

Clarifying the dubious identity of *Vanilla dubia* Hoehne (Orchidaceae) and its relatives

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Abstract

Background and aims – Brazil has more than 40 species of *Vanilla*. Some taxa have been considered to be obscure since they are known only from the type specimen. This is the case of some south-eastern Brazilian species such as *V. dubia* and *V. dungsii*. While studying the diversity of Brazilian *Vanilla*, plants of an unidentified species were found in Ubatuba, São Paulo, Brazil. After flowering, it was confirmed that the species corresponded to the obscure *V. dubia*.

Material and methods – A redescription of *V. dubia* is provided and a morphological comparison with the related taxa *V. dungsii* and *V. paulista* is presented. An illustration of *V. dubia* is provided, and its geographic distribution is presented. The identity of the enigmatic *V. dungsii* is also clarified based on holotype examination and on images of living specimens.

Key results – *Vanilla dubia* is recognized by its coriaceous leaves with prominent vascular bundles in transverse section, by its white 3-lobed labellum with yellow lobes and undulate-crenulate margins, and by its tuberculate appendages on the apical portion of the labellar crest. Morphological characteristics suggest a close relationship of *V. dubia* with *V. dungsii* and *V. paulista*. *Vanilla dubia* occurs in the Dense Ombrophilous Forest, while *V. paulista* occurs in the Semi-deciduous Mesophytic Forest, and *V. dungsii* is endemic to the Dense Montane Ombrophilous Forest. Based on current data, *V. dubia* is assessed as Endangered.

Conclusion – Evaluation of vegetative and reproductive characters from living and herbaria specimens is crucial to understanding the identity and species boundaries of *Vanilla*. The data presented here show that the species occurring in the Atlantic Forest, *V. dubia* and *V. dungsii*, are more closely related to each other than to *V. paulista*, which occurs in Semi-deciduous Mesophytic Forests. A proposal to change the status of *V. dungsii* as a subspecies of *V. dubia* can be further considered.

Keywords

Atlantic Forest, Neotropics, systematics, taxonomy, Vanilleae, Vanilloideae

INTRODUCTION

Vanilla Mill. encompasses more than 100 species distributed throughout tropical and subtropical regions of the Americas, Asia, and Africa (Cameron 2003; Pansarin and Menezes 2023). The genus is monophyletic with three main lineages, two of which are distributed throughout the Neotropics (Bouetard et al. 2010; Pansarin and Menezes 2023; Pansarin 2024). Among *Vanilla*, a Neotropical clade with thin and membranaceous leaves

emerges as sister to a clade that includes two subclades, a predominantly African/Asian/Caribbean clade and a second, strictly Neotropical one. In the latter, epiphytic *Vanilla* (i.e. *V. palmarum* (Salzm. ex Lindl.) Lindl. and *V. bicolor* Lindl.) emerge as sisters to a large clade including mainly nomadic vines (Pansarin and Ferreira 2022; Pansarin and Menezes 2023; Pansarin 2024). With more than 40 species, Brazil is the centre of diversity for *Vanilla*, with a number of endemic and obscure species (Pansarin and Miranda 2016; Pansarin and Menezes 2023; Pansarin

2024). This is the case for *V. dubia*, described by Hoehne (1944) on the basis on a single specimen collected in Brazil by Carl August Wilhelm Schwacke (*Schwacke 11107*) and deposited in the Damasio Herbarium. Although C.A.W. Schwacke did not specify the location, Hoehne (1944) suspected that the specimen had been collected in the state of Minas Gerais, presumably based on the fact that the Damasio Herbarium was located in the municipality of Ouro Preto, before being transferred to the Herbarium of the Rio de Janeiro Botanic Garden. In addition to the obscure location of the type specimen, Hoehne (1944) suggested that the *Schwacke 11107* material may be a mixture of plant parts from different *Vanilla* species, in a similar way as he proposed for *Vanilla schwackeana* Hoehne, which he described based on a single specimen collected by C.A.W. Schwacke (Hoehne 1944). Recently this species was recollected in semi-deciduous forests of the Rio Doce Valley, in the State of Minas Gerais (Pansarin 2024). Although the origin of the holotype of *V. dubia* is unknown, based on the examination of herbarium specimens, Fraga et al. (2017) have suggested that this obscure taxon is distributed in Atlantic Forest areas of south-eastern Brazil (i.e. Minas Gerais, Espírito Santo, and São Paulo). Furthermore, although *V. dubia* has been considered distinct from its related congeners *V. paulista* Fraga & Pansarin and *V. dungsii* Pabst, the circumscription of these species has been debated since studies have been carried out exclusively on herbarium specimens (e.g. Fraga et al. 2017).

