus, on the positive side of the axis 1, the hybrid, *G. petraea* and *M. hirsuta* (specimens 1063 and 1064) were concentrated, because they have the smallest values of these variables. In contrast, the other specimens of *M. hirsuta* (specimens 1066, 1068 and 1073) are positioned on the negative side of this axis as they presented the highest values of ECLG and WCI (Fig. 5).

The second axis of PCA explains 21.26% of the metric variability of the pollen grains of hybrid and parental species analyzed here, presenting the PDEV and width of ectoaperture (ECWD) as more significant variables (Table 6). On the positive side of axis 2, the hybrid, *G. petraea* (specimens 1061 and 1062) and *M. hirsuta* (specimens 1063 and 1073) have the smallest values of polar diameter in equatorial view, mainly. While that, on the negative side this axis, this variable (PDEV) was responsible for ordering the other specimens of *G. petraea* and *M. hirsuta* (Fig. 5).

The analysis of similarity (UPGMA based on Euclidean distance) produced a dendrogram with a linkage value of 33.33% of the utilized data (Fig. 6). In this analysis, we observed that the first group consists of *M. hirsuta* 1068 and 1066 (about 80% of similarity), and the second group comprises the other specimens of *M. hirsuta* (specimens 1073, 1064 and 1063), hybrid and *G. petraea* (about 35% of similarity). In this same group, it was noted that, with about 73% of similarity, the metric variables of the hybrid pollen grains are more related to the variables of *G. petraea*, including *G. petraea* 1062 (100% of similarity).



**FIGURE 5.** Principal component analysis (PCA) performed with the pollen metrical variables from hybrid (*Goydirola*) and parental species (*Goyazia petraea* and *Mandirola hirsuta*) studied. Goy = *Goyazia petraea*; Man = *Mandirola hirsuta*; EDPV = equatorial diameter in polar view; PDEV = polar diameter in equatorial view; EDEV = equatorial diameter in equatorial view; ECLG = ectoaperture length; ECWD = ectoaperture width; ENLG = endoaperture length; ENWD = endoaperture width; EXIN = exine total; SEXI = sexine; NEXI = nexine; SHAP = shape; P.A.I = polar area index; WCI = width colpus index.



**FIGURE 6.** Cluster analysis (UPGMA based on Euclidean distance) performed with the pollen metrical variables from hybrid (*Goydirola*) and parental species (*Goyazia petraea* and *Mandirola hirsuta*) studied. Goy = *Goyazia petraea*; Man = *Mandirola hirsuta*.

## Discussion

**Cytogenetic Analysis, Pollen Viability:**—The low pollen viability observed in the hybrid is common in individuals formed from non-homologous genomes, as discussed by Fishman & Sweigart (2018). This common feature of hybrids may be due to problems in the meiotic process, such as non-chromosomal disjunction in anaphase I and II, or the formation of anaphasic bridges, due to problems in the pairing of chromosomes with low similarity (homeologous), that lead to the formation of unbalanced gametes (McDermott & Noor 2010). Despite the structural abnormalities observed after the microsporogenesis of the natural hybrid, the high meiotic index registered for this individual probably indicates a genomic proximity between parental species, as observed by Monteiro *et al.* (2011) in interspecific hybrids of *Capsicum* Linnaeus (1753: 188).

The low pollen viability may also result from problems in microgametogenesis, due to the condition known as cytoplasmic male sterility (CMS). The CMS is the result of an inability in harmonizing the nuclear and mitochondrial genomes in the pollen, leading to the production of non-functional pollen grains (Bohra *et al.* 2016). Although the pollen viability recorded for the hybrid represents approximately one third of that observed for the putative parental species, it could still be sufficient to enable its sexual reproduction, as observed in interspecific *Jatropha* Linnaeus (1753: 1006) hybrids (Amkul *et al.* 2016). The functionality and reproductive capacity of this hybrid could only be confirmed in controlled pollination assays.

**Chromosome Numbers:**—Even though diverse haploid chromosome numbers were observed for *Goydirola*, the most frequent count was  $n = 13$  bivalents. The diploid number observed confirms this trend. The divergent haploid numbers reinforce the individual's hybrid origin, since the occurrence of univalent and multivalent chromosomes that causes the chromosome counts variation is common in meiotic divisions in hybrids (McKenzie *et al.* 2015). The presence of multivalent chromosomes indicates the homeology of genomic segments among parental species (Mason & Batley 2015), and it is an expected result due to the evolutionary proximity between the genera *Goyazia* and *Mandirola* (Perret *et al.* 2013).

Despite the fact that there are no records in the literature of chromosome numbers counts for *Mandirola* or *Goyazia* species, the basic chromosome numbers recorded here for both *M. hirsuta* ( $x = 12$ ) and the natural hybrid ( $x = 13$ ) are consistent with the records obtained previously for the Gloxinieae tribe of the Gesneriaceae family, being present in nine of the 15 genera of this tribe already studied (Rice *et al.* 2014).

**Pollen Morphology:**—Natural hybrids are rarely registered in Gesneriaceae (Perret *et al.* 2007, Puglisi *et al.* 2011, Ferreira *et al.* 2013). For that reason, there are few comparative studies between pollen grains of natural hybrids and parental species in the palynological literature. In general, the studies are focused on the morphological analysis of pollen grains, considering only some quantitative (pollen size and shape) or qualitative (exine ornamentation) characteristics (Wrońska-Pilarek *et al.* 2013). The pollen studies of natural hybrids have recorded variation in the numbers of apertures (van der Walt & Littlejohn 1996, Franssen *et al.* 2001), as well as anomalies may be present in the pollen grains of hybrid and parental species (Karlsdóttir *et al.* 2008).

