

Rediscovery and revalidation of the Brazilian endemic *Vanilla schwackeana* Hoehne (Orchidaceae): its distribution and phylogenetic position

Emerson R. Pansarin¹

¹ University of São Paulo, FFCLRP, Department of Biology, Ribeirão Preto, SP, Brazil

Corresponding author: Emerson R. Pansarin (epansarin@ffclrp.usp.br)

Academic editor: Brecht Verstraete ♦ Received 3 August 2023 ♦ Accepted 27 November 2023 ♦ Published 24 January 2024

Abstract

Background and aims – With 40 species, Brazil is the centre of diversity for *Vanilla*. Frederico Carlos Hoehne described eight *Vanilla* species for the Brazilian flora, including *Vanilla schwackeana*, an obscure taxon currently assumed to be a synonym of *V. planifolia*. While studying Neotropical *Vanilla*, plants were found in gallery forests of the Vale do Rio Doce region, Minas Gerais, south-eastern Brazil. Based on the examination of the protologues of the Brazilian species, in addition to the study of herbarium specimens, it was verified that the plants correspond to the rare *V. schwackeana*.

Material and methods – A redescription of *V. schwackeana* is provided, and a morphological comparison with other Neotropical taxa is presented. An illustration of *V. schwackeana* based on living specimens is provided, and its geographic distribution is presented based on recent findings. The position of *V. schwackeana* within *Vanilla* is discussed based on both morphological data and a molecular phylogeny.

Key results – *Vanilla schwackeana* is distinguishable by its pseudopetiolate leaves, by its largely elliptic to obelliptic leaf blades with attenuate base and acuminate apex, by its apical racemes with small flowers, by its yellow labellum with white undulate-crenulate margins, and by its papillose appendages on the apical portion of the labellar crest. Both morphological and molecular data suggest a close relationship of *V. schwackeana* with *V. appendiculata*, *V. hartii*, and the Brazilian endemic *V. rupicola*. Based on current data, *V. schwackeana* is assessed as Critically Endangered.

Conclusion – Integrative taxonomy was crucial to understand the identity and species boundaries of *V. schwackeana*. The *V. appendiculata/V. schwackeana/(V. hartii/V. rupicola)* clade comprises an unrecognized infrageneric *Vanilla* group. It seems plausible that the formation of a new infrageneric group will be necessary. As the habitat of *V. schwackeana* has been devastated by iron ore extraction, conservation strategies for this Brazilian endemic species will be necessary.

Keywords

Atlantic Forest, Cerrado, phylogeny, systematics, taxonomy, Vanilleae, Vanilloideae

INTRODUCTION

Frederico Carlos Hoehne (1882–1959), a Brazilian botanist who devoted his life to the study of the Brazilian flora, was the first director of the Botanical Garden and Botanical Institute of São Paulo. During his fieldworks in Brazil, Hoehne collected more than 10,000 plant specimens, leading to the description of at least 200 species new to science. He wrote more than 200 scientific papers, mostly

regarding the plants he collected on his field trips (Franco and Drummond 2005). Frederico C. Hoehne had a special interest in orchids. In addition to the volumes dedicated to orchids in his important work *Flora Brasílica* (Hoehne 1945), he published the impressive book *Iconografia de Orchidaceas do Brasil* (Hoehne 1949). Among the Brazilian orchids, Hoehne studied many genera, with special attention to the groups currently included among the Vanilloideae, such as *Cleistes* Rich. ex Lindl. and

Vanilla Plum. ex Mill. Hoehne (1945) provided detailed descriptions and illustrations of 38 species of *Vanilla* in Flora Brasílica, eight of them described by him, including the enigmatic *V. schwackeana* Hoehne.

The description of *V. schwackeana* was based on a single specimen collected in Minas Gerais, south-eastern Brazil, by Carl August Wilhelm Schwacke (*Schwacke 11106*, RB), who, however, did not specify the locality. Hoehne (1944) suspected that the specimen might have been a mixture of plant parts from different *Vanilla* species. Soto Arenas and Cribb (2010) also recognized *V. schwackeana* as a distinct taxon, but agreed with Hoehne's observation that the specimen could be a mixture of plant elements. Currently, based on examination of an image of the type specimen, *V. schwackeana* has been synonymized under *V. planifolia* Andrews (Karremans et al. 2020). In addition, based on Hoehne's premise that the type of *V. schwackeana* could be a mixture of plant elements from distinct *Vanilla* species, a dissected flower of the holotype (*Schwacke 11106*, RB) was designated as a lectotype (Karremans et al. 2020).

