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Original article

A new endangered species of *Mollinedia* (Monimiaceae, Laurales) endemic to the Atlantic forest in the state of Espírito Santo, Brazil, supported by morphology and genome size estimation

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ABSTRACT

Mollinedia (Monimiaceae) presents numerous microendemic species, and its centre of diversity is in the Brazilian Atlantic rainforest, where more than half of the species occur. The taxonomy of microendemic species can be challenging because their morphological and genetic variations can be interpreted as a response to geographic isolation rather than a circumscription for different species. In this paper, we describe *Mollinedia pignalii* Lírio & Pauli, a microendemic species from the Espírito Santo state, Brazil. Vegetatively, *M. pignalii* is similar to *Mollinedia elegans* Tul. and *Mollinedia schottiana* (Spreng.) Perkins; however, it presents the following differences: white-puberulous leaves, staminate flowers with a flat receptacle and 6 to 14 stamens, white-puberulous pistillate flowers with a cupuliform receptacle and 8 to 22 carpels, and white-puberulous drupelets. Due to the similarity between the three species, *M. pignalii* has been collected and deposited in herbaria under different names. Here, we describe the new species based on morphology and genome size, a comparison with similar species, and ecological comments in an integrative approach. We also provide its conservation status and an identification key for the species of Monimiaceae occurring in the state of Espírito Santo.

Keywords: Flora of Brazil, Flora of the state of Espírito Santo, Tropical biodiversity, Endemism, Plant conservation.

Introduction

Monimiaceae occurs predominantly in tropical regions of the world and has a disjunct distribution, with records in Central and South America, Tropical Africa, Oceania, Sri Lanka and Southeast Asia (Philipson 1993; Renner *et al.* 2010; Lorence in press; Lírio *et al.* 2020a). It comprises 28 genera and c. 250 species (Whiffin & Foreman 2007; Renner *et al.* 2010; Lírio *et al.* 2020a) grouped in three subfamilies – Hortonioideae, Monimioideae and Mollinedioideae – supported by molecular and morphological evidence (Doyle

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& Endress 2000; Romanov *et al.* 2007; Renner *et al.* 2010). Six genera are recorded in the Neotropics: the monotypic *Peumus boldus* Molina (subfamily Monimioideae; Renner *et al.* 2010), and five genera of the subfamily Mollinedioideae: the monotypic *Grazielanthus arkeocarpus* Peixoto & Per.-Moura (Peixoto & Pereira-Moura 2008) and *Hennecartia omphalandra* J.Poiss. (Poisson 1885; Lírio *et al.* 2020a); *Macrotorus* Perkins with two species (Lírio *et al.* 2020b); *Macropeplus* Perkins with four species (Santos & Peixoto 2001); and *Mollinedia* Ruiz & Pav. with about 60 species (Lírio *et al.* 2020a; Lorence in press).

In Brazil, there are 46 species of Monimiaceae, 41 of them occurring in the Atlantic forest and 36 endemic to this domain (Lírio *et al.* 2020a; 2021; Molz & Silveira 2021). Many of these species, such as *G. arkeocarpus*, *Macropeplus friburgensis* (Perkins) I.Santos & Peixoto, *Macrotorus genuflexus* Lírio & Peixoto, *Mollinedia longicuspidata* Perkins, *Mollinedia lowtheriana* Perkins, *Mollinedia myriantha* Perkins, *Mollinedia ruschii* Lírio & Peixoto and *Mollinedia stenophylla* Perkins have microendemic distribution (Peixoto & Pereira-Moura 2008; Lírio *et al.* 2015; 2018; 2020a; 2020b; 2021; 2023a; 2023b). Of the Monimiaceae species registered in Brazil, 38 are representatives of *Mollinedia*, which is one of the most diverse genera in the family (Philipson 1993; Lírio *et al.* 2020a; 2021; 2023b; Lorence in press; Molz & Silveira 2021), occurring from South Mexico to South America, except in Argentina, Chile and Uruguay (Lírio & Peixoto 2017; Lírio *et al.* 2020a; Molz & Silveira 2021).

Mollinedia was first described by Ruiz and Pavon (1794) and later placed in the Mollinedioideae tribe by Perkins (1898). The tribe was delimited by the presence of pistillate flowers with circumscissile apex, a classification accepted by other authors (Philipson 1987; 1993; Peixoto & Pereira-Moura 2008; Lírio *et al.* 2015). *Mollinedia* presents concave, flat or urceolate staminate receptacles with almost rounded buds, four tepals, tepal length to receptacle length ratio of ca. 1:1, stamens numerous and hippocrepiform, anthers ovate, elliptical or oblong , pistillate flowers with the upper part undergoing abscissions, such as a calyptra, numerous free carpels, and fruits consisting of free drupelets (Perkins 1900; Philipson 1993; Lírio *et al.* 2015; 2020a).

In the works of Perkins (1898; 1900; 1902; 1905; 1911; 1927) and Perkins and Gilg (1901), 52 species of *Mollinedia* were described, and seven had their status changed. Although Perkins examined exsiccatae available in the large herbaria of her time, the diversity of samples from Brazil did not depict the ample morphological diversity of some of the species described by her. For this reason, many taxa described by the author are today considered as morphological variations since these species present plasticity in their development and phenotype, mainly due to their geographic distribution. To date, 36 of the species described by Perkins have been considered synonyms (Peixoto 1979; 1981; Peixoto & Pereira-Moura 1996; Peixoto *et al.* 2001; Renner & Haussner 1997; Lírio *et al.* 2020a; Lorence in press). The same occurs with other authors, such as Tulasne (1855; 1856; 1857), who described 15 species of *Mollinedia* and altered the status of other eight, 10 of which are now considered synonyms (Perkins 1900; Renner & Haussner 1997; Lírio *et al.* 2020a; Lorence in press).

The above data show the difficulty in delimiting species in *Mollinedia*. Despite the notorious morphological variation in species of this genus, some characters such as the pubescence on the abaxial surface of leaves, scars left by indumenta on the adaxial surface of leaves, the colour of leaves when dried, the shape of the receptacle in staminate flowers, and the shape and pubescence of the fruits have been considered significant for the circumscription of species of the family (Lorence 1999; Whiffin & Foreman 2007; Renner & Takeuchi 2009; Lírio *et al.* 2015; 2020a; 2020b; 2021; 2023b; Molz & Silveira 2021). However, other techniques have been incorporated to corroborate or reject hypotheses of new taxa delimitation based on morphological data. Among these techniques, genome size (GS) estimation has proved very useful (Ohri 1998; Zonneveld 2009; Jedrzejczyk & Rewers 2018), including in Monimiaceae, in the genus *Macrotorus* (Lírio *et al.* 2020b). Thus, this study provides an opportunity to test the GS estimation method in the delimitation of *Mollinedia* species.

