

Systematics of the *Vanilla chamissonis* clade (Orchidaceae): a study based on integrative taxonomy

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Abstract

Background and aims – The Brazilian biomes include the world's greatest diversity of *Vanilla*. This richness has required studies on species boundaries, as many taxa have been described or erroneously synonymized. This is the case for *V. argentina*, a species described from the Dry Chaco synonymized under *V. chamissonis*. While studying the diversity of Neotropical *Vanilla*, differences between plants from the Cerrado/Dry Chaco, the Atlantic Forest, and the Caatinga became evident.

Material and methods – Descriptions of members of the *Vanilla chamissonis* clade, i.e. *V. argentina*, *V. calyculata*, and *V. chamissonis* are provided and a morphological comparison is given. An identification key and illustrations are provided. Geographic distributions and the reproductive strategies are presented. The phylogenetic relationships among the members of the *V. chamissonis* clade are discussed based on an ITS (nrDNA) phylogeny.

Key results – Plants occurring in the Cerrado and Dry Chaco correspond to *V. argentina*, while *V. calyculata* and *V. chamissonis* occur in dry environments and in the Atlantic Forest, respectively. *Vanilla argentina* is distinguished from the other species by several characteristics, such as the size of the leaves and the flower structures, and fruit features. *Vanilla argentina* is self-compatible, while *V. calyculata* and *V. chamissonis* have a reproductive system based on late-acting self-sterility. All three species are assessed as Endangered. The identities of *V. carinata*, *V. gardneri*, and *V. vellozii* are clarified. The name *V. argentina* is revalidated.

Conclusion – The evaluation of vegetative and reproductive characteristics of both living and dried specimens is crucial to understand the species delimitation among *Vanilla*. All evidence reveals that *V. argentina* is distinct from *V. chamissonis*. *Vanilla chamissonis* is endemic to Brazil, while *V. argentina* is distributed throughout Brazil, Argentina, and Paraguay. *Vanilla calyculata* occurs in dry regions of South and Central Americas. The *Vanilla chamissonis* clade comprises a lineage of three species within the *Vanilla pompona* group.

Keywords

Atlantic Forest, Caatinga, Cerrado, Dry Chaco, phylogeny, taxonomy, Vanilloideae

INTRODUCTION

The genus *Vanilla* (Orchidaceae) encompasses more than 100 species distributed throughout tropical regions of Asia, Africa, and Americas (Cameron 2003; Pansarin 2024a; Pansarin and Menezes 2023). With more than 40 species, Brazil is the centre of diversity for *Vanilla* (Hoehne 1945; Pansarin 2010; Pansarin and Miranda 2016; Pansarin and Menezes 2023; Pansarin 2024a). *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; Pansarin 2024a). These phylogenetic approaches partially recover the infrageneric classification in groups by Soto Arenas and Cribb (2010). Among the infrageneric *Vanilla* groups, Soto Arenas and Cribb (2010) recognize six species in the *Vanilla pompona* group: *V. calyculata* Schltr., *V. chamissonis* Klotzsch, *V. columbiana* Rolfe, *V. grandiflora* Lindl., *V. pompona* Schiede, and *V. vellozii* Rolfe, as the name *V. pseudopompona* is not validly

published. Recent studies involving Neotropical *Vanilla* have shown that the inclusion of specimens from the Cerrado (Brazilian savanna) areas in phylogenetic analyses make *V. chamissonis* paraphyletic. Specimens from the Cerrado are more related to *V. calyculata*, a species distributed throughout dry environments of South America, including the Caatinga (Brazilian dry forest), than to plants occurring in the Atlantic Forest (Pansarin and Menezes 2023; Pansarin 2024a). In addition, a phylogenetic inference using plants from the Dry Chaco (tropical dry forest) (Misiones, Argentina) recovered the paraphyly of *V. chamissonis* as well (Cascales et al. 2023). Besides molecular, geographical, and morphological evidences, preliminary results on the breeding systems reveal differences with regard to the reproductive strategies among species belonging to the *V. chamissonis* clade, as well as between plants from the Atlantic Forest (tropical rainforest) (i.e. *V. chamissonis*) and from the Cerrado. Vegetative and floral characteristics of specimens collected within the Cerrado strongly agree with those of *V. argentina* Hicken and *V. chamissonis* var. *longifolia* Hoehne, both taxa currently considered to be conspecific with *V. chamissonis* (BFG 2025). *Vanilla chamissonis* was described based on a specimen collected in the state of Santa Catarina, a region inserted in the Atlantic Forest. The name *V. chamissonis* var. *longifolia* was described based on a specimen collected in a Cerrado area in the municipality of Itú, São Paulo. However, the latter taxon was never validly published, as the diagnosis provided by Hoehne (1945) was written in Portuguese instead of Latin (International Code of Nomenclature for algae, fungi, and plants, Art. 39.1: Turland et al. 2018). Conversely, *Vanilla argentina* is a validly published taxon described based on specimens collected by J.A. Martinez in a Dry Chaco area in Formosa, northwestern Argentina (Hicken 1917).

Vanilla columbiana was described by Rolfe (1896) from a specimen collected in the Magdalena River valley, in Colombia (i.e. *Goudot s.n.*). Both the holotype in K (K000463745) and the isotype in P (P00367038) are degraded, unsatisfactory material (Flanagan et al. 2025). Based on the analysis of the holotype, Soto Arenas and Cribb (2010) stated that the dissected flower of the type is likely an immature bud. However, in a revision of several species, Karremans et al. (2020) proposed further synonyms. Amongst these species, *V. calyculata* has been synonymized under *V. columbiana*. Based on an accurate examination of herbaria specimens, including the types of *Vanilla calyculata*, *V. columbiana*, and *V. phaeantha* Rchb.f., the synonymization of *V. columbiana* under *V. calyculata* has been reassessed (Flanagan et al. 2025). This study strongly agrees with the previous assertion that the flower in the holotype of *V. columbiana* is an immature bud, as previously proposed by Soto Arenas and Cribb (2010). As a consequence, the name *Vanilla calyculata* has been revalidated, and *V. columbiana* synonymized under *V. phaeantha* (Flanagan et al. 2025).

Recent investigations have pointed out problems when forming conclusions about species delimitation based

solely on herbaria samples in Vanilloideae (Batista et al. 2023; Pansarin 2024a, 2025). Species boundaries based on molecular, chemical, morpho-anatomical and ecological divergencies, besides geographical disjunctions (i.e. integrative taxonomy), have critically advanced the current appraisal and understanding of plant diversity (Barrett and Freudenstein 2011; Batista et al. 2023; Suetsugu et al. 2023; Pansarin 2024a, 2025). Here, I have used an integrative taxonomic approach to determine the species boundaries and the phylogenetic relationships within the *Vanilla chamissonis* clade. I investigated the species circumscription based on specimens from the Atlantic Forest (i.e. *V. chamissonis*) and dryer environments (i.e. *V. argentina* and *V. calyculata*) by using vegetative and reproductive data from both herbarium and fresh plant material, and also the analysis of breeding systems in order to elucidate their reproductive strategies. The data obtained here are discussed on light of a phylogenetic inference for *Vanilla*.

MATERIAL AND METHODS

Plant features, habitat, and distribution

Fresh and herbarium material of flowering and fruiting plants were used for the investigations. The living material was collected from Brazilian populations and maintained in the *Vanilla* germplasm bank from the LBMBP Orchid House (Orchidarium of the Laboratory of Molecular Biology and Systematics of Plants, University of São Paulo (FFCLRP-USP), Brazil). Flowering and fruiting specimens were vouchered according to usual techniques (Fidalgo and Bononi 1989) and then deposited in the LBMBP herbarium. In addition, I undertook a revision of *Vanilla* specimens present in the Brazilian herbaria: BHCB, CEN, CVRD, F, FLOR, FURB, HCF, HRCB, HUEFS, HUENF, HUNI, IBGE, ICN, JOL, LBMBP, MBM, MBML, RBR, RFA, SJRP, SP, UB, UEC, UPCB, and VIES. International herbaria were also consulted: AFP, AMES, BM, BIGU, BOG, COL, CUVC, FMB, HUA, ICESI, K, LAGU, MEDEL, MEXU, NY, UDBC, and US (acronyms according to Thiers 2024).

Plant structures were measured using a Vernier Caliper. The vegetative and reproductive structures 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 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* groups followed Soto Arenas and Cribb (2010). The original description and digital images

from the types of *Vanilla chamissonis* and related species were consulted.

The distribution map of all species belonging to the *V. chamissonis* clade was based on both herbaria material and living specimens from the *Vanilla* germplasm bank (LBMBP Orchid House). The map with the geographic distribution of *Vanilla* species was produced with QGIS v.3.32 (QGIS Development Team 2023). The conservation statuses of the *Vanilla* species were assessed according to the IUCN Red List categories and criteria, and guidelines (IUCN Standards and Petitions Committee 2024). The area of occupancy (AOO), estimated using a 2×2 km grid, and the extent of occurrence (EOO) were calculated using GeoCAT (Bachman et al. 2011).

Taxon sampling for phylogenetic analysis

A total of 64 *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 GenBank and obtained during the development of this study. A list of ingroup and outgroup species, vouchers, and GenBank accession numbers is given in Suppl. material 1.

DNA extraction, amplification, and sequencing

DNA of *Vanilla* specimens were extracted from fresh material according to a modified CTAB method (Doyle and Doyle 1987). The amplifications were carried out using 50 µL PCR volumes. Denaturation of the DNA strands was facilitated by the addition of a 5 M betaine solution to the PCR reactions. 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 BigDye v.3.1 (ABI), purified PCR products, and the same aforementioned primers. Samples were dehydrated and re-suspended with loading dye. Sequences were obtained using an Applied Biosystems automated sequencer model 3100. Sequence Navigator and Autoassembler (Applied Biosystems) were used for sequence editing and the assembly of complementary and overlapping sequences. DNA sequences were aligned using BioEdit v.5.0.9 (Hall 1999).

Phylogenetic analyses

Maximum parsimony analysis (MP) was run with PAUP* v.4.0b5 (Swofford 2001). For the ITS matrix, a heuristic

search was conducted on 64 *Vanilla* accessions 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, an ITS data matrix containing 42 *Vanilla* accessions 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. Tracer v.1.7.2 (Rambaut et al. 2018) was used to assess chain convergence to check if the effective sample sizes (ESS) are above 200 for all parameters. 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.

Breeding systems

Here, I carried out experimental pollinations in order to investigate the reproduction strategies of species belonging to the *V. chamissonis* clade. The specimens of *V. chamissonis* (N = 10) and *V. argentina* (N = 10) used for breeding system investigations were collected from natural populations of the southeastern Brazil (municipalities of Ubatuba and Itirapina, respectively) and cultivated at the LBMBP Orchid House. The LBMBP Orchid House is completely covered with 50% black shade cloth to prevent any possible insect visitors coming into contact with the experimental plants. The plants of *V. chamissonis* and *V. argentina* used in this study are part of the *Vanilla* germplasm bank of the LBMBP Orchid House. The experimental treatments for *V. calyculata* were performed from natural populations occurring in the municipality of Unai, in the state of Minas Gerais, southeastern Brazil.