With more than 100 species distributed throughout tropical regions of Americas, Africa, and Asia, *Vanilla* is the most species-rich genus among the Vanilloideae (Cameron 2003; Pansarin and Menezes 2023). *Vanilla* is monophyletic, with three main lineages, two of which occur throughout the Neotropics (Bouetard et al. 2010; Pansarin and Ferreira 2022; Pansarin and Menezes 2023). 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 sister to a large clade including mainly nomadic vines (Pansarin and Ferreira 2022; Pansarin and Menezes 2023). With more than 40 species, Brazil is the centre of diversity for *Vanilla*, with a number of endemisms (Hoehne 1945; Pansarin 2010; Pansarin and Miranda 2016; Pansarin and Menezes 2023).

In the course of studies on Neotropical *Vanilla*, an unidentified species was found in gallery forests of the Vale do Rio Doce region, Minas Gerais, south-eastern Brazil. Based on the examination of the protologues of the Brazilian species, in addition to a study of herbarium specimens, it was verified that the plants correspond to the rare *V. schwackeana*. A redescription of this *Vanilla* is presented, and a morphological comparison with other Neotropical taxa is provided. An illustration of *V. schwackeana* based on living specimens is presented, and the geographic distribution of this rare taxon is provided based on recent findings. The position of *V. schwackeana* within *Vanilla* is discussed based on both morphological data and a molecular phylogeny for *Vanilla*.

MATERIAL AND METHODS

Study area

The fieldwork was performed along the Vale do Rio Doce, in the state of Minas Gerais, south-eastern Brazil. The climate of the region is classified as "Aw", namely semi-humid warm tropical (Köppen 1948). Rainfall is irregularly distributed, with a rainy season from November to April (800–1300 mm) and a dry season from May to October (150–250 mm). The Vale do Rio Doce is located within the limits of the Atlantic Forest and Cerrado. Over the last centuries, the natural vegetation of the region has been reduced due to land degradation caused by anthropogenic disturbance as a consequence of intensive iron ore exploitation (Consórcio ECOPLAN - LUME 2010).

Taxonomic treatment

Fresh and herbarium material of flowering and fruiting plants was used for the study. Photographs were based on specimens collected in the field. Measurements were made directly on the floral structures using a Vernier caliper. The vegetative structures, inflorescence, and flowers were photographed with a Nikon D-SLR D800 camera and a Micro Nikkor 105 mm f2.8 lens. Floral details were analysed with a Stereozoom Leica S8 APO stereomicroscope with an integrated photo output. Digitized images were used for diagramming a template over a black background, following 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* followed Soto Arenas and Cribb (2010). The original descriptions and digital images from types of related species of *Vanilla* were consulted. Plant specimens were vouchered according to the usual techniques (Fidalgo and Bononi 1989) and then deposited in the LBMBP and SPFR herbaria. In addition, the BHCb, DIAM, HDJF, LBMBP, R, RB, SPFR, SPF, and UEC herbaria (acronyms according to Thiers 2023) were also examined in order to study the Brazilian diversity of *Vanilla*. Living plants were collected in the field 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>.

The map with the geographic distribution of *V. schwackeana* was produced with QGIS v.3.32.2 (QGIS Development Team 2023). The occurrence map is based on the new records of *V. schwackeana* since the locality of the type (*Schwacke 11106*) is unknown (Hoehne 1944).

The preliminary conservation status of *V. schwackeana* was defined according to the IUCN Red List categories

and criteria, and guidelines (IUCN Standards and Petitions Committee 2022). The geographical parameters of Area of Occupancy (AOO) estimated using a 2×2 km grid and Extent of Occurrence (EOO) were calculated using GeoCAT (Bachman et al. 2011).

Phylogenetic relationships

Taxon sampling for phylogenetic analysis

A total of 57 *Vanilla* accessions (41 species) were analysed and are referred to here as the ingroup. *Lecanorchis multiflora* J.J.Sm. was selected as an outgroup according to previous phylogenetic studies on Vanilloideae (e.g. Pansarin and Ferreira 2022; Pansarin and Menezes 2023). A data matrix was built based on sequences available in the GenBank database and obtained during the course of this study. A list of ingroup and outgroup species, vouchers, and GenBank accession numbers is given in Supplementary material 1.