In this work, we describe *Mollinedia pignalii*, a new species endemic to the mountainous region of the Espírito Santo state, Brazil, based on morphological data and GS estimation. The data are discussed comparatively with *Mollinedia elegans* Tul. and *Mollinedia schottiana* (Spreng.) Perkins. We provide ecological comments, the identification key for species of Monimiaceae occurring in the state mentioned, and assess the extinction risk of the new species.

Material and methods

Taxonomic Description and Herbaria Nomenclature: Morphological terms follow Harris and Harris (2001) except for those used for trichomes, which follow Payne (1978), and the specific terminology of Monimiaceae that follows Perkins (1898, 1900). Herbarium acronyms follow Thiers (2022, continuously updated). The work was based on field observations, herbarium collections (CEPEC, HUEFS, HUFABC, K, MBML, P, NY, RB, SPF), data available in virtual herbaria and online databases (CRIA 2022; JBRJ 2022; Reflora 2022), and bibliographies (Lírio & Peixoto 2017; Lírio *et al.* 2020a).

Genome Size Estimation By Flow Cytometry: Six individuals from each species were analysed in triplicate. For each sample, approximately 50 mg of young leaf tissue was macerated with 25 mg of the internal reference standard, *Pisum sativum* var. Ctirad (2C = 9.09pg, Dolezel *et al.* 1998), in 0.5 ml of cold Ebihara buffer (Ebihara *et al.* 2005) supplied with 0.025 μg mL^{-1} RNAse. Nuclei suspensions were

stained by adding 12.5 µL to 1 mg mL^{-1} of propidium iodide solution (PI, Sigma). The analysis was performed using a FACSCanto II cytometer (Becton Dickinson, San Jose, CA, USA) kindly made available by the Microbiology and Immunology Department of IBB-UNESP (Botucatu, Brazil). The histograms were obtained with FACSDiva software based on 5,000 events, and the statistical evaluation was performed using the Flowing Software 2.5.1 (http://www.flowingsoftware.com/). The quality control of samples was based on the coefficient of variation (CV) of each measurement, which should be below 5%, and the standard deviation (SD) among 2C-values, which should be below 3%. These limits ensure that the variations observed within and among measurements are due to technical factors and should not represent intraspecific variation among individuals (Pellicer & Leitch 2014). The species were differentiated by One-Way ANOVA followed by Tukey Test in R.

Conservation Status Assessment: To assess the conservation status of the species, we followed the Categories and Criteria of IUCN Red List of Threatened Species (thereafter, IUCN Red List) (IUCN 2012; 2022). We calculated the Extent of Occurrence (EOO) using the area of the minimum convex polygon and the Area of Occupancy (AOO) using a grid of 4 km^2 cells (IUCN 2022). The analyses were performed using the geospatial conservation assessment tool GeoCat (Bachmann *et al.* 2011).

Results

Taxonomic treatment

Mollinedia pignalii Lírio & Pauli, *sp. nov.* (Figs. 1 and 2)

Type: BRAZIL. Espírito Santo: Santa Maria de Jetibá, Fazenda Azaléa, Bonito River, fragment near the coffee plantation, 20º3'21.953''S, 40º39'5.810''W, small tree of 3 m, yellow flowers, 10 August 2015, sta. fl., *E. J. Lírio 1276* (Holotype: RB! [bc] RB01108310; Isotypes: P, SPF and MBML).

Etymology: The specific epithet pays homage to Marc Pignal (1964-), French botanist of the Muséum National d'Histoire Naturelle, Paris, who has extensively contributed to research on the taxonomy, morphology, botanical history and conservation, including of Monimiaceae. He also works on developing the knowledge of the Brazilian flora, participating in significant expeditions, projects and scientific works in Brazil.

Diagnosis: The new species is similar to *M. elegans* and *M. schottiana* but can be distinguished by the combination of the following characters: white-puberulous leaves on the abaxial surface, staminate flowers with a flat receptacle, 6 to 14 stamens, white-puberulous pistillate flowers with a cupuliform receptacle, 8 to 22 carpels, and white-puberulous drupelets (Table 1).

Description: Shrubs or treelets, 3-9 m tall, dioecious, rhytidome smooth, twigs cylindrical, striate, adpressed-pilose then glabrescent. Leaves 7.3-15.5 × 1.2-3.8 cm, opposite, elliptical to oblong, apex obtuse to attenuate, generally falcate, base acute, rarely cuneate, margin with 4-6 pairs of irregular teeth in the distal half, chartaceous, discolorous, green-olive when dry, lighter on the abaxial surface, lustrous on the adaxial surface, pellucid-punctate, young leaves white-strigillose on both surfaces, then glabrescent on both surfaces, or glabrous on the adaxial surface, secondary veins 6-10 pairs, apparent on the adaxial surface and prominent on the abaxial surface, petiole 0.5-1.8 cm long, canaliculate. Staminate flowers yellow, 4-5 × 3-5 mm in thyrses or fascicles up to 5 cymes (3-florous), axillary or terminal, white-puberulous, rachis 0-1 cm, peduncle 0.3-0.8 cm, pedicel 0.2-1.3 cm, bracts ovate, apex acute, c. 1 mm long, bracteoles ovate, apex acute, c. 1 mm long, receptacle flat, tepals c. 3/4 of the flower length, externals ones ovate or oblong with an acute apex, internals ones oblong with a rounded apex, unequal, one with an entire margin, the other toothed, stamens 6-14, hippocrepiform, locules confluent at the apex, filament short. Pistillate flowers yellow, 3-5 × 4-5 mm, solitary or in fascicles up to 6 flowers, white-puberulous, rachis 0-0.65 cm, peduncle 0-0.5 cm, pedicel 0.4-1.3 cm, bracts ovate, apex acute, 1.1-1.5 mm long, bracteoles ovate, apex acute, 1.5-1.8 mm long, receptacle cupuliform, internally ferruginous-puberulous, tepals 1/3 of the receptacle length, externals ones ovate with a rounded apex, margin entire, internals ones oblong, apex truncate, irregular margin, carpels 8-22, ovary oblong or elliptical, stigma c. 1/3 of the carpel length. Drupelets reddish then blackish-purple, ellipsoid or orbicular, 1.3-2.1 × 0.9-1.3 cm, sessile, apex rounded, stigma persistent, white-puberolous, brown when dried, asperulous, fruiting receptacle 0.4-0.8 cm wide, reflexed, fruit scars not prominent, peduncle plus pedicel 0.6-1.1 cm long (Fig. 1).