In the course of studies on Neotropical *Vanilla*, we found populations of a *Vanilla* species whose characteristics strongly agree with those of *V. dubia*. Based on the examination of the protologues of the Brazilian species, in addition to a study of herbarium specimens, the plants were found to correspond to the obscure *V. dubia*. A redescription of this rare taxon is presented, and a morphological comparison with morphologically related taxa (*V. dungsii* and *V. paulista*) is provided. A detailed illustration of *V. dubia* based on living specimens is presented, and the geographic distribution of this rare taxon is provided based on our recent findings. The identity of the enigmatic *V. dungsii* is also clarified based on holotype examination and on images of living specimens.

MATERIAL AND METHODS

Fresh and herbarium material of flowering and fruiting plants from *V. dubia* and its relatives was used for the study. The living material was collected from Brazilian populations and maintained in the *Vanilla* germplasm bank of the LBMBP Orchid House (Orchidarium of the Laboratory of Molecular Biology and Systematics of Plants), University of São Paulo (FFCLRP-USP), Brazil, available at <https://www.lbmbplab.net/vanillacollection>. Flowering and fruiting specimens were vouchered according to usual techniques (Fidalgo and Bononi 1989)

and then deposited in the LBMBP herbarium. In addition, BHC, BGE, CEN, DIAM, HDJF, LBMBP, MBM, NY, R, RB, SJRP, SP, SPFR, SPF, UB, UEC, and V herbaria (acronyms according to Thiers 2023) were also examined in order to study the Brazilian diversity of *Vanilla*.

Plant structures were measured using a vernier calliper. The vegetative structures, inflorescences, and flowers were photographed with a Nikon D-SLR D800 camera and a Micro Nikkor 105 mm f2.8 lens. Floral details were analyzed with a Stereozoom Leica S8 APO stereomicroscope with an integrated photo output. Digitized images were used for diagramming a template over a black background according to the model presented by Hoehne (1945), using Microsoft PowerPoint. The terminology for describing shapes followed Radford et al. (1974). Features specific to Orchidaceae were based on Dressler (1993) and Pridgeon et al. (1999). The infrageneric classification of Neotropical *Vanilla* groups followed Soto Arenas and Cribb (2010). The original description and digital images from types of related *Vanilla* species, *V. paulista* and *V. dungsii*, were consulted.

Comparative leaf anatomy between *V. dubia* and *V. paulista* was carried out using fresh material. Adult leaves from the third apical node were collected from individuals maintained under cultivation at the LBMBP Orchid House. Plants were collected in the field and kept under the same cultivation conditions for at least three years. The median portion of the leaves were manually sectioned and examined with a light microscope. Images of transversal cuts were captured with a Leica DM500 light microscope using a Leica ICC50 video camera attached to a PC and IM50 image analysis software.

The distribution map of *V. dubia* and morphologically related species was based on both herbarium material and living specimens from the *Vanilla* germplasm bank (LBMBP Orchid House). The map with the geographic distribution of *V. dubia* was produced with QGIS v.3.32 (QGIS Development Team 2024). To evaluate the conservation status of *V. dubia*, the coordinates of the points were inserted in the online platform Geospatial Conservation Assessment Tool (GeoCAT - <https://geocat.iucnredlist.org>). The geographical parameters of the Area of Occupancy (AOO), estimated using a 2 × 2 km grid, and of the Extent of Occurrence (EOO) were calculated using GeoCAT (Bachman et al. 2011). The conservation status of *V. dubia* was defined according to the IUCN Red List categories, criteria, and guidelines (IUCN Standards and Petitions Committee 2022).