DNA extraction, amplification, and sequencing

DNA of *Vanilla schwackeana* was extracted from fresh material according to a modified CTAB method (Doyle and Doyle 1987). The amplifications were carried out using 50 µL PCR volumes. Relaxation of the DNA strands was achieved by the addition of a 5M betaine solution to the PCR preparations. Primers of the nuclear ribosomal transcribed spacer region (ITS), including the 5.8S gene, were used for amplification and sequencing (Sun et al. 1994). Taq DNA polymerase was added to the PCR mixture at 80°C following a 10 min period of denaturation at 99°C in the thermocycler. Thirty-five cycles were run according to the following program: denaturation, 1 min, 94°C; annealing, 45 s, 64°C; extension, 1 min, 72°C; final extension, 5 min, 72°C. Amplified PCR products were purified using GFX PCR columns (GE Health Care). Sequencing reactions were prepared using Big Dye™ Terminator v.3.1 (ABI), purified PCR products, and the same aforementioned primers. Samples were dehydrated and resuspended with loading dye. Sequences were obtained using an Applied Biosystems automated sequencer model 3100. Sequence Navigator and Autoassembler (Applied Biosystems) software was used for sequence editing and assembly of complementary and overlapping sequences. DNA sequences were aligned using BioEdit v.5.0.9 (Hall 1999).

Phylogenetic analyses

A data matrix of ITS containing 58 accessions was used for phylogenetic analyses. Maximum parsimony analysis (MP) was run with PAUP* v.4.0b5 (Swofford 2001). A heuristic search was conducted with 1000 replicates of random taxon entry additions, MULTREES option, and the tree bisection-reconnection (TBR) swapping algorithm, holding 10 trees per replicate and saving all the shortest trees. Support for clades was assessed using 1,000 bootstrap replicates (Felsenstein 1985). Bootstrap support

(BS) values above 50% were calculated and mapped above the branches of the consensus tree. For bootstrap support levels, we considered bootstrap percentages of 50–70% as weak, 71–85% as moderate, and > 85% as strong (Kress et al. 2002). Additionally, the ITS data matrix was analysed by Bayesian Inference (BI) using MrBayes v.3.1 (Ronquist and Huelsenbeck 2003). The optimal model of sequence evolution was selected using MEGA X (Kumar et al. 2018) based on the Bayesian Information Criterion (BIC). The software selected HKY+G as the best evolution model for the ITS region. Four Markov chains were run simultaneously for three million generations, with parameters sampled every 100 generations. The consensus tree was calculated after removal of the first 3,000 trees, which were considered to be the burn-in. Posterior probability (PP) values above 0.5 were calculated and mapped above the branches of the consensus tree.

RESULTS

Taxonomic treatment

Vanilla schwackeana Hoehne

Figs 1–2, Table 1

Type. BRAZIL – Minas Gerais • s.loc.; s.d.; *Schwacke 11106*; holotype: RB [RB00542725, accession n° 37015].

Description. Nomadic vines, up to 8 m in length. Roots axillary, 1.8–2.2 mm diam., whitish to brownish, one per node. Stem climbing, sinuous, cylindrical, glabrous, fleshy, and green; internodes 42–80 × 3–4 mm. Leaves 5.5–11 × 3–5.5 cm, alternate, distichous, elliptic to obelliptic, symmetric to asymmetric, slightly fleshy, glabrous, green, pseudopetiolate, margin entire, base attenuate, apex acuminate; pseudopetiole 5.5–8 mm. Inflorescence 3.5–6 cm long, apical or axillary, racemose, with up to 10 flowers opening in succession; 1–2 flowers opening each morning; bracts 4–8.5 × 3.5–7 mm, progressively smaller toward the apex, alternate, triangular to ovate, coriaceous, concave, patent. Flowers resupinate, pedicellate, abscission layer between perianth and ovary present; pedicel with ovary 3–3.5 × 0.35–0.42 cm, green, straight to incurved, dilated at the apex, subcylindrical in transverse section. Sepals 4.2–4.6 × 0.8–1.1 cm, free, oblanceolate, fleshy, slightly concave, pale green, margin entire, slightly incurved, apex acute to obtuse; dorsal sepal symmetric; lateral sepals asymmetric. Petals 4–4.4 × 0.7–0.9 cm, free, slightly spatulate, asymmetric, membranous, pale green, apex acute, adaxial surface with a central and longitudinally disposed keel. Labellum 1-lobed to slightly 3-lobed, 4.2–4.7 × 2.5–2.7 cm, yellowish, white in distal portion, inner surface with longitudinal yellow stripes, with a central crest near the apex, and with a penicillate callus just below the anther; central crest ca 4 mm wide, with yellow protrusions arranged in 3–5 longitudinal lines near the apex; penicillate callus 4.5–5 × 3.7–4.2 mm,



Figure 1. *Vanilla schwackeana*. **A.** Part of a plant showing the sinuous stem and distichous leaves. **B.** Leaf. Note the elliptical shape of the leaf blade. **C.** Detail of a leaf showing the acuminate apex. **D.** Apical inflorescence. **E.** Flower in diagonal view. **F.** Flower in lateral view. **G.** Longitudinal section of a flower. The detail (dashed area) shows the labellum base. **H.** Longitudinal section of part of the labellum. Note the penicillate callus (arrow), the anther (a), and the rostellum (arrowhead). **I.** Detail of the labellum and column. Note the central crest of the labellum, the penicillate callus, and the anther. **J.** Dissected perianth. The detail (dashed area) shows the penicillate callus and the central labellar crest. **K.** Labellum in lateral view. **L.** Column in lateral view. **M.** Apex of the column: in lateral view with an articulated anther (above), in lateral view with a disarticulated anther (mid), and in abaxial view (below). Based on E.R. Pansarin 1568 (LBMBP).