Distribution and habitat: *Mollinedia pignalii* is found in four municipalities in the state of Espírito Santo (Fundão, Santa Leopoldina, Santa Maria de Jetibá and Santa Teresa), in Mountainous Dense Ombrophilous Forest, at altitudes of 575 to 875 m (Fig. 2). The species is distributed in the Central Corridor of the Atlantic forest (CCAF), which extends from the southern portion of Bahia throughout Espírito Santo. This corridor aims to maintain and restore the connection between forests, mainly to facilitate the genetic flow between populations and ensure the survival of several biological species. The CCAF is considered a priority region due to the high degree of vulnerability and fragmentation of its ecosystems when compared to others (Brasil 2007).

Phenology: The species is perennial with persistent and evergreen leaves. Flowers were collected from June to September and fruits in October, December and January to March.

Genome size: The three analysed species exhibited differences in genome size ($F = 69.83$, $p = 1.7e^{-10}$; Fig. 3).

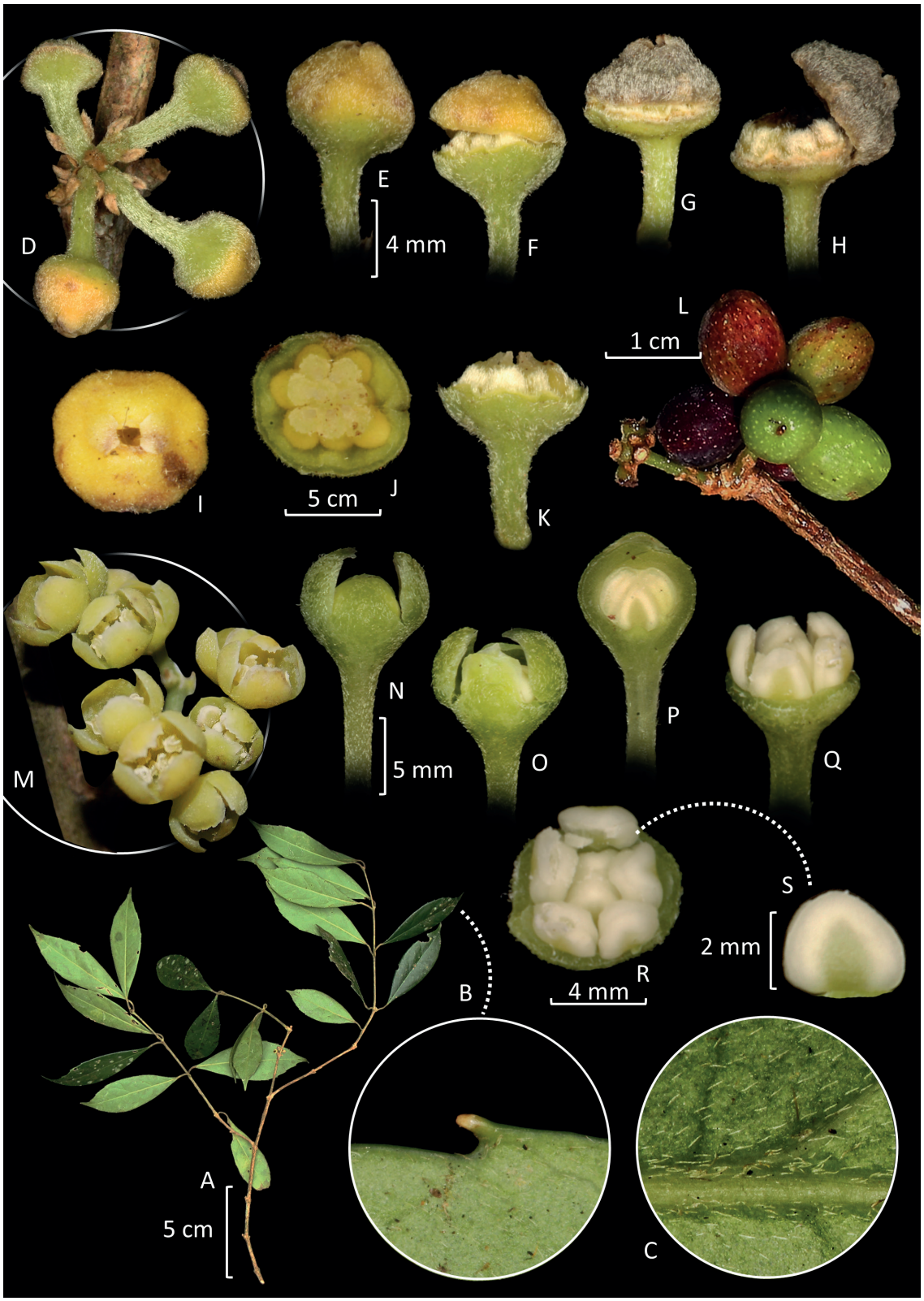

Figure 1. *Mollinedia pignalii*. (**A**) Branch. (**B**) Amplification of the monimioid teeth. (**C**) Amplification of the abaxial leaf surface. (**D**) Pistillate flowers inflorescence. (**E**) Pistillate flower. (**F**) Pistillate flower initiating the calyptra opening. (**G**) Pistillate flower with a dry calyptra. (**H**) Pistillate flower with the calyptra opened and fertilised carpels. (**I**) Front view of the pistillate flower. (**J**) Front view of the carpels. (**K**) Side view of the carpels. (**L**) Drupelets. (**M**) Staminate flowers inflorescence. (**N**, **O**) Young staminate flowers. (**P**) A longitudinal cut of a young staminate flower. (**Q**) Young staminate flower with its tepals removed. (**R**) Front view of the stamens. (**S**) Isolated stamen. Based on the vouchers: Lírio 1276 (M) and 1314 (L) and Zavatin *et al* 861 (N-S) and 908 (A-K). Photos: E.J. Lírio (L-M) and D. A. Zavatin (A-K/N-S).