The following treatments were conducted to check the breeding system in members of the *V. chamissonis* clade: spontaneous self-pollination, manual self-pollination to test the occurrence of self-compatibility, cross-pollinations, and emasculations for apomixis. A total of 120 flowers (10 plants; 30 inflorescences; 30 flowers per treatment) were used. All treatments were randomly applied to each inflorescence using one-day flowers, as flowers of *Vanilla* commonly last one day (Pansarin 2022; Pansarin and Ferreira 2022). The number of manipulations per inflorescence varied depending on the availability of flowers. Treatments were performed in the

morning hours (08:00–09:00 am), during the flowering periods of studied species; i.e. from October 2021 to December 2021, and from October 2022 to November 2022. The pollen content of an anther was used in each experimental pollination event (manual self- and cross-pollinations). Cross-pollinations were performed with previously emasculated flowers and using pollen

from a distinct accession. Fruit set from experimental treatments was recorded when pods had ripened. Fruit set in successful treatment of manual self-pollination was compared with the treatment of cross-pollination using a t-test for independent samples with Statistica v.6.0 (StatSoft 2003).

RESULTS

Taxonomic treatment

Identification key to the species belonging to the *Vanilla chamissonis* clade

1. Plants occurring in dry environments (dry forests and Caatinga vegetation), leaf blade linear to lanceolate, sepals 7.5–8 cm long, sepals revolute, petals linear-oblongate, labellum 8.8–9.5 cm long, nectar chamber 3.2–3.6 cm long, column 55–60 mm long, fruit dehiscent, mature fruit yellowish, pericarp hard..... *V. calyculata*
- Plants occurring in the Atlantic Forest, Dry Chaco, and Cerrado, leaf blade elliptic to oblong (rarely lanceolate), sepals < 7.5 cm long, sepals spreading, petals linear to oblong-elliptic, labellum < 8 cm long, nectar chamber < 3 cm long, column up to 40 mm long, fruit indehiscent, mature fruit brown, pericarp soft to half hard **2**
2. Plants occurring in the Dry Chaco and Cerrado, leaf blade elliptic (rarely lanceolate), sepals 6.8–7.4 cm long, petals 6.6–7.1 cm long, linear, labellum 6.7–7.4 cm long, nectar chamber 1.8–2 cm long, column 38–40 mm long, pericarp half hard..... *V. argentina*
- Plants occurring in the Atlantic Forest, leaf blade elliptic to oblong, sepals 4.9–6.1 cm long, petals 4.9–6.1 cm long, oblong-elliptic, labellum 5.3–6.2 cm long, nectar chamber 1.4–1.6 cm long, column 36–38 mm long, pericarp soft..... *V. chamissonis*

Vanilla argentina Hicken (Hicken 1917: 235)

Figs 1, 4–7, Table 1

Type. ARGENTINA – **Formosa** • Pilcomayo; 23 Sep. 1915; J.A. Domínguez 305; lectotype (**designated here**): SP! [SP45706B]; isoelectotype: SI not found.

Description. Nomadic vines, long scandent. Roots axillary, one per node; terrestrial roots up to 8 mm diam., fleshy, whitish, with hyaline absorbing hairs; aerial roots 2.2–2.5 mm diam., whitish to brownish. Stem climbing, cylindrical, fleshy, straight to sinuous, glabrous, glaucous to dark green, strongly furrowed under arid conditions; internodes of ascendant stems 4–16 × 0.5–10 cm. Leaves 6–22 × 3–5.5 cm, alternate, distichous, elliptic to lanceolate, symmetric to asymmetric, fleshy, glabrous, green, pseudopetiolate, margin entire, base attenuate, apex acuminate; pseudopetiole 5.5–8 mm concave. Inflorescence axillary, racemose, with up to 14 flowers opening in succession; 1–2 flowers opening each morning; rachis 5–15.5 × 0.8–1.3 cm, terete, glaucous to dark green; bracts 5–9 × 4–7.5 mm, progressively smaller toward the apex, triangular to ovate, coriaceous, concave, green, erect to patent, apex acute, not incurved. Flowers resupinate, pedicellate, abscission layer between perianth and ovary present; pedicel with ovary 45–50 × 3–4 mm, trigonous in transverse section, white at the base, green to the apex, with a calyculus (6–6.5 × 2.5–3) at the apex. Sepals 6.8–7.4 × 1.2–1.4 cm, free, oblanceolate, fleshy, slightly concave, spreading, pale green to yellowish, margin entire to the apex and involute at the base, base attenuate, apex acute, somewhat thickened; dorsal sepal symmetric; lateral sepals asymmetric; base slightly incurved. Petals 6.6–7.1 × 1.2–1.5 cm, free, obliquely linear, asymmetric,

lower margin more arcuate, membranous, pale green, base attenuate, apex acute to obtuse, adaxial surface with a central and longitudinally disposed keel. Labellum 1-lobed to slightly 3-lobed, 6.7–7.4 × 3.8–4.2 cm, tubular, deepening near the middle, yellowish to the base, white in distal portion, unguiculate, with a central crest from the unguiculus to the apex, and a penicillate callus just below the anther; unguiculus fused along the margins of the basal half (ca 30–35 mm) of column length forming a nectar chamber, nectar chamber 1.8–2 cm long, tubular; central crest yellowish from the nectar chamber to the penicillate callus, dark yellow to the apex; distal portion of the central crest swollen, low cushion, rugose-papillose at the apex, with a group of transversal yellow-orange scales near the penicillate callus, with five yellow longitudinal lines near the apex; penicillate callus 5.2–6 × 4.4–4.7 mm, made by yellow-hyaline lacerate-laciniate scales and clusters of trichomes; lateral lobes not much evident, rounded, overlapping the column apex, margin undulate; midlobe deeply emarginated; margin undulate. Column 38–40 × 4.5–5 cm, trigonous, arched to the base, forming an angle ca 90° with the ovary, strait to the apex, ventral surface flat with white to yellowish hyaline trichomes over the distal half, attenuate to the base, dilated to the apex, with two lateral wings; lateral wings rounded, bilobed, undulate. Stigma bilobed; rostellum 5–5.2 × 3.7–3.9 mm, trapezoidal, membranous, white. Anther 5.8–6 × 4.7–4.9 mm, rectangular to trapezoidal, white, versatile, apex truncate; pollen mass 3.2–3.3 × 3.5–3.8 mm, triangular, bipartite, yellowish. Fruits 11–16 × 2.3–2.8 cm, oblong, straight, transversally trigonous, fleshy, brown and indehiscent when mature, fragrant; pericarp half hard; fruit cavity hollow. Seeds ca 0.5 mm, ovoid, black.



Figure 1. *Vanilla argentina* Hicken. **A.** Part of a flowering plant showing the stem, leaves, and inflorescence. **B.** Flower in diagonal view. **C.** Flower in lateral view. **D.** Flower in front view. **E.** Dissected perianth. The details (dashed areas) show the central labellar crest (left) and the penicillate callus (right). **F.** Detail of the adaxial surface of a petal showing the longitudinal keel. **G.** Detail of the apex of the labellum showing the yellowish longitudinal crest with five longitudinal ribs and the lateral lobes overlapping the column apex. **H.** Pedicel/ovary, column, and labellum in lateral view. **I.** Pedicel/ovary and column in lateral view. **J.** Apex of the column: in lateral view with an articulated anther (above), in abaxial view (mid), and in lateral view with a disarticulated anther (below). **K.** Anther in dorsal view (left) and ventral view (right). **L.** Pollen mass in dorsal view (left) and ventral view (right). **M.** Mature fruit. **N.** Transversal section of a mature fruit. Note the hollow fruit cavity. Based on *E.R. Pansarin 1570* (LBMBP).

Distribution and ecology. *Vanilla argentina* occurs in Cerrado areas in Argentina, Brazil, and Paraguay (Fig. 4). The species is commonly found in gallery forests, at elevation is from 657 to 850 m a.s.l. *Vanilla argentina* has a nomadic vine habit, commonly rooting on the forest litterfall, and climbing on tree trunks. The flowers are fragrant. Each flower lasts ca 1 day. The fruits ripen 18 months after pollination. The fruits are indehiscent and have an unpleasant aroma and a bitter flavour.

Phenology. *Vanilla argentina* blooms from October to December. The fruits ripen from May to July.

Preliminary IUCN conservation assessment. Endangered: EN B2ab(i,ii,iii). *Vanilla argentina* is an uncommon and rarely collected species currently known to grow in Cerrado areas of southeastern and central-western Brazil and Dry Chaco vegetation in Argentina and Paraguay. The populations are composed of few sparse specimens. The extent of occurrence (EOO) is estimated to be 1,344,031 km², which falls within the limits for Least Concern (LC) under criterion B1, according to the IUCN Red List categories and criteria. The area of occupancy (AOO) is estimated to be 80 km², which falls within the limits for Endangered (EN) under criterion B2. Considering that the Cerrado areas have been reduced to scattered fragments due to the expansion of soybean and sugar cane crops and the transformation of native areas into pastures lands, I project a continuing decline in (i) extent of occurrence, (ii) area of occupancy, and (iii) extent and/or quality of habitat for *V. argentina*. Based on these threats and the fact that the species is distributed in less than five locations, *V. argentina* is assessed as Endangered: EN B2ab(i,ii,iii).

Additional material examined. ARGENTINA – Misiones

• San Pedro, Catamarca; 26°37'45"S, 54°06'48"W; 548 m; Dec. 1916; T. Rojas 2040; SP.

BRAZIL – Distrito Federal • Brasília, Parque Nacional de Brasília, Cascalheira do Exército; 15°46'48"S, 47°55'45"W; 16 Sep. 2018; C.R. Martins 3054; CEN • FERCAL - APA da Cafuringa, a 38 km do CENARGEN; 15°46'48"S, 47°55'45"W; 910 m; 9 Aug. 1990; T.B. Cavalcanti 598; CEN • Taguatinga, Floresta Nacional de Brasília (Área 1); 15°50'00"S, 48°03'23"W; 1200 m; 23 Nov. 2007; J.H. Lima 32; UB. – Goiás • Campos Belos, Estrada velha de São Domingos para Campos Belos; 13°01'31"S, 46°45'54"W; 652 m; 22 Oct. 2001; M.L. Fonseca 3002; IBGE • Cristalina, Margem direita do Rio Preto; 16°46'4"S, 47°36'47"W; 1237 m; 12 Aug. 2002; A. Amaral-Santos 1367; CEN • Formosa, Ribeirão Bezerra; 15°32'13"S, 47°20'09"W; 911 m; 8 Oct. 2002; E. Tameirão Neto 3567; BHCB • Morrinhos, Rodovia Morrinhos-Caldas Novas; 17°43'16"S, 49°06'29"W; 768 m; 4 Sep. 1976; G. Hatschbach 38915; MBM. – Mato Grosso • Nova Xavantina, ca 70 km N of Xavantina; 14°22'S, 52°37'W; 300–400 m; 10 Oct. 1964; H.S. Irwin 6750; NY. – Mato Grosso do Sul • Campo Grande; 20°26'37"S, 54°38'52"W; 612 m; 15 Nov. 2023; E.R. Pansarin 1573; LBMBP. – Minas Gerais • Belo Horizonte, Campus da UFMG; 19°48'57"S, 43°57'15"W; 5 Nov. 1994; V.A. de Sousa s.n.; BHCB

24796 • Diamantina, Serra do Espinhaço; 18°14'17"S, 43°36'40"W; 975 m; 17 Apr. 1972; H.S. Irwin 28146; NY • Serra do Espinhaço, Mata Ciliar; 18°14'17"S, 43°36'40"W; 970 m; 25 Oct. 2023; E.R. Pansarin 1571; LBMBP • Mariana, Mina da Samitri; 20°22'41"S, 43°25'00"W; 718 m; 28 Nov. 2000; R.C. da Mota & L. Viana 570; BHCB • Paracatú, Cerrado, ca 5 km S.E. of Paracatú; 17°13'21"S, 46°52'31"W; 650m; 6 Feb. 1970; H.S. Irwin et al. 26210; UB • Pimhuí; 20°27'42"S, 45°56'45"W; 818 m; 1 Nov. 2023; E.R. Pansarin 1572; LBMBP • São Gonçalo do Rio Abaixo; 19°49'41"S, 43°22'55"W; 743 m; 21 Oct. 1993; E.L. Borba 79; BHCB. – São Paulo • Itirapina, Itaqueri da Serra, Mata de Galeria; 22°15'10"S, 47°49'22"W; 817 m; 22 Oct. 2023; E.R. Pansarin 1569; LBMBP • Itirapina, Itaqueri da Serra, Mata de Galeria; 22°15'10"S, 47°49'22"W; 817 m; 29 Oct. 2023; E.R. Pansarin 1570; LBMBP • Itú, Capão Sombrio; [23°15'57"S, 47°17'57"W]; 577 m; 20 Dec. 1897; A. Russel CGG3840; SP • São José do Rio Preto, Estação Ecológica do Noroeste Paulista; 20°43'36"S, 49°22'50"W; 410 m; 10 Oct. 1996; W. Forster 9; SJRP.