RESULTS

Vanilla dubia Hoehne (Hoehne 1944: 126)

Figs 1–4, Table 1

Type. BRAZIL • s.loc.; s.d.; *Schwacke 11107*; holotype: RB [RB00542722, accession n° 37014].

Description. Nomadic vines, long scandent. Roots axillary, 1.6–2.1 mm diam., whitish to greenish brown, one per node. Stem climbing, sinuous, cylindrical, fleshy, slightly sulcate, glabrous dark green; internodes 55–130 × 3–6 mm. Leaves 5.5–22 × 2–4.8 cm, alternate, distichous, lanceolate to oblanceolate, rarely elliptic, asymmetric, coriaceous, glabrous, dark green, with prominent and parallelly arranged vascular bundles in transverse section, margin entire, apex acuminate, pseudopetiolate; pseudopetiole sulcate, up to 1.8 cm long. Inflorescence 3.7–7 cm long, axillary, racemose, with up to 20 flowers opening in succession; 1 flower opening each morning; bracts 3–5.2 mm long progressively smaller toward the apex, alternate, triangular to ovate, coriaceous, concave, patent, apex acute. Flowers resupinate, pedicellate, with an abscission layer between perianth and ovary; pedicel with ovary 3.5–3.9 × 0.3–0.35 cm, straight to incurved, dilated at the apex, subcylindrical in transverse section, white along the pedicel and green along the ovary. Sepals 6–6.2 × 1.1–1.3 cm, free, oblanceolate, fleshy, slightly concave, internally cream-coloured, externally greenish, apex obtuse, margin entire, reflexed at apex; dorsal sepal symmetric; lateral sepals asymmetric. Petals 5.8–6.1 × 0.8–1.2 cm, free, lanceolate to slightly spatulate, asymmetric, membranous, apex acute, slightly reflexed, internally cream-coloured, externally greenish, adaxial surface with a central and longitudinally arranged keel. Labellum 3-lobed, 5.8–6.2 × 3–3.2 cm, unguiculate, tubular, fused to the column along the margins from the base, up to 3.6 cm long, white, yellowish in the distal portion, inner surface with longitudinal brownish stripes, with a central crest near the apex, and with a penicillate callus just below the anther; central crest ca 5 mm wide, with large yellow and tuberculate protrusions near the apex, on the midlobe; penicillate callus 4–4.5 × 3.5–3.8 mm, white; labellum margins fused from the base to ca 3/4 of the column length forming a tubular nectar chamber; nectar chamber 2.2–2.4 cm long; lateral lobes obliquely rounded, overlapping above the column, margins undulate to crenulate; apical lobe emarginated, reflexed, margin undulate to slightly crenulate. Column 4.5–4.7 × 0.3–0.35 cm, semi-cylindrical, slender, base attenuate, dilated to the apex, with white-hyaline hairs close to the stigma, apex with two lateral wings; anther 4.7–5 × 3.7–4.2 mm, rhomboid, white, versatile; rostellum 4.5–4.7 × 3.4–3.6 mm, trapezoidal, membranous, white. Fruits 5–7 × 1.2–1.5 cm, linear, incurved, subcylindrical in transverse section, fleshy, indehiscent, green when immature, black when mature. Seeds ovate, black.

Distribution and ecology. *Vanilla dubia* occurs in primary forests of the Atlantic Rainforest in south-eastern Brazil (Fig. 2). It shows a nomadic vine habit, rooting on the forest litterfall and climbing on trees. The elevation is from 30 to 810 m a.s.l. Flowers open in morning hours. Each flower lasts ca 12 hours. The fruits are indehiscent, black, and ripen in nine months from pollination.

Phenology. *Vanilla dubia* blooms from October to December. The fruits ripen between May and June.

Preliminary IUCN conservation assessment.