**TABLE 5.** Morphological characterization and metric data of ectoapertures, endoapertures and exine of pollen grains from hybrid (*Goydirola*) and parental species (*Goyazia petraea* and *Mandirola hirsuta*) in  $\mu\text{m}$ .

Species	Voucher	Size	P/E	Shape	P.A.I.	Ectoaperture	WCI	Endoaperture		Exine layers		Exine
								Enlg $\times$ Ecwd	Enlg $\times$ Enwd	Total	Sexine	
<i>G. petraea</i>	A.O. Araujo 1061	S	0.88	OS	0.11	13.90 $\times$ 3.50	6.22	5.32 $\times$ 3.70	1.82	1.53	0.29	MIC/HO
<i>G. petraea</i>	A.O. Araujo 1062	S	0.89	OS	0.14	13.31 $\times$ 3.26	5.81	4.80 $\times$ 3.63	1.37	1.08	0.29	MIC/HO
<i>G. petraea</i>	A.O. Araujo 1067	S-M	1.14	PS	0.22	13.90 $\times$ 3.50	6.06	5.32 $\times$ 3.70	1.82	1.53	0.29	MIC/HO
<i>Goydirola</i>	A.O. Araujo 1170	S	0.87	SO	0.10	13.62 $\times$ 3.15	6.73	5.32 $\times$ 3.70	1.39	1.10	0.29	MIC/HE
<i>M. hirsuta</i>	A.O. Araujo 1063	S	1.30	SP	0.13	17.39 $\times$ 3.06	5.90	4.74 $\times$ 3.08	1.69	1.40	0.29	MIC/HE
<i>M. hirsuta</i>	A.O. Araujo 1064	S	1.39	P	0.13	13.90 $\times$ 3.50	4.88	5.32 $\times$ 3.70	1.56	1.27	0.29	MIC/HE
<i>M. hirsuta</i>	A.O. Araujo 1073	S	1.00	PS	0.17	18.35 $\times$ 2.01	9.35	4.70 $\times$ 2.54	1.73	1.44	0.29	MIC/HE
<i>M. hirsuta</i>	A.O. Araujo 1066	S-M	1.11	PS	0.24	23.25 $\times$ 2.99	7.72	6.05 $\times$ 3.03	1.07	0.69	0.38	MIC/HE
<i>M. hirsuta</i>	A.O. Araujo 1068	S	0.99	OS	0.25	20.10 $\times$ 2.84	8.23	4.36 $\times$ 2.67	1.06	0.63	0.43	MIC/HE

NOTE. – S = small, S-M = small to medium, P/E = ratio between polar and equatorial diameter, SO = suboblate, OS = oblate spheroidal, PS = prolate spheroidal, SP = subprolate, P = prolate, P.A.I. = polar area index, Ecwg = ectoaperture length, Ecwd = ectoaperture width, Enlg = endoaperture length, Enwd = endoaperture width, WCI = width colpus index, MIC = microreticulate, HO = homobrochate on the mesocolpium, HE = heterobrochate on the mesocolpium.

Pollen grains of hybrids and species of *Cyrtandra* (Gesneriaceae) from Hawaiian and South Pacific were analyzed using light microscopy, scanning and/or transmission electron microscopy, but not significant palynological differences were found among them, not even abnormality or infertility for this genus (Luegmayer 1993, Schlag-Edler & Kiehn 2001). In contrast, the pollen grains from natural intergeneric hybrids between *Rhytidophyllum* Martius (1832: 196) and *Gesneria* Linnaeus (1753: 612) have been reported to be deformed and collapsed. However, when it comes to an interspecific hybrid (*Gesneria pedicellaris* Alain (1971: 146) × *G. pedunculosa* (Candolle (1838: 525)) Fritsch (1894: 184)), the pollen grains are not collapsed (Skog 1976).

In the literature, pollen morphology of the genera *Goyazia* and *Mandirola* has similar qualitative and quantitative characteristics, which are difficult to distinguish (Souza *et al.* 2018). Although, *Goydirola* and *M. hirsuta* have pollen grains with circular or triangular amb, while *G. petraea* has circular amb, pollen grains from *Goyazia* and *Mandirola* species may vary in terms of amb and shape (Souza *et al.* 2018). Our results reveal that the *Goydirola* has intermediate characteristics between *G. petraea* and *M. hirsuta*: the hybrid is quantitatively more related to *G. petraea* by the measures of diameters and ectoapertures, while qualitatively it has pollen characters similar to *M. hirsuta*, mainly regarding the microreticulum on the mesocolpium region.

**TABLE 6.** Pearson and Kendall correlation coefficients for pollen grains metric variables of the first and the second axes of PCA ordination in hybrid and parental species.

Variables	Principal components	
	Axis 1	Axis 2
EDPV (Equatorial diameter in polar view)	-0.1361	-0.2172
PDEV (Polar diameter in equatorial view)	-0.1513	-0.4750
EDEV (Equatorial diameter in equatorial view)	-0.1890	-0.1450
ECLG (Ectoaperture length)	-0.5656	-0.0718
ECWD (Ectoaperture width)	0.2386	-0.4563
ENLG (Endoaperture length)	0.0271	-0.2079
ENWD (Endoaperture width)	0.2987	-0.2345
EXIN (Exine total)	0.2722	0.2456
SEXI (Sexine)	0.3916	0.3344
NEXI (Nexine)	-0.1025	-0.0699
SHAP (Shape)	0.0272	-0.1804
P.A.I (Polar area index)	-0.1116	-0.0667
WCI (Width colpus index)	-0.4527	0.4250

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