yellow; margins fused from the base to ca $\frac{3}{4}$ of the column length forming a tubular nectar chamber; nectar chamber 1–1.2 cm long; lateral lobes rounded, margins undulate to crenulate; apical lobe rounded to emarginated, reflexed, margin undulate to slightly crenulate. **Column** 3.2–3.5 \times 0.3–0.32 cm, semicylindrical, slender, sinuous, white, attenuate base, dilated to the apex, with white-hyaline hairs close to the stigma, apex ending in a membranous ochrea; anther 2.9–3 \times 2.1–2.5 mm, ovate, white, versatile; rostellum 3.8–4.1 \times 2.3–2.6 mm, trapezoidal, membranous, white. **Fruits** 11–15 \times 2.8–3.2 cm, linear, incurved, slightly triangular in transverse section, fleshy.

Distribution and ecology. *Vanilla schwackeana* occurs in both gallery forests and mesophytic semideciduous forests from the south of Vale do Rio Doce, eastern Minas Gerais, south-eastern Brazil (Fig. 2). *Vanilla schwackeana* shows a nomadic vine habit, rooting on the forest litterfall, and

climbing on tree trunks. The elevation is from 730 to 810 m a.s.l. Each flower lasts ca 12 hours.

Phenology. *Vanilla schwackeana* blooms from September to October.

Preliminary IUCN conservation assessment. Critically Endangered: CR B2ab(i,ii,iii). *Vanilla schwackeana* is a rare species growing in mesophytic forests in the municipalities of Timóteo and Antônio Dias, southern region of Vale do Rio Doce. The populations only contain a few specimens. The species is known from only three herbarium collections, the holotype without any locality information and two more recent specimens. Since only two specimens have locality data, the EOO (extent of occurrence) cannot be calculated. The area of occupancy (AOO) is estimated to be 8 km² (cell width 2 km), which falls within the limits for Critically Endangered (CR) under criterion B2 (area of occupancy). The two recent