Table 1. Comparison between *Mollinedia pignalii sp. nov.* and the similar species *M. elegans* Tul. and *M. schottiana* (Spreng.) Perkins. Characters are based on the examined material and bibliographies (Peixoto *et al.* 2001, 2002; Lírio & Peixoto 2017; Lírio *et al.* 2020a; Molz & Silveira 2021).

	M. pignalii, sp. nov.	*M. elegans*	*M. schottiana*
Habit	Shrub or treelet	Shrub or treelet	Shrub or tree
Distribution	Espírito Santo, Montane Ombrophilous Dense Forest	Minas Gerais, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul, Ombrophilous Mixed and Dense Forest and Seasonal Semideciduous Forest	Bahia, Minas Gerais, São Paulo, Espírito Santo, Rio de Janeiro, Paraná, Santa Catarina and Rio Grande do Sul, Ombrophilous Mixed and Dense Forest and Seasonal Semideciduous Forest
Leaves	Elliptical or oblong; abaxial face white-puberulous	Rhombic-lanceolate, rhombic, rarely elliptical ou lanceolate; abaxial face glabrescent	Ovate or elliptical , rarely obovate; abaxial face adpress-flavescents or ferruginous
Staminate flowers	Flat receptacle, 10 to 14 stamens	Cupuliform receptacle, 8 to 19 stamens	Campanulate receptacle, 20 to 42 stamens
Pistillate flowers	Cupuliform receptacle, 11 to 22 carpels	Cupuliform receptacle, 6 to 10 carpels	Cupuliform receptacle, 30 to 80 carpels
Drupelets	Reddish then blackish-purple drupelets; white-puberulous	Orangish then blackish-purple drupelets; glabrescent to glabrous	Reddish then blackish-purple drupelets; fawn-puberulous then glabrescent
Genome size	2C= 2.72 pg	2C= 3.19 pg	2C= 2.49 pg

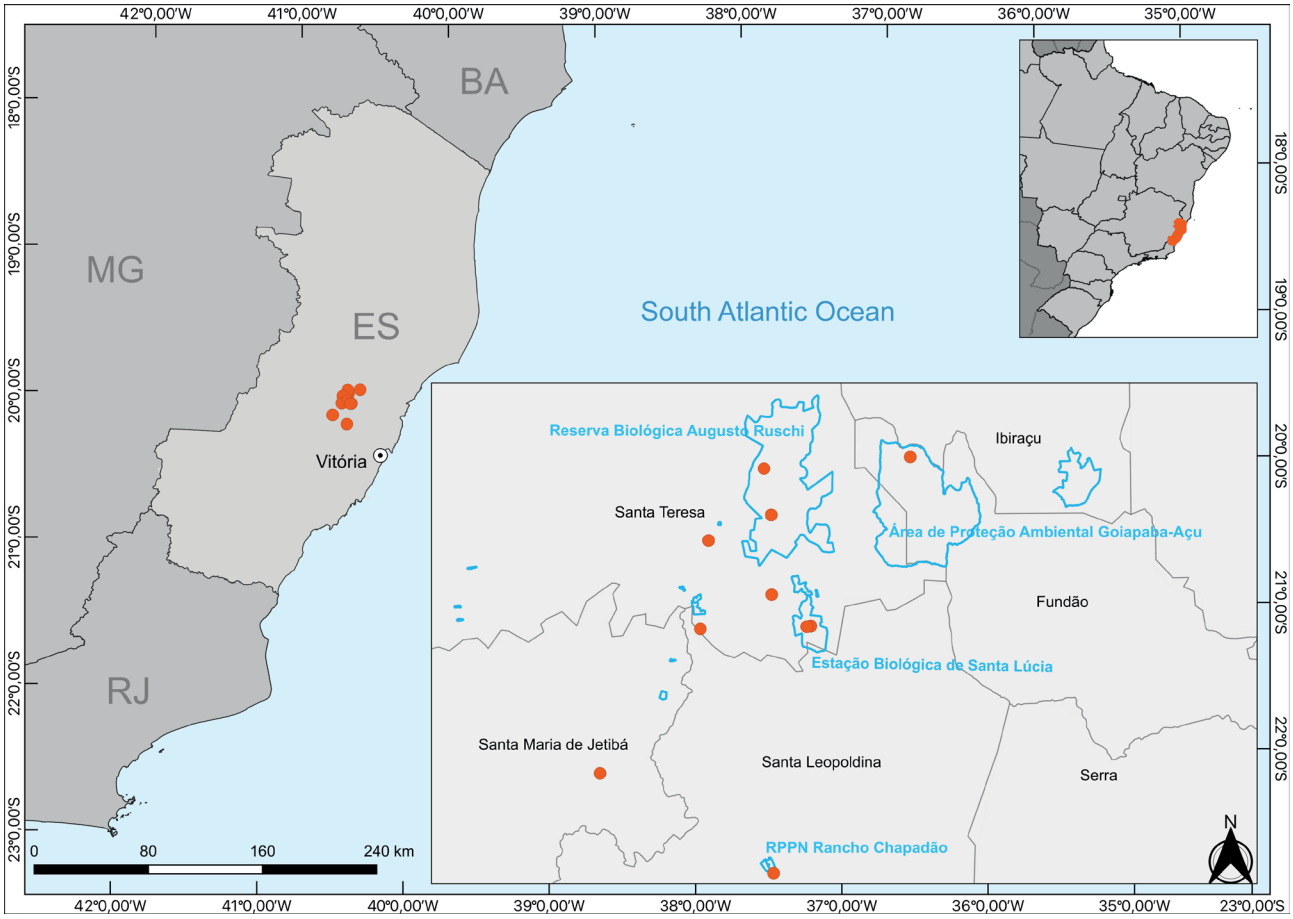

Figure 2. Geographic distribution map of *Mollinedia pignalii*. Orange dots represent occurrence records and blue polygons represent Conservation Units.

The variation in genome size was as follows: *M. pignalii* 2C = 2.72 pg ± 0.04 (CV = 3.39, SD = 1.47%); *M. elegans* 2C = 3.19 pg ± 0.09 (CV = 2.65, SD = 2.8%); and *M. schottiana* 2C = 2.49 pg ± 0.2 (CV = 3.61; SD = 8%). Notably, *M. elegans* exhibited a significantly larger genome size (p = 0.00) compared to both *M. pignalii* and *M. schottiana*, which also displayed distinct genome sizes (p = 0.002; Fig. 3A). The large SD observed in *M. schottiana* can be observed on the stretch violin format (Fig. 3B).