Endangered: EN B2ab(i,ii,iii). *Vanilla dubia* is an uncommon and rarely collected species currently known to grow in the Atlantic Forest areas of south-eastern Brazil. The populations found are composed of few sparse specimens. The extent of occurrence (EOO) is estimated to be 7,953.845 km², which falls within the limits for Vulnerable (VU) under subcriterion B1, according to the IUCN red list categories and criteria and guidelines. The area of occupancy (AOO) is estimated to be 24 km² (cell width 2 km), which falls within the limits for Endangered (EN) under criterion B2 (area of occupancy). Considering that the Atlantic Forest areas have been reduced to scattered fragments due to extensive human occupation, we project a continuing decline in (i) extent of occurrence, (ii) area of occupancy, and (iii) extent and/or quality of habitat of *V. dubia*. Based on these threats and on the fact that the species is distributed in less than five locations, *V. dubia* is assessed as Endangered (EN B2ab(i,ii,iii)).

Additional material examined. BRAZIL – Espírito

Santo • Santa Leopoldina, Rio das Farinhas; 20°6'7"S, 40°36'58"W; 40 m; 16 Oct. 2003; A.P. Fontana 626; MBML • Moxafongo; 20°6'53"S, 40°31'54"W; 150 m; 19 Nov. 2005; A.P. Fontana, C. Esgario, A.M. de Assis, K.F.O. Faria & V. Pagund 1797; MBML • Santa Teresa, Topo do Canaã; 19°55'5"S, 40°35'58"W; 685 m; 4 Apr. 2004; R.R. Vervloet *et al.* 1841; MBML • Alto Santo Antônio; 19°53'14"S, 40°34'32"W; 25 Feb. 2005; A.P. Fontana; *et al.* 1131; MBML • Estrada da Pedra da Onça; 19°53'29"S, 40°49'8"W; 10 Sep. 1998; L. Kollmann, E. Bausen & W. Pizziolo 519; MBML. – **São Paulo** • Ubatuba, Praia Dura; 23°29'35"S, 45°10'30"W; 30 m; E.R. Pansarin & M.R. Miranda 1574; LBMBP.

Notes. Hoehne (1944) assumed that the holotype of *Vanilla dubia* was collected in Minas Gerais based on the fact that the Damasio Herbarium was located in Ouro Preto. However, to date, there is no evidence of the occurrence of this species in Minas Gerais. If the holotype of *V. dubia* (Schwacke 11107) was collected in Minas Gerais, it was most likely from the east of the State in areas of Atlantic Forest *sensu stricto*, on the borders with Espírito Santo State, where this species has been most commonly found. Hoehne (1944) also suggested that the holotype of *V. dubia* can be a mixture of elements from distinct *Vanilla* species. However, according to our data based on live plants plus herbarium material, there is no mixture of elements in the specimen Schwacke 11107. Considering that the description by Hoehne (1944) was based on a single collection, we conclude here that the Schwacke 11107 (RB) specimen is the holotype of *V. dubia*.

Morphological affinities. *Vanilla dubia* is easily recognized by its coriaceous and dark-green leaves with prominent and parallelly-arranged vascular bundles in transverse section (Fig. 3), by its lateral inflorescence with small flowers whose sepals and petals are internally cream-coloured and externally greenish, by its white 3-lobed labellum with yellow lobes and undulate-



Figure 1. *Vanilla dubia* Hoehne. **A.** Inflorescence. **B.** Leaf. Note the asymmetrical shape of the leaf blade and the acuminate apex. **C.** Flower in front view. **D.** Flower in lateral view. **E.** Flower in diagonal view. **F.** Dissected perianth. **G.** Detail of the adaxial surface of a petal showing the longitudinal keel. **H.** Detail of the apex of the labellum showing the brown longitudinal lines and the central crest with yellow projections. **I.** Detail of the labellum showing the penicillate callus and the central labellar crest. **J.** Labellum in dorsal view. **K.** Labellum in lateral view. **L.** Longitudinal section of the labellum. Note the penicillate callus (arrow), the anther (a), and the rostellum (arrowhead). **M.** Column in lateral view. **N.** Apex of the column: in abaxial view with an articulated and disarticulated anther (above), in lateral view with an articulated anther (mid), in lateral view with a disarticulated anther (below). **O.** Anther in dorsal view (left) and ventral view (right). **P.** Immature fruit. Based on E.R. Pansarin & M.R. Miranda 1574 (LBMBP). Photographs by Emerson Pansarin.

