European Journal of Taxonomy 284: 1–26
http://dx.doi.org/10.5852/ejt.2017.284
This work is licensed under a Creative Commons Attribution 3.0 License.

ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2017 · Azofeifa-Bolaños J.B. *et al.*

DNA Library of Life, research article

# A new vanilla species from Costa Rica closely related to *V. planifolia* (Orchidaceae)

José B. AZOFEIFA-BOLAÑOS [1], L. Rodolphe GIGANT [2], Mayra NICOLÁS-GARCÍA [3], Marc PIGNAL [4], Fabiola B. TAVARES-GONZÁLEZ [5], Eric HÁGSATER [6], Gerardo A. SALAZAR-CHÁVEZ [7], Delfino REYES-LÓPEZ [8], Fredy L. ARCHILA-MORALES [9], José A. GARCÍA-GARCÍA [10], Denis da SILVA [11], Agathe ALLIBERT [12], Frank SOLANO-CAMPOS [13], Guadalupe del Carmen RODRÍGUEZ-JIMENES [14], Amelia PANIAGUA-VÁSQUEZ [15], Pascale BESSE [16], Araceli PÉREZ-SILVA [17] & Michel GRISONI [18,*]

[1,15] Universidad Nacional de Costa Rica, Instituto de Investigación y Servicios Forestales, Heredia, Costa Rica.
[2,11,16] Université de La Réunion, UMR PVBMT, Saint Denis, La Réunion, France.
[3,17] Instituto Tecnológico de Tuxtepec, Tuxtepec (Oax), Mexico.
[4] Muséum national d'Histoire naturelle, UMR 7205 – ISYEB CNRS-MNHN-UPMC-EPHE, Paris, France.
[5,14] Instituto Tecnológico de Veracruz, Veracruz (Ver), Mexico.
[6] Herbario AMO, México (DF), Mexico.
[7] Universidad Autónoma de México, Instituto de Biología, México (DF), Mexico.
[8] Benemérita Universidad Autónoma de Puebla, Facultad de Ingeniería Agro-hidráulica, Teziutlán (Pue), Mexico.
[9] Herbario BIGU, Universidad de San Carlos de Guatemala, Guatemala.
[10] Universidad Nacional de Costa Rica, Escuela de Ciencias Agrarias, Heredia, Costa Rica.
[12,18] CIRAD, UMR PVBMT, Saint Pierre, La Réunion, France.
[13] Universidad Nacional de Costa Rica, Laboratorio de Biotecnología de Plantas, Heredia, Costa Rica.

[*] Corresponding author: michel.grisoni@cirad.fr
[1] Email: jb12azo@gmail.com
[2] Email: rodolphe.gigant@cirad.fr
[3] Email: aryam_89_01@hotmail.com
[4] Email: pignal@mnhn.fr
[5] Email: arjfabby@hotmail.com
[6] Email: herbamo@prodigy.net.mx
[7] Email: gasc@ib.unam.mx
[8] Email: delfino_reyes2001@yahoo.com.mx
[9] Email: archilae@hotmail.com
[10] Email: dendrogar@yahoo.com
[11] Email: denis.dasilva@univ-reunion.fr
[12] Email: agathe.allibert@cirad.fr
[13] Email: frank.solano.campos@una.cr
[14] Email: lupitarj@itver.edu.mx
[15] Email: amelia.paniagua.vasquez@una.cr

[16] Email: pascale.besse@univ-reunion.fr
[17] Email: apsilva30@hotmail.com

**Abstract.** We describe a new vanilla species growing in sympatry with *Vanilla planifolia* Jacks. ex Andrews (Orchidaceae) in the province of Limón, Caribbean coast of Costa Rica. The morphology of the reproductive and vegetative organs observed on vines cultivated under shade-house, the nuclear (Internal Transcribed Spacer) and plastid (*mat*K) nucleotide sequences, as well as the contents of aromatic compounds measured in ripe fruits, show that this species is close to but distinct from *V. planifolia.* The name *V. sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. is proposed for this new *Vanilla* species endemic in Costa Rica. It is especially distinguished from *V. planifolia* by a reduction of about 30% of the size of the fruits and flowers, by a divergence of ITS sequences for at least two species-conserved nucleotides compared to seven other species of the *V. planifolia* group, and by the presence of anisic compounds and low content of phenolic compounds (including vanillin) in the fruits. These results confirmed the extension of the area of distribution of *V. planifolia* southward to Costa Rica, where a recent speciation process occurred. Because of its particular agronomic and aromatic properties, *V. sotoarenasii* sp. nov. could represent a valuable biological resource for the vanilla industry.