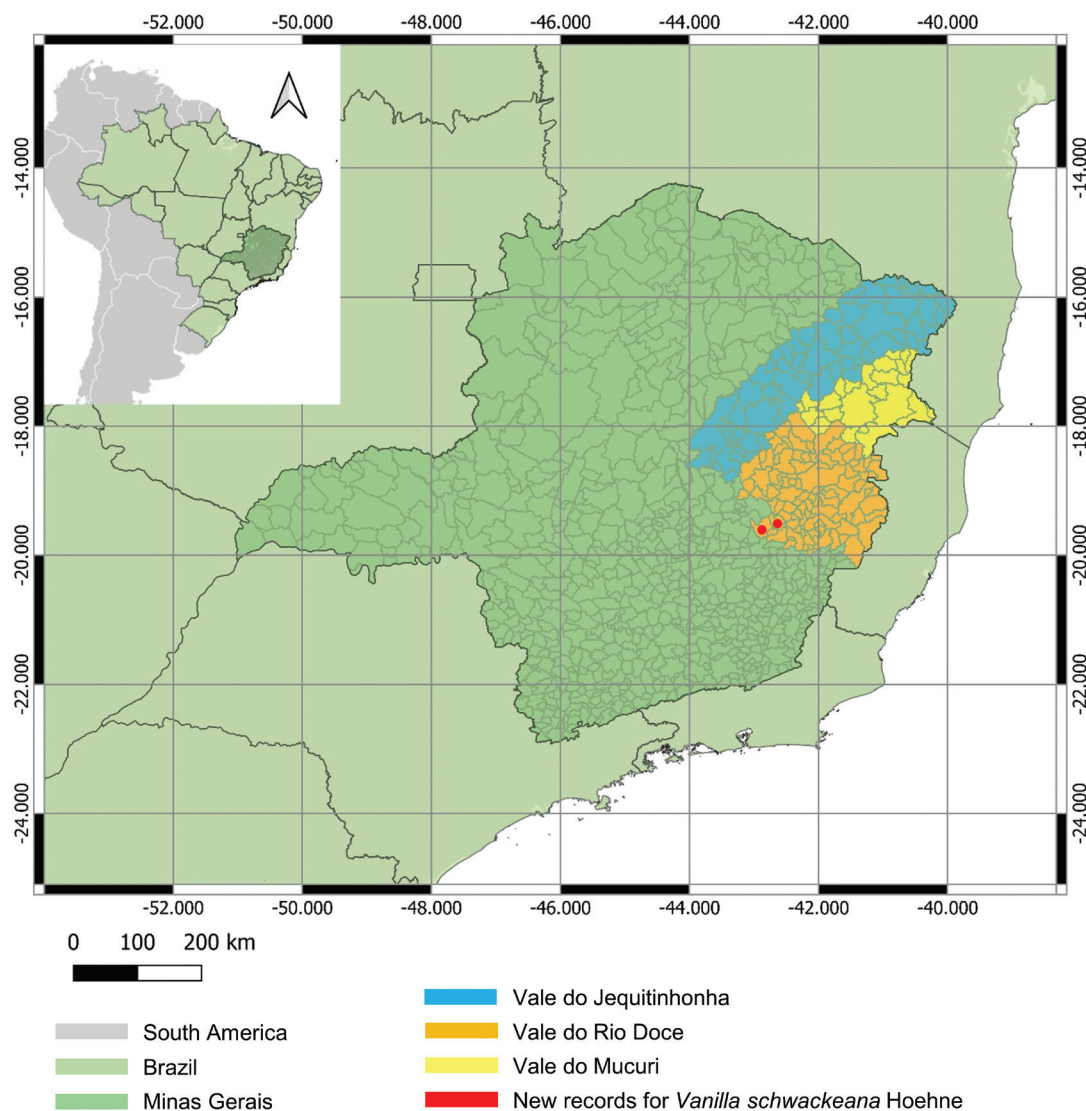


Figure 2. Occurrence map of *Vanilla schwackeana* based on new records from the Vale do Rio Doce region, Minas Gerais, south-eastern Brazil.

Table 1. Comparison of the morphological features of *Vanilla schwackeana* and related species. *Vanilla rupicola* data were obtained from Pansarin and Menezes (2023). The characteristics of *V. hartii* were obtained from Ferreira et al. (2020), while the characteristics of *V. appendiculata* were obtained from Engels and Ferneda Rocha (2016)^a and Barona-Colmenares (2018)^b.

| | <i>V. schwackeana</i> | <i>V. rupicola</i> | <i>V. appendiculata</i> | <i>V. hartii</i> |
|-----------------------------|------------------------|--------------------|--|-------------------|
| Habit | hemiepiphytic | rupicolous | hemiepiphytic ^{a,b} | hemiepiphytic |
| Stem | scandent | reptant | scandent ^{a,b} | scandent |
| Leaves (cm) | 5.5–11 × 3–5.5 | 3.2–9 × 2.8–5.2 | 13–19 × 3.9–5.7 ^a 14–17.7 × 4.2–4.9 ^b | 6.5–8 × 2.5–3.5 |
| Leaves | pseudopetiolate | sessile | petiolate ^{a,b} | petiolate |
| Leaf blade | elliptic to obelliptic | ovate to rounded | obovoid ^a spatulate ^b | elliptic |
| Inflorescence | apical/lateral | apical/lateral | apical ^{a,b} | apical/lateral |
| Sepals (cm) | 4.2–4.6 × 0.8–1.1 | 5.8–6.3 × 1–1.3 | 6.6 × 0.7 ^a 7.5–7.8 × 0.8–1.1 ^b | 4.9–5.3 × 0.8–1.1 |
| Petals (cm) | 4–4.4 × 0.7–0.9 | 5.7–6.2 × 0.7–1.2 | 6.5 × 0.6 ^a 7.6–7.7 × 0.7–0.8 ^b | 4.9–5.1 × 0.7–0.9 |
| Labellum (cm) | 4.2–4.7 × 2.5–2.7 | 5.6–6.3 × 3.2–3.5 | 6.8 × 1.6 ^a 3.8 × 2.9 ^b | 4.4–4.7 × 2.3–2.4 |
| Labellar protrusions | papillous | papillous | finger-like ^b | verrucose |
| Column (cm) | 3.2–3.5 × 0.3–0.32 | 4.2–4.8 × 0.3–0.4 | 5.4 × 0.2 ^a ; 6.1 × 0.3 ^b | 3.8–4 × 0.2–0.3 |

specimens are found in one location, which also falls within the limits of Critically Endangered under subcriterion a. The region where individuals of *V. schwackeana* are found is being studied for the extraction of iron ore by a mining company. The Vale do Rio Doce has known a long history of deforestation. The region comprises 81 municipalities, 24 of which comprise the “Vale do Aço” (Steel Valley), including Timóteo and Antônio Dias, where exploitation of iron ore has drastically reduced the native flora of the region to sparse forest fragments. I therefore project a continuing decline in (i) extent of occurrence, (ii) area of occupancy, and (iii) extent and/or quality of habitat. Considering these threats and the fact that the species is distributed in one location, *V. schwackeana* is assessed as Critically Endangered (CR).