PARAGUAY • Cordillera de Altos; 25°16'55"S, 57°38'6"W; 7 Dec. 1902; K. Fiebrig 564; F.

Notes. *Vanilla argentina* has been considered synonymous with *V. chamissonis*, the latter assumed to be a species widely distributed throughout South America. However, some authors have observed differences between specimens collected inland compared to those occurring on the coast. Such differences have resulted in the publication of two taxa, *Vanilla argentina* and *V. chamissonis* var. *longifolia*, the latter being invalidly published, as F.C. Hoehne providing a diagnosis in Portuguese instead of Latin (Turland et al. 2018). My data, based on specimens collected in Cerrado areas in the states of São Paulo, Minas Gerais, and Goiás, show that the plants of some populations have long and dark green leaves that are quite distinct from those from populations in the Gran Chaco. Specimens with long leaves strongly agree with those of *V. chamissonis* var. *longifolia*. However, the flowers and fruits are identical. In addition, ITS DNA sequences align perfectly with those of *V. argentina*. For this reason, the long- and short-leaved plants are treated here as single taxon, *V. argentina*.

Morphological affinities. *Vanilla argentina* is distributed in the Dry Chaco and Cerrado (Fig. 4). The species is recognized by its commonly elliptic leaf blades (Fig. 5). Plants from some Cerrado areas in São Paulo, Goiás, and Minas Gerais (southwestern and central-western Brazil) commonly have long lanceolate leaves. However, this appears to be intraspecific variation, as populations with leaves morphologically similar to those plants found in the Dry Chaco are also found in the Brazilian Cerrado. Apart from vegetative variations, flowers from plants of both biomes are morphologically indistinguishable. Flowers of *V. argentina* are recognized from the remaining species of the *V. chamissonis* clade by its sepals 6.8–7.4 cm long, linear petals 6.6–7.1 cm long, labellum 6.7–7.4 cm long with a nectar chamber 1.8–2 cm long and a column 38–40 mm long, and fruits with a half hard pericarp

Table 1. Comparison of the morphological features of *Vanilla argentina* and the related species, *V. chamissonis* and *V. calyculata*.

Characteristic	<i>V. argentina</i>	<i>V. chamissonis</i>	<i>V. calyculata</i>
Vegetation	Cerrado, Dry Chaco	Atlantic Forest	Dry forests, Caatinga
Leaf blade	elliptic to lanceolate	elliptic to oblong	linear to lanceolate
Rachis size (cm)	5–15.5 × 0.8–1.3	5–9 × 0.9–1.3	6–11 × 1.1–1.8
Sepal orientation	spreading	spreading	revolute
Sepals size (cm)	6.8–7.4 × 1.2–1.4	4.9–6.1 × 0.9–1.3	7.5–8 × 1.1–1.4
Sepal shape	oblanceolate	oblanceolate	oblanceolate to oblong-elliptic
Petals size (cm)	6.6–7.1 × 1.2–1.5	4.9–6.1 × 0.9–1.2	7.5–8 × 0.9–1.3
Petal shape	linear	oblong-elliptic	linear-oblanceolate
Labellum size (cm)	6.7–7.4 × 3.8–4.2	5.3–6.2 × 3.3–4.2	8.8–9.5 × 3.7–4.5
Nectary length (cm)	1.8–2	1.4–1.6	3.2–3.6
Labellar crest	five conspicuous lines	three conspicuous lines	inconspicuous lines
Column size (mm)	38–40 × 4.5–5	36–38 × 3.2–3.5	55–60 × 4–4.5
Fruit size (cm)	11–16 × 2.3–2.8	12–17 × 2.6–3.5	15–21 × 2–3
Fruit dehiscence	indehiscent	indehiscent	dehiscent
Fruit transverse section	trigonous	subtrigonous	terete to subtrigonous
Fruit pericarp	half hard	soft	hard
Fruit colour	brown	brown	yellowish
Fruit maturation	18 months	18 months	9 months
Breeding system	self-compatible	self-incompatible	self-incompatible

(Figs 6–7). All morphological characteristics support *V. argentina* as a member of the *V. chamissonis* clade, which is corroborated by the phylogenetic analysis (see further).

Vanilla calyculata Schltr. (Schlechter 1920: 42)

Figs 2, 4–7, Table 1

Vanilla esquipulensis Archila & Chiron (Archila and Chiron 2012: 6) – Type: GUATEMALA • El Barrial, Esquipulas, Chiquimula; 900 m; Apr. 2012; *F. Archila* 1300; holotype: BIGU!.

Type. COLOMBIA – Cauca • 1000 m; *M. Madero s.n.*; holotype: B†. – Valle del Cauca • Tuluá, Corregimiento Mateguadua, Jardín Botánico Juan Maria Céspedes, laderas en vía de repoblación natural, 1100 m a.s.l.; 28 Sep. 1984; *W. Devia* 815; neotype (designated by Soto Arenas and Dressler 2010): TULV! [TULV003790]; isoneotype NY! [NY04170480].

Description. Nomadic vines, long scandent. Roots axillary, one per node; terrestrial roots up to 10 mm diam., fleshy, whitish, with white-hyaline absorbing hairs; aerial roots 2.2–2.8 mm diam., creamy to brownish. Stem climbing, cylindrical, fleshy, sinuous, glabrous, green, strongly furrowed under arid conditions; internodes of ascending stems 7–16 × 0.6–1.2 cm. Leaves 8–21 × 2–4 cm, alternate, distichous, linear to lanceolate, symmetric, fleshy, glabrous, green, pseudopetiolate, margin often revolute, base rounded, apex acute; pseudopetiole 6–10 mm long, concave. Inflorescence axillary, racemose, with up to 20 flowers opening in succession; 1–3 flowers opening each morning; rachis 6–11 × 1.1–1.8 cm, terete, green; bracts 6.5–10 × 4.5–8 mm, progressively smaller to

the apex, widely deltoid, coriaceous, concave, green, apex acute, involute. Flowers resupinate, pedicellate, abscission layer between perianth and ovary present; pedicel with ovary 42–56 × 4.5–5.5 mm, cylindrical, sulcate, whitish at the base, green to the apex, with a calyculus (7.5–8.5 × 3.5–4.5 mm) at the apex. Sepals 7.5–8 × 1–1.4 cm, free, narrowly oblanceolate to oblong-elliptic, slightly concave, revolute, white at the base, green to the apex, margin entire, base attenuate, apex subacute to obtuse, somewhat thickened; dorsal sepal symmetric; lateral sepals asymmetric. Petals 7.5–8 × 0.9–1.3 cm, free, obliquely linear-oblanceolate, asymmetric, lower margin more arcuate, membranous, white at the base, green to the apex, base attenuate, apex acute to rounded, adaxial surface with central and longitudinally disposed keel. Labellum 3-lobed, 8.8–9.5 × 3.7–4.5 cm, tubular, deepening near the middle, yellowish to the base, dark yellow at the distal half and with white lobes, unguiculate, with a central crest from the unguiculus to the apex, and a penicillate callus just below the anther; unguiculus fused along the margins of the basal half (ca 41–46 mm) of column length forming a nectar chamber; nectar chamber 3.2–3.6 mm long, tubular; central crest whitish from the nectar chamber to the penicillate callus, yellow to the apex; distal portion of central crest swollen, low cushion, rugose-papillose at the apex, with a group of transversal and yellow-orange hairs near the penicillate callus, longitudinal lines not much evident; penicillate callus ca 4.5 × 3.8 mm, made up by 8–10 flabellate, shortly lacerate-laciniate and retrorse scales; lateral lobes rounded, bilobed, overlapping the column apex, margins entire; midlobe deeply emarginate

to bilobed, margins crenulate-undulate. Column 55–60 × 4–4.5 mm, subterete, subclavate, sigmoid, ventral surface flat with white to yellowish hyaline trichomes over the distal half, dilated to the apex, with two lateral wings; lateral wings with two triangular-flabellate protrusions, narrow, undulate. Stigma bilobed; rostellum 4.2–4.6 × 2.8–3.2 mm; trapezoid, flabellate. Anther 5.2–6 × 4–4.3 mm, rectangular, apex truncate to slightly emarginate; pollen mass 1.9–2.1 × 3.7–4.2 mm, triangular, bipartite, yellow. Fruits 15–21 × 2–3 cm, oblong, transversally terete to subtrigonal, fleshy, yellowish and dehiscent when mature, fragrant; pericarp hard; fruit cavity hollow. Seeds ca 0.5 mm, ovoid, black.

Distribution and ecology. *Vanilla calyculata* is distributed throughout dry forests of Brazil, El Salvador, Guatemala, Honduras, and Colombia (Fig. 4). In Colombia, this species is widely distributed in the inter-Andean valleys in the western and central Andean cordilleras, at elevations between 500 and 1750 m a.s.l. When found at lower elevations, the vegetation is sub-xerophytic. In Brazil, this species occurs predominantly in the Caatinga.

Phenology. *Vanilla calyculata* blooms from September to November in Brazil. The fruits ripen from June to July. Flowering in Colombia corresponds to two annual periods with higher precipitation: April–May and September–October. Flowers were recorded in April in El Salvador, Guatemala, and Honduras.

Preliminary IUCN conservation assessment. Endangered: EN B2ab(i,ii,iii). *Vanilla calyculata* is a species distributed throughout dry forests of Brazil, Colombia, El Salvador, Guatemala, and Honduras. The species is rarely collected in Brazil and the populations are composed of few sparse specimens. The extent of occurrence (EOO) is estimated to be 4,145,682 km², which falls within the limits for Least Concern (LC) under criterion B1, according to the IUCN Red List categories and criteria. The area of occupancy (AOO) is estimated to be 92 km², which falls within the limits for Endangered (EN) under criterion B2. In Colombia, the tropical dry forest is distributed across the Caribbean and Eastern (Llanos) plains, and in the inter-Andean valleys in the Cauca and Magdalena watersheds. This ecosystem has been highly transformed for agriculture, cattle grazing, and urbanization, with only 8% of the original landcover remaining. The tropical dry forest ecosystem in Colombia is categorized as CR – critically endangered, with a representativity in the Colombia National Protected Area System (SINAP) of only 5%. In Brazil, the Caatinga vegetation has been reduced to the expansion of human activities in northeastern Brazil. Considering habitat fragmentation, besides the climatic changes that have reduced the raining period in the habitat of *V. calyculata* specially in Brazil, I project a continuing decline in (i) extent of occurrence, (ii) area of occupancy, and (iii) extent and/or quality of habitat for this species. Based on these threats and the fact that the species is distributed in less than five locations, *V. calyculata* is assessed as Endangered: EN B2ab(i,ii,iii).