**Keywords.** Barcoding, Costa Rica, Limón, radiation, *Vanilla planifolia*, *V. sotoarenasii*.

Azofeifa-Bolaños J.B., Gigant L.R., Nicolás-García M., Pignal M., Tavares-González F.B., Hágsater E., Salazar-Chávez G.A., Reyes-López D., Archila-Morales F.L., García-García J.A., da Silva D., Allibert A., Solano-Campos F., Rodríguez-Jimenes G.d.C., Paniagua-Vásquez A., Besse P., Pérez-Silva A. & Grisoni M. 2017. A new vanilla species from Costa Rica closely related to *V. planifolia* (Orchidaceae). *European Journal of Taxonomy* 284: 1–26. http://dx.doi.org/10.5852/ejt.2017.284

## Introduction

The *Vanilla* genus, *Vanilla* Plum. ex Miller (Miller 1754), belongs to the subfamily Vanilloideae, tribe Vanillinae (Cameron 2010). It is an ancient group of tropical orchids that originated in America about 70 million years ago and differentiated in America, Africa and Asia (Ramírez *et al.* 2007; Bouétard *et al.* 2010). The classification of the species of *Vanilla* was recently reviewed by Soto Arenas & Cribb (2010), who divided the genus into two subgenera, *Vanilla* and *Xanata* Soto Arenas & P.J.Cribb, and further split the subgenus *Xanata* into two sections *Xanata* and *Tethya* Soto Arenas & P.J.Cribb. Currently, about 110 *Vanilla* species are reported and clustered into 20 groups (Portères 1954; Soto Arenas & Cribb 2010; Cameron 2011; Pansarin *et al.* 2012). The *V. planifolia* group is the most important by the number of species (16) and, economically, because it contains the commercial species *V. planifolia* Jacks. ex Andrews (Andrews 1808) and *V.* × *tahitensis* J.W.Moore (Moore 1933), the aromatic fruits of which provide the vanilla flavor used by the food and perfumes industries. The taxonomy of the species from Mexico and Central America was reviewed by Soto Arenas & Dressler (2010) on the basis of their morphology and Internal Transcribed Spacer (ITS) DNA sequences. Recently, two new species have been described in the region, *V. esquipulensis* Archila & Chiron (Archila & Chiron 2012) in Guatemala and *V. rivasii* Molineros, R.T.González, Flanagan & J.T.Otero (Molineros-Hurtado *et al*. 2014) in Chocó, northern Colombia, plus two more in French Guiana, *V. inornata* Sambin & Chiron (Sambin & Chiron 2015) and *V. aspericaulis* Sambin & Chiron (Sambin & Chiron 2015).

Ten out of the 17 species recorded in Mexico and Central America have been reported in Costa Rica (Soto Arenas & Dressler 2010), namely: *V. costaricensis* Soto Arenas (Soto Arenas & Dressler 2010), *V. dressleri* Soto Arenas (Soto Arenas & Dressler 2010), *V. hartii* Rolfe (Rolfe 1899), *V. helleri* A.D.Hawkes (Heller & Hawkes 1966), *V. inodora* Schiede (Schiede 1829), *V. odorata* C.Presl (Presl

1827), *V. planifolia*, *V. pompona* Schiede (Schiede 1829), *V. sarapiquensis* Soto Arenas (Soto Arenas & Dressler 2010) and *V. trigonocarpa* Hoehne (Hoehne 1944).

During surveys carried out in Costa Rica since 2012 (Azofeifa-Bolaños *et al*. 2014) abundant populations of vanilla which did not fit any species previously described in the country were observed in Limón Province (Caribbean coast of Costa Rica). This vanilla, called "vanilla Limón" (VanL), had strong morphological similarities with a vanilla accession collected in Cahuita (Limón, Costa Rica) in 1993, referenced *Pignal 396b* (P00075132) in the herbarium of the Muséum national d'Histoire naturelle in Paris, P (herbarium acronym following Thiers continuously updated). Living specimens of this accession were preserved in the Emmanuel Liais park (Cherbourg-en-Cotentin, France) under accession number CH554, and subsequently in the Biological Resources Centre (BRC) Vatel (Saint Pierre, La Réunion) under accession number CR0068, and were tentatively classified as *V.* aff. *planifolia*.

Molecular approaches have enhanced plant taxonomy by allowing reliable genealogy-based classifications (Besse 2014). In the last decade, nuclear, mitochondrial and chloroplastic DNA sequencing has been used to study plant diversity and resolve taxonomic positions in all plant families, including the puzzling group of vanilloid orchids (Cameron 2009, 2010; Soto Arenas & Dressler 2010).

The aim of this study was thus to investigate the taxonomic status of VanL by using complementary approaches involving morphology of reproductive and vegetative organs, molecular barcoding and the accumulation of secondary metabolites in fruits.