Conservation status: *Mollinedia pignalii* has estimated EOO of ca. 257.665 km² and an AOO equal to 40 km², thus falling into the Endangered category, under the criterion B. There are four known locations and all of them are under different ongoing threats. Habitat loss is the main threat for this species, considering that the Atlantic forest has only 12.4% of its original vegetation cover (Fundação SOS Mata Atlântica & INPE 2021). Fragmentation and habitat degradation due to urban expansion, agriculture and livestock farming are also important threats. Pasturelands cover a great proportion of the municipalities where the species occurs, representing 38.6% of land use in Fundão, 22% in Santa Teresa, 17.5% in Santa Maria de Jetibá, and 17.3% in Santa Leopoldina (LAPIG 2022). In addition, the increase of droughts and fires has been negatively impacting the endemic species of the region (Fraga *et al.* 2019). Finally, defaunation is a relevant threat for plant species which rely on threatened fauna for pollination and/or dispersion, which is the case for many Monimiaceae species. Therefore, a continuing decline in habitat quality is expected in view of all the above mentioned factors.

The global population of *M. pignalii* is also considered small: restricted to less than 50 herbarium records or observations. An effort to quantify the population size based on the number of mature individuals is needed in order to confirm if it would fall into the Critically Endangered category threshold. The species occurs in four protected areas with different levels of protection and permitted uses [Área de Proteção Ambiental de Goiapaba-Açu (Fundão), Estação Biológica de Santa Lúcia (Santa Teresa), Reserva Particular do Patrimônio Natural Rancho Chapadão (Santa Leopoldina), and the integral protection conservation unit Reserva Biológica Augusto Ruschi (Santa Teresa)]. Despite that, phytosociological studies have indicated that the number of individuals is decreasing. In a floristic and phytosociological study carried out at the Estação Biológica de Santa Lúcia covering sections of 3,400 m² at valley, slope and mountain top areas totaling 1.02 ha, and considering individuals with a CAP equal to or greater than 20 cm, Thomaz and Monteiro (1997) found two individuals of the species, one in the slope area (between 675 and 700 m) and another in the top of the hill (between 820 and 855 m). In a review of the study carried out in 2014, only one individual of *M. pignalii* was found in the same area, which demonstrates a decrease in the number of individuals with

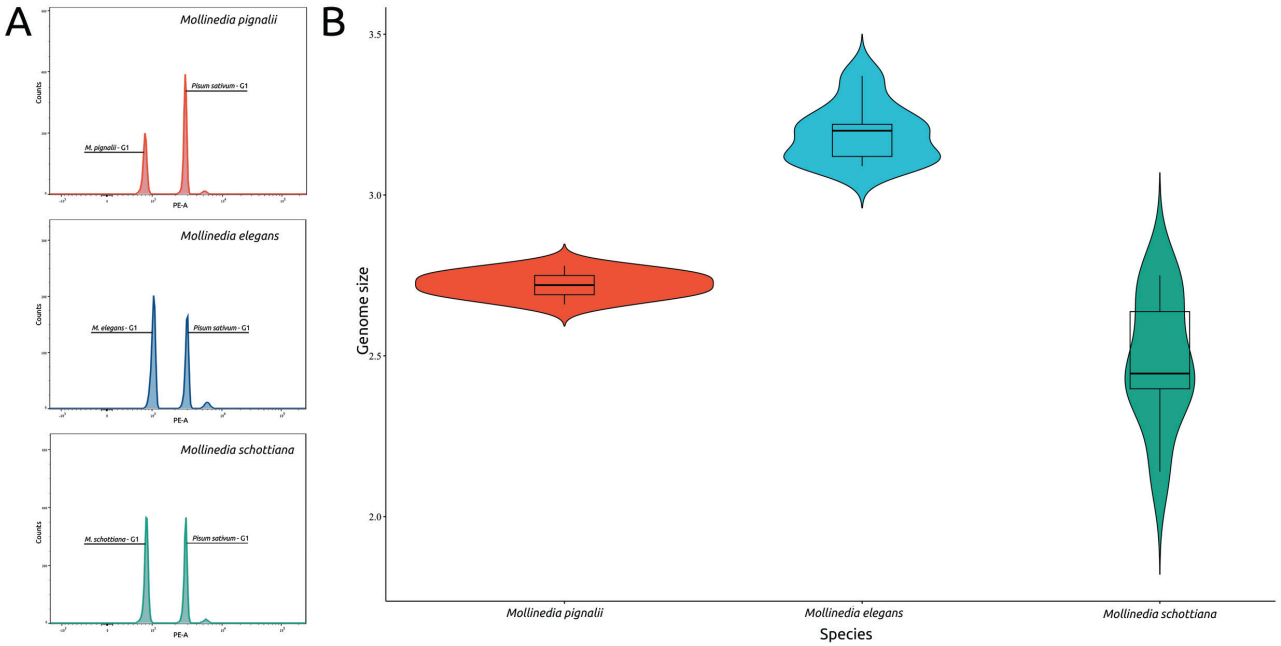

Figure 3. Genome size analysis of *Mollinedia pignalii*, *Mollinedia elegans*, and *Mollinedia schottiana* by Flow Cytometry. (**A**) Flow cytometry histograms illustrating the analysis of *M. pignalii* (top, in red), *M. elegans* (middle, in blue), and *M. schottiana* (bottom, in green). The G1 peaks of both *Pisum sativum* (a reference) and the samples are indicated. (**B**) The violin plot showcases the differences in genome size (Y-axis) among the three species (X-axis): *M. pignalii* (red), *M. elegans* (blue), and *M. schottiana* (green). The height of each violin corresponds to the internal standard deviation, while the width indicates the internal coefficient of variation. Each violin includes an internal box plot representing the interquartile range, spanning from the lower to the upper quartile, indicating 50% of the data. Additionally, a line within the violin represents the mean 2C value.

no recruitment of new individuals (Saiter *et al.* 2011; Saiter & Thomaz 2014). Due to its restricted geographic range and the inferred continuing decline of habitat quality and population size, the species is assessed here as Endangered (EN) as it meets the criteria B1ab(iii) + B2ab(iii); D.