Additional material examined. BRAZIL – Minas Gerais

• Antônio Dias, Comunidade Japao, semi-deciduous forest; 19°61'15"S, 42°77'26"W; 850 m; 6 Oct. 2022; E.R. Pansarin 1567; SPFR • Timóteo, semi-deciduous forest; 19°54'59"S, 42°69'56"W; 840 m; 7 Oct. 2022; E.R. Pansarin 1568; LBMBP.

Notes. Based on Hoehne's hypothesis that the holotype of *V. schwackeana* is a mixture of elements from distinct *Vanilla* species and due to the size of the similar floral parts, a dissected flower from the material *Schwacke 11106*, deposited in the RB herbarium, was designated as the lectotype of *V. schwackeana* and synonymized under *V. planifolia* (Karremans et al. 2020). However, according to my data based on live plants, except for the immature fruit whose characteristics may overlap with those of many Neotropical *Vanilla*, all other elements present in the material collected by Carl August Wilhelm Schwacke agree with those of the specimens found in the Rio Doce

Valley, in the state of Minas Gerais. Therefore, according to the data presented here, there is no mixture of elements in the specimen *Schwacke 11106*. Based on the fact that Hoehne (1944) described *V. schwackeana* on the basis of a single collection, I conclude that the collection *Schwacke 11106* (RB) is the holotype of *V. schwackeana*.

Morphological affinities

Vanilla schwackeana is easily recognized by its pseudopetiolate leaves, by its largely elliptic to obelliptic leaf blades with attenuate base and acuminate apex, by its apical inflorescence with small flowers, by its yellow labellum with white undulate-crenulate margins, and by its papillous appendages on the apical portion of the labellar crest. These characteristics suggest a close relationship of *V. schwackeana* with *V. rupicola* Pansarin & E.L.F.Menezes and with some species currently recognized in the *V. planifolia* group and *V. trigonocarpa* group, i.e. *V. appendiculata* Rolfe and *V. hartii* Rolfe, respectively (Table 1). This hypothesis is corroborated by phylogenetic results (see further).

Phylogenetic relationships

Phylogenetic analysis of the ITS region resulted in strongly congruent MP and BI trees (Fig. 3). In both analyses, the clade with Neotropical *Vanilla* with membranaceous leaves (PP 1, BS 100%; Fig. 3) emerges as sister to a large clade (PP 0.86, BS 99%) with two subclades: a predominantly Old World/Caribbean clade (PP 0.95, BS 99%) and a well-supported clade including the remaining Neotropical *Vanilla* (PP 0.99, BS 98%; Fig. 3). Within