Table 1. Comparison of the morphological features of *Vanilla dubia* and related species. *Vanilla paulista* data were obtained from Fraga et al. (2017). The characteristics of *V. dungsii* were obtained from Pabst (1975).

Characters	<i>V. dubia</i>	<i>V. dungsii</i>	<i>V. paulista</i>
Habitat	Dense Ombrophilous Forest	Dense Montane Ombrophilous Forest	Semi-deciduous Mesophytic Forest
Internode length (cm)	5.5–13	16–18	4.2–10
Leaf texture	coriaceous	coriaceous	fleshy
Leaf size (cm)	5.5–22 × 2–4.8	20–21 × 4–5	9.2–16.2 × 2.5–3.2
Inflorescence length (cm)	2.7–7	ca 5	4–5.2
Bract length (mm)	3–5.2	1.3–2.6	3.5–5
Sepals (cm)	6–6.2 × 1.1–1.3	5–6.5 × 0.8–1	5.7–7.8 × 0.7–1.4
Petals (cm)	5.8–6.1 × 0.8–1.2	5.5 × 1–1.1	6.5–7.1 × 0.8–1.3
Labellum (cm)	5.8–6.2 × 3–3.2	5 × 2.5	5.7–6.9 × 3.3–3.5
Longitudinal labellar bands	brown	brown	yellow
Arrangement of labellar protrusions	3–5 rows	3–5 rows	1–3 rows
Shape of labellar protrusions	tuberculate	tuberculate	papillous
Colour of labellar protrusions	yellow	brownish	white
Column length (cm)	4.5–4.7	ca 3.5	4.4–4.8