Taxonomic comments: *Mollinedia pignalii* is vegetatively similar to *M. elegans* and *M. schottiana* in the habit and leaf shape but differs from them by the characteristics shown in Table 1. The new species was previously treated as *Mollinedia fruticulosa* Perkins or *Mollinedia* aff. *fruticulosa* in herbaria and the literature (Thomaz & Monteiro 1997; Saiter & Thomaz 2014) due to the morphological similarity of its leaves to those of the type material of *M. fruticulosa*. However, in the analysis of the original descriptions, type materials, and herbarium specimens during the preparation of the treatment of Monimiaceae in the Flora do Brasil 2020 project (Lírio *et al.* 2020a), *M. fruticulosa* was synonymized under *M. elegans* because its morphological characters are consistent with the circumscription of that species. Like *M. schottiana, M. elegans* has a wide distribution, occurring in all states of the South and in Minas Gerais, São Paulo and Rio de Janeiro in the Southeast of Brazil, and morphological variations are expected. These variations led to the description of distinct species, such as *M. fruticulosa, Mollinedia micrantha* Perkins and *Mollinedia hatschbachii* Peixoto, all currently considered to be synonyms.

Additional specimens examined (Paratypes) – BRAZIL. Espírito Santo: Fundão, Piabas, Albino's property, Hillside Atlantic Forest (19°52'27.001''S, 40°28'23.002''W) alt. 576 m, small tree of 6 m, yellow flowers, 04 June 2011, pis. fl., *C. N. Fraga 3399* (MBML!, NY [bc] NY03082202, RB!); **Santa Leopoldina**, Bragança, RPPN Rancho Chapadão, inside the preserved hillside forest (20°06'48.6''S, 40°33'06.1''W) shrub of 4 m, DBH of 3 cm, green fruits, 09 Mar. 2013, fr., *A. M. Assis 3777* (MBML [bc] MBML050142); **Santa Maria de Jetibá**, Fazenda Azaléa, Bonito River, fragment near the coffee plantation (20°03'21.953''S, 40°39'5.810''W) shrub of 2 m, staminate yellow flowers, 20 June 2014, sta. fl., *E. J. Lírio 1262* (RB! [bc] RB01108578); **Santa Teresa**, Mata da Penha (19°57'11.999''S, 40°33'10.001''W) 10 Apr. 1984, shrub of 3 m, reddish soil, fr., *W. Boone 39* (MBML! [bc] MBML000046); *idem*, Aparecidinha, land of Luiz Bringuenti, (19°58'23.002''S, 40°35'38.000''W) bindweed ca. 4 m, chartaceous leaves slightly discoloured, ripe vinaceous fruits, immature green, 13 Apr. 2003, fr., *R. P. Oliveira 870* (CEPEC!, HUEFS! and MBML! [bc] MBML021579); *idem*, Estação Biológica de Santa Lúcia (19°58'17.000''S, 40°31'49.001''W) alt. 700 m, shrub of 4 m, green fruits, 04 February. 1999, fr., *L. Kollmann 1835* (MBML! [bc] MBML008322); *idem*, (19°58'18.257''S, 40°31'57.277''W) small tree of 7 m, 6 cm DBH, green flowers, 03 August 2004, pis. fl., *L. Kollmann 6917* (RB! [bc] RB00237661); *idem*, (19°58'18.257''S, 40°31'57.277''W) small tree of 2 m, ripe purple fruits, immature green, 11 September 2013, fr., *E. J. Lírio 1314* (RB! [bc] RB01120134); *idem*, (19°58'18.257''S, 40°31'57.277''W) small tree 2 m, green immature fruits, 11 September 2013, fr., *E. J. Lírio 1315* (RB! [bc] RB01120135); *idem*, (19°58'17.000''S, 40°31'49.001''W) hillside forest, shrub of 3 m, green fruits, 25 November 1998, fr., *L. Kollmann 1112* (MBML! [bc] MBML008081); *idem* (19°58'17.000''S, 40°31'49.001''W) alt. 650 to 800 m, hillside Atlantic Forest, 5 m individual, green fruits, 22 Mar. 1994, fr., *L. D. Thomaz 1660* (MBML! [bc] MBML010656 and RB [bc] RB00792278); *idem*, (19°58'17.000''S, 40°31'49.001''W) Ombrophilous Dense Forest, shrub 6 m, green fruits, 11 February. 2012, fr., *E. J. Lírio 58* (MBML! [bc] MBML044444); *idem*, Indaiaçu trail (19°58'17.000''S, 40°31'49.001''W) small tree of 7 m, green immature fruits, 25 January. 2000, fr., *V. Demuner 605* (MBML! [bc] MBML011537 and RB! [bc] RB00792351); *idem*, (19°58'17.000''S, 40°31'49.001''W) Ombrophilous Dense Forest, 9 m tree, white flowers, mild odour, alt. 773 m, 08 July 2011, sta. fl., *E. J. Lírio 32*, (MBML! [bc] MBML043838); *idem*, Sagui trail (19°58'17.000''S, 40°31'49.001''W) small tree of 5 m, green immature fruits, 29 June 2000, fr., *V. Demuner 1213* (MBML! [bc] MBML012896); *idem*, (19°58'17.000''S, 40°31'49.001''W) alt. 839 m, Ombrophilous Dense Forest, small tree 6 m, greenish-white floral buds, 22 July 2011, sta. fl., *E. J. Lírio 36* (MBML! and RB! [bc] RB00792283); *idem*, Seca trail (19°58'18.257''S, 40°31'57.277''W) small tree of 3 m, with flowers, 09 August 2006, pis. fl., *M. C. Souza 359* (RB! [bc] RB00485747); *idem*, Pinguela climb (19°58'17.000''S, 40°31'49.001''W) Ombrophilous Dense Forest, shrub 4 m, green fruits, 11 February. 2012, fr., *E. J. Lírio 53* (MBML! and RB! [bc] RB00792284); *idem*, Santo Antônio (19°55'19.999''S, 40°35'21.001''W) small tree of 6 m, light green immature fruits, 15 February. 2000, fr., *V. Demuner 718* (MBML! [bc] MBML012021 and RB! RB00792299); *idem*, Boza's Terrain (19°55'19.999''S, 40°35'21.001''W) hillside forest, shrub of 4 m, green floral buds, 15 June 1999, sta. fl., *L. Kollmann 2561* (MBML! [bc] MBML010768, RB! [bc] RB00792297 and SPF); *idem*, (19°55'19.999''S, 40°35'21.001''W) on the side of the road, small tree of 5m, green flowers, 04 August 2004, sta. fl., *L. Kollmann 6921*, (RB! [bc] RB00237669); *idem*, REBIO Augusto Ruschi (19°52'50.999''S, 40°33'25.999''W) entrance above the researcher's home, 3 m individual, ripe vinascious fruits, green immature fruits, 18 September 2012, fr., *E. J. Lírio 186*, (MBML! and RB! [bc] RB00824786); *idem*, (19°54'27.000''S, 40°33'11.002''W) alt. 875 m, landmark (78, 77, 76), hilltop, 18 m canopy, shrub of 2 m, immature green fruits, 29 October 2002, fr., *R. R. Vervloet 1330*, (MBML! [bc] MBML018209 and RB! [bc] RB00792350); *idem*, (19°54'27.000''S, 40°33'11.002''W) alt. 870 m, hilltop, 15 m canopy, small tree of 4 m, light green fruits, 18 December 2002, fr., *R. R. Vervloet 1561* (MBML [bc] MBML018521 and RB [bc] RB00792304); *idem*, forest interior by the right side of the trail, after the administration headquarters (19°54'47.05''S, 40°33'10.07''W) alt. 822 m, small tree of 2 m, discolour leaves, green young staminate