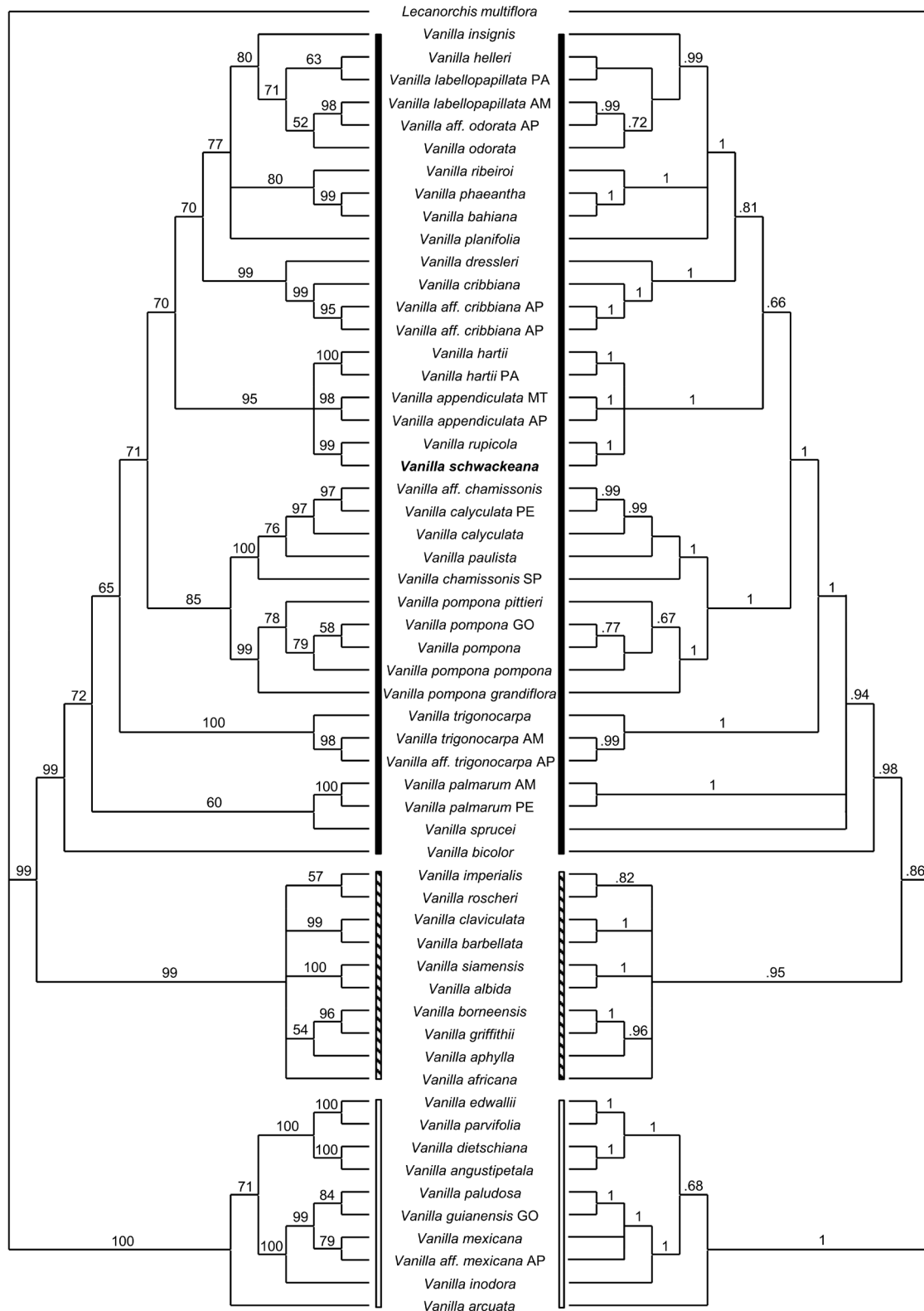


Figure 3. Bayesian inference (right) and Maximum parsimony (left) analyses of *Vanilla* (Orchidaceae) based on ITS (nrDNA) showing the phylogenetic relationships among *V. schwackeana* (bold) and Neotropical congeners. Bootstrap values > 50% (MP) and posterior probability values > 0.5 (BI) are given above the branches. Acronyms after species names are Brazilian states: AM = Amazonas, AP = Amapá, GO = Goiás, MT = Mato Grosso, PA = Pará, PE = Pernambuco, SP = São Paulo. Vertical bars refer to the main *Vanilla* lineages: white bar = Neotropical *Vanilla* with membranaceous leaves, dashed bar = Old World/Caribbean clade, black bar = Neotropical *Vanilla* with non-membranaceous leaves.

the non-membranaceous Neotropical *Vanilla*, species currently recognized among the *V. palmarum* group, i.e. the epiphytes *V. palmarum* and *V. bicolor*, besides *V. sprucei* Rolfe, emerge in a basal position as sisters to a large clade whose species are mostly hemiepiphytes or nomadic vines (PP 1, BS 65%). In the latter, the Amazonian *V. trigonocarpa* Hoehne emerges as sister to a clade with two subclades: one clade including the members of the *V. pompona* group, i.e. *V. calyculata* Schltr., *V. chammissonis* Klotzsch, *V. paulista* Fraga & Pansarin, and *V. pompona* Schiede (PP 1, BS 85%), while the other large clade includes the remaining Neotropical species (Fig. 3). In the latter, a strongly supported clade (PP 1, BS 95%), including *V. hartii*, *V. appendiculata*, and *V. rupicola/V. schwackeana* (PP 1, BS 99%), emerges as sister to the remaining non-membranaceous *Vanilla* with moderate support (PP 0.81, BS 70%; Fig. 3).