Additional material examined. BRAZIL – Bahia • Conceição do Coité, Salgadália. Próximo às casas populares; 28 Nov. 2012; D.N. Carvalho 173; HUEFS • s.loc.; 28 Oct. 2008; C.E. Ramos et al. 528; US. – Minas Gerais • Unaí, Ilha a montante do túnel de desvio, cerca de 500 m da ponte de madeira, Margem esquerda do Rio Preto; 12 Sep. 2002; A. Amaral-Santos 1508; CEN • Unaí, Fazenda Saco Grande, margem do córrego, próximo à casa do Sr. Teodorim; 16 Oct. 2019; A. Amaral-Santos 3758; CEN.

COLOMBIA – Cauca • El Socavón, Mercaderes; 1200 m; 3 Feb. 1990; M. Rocio Galindez 103; AFP • Piedrasentada, vereda Piedra Rica; 980 m; 4 May 2002; R.J.C. Muñoz 010; AFP • Vereda Potrerillo, Patía; 624 m; 25 Jan. 2015; G. Reina Rodriguez, I. Nichols, F. Lopez & J. Reyna 2122; FMB. – Cundinamarca • Finca “El Descanso”, vereda Chinauta, Fusagasugá; 1200 m; 3 Apr. 1988; F. Sarmiento 2140; BOG. – Huila • Between La Jagua & Altamira; 880 m; 23 Jul. 1961; L.A. Garay, C.E. McClennen & A. Kapuler 277; AMES. – Nariño • Vía Mojarras-Leyva, vereda Puerto Nuevo, Finca La Sortija, Leyva, Vegetación de Bosque seco; 576 m; 24 Jan. 2015; G. Reina-Rodriguez, I. Nichols, F. Lopez & J. Reyna 2101; FMB. – Santander • Reserva natural de la sociedad civil La Montaña Mágica – El Pole, Zapatoca; 1750 m; 17 May 2017; D. Díaz Rueda, R. Díaz Rueda & L. Rivera 1369; MEDEL. – Tolima • La Plata; 800–1500 m; 3 Dec. 1882; F.C. Lehmann 2263; BM. – Valle del Cauca • Hacienda El Carmen, carretera La Uribe-Sevilla; 1140 m; 19 Jul. 1994; P. Silverstone-Sopkin & N. Paz 6933; CUVC • Finca la Josefina, La Herradura, Bolívar; 1076 m; 5 Aug. 2010; G. Reina Rodriguez & M. Moreno 1344; FMB • Corregimiento Loboguerrero, Dagua; 730 m; 31 Jul.–4 Aug. 1998; W.G. Vargas 4643; COL, HUA • Atuncela, Corregimiento Loboguerrero, Dagua; 950 m; Jul. 1998; W.G. Vargas 6087; ICESI • Reserva Forestal Bosque Yotoco; 27 May 2007; F. Rojas 62; UDBC • Cultivated, Cali; E. Dryander 2379; BM • Roldanillo, Valle; 1000 m; F.C. Lehmann 8378; K.

EL SALVADOR – Depto. Morazán • Mpio. Arambala; 688 m; 7 Mar. 2018; Y. Ruiz s.n.; LAGU [LAGUJBL07763] • Mpio. Perquín, Crio. La Tejera; 1038 m; 19 Jun. 2018; Y. Ruiz 720; LAGU • La Palma, Finca El Refugio, Río Nanuapa; 1000 m; 3 Apr. 1969; O. Pank & F. Hamer 203; AMES.

GUATEMALA – El Barrial • Esquipulas, Chiquimula; 600 m; Mar. 1999; F. Archila s.n.; illustrated in Archila and Chiron (2012); BIGU • Río Jocotan, Chiquimula; 500 m; Apr. 2000; F. Archila s.n.; BIGU.

HONDURAS – Comayagua • Siguatepeque; 1050 m; 23 Jul. 1936; T.G. Yuncker, R.F. Dawson & H.R. Youse 6045; K, NY, AMES • Quebrada Santa Clara, ca 2 km al norte del Zamorano, Mpio de San Antonio de Oriente; 800 m; 19 Jun. 1996; J.L. Linares 3386; MEXU • El Paraiso, Quebrada El Cajocote, 8.7 km al N de Moroceli, por el camino a Mata de Plátano; 680 m; 29 Apr. 2004; J.L. Linares 7313; MEXU • Las Mesas region near Yuscarán; Aug. 1960; H.W. Pfeifer 1454; US.

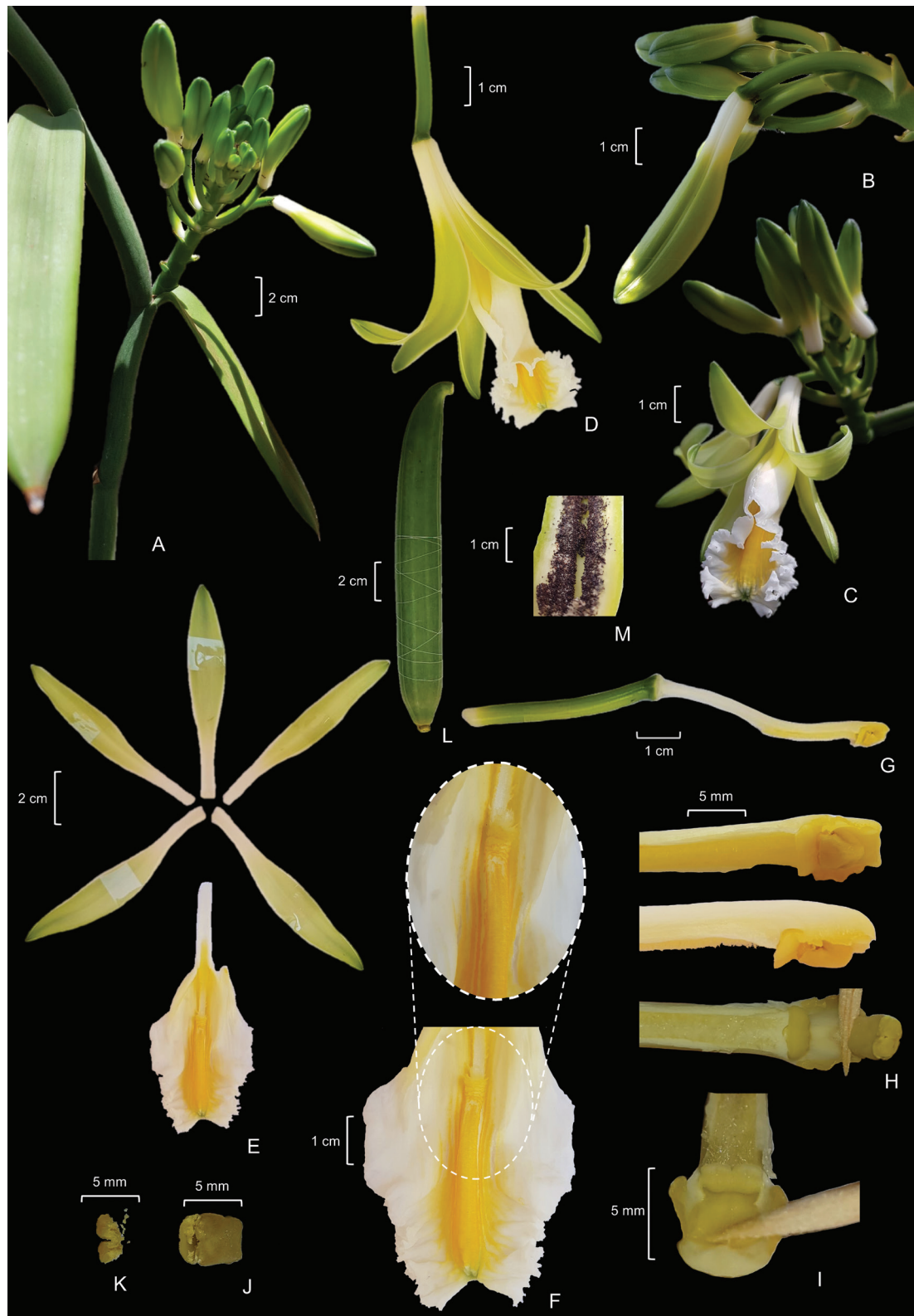


Figure 2. *Vanilla calyculata* Schltr. A. Part of a flowering plant showing the stem, leaves, and inflorescence. B. Inflorescence in lateral view showing a mature bud. C. Detail of an inflorescence showing an open flower in diagonal view. D. Flower in upper view. E. Dissected perianth. F. Detail of the apex of the labellum showing the yellowish longitudinal crest. The detail (dashed area) shows the penicillate callus. G. Pedicel/ovary and column in lateral view. H. Apex of the column: in diagonal view with an articulated anther (above), in lateral view with an articulated anther (mid), and in ventral view with a disarticulated anther (below). I. Apex of the column in ventral view showing the bilobed rostellum. J. Anther in ventral view. K. Pollen mass in dorsal view. L. Mature fruit. M. Transversal section of a mature fruit. Note the hollow fruit cavity. Based on VAN 206 (*Vanilla* germplasm bank, LBMBP Orchid House).

Notes. *Vanilla calyculata* has been considered synonymous with *V. columbiana*, a species described based on a specimen collected in the Magdalena River valley, Colombia. However, the holotype of *V. columbiana* is clearly a *V. phaeantha* with immature flowers. For this reason, the name *V. calyculata* has been re-established and *V. columbiana* has been synonymized under *V. phaeantha* (Flanagan et al. 2025). *Vanilla calyculata* is one of four species found in tropical dry forest ecosystems in Colombia. In the Cauca valley, it occurs in sympatry with *V. odorata*, *V. phaeantha*, and *V. pompona*. Herbarium specimens of *V. calyculata* have been historically referred to as *V. phaeantha*. However, *V. calyculata* can be distinguished from *V. phaeantha* by its long leaves larger than the internodes, its pendant flowers, by its pedicels, petals and sepals with white bases, and by its ovary ending in a calyculus.

Morphological affinities. *Vanilla calyculata* occurs in dry forests. The species is recognized from the remaining species of the *V. chamissonis* clade by the following characters: long and linear to lanceolate leaf blades (Fig. 5), revolute sepals 7.5–8 cm long, labellum 8.8–9.5 cm long with a nectar chamber 3.2–3.6 cm long, column 55–60 mm long, and dehiscent fruit yellowish when mature with a hard pericarp (Figs 6–7). Several vegetative and reproductive characteristics support *V. calyculata* as a member of the *V. chamissonis* clade, such as the presence of an ovary ending in a calyculus, a labellum with emarginate midlobe, and a yellow-orange elevated central crest. The phylogenetic analysis supports *V. calyculata* as a member of the *V. chamissonis* clade (see further).

Vanilla chamissonis Klotzsch (Klotzsch 1846: 564)

Figs 3–7, Table 1

Vanilla chamissonis var. *brevifolia* Cogn. (Cogniaux 1893: 149). Based on *Epidendrum vanilla* Vell. (Vellozo 1827: ic. 9, t. 1), nom. illeg.

Vanilla gardneri Rolfe (Rolfe 1895: 177), **syn. nov.** – Type: BRAZIL • Rio de Janeiro, Morro do Flamengo, near Rio de Janeiro; *G. Gardner* 245; lectotype (designated by Soto Arenas and Dressler 2010): K! [K000463752, K000463753].