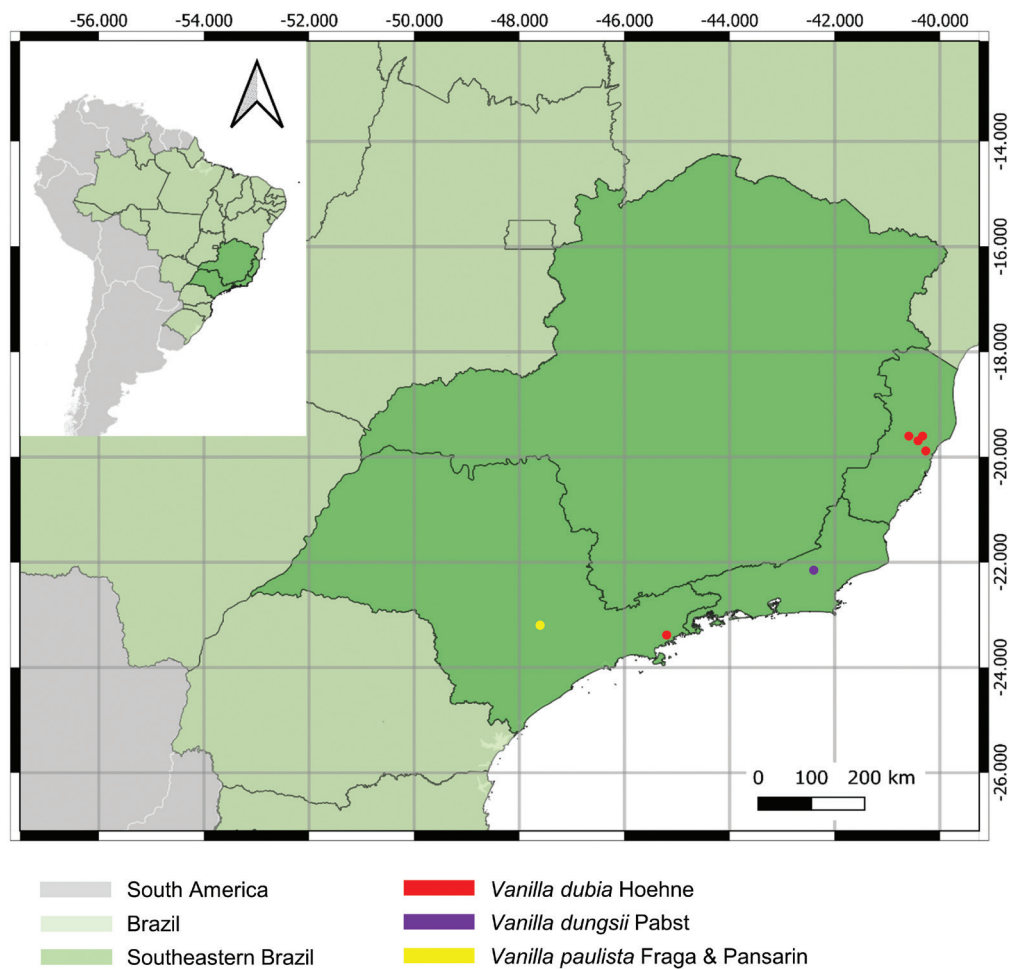


Figure 2. Map with records of occurrence of *Vanilla dubia* Hoehne and related species, *Vanilla dungsii* Pabst and *Vanilla paulista* Fraga & Pansarin. Records of occurrence of *V. paulista* and *V. dungsii* are based on the locality of type specimens. Map created with QGIS v.32.2 (QGIS Development Team 2024).

crenulate margins, and by its tuberculate appendages on the apical portion of the labellar crest (Fig. 4). These characteristics suggest a close relationship of *V. dubia* with *V. dungsii* and *V. paulista*, as all three species have a long-unguiculate labellum with a ventral penicillate callus and labellar protrusions on the midlobe. *Vanilla dubia* is more related to *V. dungsii* than to *V. paulista*. In fact, *V. dubia* and *V. dungsii* occur in Atlantic Forest areas, while *V. paulista* occurs in drier environments (i.e. semi-deciduous forests). Although *V. dubia* and *V. dungsii* occur in the Atlantic Forest, the habitat of *V. dungsii* differs from that of *V. dubia*. *Vanilla dungsii* occurs in high altitude areas of Rio de Janeiro, above 1000 m a.s.l., in the Dense Montane Ombrophilous Forest (Fig. 2). With regards to the vegetative characteristics, both *V. dungsii*

and *V. dubia* have coriaceous and asymmetric leaves with prominent veins, while *V. paulista* show fleshy and more commonly symmetric leaf blades (Fig. 3). Although leaf size and thickness have been intriguing factors regarding the identity of *V. dungsii*, our data show that these characteristics overlap with those of *V. dubia*. The floral characteristics of *V. dubia* also suggest close affinity with *V. dungsii*. Both taxa have brown longitudinal bands over a white labellum and tuberculate appendages on the apex of the labellum. However, the labellar appendages of *V. dungsii* are much larger and fewer in number than in *V. dubia*. In *V. paulista*, the longitudinal bands are yellow and the labellar protrusions are white papillae arranged in 1–3 rows (Fig. 4).