DISCUSSION

Batista et al. (2023) have pointed out the problems resulting from the decision to base species boundaries exclusively on dried specimens of Vanilloideae. Since its description about 80 years ago, *Vanilla schwackeana* has been regarded as an obscure taxon. In fact, *V. schwackeana* was described on the basis of a single specimen (Hoehne 1944, 1945; Soto Arenas and Cribb 2010). Currently, based on the image of this specimen, *V. schwackeana* has been assumed to be conspecific with *V. planifolia* (Karremans et al. 2020). Here, the mystery about *V. schwackeana* is finally unravelled with the use of integrative taxonomy. Conclusions about the circumscription of this rare species are based on extensive fieldwork, on data about geographical distribution, on morphological features on both living and dried specimens, and on a molecular phylogeny. Although the sizes of floral parts overlap, the vegetative and floral morphology of *V. schwackeana* strongly differs from that of *V. planifolia* (e.g. Azofeifa-Bolaños et al. 2017; Maceda et al. 2023). In addition, the type of *V. schwackeana* has been considered to be a mixture of plant elements (see Hoehne 1944, 1945; Soto Arenas and Cribb 2010; Karremans et al. 2020). This assumption resulted in the lectotypification of a single flower from the type specimen (Karremans et al. 2020). According to the data presented here, there is no mixture of elements in the specimen collected by Carl August Wilhelm Schwacke (*Schwacke 11106*). Plant materials collected in the southern region of Vale do Rio Doce strongly agree with the type specimen of *V. schwackeana* (*Schwacke 11106*). Besides its distinctive morphology, in Brazil, *V. planifolia* is considered to occur in the Amazon region, while *V. schwackeana* occurs in Minas Gerais, south-eastern Brazil. As far as is known, *V. planifolia* does not occur in Minas Gerais (Emerson R. Pansarin pers. obs.). The results presented here indicate the occurrence of *V. schwackeana* in the south of Vale do Rio Doce. However, it seems plausible that *V. schwackeana* had a

wider distribution in the past, as the Vale do Rio Doce has a long history of vegetation destruction due of iron ore exploitation (Consórcio ECOPLAN - LUME 2010).

My results reveal that *V. schwackeana* is easily distinguishable by its pseudopetiolate leaves, by its largely elliptic to obelliptic leaf blades with an attenuated base and acuminate apex, by its apical inflorescences with small flowers, by its yellow labellum with white undulate-crenulate margins, and by its papillous appendages on the apical portion of the labellar crest. Such characteristics suggest a close relationships of *V. schwackeana* with *V. appendiculata*, *V. hartii*, and the Brazilian endemic *V. rupicola* (Engels and Ferneda Rocha 2016; Barona-Colmenares 2018; Ferreira et al. 2020; Pansarin and Menezes 2023). In addition to the morphological data, molecular phylogenetic data strongly suggests a close relationship between *V. schwackeana* and *V. rupicola*, a species belonging to the Espinhaço Range in the Vale do Jequitinhonha (Fig. 2), Minas Gerais (Pansarin and Menezes 2023). Both sister species *V. schwackeana/V. rupicola* emerge as belonging to a clade that also includes *V. appendiculata* and *V. hartii* (Fig. 3). According to the classification proposed by Soto Arenas and Cribb (2010), *V. schwackeana* and *V. appendiculata* are members of the *V. planifolia* group, while *V. hartii* is a member of the *V. trigonocarpa* group. As pointed out by Pansarin and Menezes (2023), infrageneric realignment will be necessary because *V. trigonocarpa* emerges in a basal position within the non-membranous nomadic vine/hemiepiphyte *Vanilla*, while *V. schwackeana* and *V. hartii* emerge among the most derived clades among non-membranaceous Neotropical *Vanilla* (Fig. 3). Consequently, both the *V. trigonocarpa* group and the *V. planifolia* group (sensu Soto Arenas and Cribb 2010) are polyphyletic (Pansarin and Menezes 2023). Besides such realignments, according to the current *Vanilla* classification by Soto Arenas and Cribb (2010), the *V. appendiculata/V. schwackeana/(V. hartii/V. rupicola)* clade comprises an unrecognized infrageneric *Vanilla* group. Based on the results presented here, it seems plausible that the formation of a group comprising species from this clade will be necessary. Although the identity of *V. schwackeana* has been clarified in this study, efforts are needed in order to clarify the identity of other obscure *Vanilla* names, such as *V. dungsii* Pabst, and another species described by Frederico Carlos Hoehne on the basis of a single specimen collected by Carl August Wilhelm, i.e. *V. dubia* Hoehne.

CONCLUSION

Integrative taxonomy was crucial to understand the identity and species boundaries of *V. schwackeana*. The *V. appendiculata/V. schwackeana/(V. hartii/V. rupicola)* clade comprises an unrecognized infrageneric *Vanilla* group. The formation of a new infrageneric group comprising species from this clade will be necessary.

ACKNOWLEDGEMENTS

The author thanks ICMBIO for permission to collect (Protocol SISBIO number 35178-1), Elettra Greene for English revision, and Cristina Lourenço for help with fieldwork. Research supported by the São Paulo Research Foundation – FAPESP (Grant 2018/07357-5) and by CNPq (Productivity Research Grant 301773/2019-0).

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SUPPLEMENTARY MATERIAL

Supplementary material 1

Species of *Vanilla* included in the molecular study, their locations, vouchers and GenBank accession numbers. VAN = *Vanilla* germplasm bank, LBMBP Orchid House, Department of Biology, FFCLRP-USP, University of Sao Paulo, Brazil, available at <https://www.lbmbplab.net/vanillacollection>. <https://doi.org/10.5091/plecevo.110331.suppl1>