Vanilla vellozoi Rolfe (Rolfe 1896: 467), as “*Vanilla vellozii*”, **syn. nov.** – Type: BRAZIL • Rio de Janeiro; 1882; *A. Glaziou* 14302; lectotype designated by Soto Arenas and Dressler 2010): K! [K000940259].

Vanilla carinata Rolfe (Rolfe 1896: 468), **syn. nov.** – Type: BRAZIL • Rio de Janeiro, Organ Mountains; 1878; *J. Miers s.n.*; holotype: K! [K000463750].

Type. BRAZIL • Santa Catarina; *A. von Chamisso s.n.*; lectotype (designated by Christenson 1995: 33): LE! [LE00011144].

Description. Nomadic vines, long scandent. **Roots** axillary, one per node; terrestrial roots up to 8 mm diam., fleshy, whitish, with hyaline absorbing hairs; aerial roots 2.2–3.2 mm diam., whitish to brownish. **Stem** climbing, cylindrical, fleshy, straight to sinuous, glabrous,

glaucous to dark green, strongly furrowed under arid conditions; internodes of ascendant stems 8–14 × 0.7–1.3 cm. **Leaves** 5.2–18 × 2.5–5.5 cm, alternate, distichous, elliptic to oblong, asymmetric, fleshy, glabrous, pale green to dark green, pseudopetiolate, margin entire, base attenuate, apex acuminate; pseudopetiole 5–10 mm concave. **Inflorescence** axillary, racemose, with up to 22 flowers opening in succession; 1–2 flowers opening each morning; rachis 5–9 × 0.9–1.3 cm, terete, pale green to dark green; bracts 4–7.5 × 4.5–8 mm, progressively smaller toward the apex, triangular/deltoid, coriaceous, concave, green, patent, apex acute, not incurved. **Flowers** resupinate, pedicellate, abscission layer between perianth and ovary present; pedicel with ovary 46–50 × 4.2–6 mm, triangular in transverse section, straight to incurved, whitish at the base, green to the apex, with a calyculus (6–6.5 × 3–3.5 mm) at the apex. **Sepals** 4.9–6.1 × 0.9–1.3 cm, free, oblanceolate, fleshy, slightly concave, spreading, pale green to yellowish, margin entire, involute at the base, base attenuate, apex acute to obtuse; dorsal sepal symmetric; lateral sepals asymmetric. **Petals** 4.9–6.1 × 0.9–1.2 cm, free, obliquely oblong-elliptic, asymmetric, lower margin more arcuate, yellowish at the base, pale green to the apex, base attenuate, apex obtuse to rounded, adaxial surface with a central and longitudinally disposed keel. **Labellum** 1-lobed to slightly 3-lobed, 5.3–6.2 × 3.3–4.2 cm, tubular, deepening near the middle, yellowish to the base, white in distal portion, unguiculate, with a central crest from the unguiculus to the apex, and a penicillate callus just below the anther; unguiculus fused along the margins of the basal half (ca 29–32 mm) of column length forming a nectar chamber, nectar chamber 1.4–1.6 cm long, tubular; central crest yellowish from the nectar chamber to the penicillate callus, dark yellow to the apex; distal portion of the central crest swollen, low cushion, rugose-papillose at the apex, with a group of transversal yellow-orange scales near the penicillate callus, with three yellow longitudinal lines near the apex; penicillate callus 5.1–5.5 × 3.8–4.2 mm, made by yellow-hyaline lacerate-laciniate scales and clusters of trichomes; lateral lobes not much evident, rounded, overlapping the column apex, margin undulate; midlobe deeply emarginated; margin undulate. **Column** 36–38 × 3.2–3.5 cm, trigonous, arched to the base, forming an angle ca 90° with the ovary, strait to the apex, ventral surface flat with white to yellowish hyaline trichomes over the distal half, attenuate to the base, dilated to the apex, with two lateral wings; lateral wings rounded, undulate. **Stigma** bilobed; rostellum 4–4.2 × 2.5–2.7 mm, trapezoidal, membranous, white. **Anther** 4.8–5.2 × 3.5–3.6 mm, rectangular to trapezoidal, white, versatile, apex truncate; pollen mass 2.9–3.2 × 2.9–3.1 mm, triangular, bipartite, whitish. **Fruits** 12–17 × 2.6–3.5 cm, oblong to clavate, arched, transversally subtrigonus, fleshy, brown indehiscent when mature, fragrant; pericarp soft; fruit cavity filled. **Seeds** ca 0.5 mm, ovoid, black.

Distribution and ecology. *Vanilla chamissonis* is endemic to the Brazilian Atlantic Forest (Fig. 4). The species is a nomadic vine found both on tall trees in the restinga

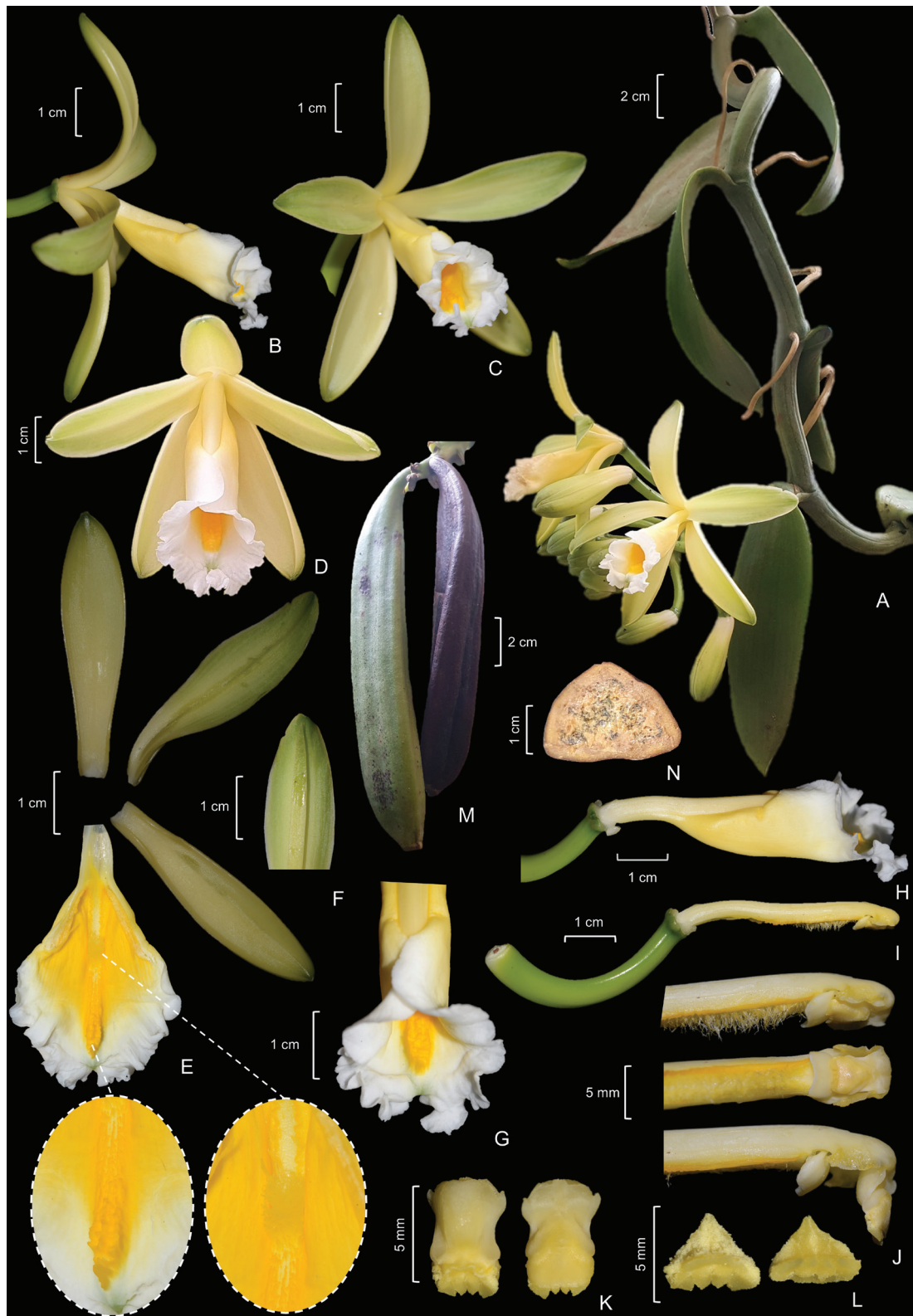


Figure 3. *Vanilla chamissonis* Klotzsch. A. Part of a flowering plant showing the stem, leaves, and inflorescence. B. Flower in lateral view. C. Flower in diagonal view. D. Flower in front view. E. Dissected perianth. The details (dashed areas) show the central labellar crest (left) and the penicillate callus (right). F. Detail of the adaxial surface of a petal showing the longitudinal keel. G. Detail of the apex of the labellum showing the yellowish longitudinal crest with three longitudinal ribs and the lateral lobes of the labellum overlapping the column apex. H. Pedicel/ovary, column, and labellum in lateral view. I. Pedicel/ovary and column in lateral view. J. Apex of the column: in lateral view with an articulated anther (above), in abaxial view (mid), and in lateral view with a disarticulated anther (below). K. Anther in dorsal view (left) and ventral view (right). L. Pollen mass in dorsal view (left) and ventral view (right). M. Mature fruits. N. Transversal section of a mature fruit. Note the filled fruit cavity. Based on E.R. Pansarin 1579 (LBMBP).

vegetation and on shrubs of the rocky outcrops close to the beach. The flowers are very fragrant and produce a small amount of nectar. The fruits have a bitter taste and an unpleasant odour. After 18 months from pollination, they turn brown and fall to the forest floor where they are consumed by agoutis.

Phenology. *Vanilla chamissonis* blooms from November to January. The fruits ripen from April to June, 18 months from pollination.

Preliminary IUCN conservation assessment. Endangered: EN B2ab(i,ii,iii). *Vanilla chamissonis* is distributed along the Brazilian coast. The species is particularly common in the Atlantic Rainforest of south and southeast Brazil. The populations are commonly composed of many individuals. The extent of occurrence (EOO) is estimated to be 229,334 km², which falls within the limits for Least Concern (LC) under criterion B1, according to the IUCN Red List categories and criteria. The area of occupancy (AOO) is estimated to be 128 km², which falls within the limits for Endangered (EN) under criterion B2. Considering that the Brazilian restingas have been reduced to scattered fragments due to the urban

occupation, I project a continuing decline in (i) extent of occurrence, (ii) area of occupancy, and (iii) extent and/or quality of habitat of *V. chamissonis*. Based on these threats and the fact that the species is distributed in less than five locations, *V. chamissonis* is assessed as Endangered: EN B2ab(i,ii,iii).