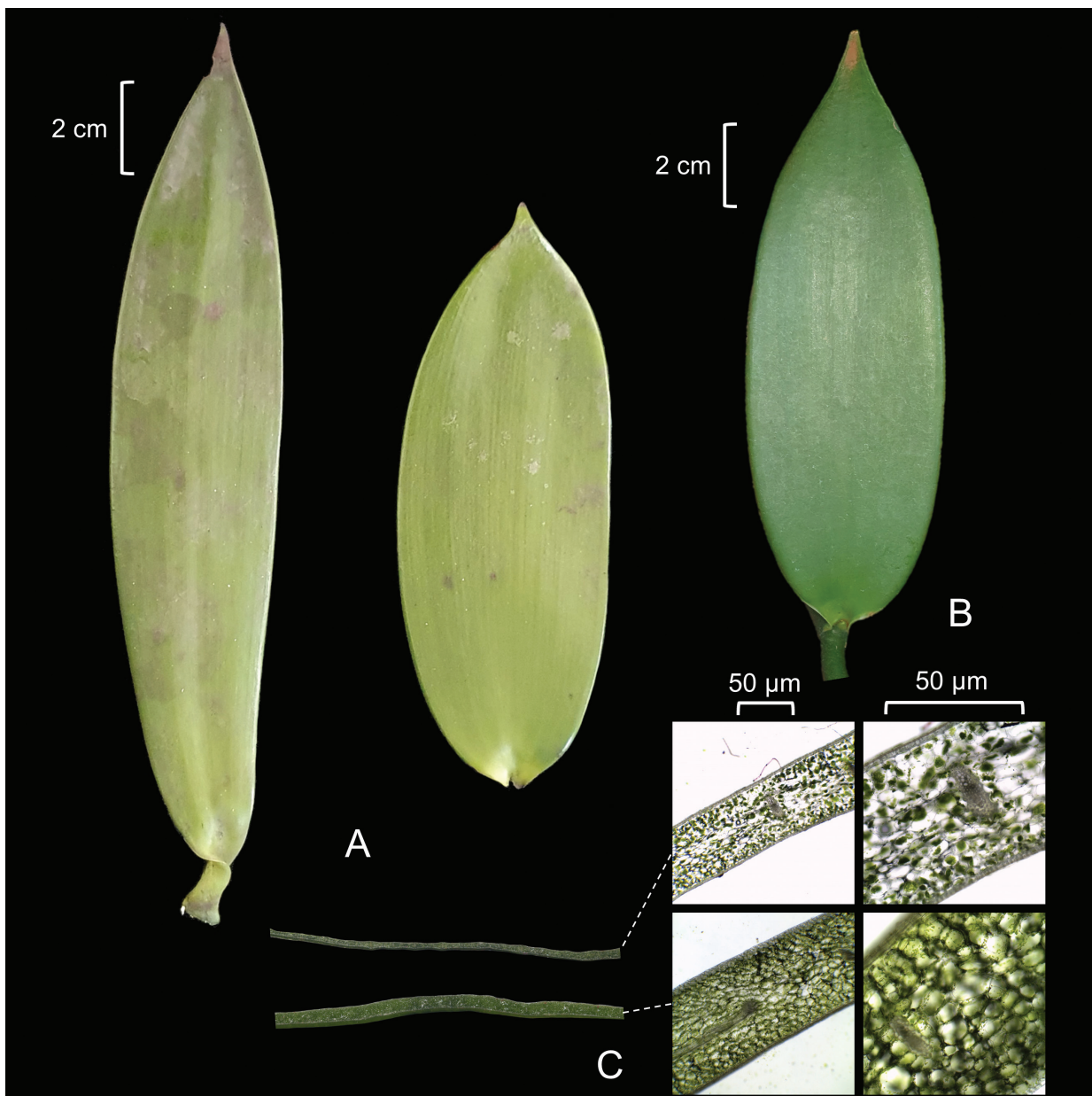


Figure 3. A. Variation in the shape of leaf blades of *Vanilla dubia* Hoehne, based on E.R. Pansarin & M.R. Miranda 1574 (LBMBP). B. Leaf of *V. paulista* Fraga & Pansarin, based on E.R. Pansarin 727 (UEC). C. Comparative anatomy of the mid portion of leaf blades of *V. dubia* (above) and *V. paulista* (below). Photographs by Emerson Pansarin.

DISCUSSION

Several studies have pointed out the need to clarify the circumscription of obscure species of *Vanilla* (e.g. Pansarin 2024). Some Brazilian species were described solely based on the holotype. This was the case for *V. schwackeana*, *V. dungsii*, and *V. dubia*. Although the identity of *V. schwackeana* has been clarified in a previous study (Pansarin 2024), more efforts are needed in order to clarify the identity of other obscure *Vanilla* names. Here, the identity of *V. dubia* is clarified and its morphological affinities with the related species *V. dungsii* and *V. paulista* is presented. With a history closely similar to that of *V. schwackeana*, *V. dubia* has been regarded as an obscure name since its description about 80 years ago since this taxon was described on the basis of a single specimen whose origin is unknown (Hoehne 1944, 1945; Pansarin 2024). Here, the conclusions about the circumscription of this rare species are based on extensive fieldwork, on data about geographical distribution, and on morphological features of both living and dried specimens. According to the data presented here, there is no mixture of elements in the specimen collected by C.A.W. Schwacke (*Schwacke 11107*), as previously proposed by Hoehne (1944). All plant elements present in the holotype of *V. dubia* (*Schwacke 11107*) strongly agree with the specimens collected in the Atlantic Forest of Ubatuba, south-eastern Brazil.