## Material and methods

### Plant and DNA samples

The plant samples used in this study were obtained from field surveys carried out in Costa Rica, Mexico and Guatemala, supplemented with lyophilized plant materials preserved in the Mexican National Collection (Reyes-López *et al*. 2014), the BRC Vatel (Roux-Cuvelier & Grisoni 2010), and DNA from the herbarium of the Instituto Chinoin, AMO (Thiers continuously updated).

The survey in Costa Rica was conducted between November 2012 and March 2016 in the entire country. Prospections were targeted on areas where vanilla were recorded based on herbarium specimen information and consulting orchid experts, local guides, and earlier project experiences, among others. The material was collected according to permit number 061-2013 issued on 12 Jun. 2013 by National System of Conservation Areas (SINAC) of the Ministry of Environment and Energy (MINAE).

All plant and DNA samples analyzed in this study are listed in Table 1.

### Morphological traits

To compare the morphology of vegetative and reproductive organs of VanL to those of *V. planifolia*, the following traits were measured on plants cultivated in controlled conditions in BRC Vatel in La Réunion: length and greater width of the third and fourth leaves, diameter and length of the third inter-node, length and width of the tube, length of the ovary, length and width of the dorsal sepal. Measurements were carried out on two to six plants per accession grown under shade-house in November, which is the optimum vegetative growth and flowering period in La Réunion. From 14 to 30 fresh organs were measured per accession according to the availability of material. Length and weight of fruits were also measured at maturity (in July). We used the experimental tool "collaboratoire" of the national French infrastructure e-ReColNat (ANR-11-INBS-0004) for specimen comparisons.

**Table 1.** Identification and origin of the accessions of species of *Vanilla* Plum. ex Miller used in this study. [continued on next 3 pages]

| Code | Species | Country of origin | Depository | Locus | Code in collection / voucher |
|---|---|---|---|---|---|
| bahi0071 | *V. bahiana* | Brazil | VATEL | *mat*K | CR0071 |
| bahi0086 | *V. bahiana* | Brazil | VATEL | ITS & *mat*K | CR0086 |
| bahi0098 | *V. bahiana* | Brazil | VATEL | ITS | CR0098 |
| bahi0099 | *V. bahiana* | Brazil | VATEL | ITS & *mat*K | CR0099 |
| bahi0668 | *V. bahiana* | Brazil | VATEL | *mat*K | CR0668 |
| caly-01 | *V. calyculata* | Honduras | UNAM | ITS | Linares_3386 |
| cham0666 | *V. chamissonis* | Brazil | VATEL | ITS & *mat*K | CR0666 |
| crib0109 | *V. cribbiana* | French Guyana | VATEL | ITS & *mat*K | CR0109 |
| crib0122 | *V. cribbiana* | French Guyana | VATEL | *mat*K | CR0122 |
| cribb-01 | *V. cribbiana* | Mexico | UNAM | ITS | Soto_7940 |
| cribb-02 | *V. cribbiana* | Mexico | UNAM | ITS | Soto8439(nueva) |
| cribb-03 | *V. cribbiana* | Mexico | UNAM | ITS | Soto_8370 |
| dres-01 | *V. dressleri* | Costa Rica | UNAM | ITS | ByrdIA6(azul) |
| dres-02 | *V. dressleri* | Costa Rica | UNAM | ITS | Byrd_IIC3 |
| dres-03 | *V. dressleri* | Costa Rica | UNAM | ITS | Byrd_ID3 |
| dres-04 | *V. dressleri* | Costa Rica | UNAM | ITS | Byrd_IIC2 |
| hart-01 | *V. hartii* | Costa Rica | UNAM | ITS | Byrd_IIF1 |
| hart-03 | *V. hartii* | ? | UNAM | ITS | MAS7956 |
| hart-04 | *V. hartii* | Costa Rica | UNAM | ITS | Salas_1 |
| hell-0 | *V. helleri* | Mexico | UNAM | ITS | Soto8818 |
| hell-01 | *V. helleri* | Costa Rica | UNAM | ITS | Byrd_IID5 |
| hell-02 | *V. helleri* | Costa Rica | UNAM | ITS | Byrd_IIF4 |
| insi-02 | *V. insignis* | Mexico | UNAM | ITS | Soto_7668 |
| insi-03 | *V. insignis* | Honduras | UNAM | ITS | Linares7 |
| insi-04 | *V. insignis* | Guatemala | UNAM | ITS | Soto_8611 |
| insi2507 | *V.* cf. *insignis* | Mexico | ITT | *mat*K | muestra-13 |
| insi2519 | *V.* cf. *insignis* | Mexico | ITT | ITS | muestra-25 |
| insi2520 | *V.* cf. *insignis* | Mexico | ITT | ITS | muestra-26 |
| insi2521 | *V.* cf. *insignis* | Mexico | ITT | ITS & *mat*K | muestra-27 |
| insi2542 | *V.* cf. *insignis* | Mexico | ITT | ITS & *mat*K | muestra-20 |
| insi2545 | *V.* cf. *insignis* | Mexico | ITT | ITS | muestra-23 |
| insi2583 | *V. insignis* | Mexico | ITT | ITS | muestra-33 |
| insi2584 | *V.* cf. *insignis* | Mexico | ITT | ITS | muestra-34 |
| insi2594 | *V.* cf. *insignis* | Mexico | BUAP | ITS | #005 |
| insi2618 | *V. insignis* | Mexico | BUAP | *mat*K | CR2618 |
| insi2672 | *V. insignis* | Guatemala | Orquideario_Archila | ITS | VG-002 |
| insig2548 | *V.* cf. *insignis* | Mexico | ITT | *mat*K | muestra-26 |
| lind0682 | *V. lindmaniana* | French Guyana | VATEL | ITS & *mat*K | CR0682 |
| odor-01 | *V. odorata* | ? | UNAM | ITS | Soto_8822 |
| odor0116 | *V. odorata* | French Guyana | VATEL | ITS & *mat*K | CR0116 |
| odor0117 | *V. odorata* | French Guyana | VATEL | *mat*K | CR0117 |