flowers, 19 July 2022, sta. fl., *D. A. Zavatin, 861*, (SPF!); *idem*, Roda d'água trail (19º54'27.000''S, 40º33'11.002''W) division on landmark 37, hilltop, 20 m canopy, small tree of 4 m, light green fruits with small white dots, 13 Mar. 2003, fr., *R. R. Vervloet 1994* (MBML [bc] MBML020007 and RB! [bc] RB00792345); *idem*, Mountain top to the right of the river Piraquê-Açu (19º52'49.04''S, 40º32'13.02''W) alt. 875 m, small tree of 3 m, discolour leaves, greenish-yellow pistillate flowers with white pilosity, 13 ago. 2022, pis. fl., *D. A. Zavatin, 908* (SPF!).

Additional Material Examined – *Mollinedia elegans* Tul. **BRASIL. Rio de Janeiro: Nova Friburgo**, Reserva Ecológica Municipal de Macaé de Cima (22º28'06.870''S, 42º53'28.950''W) source of the Rio das Flores, 07 November 1988, sta. Fl., *R. Guedes* 2189 (BHCB, CEPEC, MO, NY, RB and UEC [bc] UEC128109). **Santa Catarina:** São Bento do Sul surroundings of the CEPA Rugendas, Rio Natal road (26º09'0.720''S, 49º13'27.480''W) 22 June 2004, fr., *F. S. Meyer 48* (JOI and RB [bc] RB00682680). **São Paulo:** Ilha do Cardoso, Captação Hill in direction to the Morro dos Três Irmãos, Perequé River (25º00'36.001''S, 47º55'11.993''W) 10 October 1980, sta. fl., *E. Forero 8780* (SP [bc] SP048157, RB and RBR). **–** *Mollinedia schottiana* (Spreng.) Perkins. **BRASIL**. **Espírito Santo: Castelo**, Forninho trail (20º30'58.000''S, 41º05'01.000''W) Dense High-Montane Ombrophilous Forest with inselbergs, 12 February. 2008, pis. fl., *L. Kollmann 10561* (MBML [bc] MBML034095, RB and UPCB). **Rio de Janeiro: Nova Iguaçu**, Reserva Biológica do Tinguá, Comércio road (22º45'33.117''S, 43º27'03.958''W) 24 Ago. 1993, pis. fl., *L. S. Sylvestre 891* (RBR [bc] RBR00025676). **Santa Catarina:** Águas Mornas, Rio Novo (27º41'44.640''S, 48º49'27.720''W) 14 December 1972, sta. fl., *R. M. Klein 10536* (FLOR, HBR, ICN and RBR [bc] RBR00007863).

Key to the Monimiaceae species from the state of Espírito Santo, Brazil

1. Leaves glabrous 2

– Leaves villous, velutinous, sericeous, tomentose, pubescent or puberulous 8

2. Flowers white, tepals with double the length of the receptacle or more ..... ***Macropeplus schwackeanus*** (Perkins) I.Santos & Peixoto

– Flowers yellowish, orangish or greenish, tepals with approximately the same length of the receptacle or less 3

3. Leaves pellucid-punctate 4

– Leaves non-pellucid-punctate 5

4. Shrubs or treelets, leaves lustrous, papyraceous or chartaceous; staminate flowers with urceolate receptacle ..... ***Mollinedia glabra*** Perkins

– Trees, leaves opaque, never papyraceous or chartaceous, staminate flowers with campanulate receptacle ..... ***Mollinedia ruschii*** Lírio & Peixoto

5. Leaves blackened when dried; puberulous drupelets ..... ***Mollinedia engleriana*** Perkins

– Leaves greenish, green-olive or brown when dry; drupelets puberulous 6

6. Staminate flowers with long urceolate receptacle; anthers of the receptacle base reniform ..... ***Macrotorus utriculatus*** (Mart. ex Tul.) Perkins

– Staminate flowers with flat receptacle; anthers of the receptacle base never reniform 7

7. Leaves green-olive, base cuneate, secondary veins 5-7 pairs ..... ***Mollinedia oligantha*** Perkins

– Leaves brown, base acute, secondary veins 11-14 pairs ..... ***Mollinedia aff. oligantha*** Perkins

8. Leaves canescent on the abaxial surface; flowers externally canescent ..... ***Mollinedia salicifolia*** Perkins

– Leaves never canescent on the abaxial surface; flowers never canescent 9

9. Leaves pubescent or puberulous 10

– Leaves villous, velutinous, sericeous or tomentose 12

10. Leaves chartaceous, staminate flowers with campanulate receptacle, ovate drupelets, 8-9 × ca. 6 mm ..... ***Mollinedia ovata*** Ruiz & Pav.