Additional material examined. BRAZIL – **Espírito Santo** • Espírito Santo, Afonso Cláudio, Serra Pelada, comunidade de Palmital, propriedade da família Brandenburg, Pedra do Sol, Proximidades da Pedra da Lajinha. Inselberg; 20°00'36"S, 41°04'30"W; 760 m; 21 Oct. 2019; C.N. Fraga & D.R. Couto 3912; MBML • Guarapari, Village do Sol; 20°33'16"S, 40°24'35"W; 27 Oct. 1984; B. Weinberg 645; MBML • Guarapari, Parque Estadual Paulo César Vinha; 20°36'19"S, 40°25'19"W; 17 Nov. 1994; C. N. de Fraga et al. 59; MBML • Jaguaré; 18°54'20"S, 40°04'33"W; 78 m; 30 Nov. 2012; D.A. Folli 6934; CVRD • Santa Leopoldina, Rio das Farinhas, propriedade da Sra. Maria Knak Ule. Mata, dossel; 20°01'21"S, 40°36'29"W; 827 m; 15 Apr. 2008; A.P. Fontana, L. Kollmann & K.A. Brahim 4969; MBML • Vila Velha, Jacarenema; 20°19'46"S, 40°17'32"W; 25 May 1990; J.M.L. Gomes &

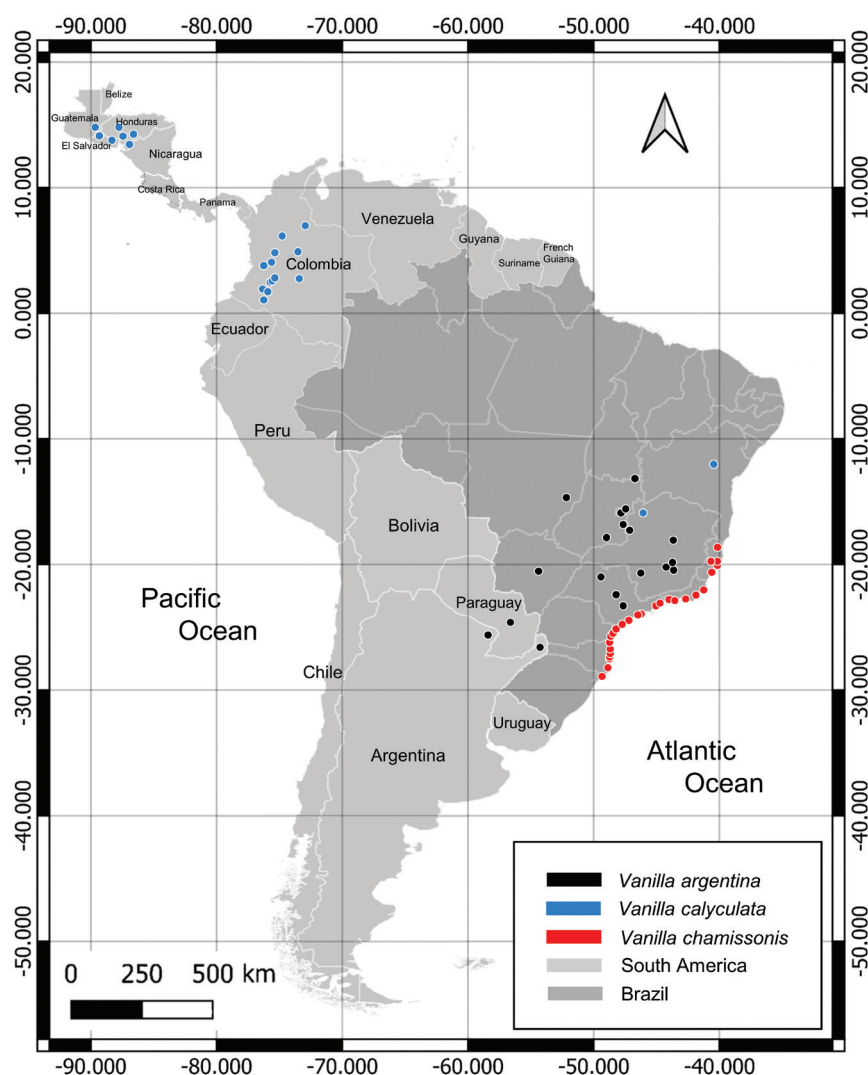


Figure 4. Occurrence map of *Vanilla argentina*, *V. chamissonis*, and *V. calyculata*.



Figure 5. Comparative morphology of leaves of related *Vanilla* species. A. *V. argentina*. B. *V. calyculata*. C. *V. chamissonis*. Scale bars = 2 cm. A from E.R. Pansarin 1570 (LBMBP), B from VAN 206 (*Vanilla* germplasm bank, LBMBP Orchid House), C from E.R. Pansarin 1579 (LBMBP).

O.J. Pereira 1129; VIES. – **Paraná** • Matinhos, ao pé do Morro do Tabaquara, perto de Matinhos; 25°49'02"S, 48°32'34"W; 3 Jan. 1967; J.C. Lindeman *et al.* 3855; MBM • Paranaguá, Ilha de Mel; 25°31'13"S, 48°30'33"W; 3–5 m; 28 Nov. 1970; G.G. Hatschbach 25679; NY • Paranaguá; 25°31'11"S, 48°30'33"W; 3–5 m; 23 Nov. 1994; J. Cordeiro 1201; HUEFS • Paranaguá, Ilha do Mel - Restinga da Praia Grande; 25°31'11"S, 48°30'33"W; 8 Nov. 1986; W.S. Souza 388; UPCB. – **Rio de Janeiro** • Angra dos Reis, Ilha Grande, Parcelas do Módulo Oeste do RAPELD Ilha Grande em Mata de restinga, Reserva Biológica Estadual da Praia do Sul; 23°10'36"S, 44°17'52"W; 13 Oct. 2016; A.C.R. Cruz & R.G. Diniz 2; RBR • Campos dos Goytacazes, Morro Itaoca - Morro do rato; 21°47'49"S, 41°26'52"W, 201 m; 23 Nov. 2008; A.S. Pessanha & M.T. Nascimento 48; HUENF • Maricá, Barra de Maricá, Área C1; 22°55'09"S, 42°49'06"W; 25 Oct. 1988; Occhioni *et al.* 9286; RFA • Macaé; 22°22'14"S, 41°47'12"W; 18 Sep. 2008; I.E. Santo & M.F. Castilho 174; R • Rio de Janeiro, Restinga da Marambaia; 23°03'11"S, 43°35'03"W; 20 May 2009; B.S. Haiad, C. Novaes & I. Soares *s.n.*; HUNI [HUNI4402]. – **Santa Catarina** • Balneário Camboriú, Taquarinhas; 26°59'26"S, 48°38'04"W; 17 Nov. 2002; C. Hering-Rinnert 244; JOI • Bombinhas, Praia de Zimbros; 27°08'16"S, 48°31'01"W; 35 m; 11 Nov. 2006; M.G. Caxambu 1270; HCF • Garopaba, Ouvidor - Praia Vermelha; 28°06'42"S, 48°38'02"W; 23 m; 7 Jul. 2018; A. Kassner-Filho 3056; FURB • Florianópolis, Morro do Ribeirão, Ilha de Santa Catarina; 27°35'48"S, 48°32'57"W; 100 m; 23 Sep. 1970; R.M. Klein & A. Bresolin 8773; FLOR • Florianópolis, Lagoa Pequena, Rio Tavares; 27°39'20"S, 48°28'18"W; 11 m; 2 Nov. 2017; G.D.S. Seger & E. Bach 843; ICN • Imaruí, Forquilha; 28°09'52"S, 48°52'10"W; 666 m; 28 Jan. 2010; J.L. Schmitt *et al.* 1111; FURB • Laguna, Praia

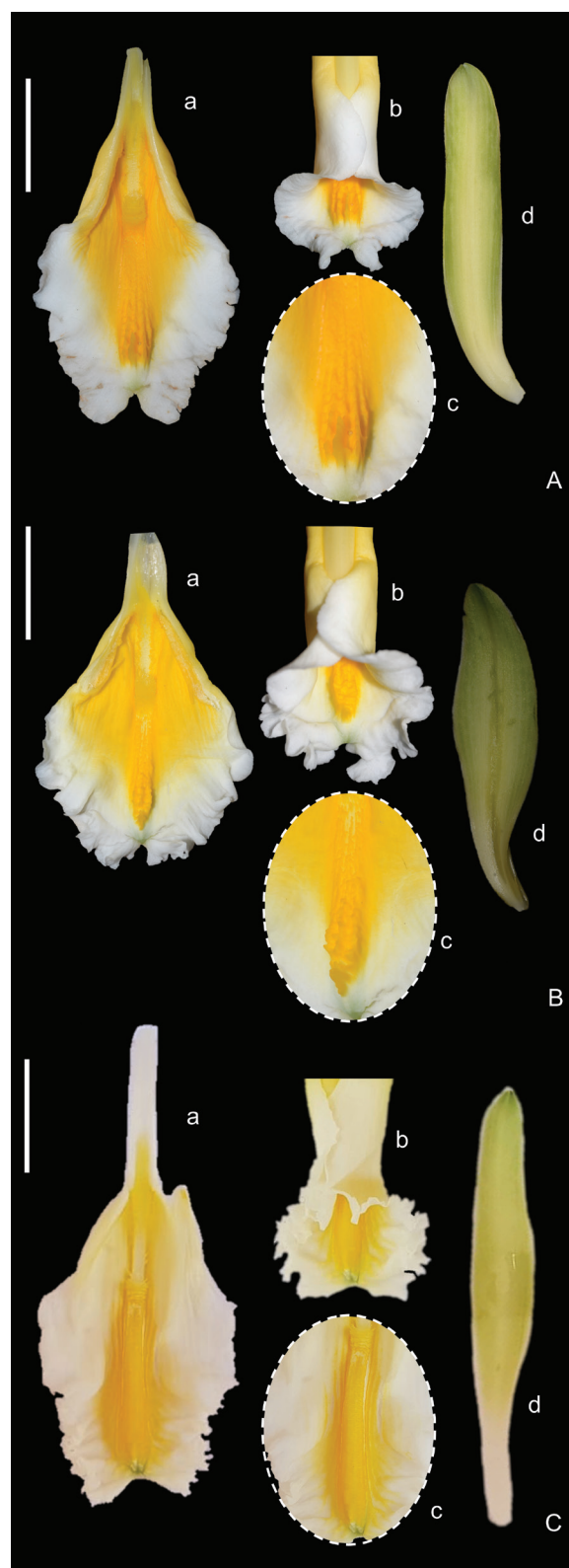


Figure 6. Comparative morphology of perianth parts of related *Vanilla* species. A. *V. argentina*. B. *V. chamissonis*. C. *V. calyculata*. Lowercase letters on the right side of the floral parts are: (a) distended labellum, (b) labellum apex, (c) detail of the labellar crest, (d) petal. Scale bars = 2 cm. A from E.R. Pansarin 1570 (LBMBP), B from E.R. Pansarin 1579 (LBMBP), C from VAN 206 (*Vanilla* germplasm bank, LBMBP Orchid House).

do Sol, Dunas fixas; 28°28'57"S, 48°46'50"W; 3–5 m; 13 Dec. 2000; *G. Hatschbach*, A.C. *Cervi* & *E. Barbosa* 71869; BHCB • Paulo Lopes; 28°01'21"S, 48°42'20"W; 123 m; 18 Nov. 2022; *M.E. Engels* 10283; UPCB • Penha, J.B. World Entretenimentos S/A; 26°48'06"S, 48°36'58"W; 7 m; 26 Dec. 2018; *A. Kassner-Filho* & *F.L.V. Bones* 4490; FURB • São Francisco do Sul, Parque Estadual do Acaraí - Praia do Ervino; 26°20'58"S, 48°33'47"W; 5 m; 29 Nov. 2010; *A. Korte* & *A.L. de Gasper* 5252; FURB • São Francisco do Sul; 26°14'35"S, 48°38'17"W; 5 m; 5 Apr. 2008; *L. Sevegnani s.n.*; FURB [FURB7432]. – **São Paulo** • Cananéia, Parque Estadual da Ilha do Cardoso, Trilha morro das almas; 25°00'36"S, 47°55'11"W; 19 Mar. 2003; *T.B. Breier* 953; UEC • Cananéia, Ilha do Cardoso, Restinga do Pereirinha; 25°00'36"S, 47°55'11"W; 2 Dec. 1990; *F. Barros* & *P. Martuscelli* 1989; SP • Iguape, Estação Ecológica Juréia-Itatins, Estrada entre o rio Una do Prelado e o Rio Verde, 18 km do rio Una, restinga junto à desembocadura do rio Verde; 24°42'00"S, 47°32'59"W; 9 Dec. 1995; *I. Cordeiro et al.* 1595; SP • Peruíbe, Estação Ecológica Juréia-Itatins, Barra do Uma; 24°19'12"S, 46°59'24"W; 20 Nov. 1990; *E.L.M. Catharino, et al.* 1503; SP • Praia Grande; 24°00'00"S, 46°24'00"W; 14 Nov. 1929; *A. Gehrt s.n.*; SP24495 • Praia Grande; 24°00'00"S, 46°24'00"W; 14 Nov. 1929; *A. Gehrt* 24495; NY • Ubatuba, Picinguaba; 23°25'47"S, 45°04'12"W; 6 Nov. 1988; *A. Furlan* 598; HRCB • Ubatuba, Picinguaba; 23°25'47"S, 45°04'12"W; 28 Dec. 2024; *E.R. Pansarin* 1579; LBMBP • Ubatuba, Estação Experimental; 23°25'47"S, 45°04'12"W; 23 Nov. 1940; *J.F. Cunha s.n.*; SP [SP44817].