A close relationship between *Vanilla dubia* and *V. paulista* has been proposed (Fraga 2002; Fraga et al. 2017). However, according to the data presented here, *V. dubia* is more related to *V. dungsii* than to *V. paulista*. The leaf blades of *V. paulista* are fleshy and symmetric, the longitudinal bands of the labellum are yellow and the labellar protrusions are white papillae. In addition, *V. paulista* is reported for the interior of state of São

Paulo, Brazil, growing in semi-deciduous mesophytic forests (Pansarin and Pansarin 2010; Fraga et al. 2017). Conversely, *V. dungsii* and *V. dubia* have coriaceous and asymmetric leaves with prominent and parallelly-arranged vascular bundles, a white and 3-lobed labellum with brown longitudinal bands, and tuberculate appendages on the apical portion of the labellar crest. Regarding distribution, both *V. dubia* and *V. dungsii* occur in the Atlantic Forest. Although morphological analyses indicate a similarity between the two species, the labellar appendages of *V. dungsii* are much larger and fewer in number than in *V. dubia*. In addition, *V. dubia* is distributed along the Atlantic Forest sensu stricto, while the distribution of *V. dungsii* is restricted to the Dense Montane Ombrophilous Forest (above 1000 m a.s.l.) of the Rio de Janeiro (Pabst 1975; Fraga 2002). A proposal to change the status of *V. dungsii* as a subspecies of *V. dubia* can be further considered after detailed studies on the reproductive isolation of their populations.

Although *V. dubia* is supposed to occur in Minas Gerais (Hoehne 1944; Fraga 2002), its presence in this Brazilian state has not been confirmed. If *V. dubia* indeed occurs in Minas Gerais, the species is probably distributed in the eastern portion of the State containing remnants of Atlantic Forest on the border with Espírito Santo, where this species has been most commonly found. *Vanilla schwackeana* was rediscovered in areas east of Minas Gerais, in the Rio Doce Valley (Pansarin 2024). Thus, the hypothesis that Schwacke collected *V. dubia* in this Brazilian state cannot be ruled out. In addition, it seems plausible that *V. dubia* had a wider distribution in the past since the east of Minas Gerais has a long history of vegetation destruction due of human occupation and iron ore exploitation (Consórcio ECOPLAN - LUME 2010). Despite habitat fragmentation, several species endemic to Brazil have been described or recollected (Pansarin



Figure 4. Comparative morphology of the flowers of *Vanilla dubia* Hoehne and related species. **A.** *Vanilla dubia* Hoehne (Ubatuba, São Paulo). **B.** *Vanilla dungsii* Pabst (Nova Friburgo, Rio de Janeiro). **C.** *Vanilla paulista* Fraga & Pansarin (Jundiá, São Paulo). Details bellow the flowers (oval frames) show the types of labellar protrusions among the three *Vanilla* species. **A.** Based on E.R. Pansarin & M.R. Miranda 1574 (LBMBP). **B.** Based on a living specimen collected in Nova Friburgo, Rio de Janeiro. **C.** Based on E.R. Pansarin 727 (UEC). Photographs by Emerson Pansarin (A and C), and Jorge Gastin (B).

and Miranda 2016; Pansarin and Menezes 2023; Pansarin 2024). Understanding the diversity of Brazilian *Vanilla* is crucial to knowledge on the evolution and the systematics of this important orchid genus.

CONCLUSION

Comparison of vegetative and reproductive characteristics between living specimens and those deposited in herbaria was crucial to understanding the identity and species boundaries of *V. dubia* and its relatives. The data presented here show that the species occurring in the Atlantic Forest, *V. dubia* and *V. dungsii*, are more closely related to each other than to *V. paulista*, which occurs in semi-deciduous mesophytic forests. A proposal to change the status of *V. dungsii* as a subspecies of *V. dubia* can be further considered after detailed studies on the reproductive isolation of their populations.

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