– Leaves rigid-chartaceous to coriaceous, staminate flowers with receptacle flat or urceolate, drupelets elliptical, bigger than 13 × 8 mm 11

11. Leaves non-pellucid-punctate, puberulous in all the abaxial surface; inflorescence ferruginous-tomentose, staminate flowers with receptacles flat ..... ***Mollinedia gilgiana*** Perkins

– Leaves pellucid-punctate, puberulous in the inferior half of the abaxial surface and in the central vein; inflorescence pubescent, staminate flowers with urceolate receptacle ..... ***Mollinedia sphaerantha*** Perkins

12. Leaves bullate or semi-bullate, margin with a continuous line of trichomes ..... ***Mollinedia lamprophylla*** Perkins

– Leaves not bullate, margin without a continuous line of trichomes 13

13. Leaves coriaceous, villous in the abaxial surface, anthers with non-confluents locules ..... ***Mollinedia glaziovii*** Perkins

– Leaves non-coriaceous, non-villous on the abaxial surface, anthers with confluents locules 14

14. Leaves with thickened trichome scars, drupelets nigrescent when dried ..... ***Mollinedia argyrogyna*** Perkins

– Leaves without thickened trichome scars, non-nigrescent drupelets when dried 15

15. Leaves velutinous in the abaxial surface; drupelets brown, velutinous ..... ***Mollinedia uleana*** Perkins

– Leaves never velutinous in the abaxial surface; drupelets never brown or velutinous 16

16. Young branches and petioles with indumentum ferruginous tomentose 17

– Young branches and petioles never ferruginous tomentose 18

17. Leaves oblong; basal anthers with short filaments, apical stamens with null filaments ..... ***Mollinedia longifolia*** Tul.

– Leaves ovate or elliptical; stamens with sessile filaments ..... ***Mollinedia schottiana*** (Spreng.) Perkins

18. Branches with an external layer that often detaches itself, leaves with erect trichomes, elongated and hyalines, especially along the central vein ..... ***Mollinedia dolichotricha*** Lírio & Peixoto

– Branches without an external layer that detaches itself, leaves with adpressed trichomes 19

19. Leaves brown when dried; flowers grey-sericeous the staminate ones with a campanulate receptacle ..... ***Mollinedia puberula*** Perkins

– Leaves green olive when dried; flowers white-puberulous the staminate ones with a flat receptacle ..... ***Mollinedia pignalii*** *sp. nov.*

Discussion

Some studies show that the number of known angiosperm species is likely lower than we imagine, and the reasons might be the deficit of chromosomal data in some plants' groups, the lack of taxonomic revisions and fieldwork. Even in well-studied areas, new species are often found: e.g. *Macrotorus genuflexus*, from the Biological Reserve of Poço das Antas, in the state of Rio de Janeiro, and *Eleocharis pedrovianae* C.S. Nunes, R.Trevis. & A.Gil (Cyperaceae), from Serra dos Carajás, in the state of Pará (Nunes *et al.* 2016); therefore, the rarity of some species need to be considered as well (Soltis *et al.* 2007; Cheek *et al.* 2020; Christenhusz & Byng 2016). *Mollinedia pignalii* is a new species with a small distribution range, and shares morphological similarities with *M. elegans* and *M. schottiana* regarding its vegetative traits. Despite these similarities, the new species can be differentiated by a set of characteristics such as leaf pubescence, staminate and pistillate flower and fruit traits, and the genome size estimation (Table 1).

In recent decades, several new species have been described within the family, including *Kairoa endressiana* W.N.Takeuchi & Renner, *Macrotorus genuflexus*, *Mollinedia leucantha* Molz & Silveira, *M. oaxacana* Lorence, *M. ruschii*, *M. arianeae* Lírio & Pignal and *M. torresiorum* Lorence (Lorence 1999; Renner & Takeuchi 2009; Lírio *et al.* 2020b; 2021; 2023b; Molz & Silveira 2021) along with three monotypic genera: *G. arkeocarpus*, *Pendressia wardellii* (F.Muell.) Whiffin and *Hemmantia webbii* Whiffin (Whiffin & Foreman 2007; Peixoto & Pereira-Moura 2008; Ford & Whiffin 2018). This indicates that taxonomic studies and field collections focused on this group are still needed. Finally, it is worth noting that many species are described based solely on morphological approaches, often neglecting the evaluation of molecular and cytogenetic data, which are very important for accurately determining new species and solving species complexes.

Since genome size estimation can be related to the number or size of chromosomes, this technique becomes particularly relevant for species description in groups with large ploidy variations, such as those in the Monimiaceae family (n = 19, 20, 22, 39, 40–42, c. 44,

c. 48, 50, 72, c. 83, 90; Morawetz 1986; Rohwer 1993; Renner & Chanderbali 2000; Oginuma & Tobe 2006; Lírio *et al.* 2022). By providing a rapid estimation of genome size, this method allows for comparisons between species and provides indications of species' ploidy levels, which are otherwise confirmed through chromosomal number determination. The genome size estimation proved to be a valuable tool in distinguishing species in the family before, as demonstrated in a previous study on the genus *Macrotorus* (Lírio *et al.* 2020b). *Macrotorus* has two species: the known tetraploid species *Macrotorus utriculatus* (Mart. ex Tul.) Perkins (2n= 80, 2C=5.545 pg) and the recently described diploid species *M. genuflexus* (2n= 40, 2C = 2.644 pg) (Lírio *et al.* 2020b).

Genome size estimation also has been employed to assign plant species to different families, demonstrating its reliability and applicability across various taxonomic groups (Ohri 1998; Zonneveld 2009; Jedrzejczyk & Rewers 2018). In our study, we were able to differentiate the three *Mollinedia* species based on their genome size. However, *M. schottiana* exhibited a large standard deviation (SD) of 8% with a low coefficient of variation (CV). This significant variation suggests that the observed SD is likely a result of biological factors rather than methodological issues. As currently circumscribed, the taxonomic entity of *M. schottiana* may be polymorphic and could potentially encompass more than one species. Furthermore, such intraspecific genome size variation could be associated with the wide geographic distribution of *M. schottiana*, as previously suggested in different species (Biémont 2008; Moraes *et al.* 2022).

Regarding the geographic distribution and conservation status, although *M. pignalii* occurs in four protected areas with different levels of protection and permitted uses, the species is restricted to regions of Mountainous Dense Ombrophilous Forest in only four municipalities of the state of Espírito Santo, distributed in fragmented areas due to deforestation, agriculture and livestock. *Mollinedia ruschii*, a recently described species, has a similar geographic distribution, occurring only in conservation units (Estação Biológica de Santa Lúcia and Reserva Biológica Augusto Ruschi). These data demonstrate the fundamental role of conservation units in the survival of species with restricted distribution (Lírio *et al.* 2021; 2023a).

The present study demonstrates the importance of integrating different research techniques to increase our knowledge of biodiversity, and also highlights the importance of field and herbarium work for the advancement of taxonomic understanding, reaffirming the fundamental role of conservation units in the protection of species that are endemic to small areas, ensuring their survival, especially in light of the unbridled environmental degradation that increases every year, transforming the conservation of these organisms into a race against time.

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