Notes. Differences between specimens identified as *Vanilla chamissonis* from coastal populations and from the interior of Brazil have been recognized. The plants that occur inland are commonly referred to as "*V. chamissonis mineira*", in reference to their occurrence in Minas Gerais, a southeastern Brazilian state inserted in the Cerrado. Morphological differences among inland and coastal plants were formally recognized twice: Hoehne (1945) published the invalid name *V. chamissonis* var. *longifolia* based on a plant collected in the municipality of Itú, southeastern Brazil. Itú has areas typical of an open Cerrado vegetation with many rocky outcrops, while Hicken (1917) described *V. argentina* based on a plant collected in the Formosa region, Argentina. The region of Formosa, besides Corrientes and Misiones, is characterized as Arid Chaco or Dry Chaco. The Dry Chaco vegetation consists of a mosaic formed by xerophytic forests, gallery forests, and savannas. Here, *V. chamissonis*, which occurs in the Atlantic Forest, is considered as a distinct species from *V. argentina*, which is distributed in the Brazilian Cerrado and Dry Chaco. Some species described based on plant material collected in the state of the Rio de Janeiro, at the Brazilian coast, clearly are synonyms of *V. chamissonis*. This is the case for *Vanilla vellozoi* (as *V. vellozii*). Although some authors consider *V. vellozoi* as an obscure taxon related to *V. calyculata*, both plant material collected in the Rio de Janeiro (hosted at K), i.e. *A. Glaziou* 11621 and *A. Glaziou* 14302 (the latter designated by Soto Arenas and Cribb (2010) as lectotype of *V. vellozoi*, as *V. vellozii*), strongly agree with *V. chamissonis*. Apart from

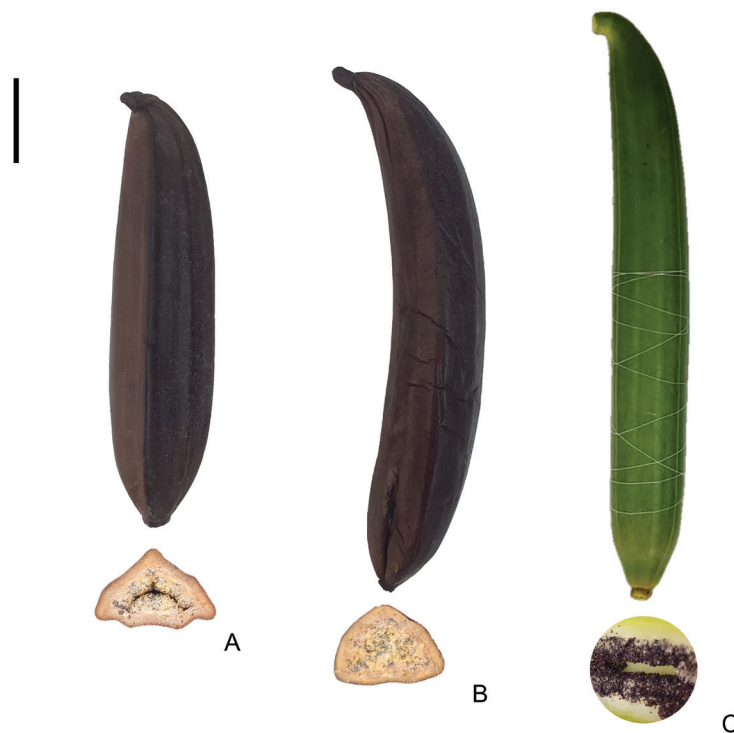


Figure 7. Comparative morphology of mature fruits of related *Vanilla* species. **A.** *V. argentina*. **B.** *V. chamissonis*. **C.** *V. calyculata*. Details in figures are transversal (A, B) or longitudinal (C) sections of mature fruits. Scale bar = 2 cm. A from *E.R. Pansarin* 1570 (LBMBP), B from *E.R. Pansarin* 1579 (LBMBP), C from VAN 206 (*Vanilla* germplasm bank, LBMBP Orchid House).

Table 2. Results of the experimental pollinations in order to know the breeding systems of *Vanilla argentea*, *V. chamissonis*, and *V. calyculata*: percentage of fruit set per treatment. The fruit set/flowers is indicated between parentheses.

Treatment	<i>V. argentea</i>	<i>V. chamissonis</i>	<i>V. calyculata</i>
Manual self-pollination	93.3% (28/30)	- (0/30)	- (0/30)
Spontaneous self-pollination	- (0/30)	- (0/30)	- (0/30)
Emasculation	- (0/30)	- (0/30)	- (0/30)
Cross-pollination	90% (27/30)	93.3% (28/30)	93.3% (28/30)

the small size of the flower structures, the shape of the leaves (symmetrically elliptic) and the short and robust inflorescence with patent triangular/rhomboidal bracts leave no doubt that both plant material are *V. chamissonis*. Soto Arenas and Cribb (2010) consider *V. argentea* to be synonymous with *V. vellozoi*. However, as presented here, *V. vellozoi* is related to *V. chamissonis*, not to *V. argentea*. The second taxon is *V. carinata*. The holotype of *V. carinata* has been considered as inadequate and conspecific with *V. planifolia* (Hoehne 1945). However, the material *J. Miers s.n.* (K000463750) was collected on the Atlantic Coast, in the state of Rio de Janeiro, where *V. planifolia* does not occur. Although I agree that the holotype is not in good condition, since it lacks vegetative structures, analysis of the reproductive characteristics, such as the presence of a calyculus, the robust inflorescence rachis with patent triangular/rhomboidal bracts, and the presence of an evident labellar keel leaves no doubt that *V. carinata* is conspecific with *V. chamissonis*. A third obscure taxon is *V. gardneri*. *Vanilla gardneri* was described based on several materials belonging to at least three *Vanilla* species (Soto Arenas and Cribb 2010). However, the material *G. Gardner 245* (K), collected at Morro do Flamengo, Rio de Janeiro, has been designated as the lectotype of *V. gardneri* (Soto Arenas and Cribb 2010). This exsiccate appears to contain a mixture of plant elements from two distinct species. While the vegetative portion and the flowers appear to be *V. phaeantha*, the fruit longitudinally sectioned strongly agree with those of *V. chamissonis*. This is corroborated by the fact that both species are sympatric in this region. Furthermore, the fruiting period of *V. phaeantha*, whose fruits ripen in nine months, does not overlap with its flowering period. On the other hand, the fruiting period of *V. chamissonis*, whose fruits take 18 months to mature, overlaps with the flowering period of both sympatric species, *V. phaeantha* and *V. chamissonis*. Therefore, based on the presence of a fruit of the latter species in the material *G. Gardner 245* (K), *V. gardneri* was synonymized here under *V. chamissonis*.

Morphological affinities. While *V. calyculata* and *V. argentea* occur in dryer environments, *V. chamissonis* is distributed throughout the Atlantic Forest sensu stricto (Fig. 4). *Vanilla chamissonis* is easily recognized by its elliptic to oblong and asymmetric leaf blades (Fig. 5), sepals 4.9–6.1 cm long, oblong-elliptic petals 4.9–6.1 cm long, labellum 5.3–6.2 cm long with a nectar chamber 1.4–1.6 cm long, column 36–38 mm long, and fruits whose pericarp is soft (Figs 6–7). Vegetative and floral

features of *V. chamissonis* suggest a close relationship with *V. calyculata* and *V. argentea*. All three species show robust climbing stems with long fleshy ascendant leaves and lateral inflorescences with whitish resupinate flowers. Flowers of *V. chamissonis* and related taxa also share several characteristics, such as an unguiculate labellum with an emarginated apical lobe, and a yellow central crest with a penicillate callus just below the anther. In addition, the inner surface of the labellum base of *V. chamissonis* and related taxa is yellow, while the distal portion is white. In particular, fruit features suggest a close relationship between *V. argentea* and *V. chamissonis*. Both species produce large brown and indehiscent fruits that take 18 months to mature. Fruits have an unpleasant fragrance and a bitter flavour. The close relationships among *V. argentea*, *V. chamissonis*, and *V. calyculata* is corroborated by molecular data (see further).

Phylogenetic relationships

The phylogenetic analysis of the ITS region resulted in strongly congruent MP (Fig. 8) and BI (Suppl. material 2) trees. In both analyses, the Neotropical *Vanilla* clade with membranaceous leaves (clade A; PP 1, BS 100%; Fig. 8, Suppl. material 2) emerges as sister to a large clade (PP 0.87, BS 100%) with two subclades: a predominantly Old World/Caribbean clade (clade B; PP 0.97, BS 100%) and a clade including the remaining Neotropical *Vanilla* (clade C; PP 0.96, BS 100%; Fig. 8, Suppl. material 2). Within the non-membranaceous Neotropical *Vanilla* (clade C), the taxa currently recognized among the *V. palmarum* group, i.e. *V. bicolor* Lindl., *V. palmarum* (Salzm. ex Lindl.) Lindl., and *V. sprucei* Rolfe, emerge in a basal position to a large clade including the remaining Neotropical species (PP 0.94, BS 59%). 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 (PP 1, BS 88%), and the other large clade includes the remaining Neotropical *Vanilla* (Fig. 8, Suppl. material 2). Within the *V. pompona* clade, *V. pompona* emerges as sister to a strongly supported clade (PP 0.99, BS 100%) including *V. chamissonis* and *V. calyculata/V. argentea* (PP 0.99, BS 65%; Fig. 8, Suppl. material 2).

Breeding systems

Vanilla chamissonis and *V. calyculata* were obligatorily self-sterile, where *V. argentea* was completely self-compatible (Table 2). Flowers setting fruits were from 90% to 93.3%

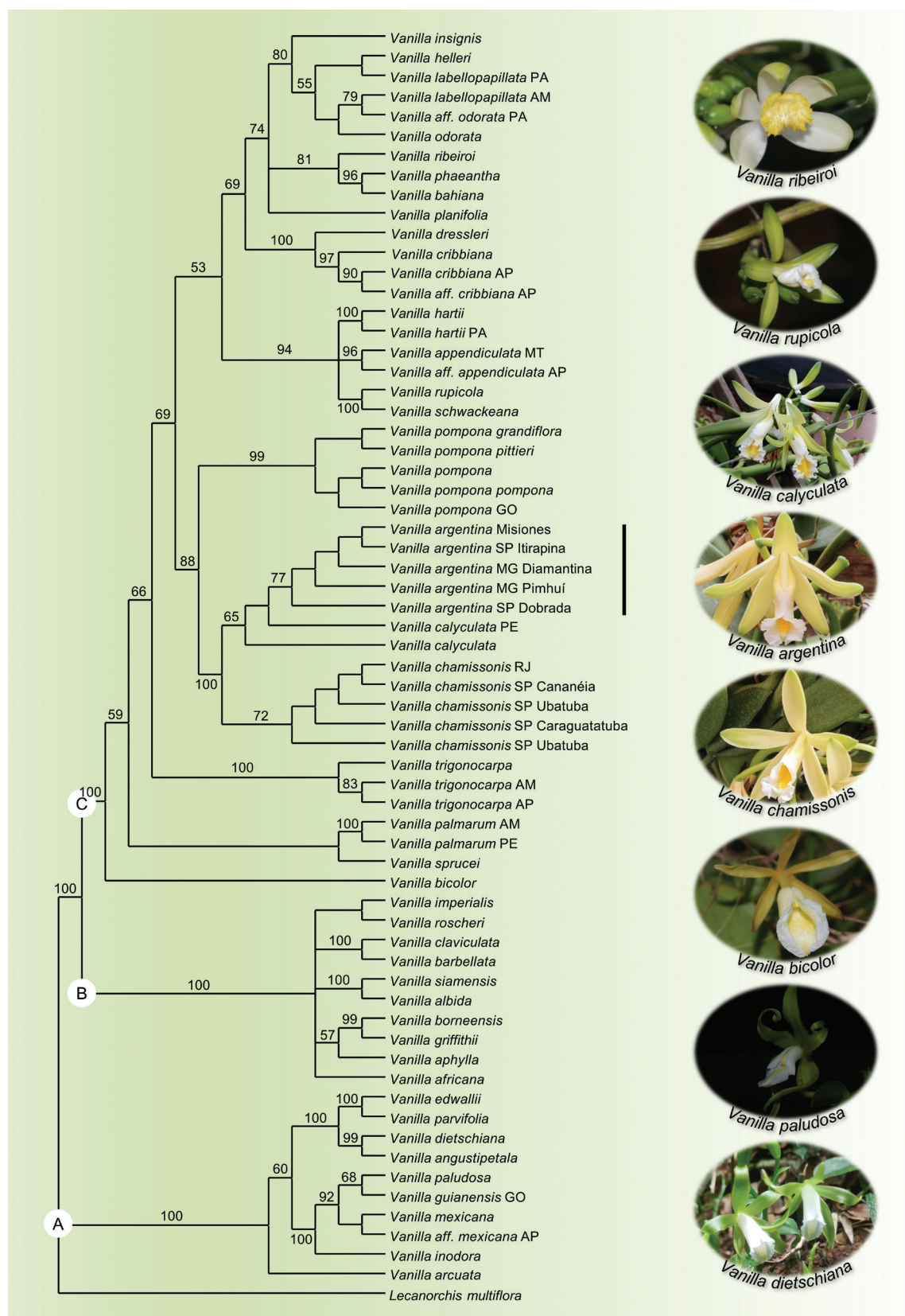


Figure 8. Maximum parsimony analysis of *Vanilla* (Orchidaceae) based on ITS (nrDNA) showing the phylogenetic relationships among *V. argentina* from multiple populations (vertical bar) and Neotropical congeners. Bootstrap values > 50% are given on the branches. A = Neotropical *Vanilla* with membranaceous leaves, B = Old World/Caribbean clade, C = Neotropical *Vanilla* with non-membranaceous leaves. Acronyms are Brazilian states: AM = Amazonas, AP = Amapá, GO = Goiás, MG = Minas Gerais, MT = Mato Grosso, PA = Pará, PE = Pernambuco, SP = São Paulo, RJ = Rio de Janeiro. Names after the acronyms MG and SP are Brazilian municipalities of Minas Gerais and São Paulo, respectively.

($n = 27\text{--}28$) in cross-pollinations, and 93.3% ($n = 28$) in manual self-pollination (*V. argentina*). No fructification was recorded in either intact (bagged) or emasculated flowers, showing that a biotic pollen vector is necessary for pollen transference. Fruits were mature from nine months (*V. calyculata*) to 18 months after pollination (*V. chamissonis* and *V. argentina*). Among species yielding fruits through self-pollination (*V. argentina*), there was no significant difference ($t\text{-test} = -1.734$, $d.f. = 0.1$, $p = 0.313$) between the mean fruit set resulting from self- and cross-pollinations. Among self-sterile species (*V. chamissonis* and *V. calyculata*), the fruits aborted three months after manual self-pollination.

DISCUSSION

All evidence presented here, i.e. morphological, molecular, and ecological data, supports *Vanilla argentina* as a taxon distinct from its Neotropical congeners. *Vanilla argentina* emerges among the members from the “*Vanilla pompona* group” (Soto Arenas and Cribb 2010), in a clade including *V. chamissonis* and *V. calyculata*. Although Pansarin (2024a) and Pansarin and Menezes (2023) show *V. paulista* among the members of the “*Vanilla pompona* group”, the DNA sample on which their analyses were carried out was obtained from a juvenile specimen of *V. chamissonis* collected in the Atlantic Forest and cultivated in the LBMBP Orchid House. After flowering, the identification of the sample as *V. chamissonis* was confirmed. Actually, *V. paulista* emerges in a clade including *V. dubia* (Emerson R. Pansarin unpubl. data).

While widely distinguished by Brazilian orchidologists and vanilla producers, the species occurring in inland South America has been considered as a synonym of *V. chamissonis* (e.g. BFG 2025; Soto Arenas and Cribb 2010). However, this taxon was formally recognized as a distinct taxon from *V. chamissonis* by Hicken (1917) and Hoehne (1945). *Vanilla chamissonis* var. *longifolia* was never validly published, as Hoehne (1945) did not provide a diagnosis in Latin for this taxon. A Latin diagnosis became mandatory from 1935, i.e. Art. 39.1 (Turland et al. 2018). Conversely, *V. argentina* was validly published by Hicken (1917). Although *V. argentina* has been synonymized under *V. chamissonis* (e.g. BFG 2025), Soto Arenas and Cribb (2010) considered *V. argentina* as conspecific to *V. vellozoi* (as *V. vellozii*). Their assertion is based on the fact that the latter species differs from *V. chamissonis* by its shorter perianth parts and a broader subentire labellum. *Vanilla vellozoi* was described based on plants collected in restinga areas from Rio de Janeiro, the habitat of *V. chamissonis*. The material on which the description of *V. vellozoi* was based (Glaziou 11621, 14302), except for the length of flower segments, strongly agrees with those of *V. chamissonis*. Thus, *V. vellozoi* seems to be conspecific with *V. chamissonis*, not with *V. argentina*. Besides *V. vellozoi*, two other *Vanilla* names were clarified here. Although the holotype of *V. carinata* is not in good condition, the presence of a calyculus, the robust inflorescence

rachis with patent triangular/rhomboidal bracts, and the presence of an evident labellar keel leave no doubt that *V. carinata* is conspecific with *V. chamissonis*. Another obscure taxon clarified here is *V. gardneri*. Although this species was described based on several materials belonging to at least three *Vanilla* species, Soto Arenas and Cribb (2010) selected the material G. Gardner 245 (K) as the lectotype of *V. gardneri*. The plant material was collected at Morro do Flamengo, Rio de Janeiro where two *Vanilla* species occur, *V. phaeantha* and *V. chamissonis*. While the vegetative portion and the flowers appear to be those of *V. phaeantha*, the fruit strongly agrees with those of *V. chamissonis*. It is very plausible, as the fruiting period of *V. phaeantha*, does not overlap with its flowering season (Pansarin 2022). Conversely, the fruits of *V. chamissonis* take 18 months to mature (Pansarin 2024b), overlapping with its flowering season.

The morphology of *V. argentina* suggests a close relationship with *V. chamissonis*. However, molecular data reveal that *V. argentina* is more closely related to *V. calyculata* than to *V. chamissonis*. The relationship between *V. argentina* and *V. calyculata* has been shown in previous studies involving Neotropical *Vanilla* (Pansarin and Menezes 2023; Cascales et al. 2023; Pansarin 2024a). *Vanilla calyculata* is clearly distinct from *V. chamissonis* (see Table 2). In addition, *V. calyculata* occurs in xeric environments (e.g. Soto Arenas and Dressler 2010; Flanagan et al. 2025), while *V. chamissonis* is distributed within the Atlantic Rainforest (Hoehne 1945). *Vanilla calyculata* has been assumed to be a synonym of *V. columbiana*, however, the latter is related to *V. phaeantha*, not to *V. calyculata*, as the description of *V. columbiana* was clearly based on a flower bud (Soto Arenas and Cribb 2010; Flanagan et al. 2025).

As far as known, the vast majority of Neotropical thick-leaved *Vanilla* are self-compatible but require a biotic vector for pollen transfer (e.g. Pansarin and Pansarin 2014; Pansarin 2023). However, most basal taxa among tick-leaved *Vanilla*, i.e. *V. bicolor*, *V. lindmaniana* Kraenzl., and *V. palmarum* (Pansarin 2024a, 2025; Pansarin and Ferreira 2022), set fruit by means of autonomous self-pollination (Gigant et al. 2011; Pansarin and Ferreira 2022; Pansarin 2025). Here, a breeding system based on total self-sterility is shown for the first time for *Vanilla*. Based on the fact that fruits of both *V. chamissonis* and *V. calyculata* abort 3–4 months from self-pollination, a late-action self-incompatibility can be involved. In a late-acting self-sterility system, the pollen from self-pollination successfully reaches the ovule, but embryos do not develop (Pansarin and Pansarin 2011).

CONCLUSION

Here, it was possible to demonstrate that species that occur in the Cerrado and Dry Chaco (*V. argentina*) correspond to a taxon distinct from *V. chamissonis*, which is distributed along the Brazilian coast. Thus, as currently known, the *Vanilla chamissonis* clade includes three

species: *V. argentina*, *V. calyculata*, and *V. chamissonis*. Beyond morphological comparisons of vegetative and reproductive characteristics that provide distinguishing features among the three *Vanilla* species, this study also includes phylogenetic inference with strong group representation, as well as ecological and biogeographic information. Each of these components contributed valuable data for making taxonomic decisions. A multisource approach (i.e. integrative taxonomy) plus extensive field work has been crucial to understand the species boundaries among Neotropical *Vanilla*. In addition, field investigations in the environments where the type specimens were collected has been critical to elucidate the circumscription of obscure *Vanilla* species.

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SUPPLEMENTARY MATERIALS

Supplementary material 1

Taxa of *Vanilla* included in the molecular study, their locations (State and country), vouchers, and GenBank accession numbers. VAN = *Vanilla* germplasm bank, LBMBP Orchid House, Department of Biology, FFCLRP-USP, University of São Paulo, Brazil.

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Supplementary material 2

Bayesian inference analysis of *Vanilla* (Orchidaceae) based on ITS (nrDNA) showing the phylogenetic relationships among members of the *V. chamissonis* clade and Neotropical congeners. Note the position of *V. argentea* (arrow). Posterior probability values > 0.5 are given on the branches. A = Neotropical *Vanilla* with membranaceous leaves, B = Old World/Caribbean clade, C = Neotropical *Vanilla* with non-membranaceous leaves. The acronym MG is the Brazilian state Minas Gerais.

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