| Code | Species | Country of origin | Depository | Locus | Code in collection / voucher |
|---|---|---|---|---|---|
| odor-02 | *V. odorata* | ? | UNAM | ITS | Soto_8797 |
| odor-04 | *V. odorata* | ? | UNAM | ITS | Soto_8365 |
| odor-05 | *V. odorata* | ? | UNAM | ITS | Soto_8356 |
| odor-06 | *V. odorata* | ? | UNAM | ITS | Soto_7955 |
| odor2154 | *V. odorata* | Mexico | CITRO | ITS | V. odorata |
| odor2671 | *V. planifolia* | Guatemala | OrquidearioArchila | ITS | VG-001 |
| odor686 | *V. odorata* | ? | VATEL | *mat*K | CR0686 |
| odor-sn | *V. odorata* | Surinam | UNAM | ITS | Hagsater11881 |
| phae-01 | *V. phaeantha* | Mexico | UNAM | ITS | Carnevali_4825 |
| phae-02 | *V. phaeantha* | ? | UNAM | ITS | Kew |
| phae-03 | *V. phaeantha* | Panama | UNAM | ITS | Soto_9920 |
| phae1522 | *V. phaeantha* | Madagascar | VATEL | *mat*K | CR1522 |
| phae1524 | *V. phaeantha* | Madagascar | VATEL | *mat*K | CR1524 |
| phae1525 | *V. phaeantha* | Madagascar | VATEL | *mat*K | CR1525 |
| phae1526 | *V. phaeantha* | Madagascar | VATEL | *mat*K | CR1526 |
| pla2506 | *V. planifolia* | Mexico | ITT | *mat*K | CR2506 |
| plan_KJ566306 | *V. planifolia* | ? | Genbank | *mat*K | KJ566306 |
| plan0001 | *V. planifolia* | La Réunion | VATEL | ITS & *mat*K | CR0001 |
| plan0020 | *V. planifolia* | La Réunion | VATEL | ITS & *mat*K | CR0020 |
| plan0024 | *V. planifolia* | La Réunion | VATEL | ITS & *mat*K | CR0024 |
| plan0027 | *V. planifolia* | La Réunion | VATEL | ITS | CR0027 |
| plan0038 | *V. planifolia* | La Réunion | VATEL | ITS & *mat*K | CR0038 |
| plan0043 | *V. planifolia* | La Réunion | VATEL | ITS | CR0043 |
| plan0196 | *V. planifolia* | La Réunion | VATEL | ITS & *mat*K | CR0196 |
| plan-02 | *V.* cf. *planifolia* | Costa Rica | UNAM | ITS | Byrd_IIA1 |
| plan-03 | *V.* cf. *planifolia* | Honduras | UNAM | ITS | Linares8BF |
| plan-05 | *V. planifolia* | Mexico | UNAM | ITS | Soto8355(nueva) |
| plan-06 | *V. planifolia* | Costa Rica | UNAM | ITS | Pupulin_1966 |
| plan0628 | *V. planifolia* | La Réunion | VATEL | ITS | CR0628 |
| plan-08 | *V. planifolia* | Mexico | UNAM | ITS | MAS_8526 |
| plan0802 | *V. planifolia* | La Réunion | VATEL | ITS & *mat*K | CR0802 |
| plan0831 | *V. planifolia* | Mayotte | VATEL | ITS | CR0831 |
| plan0836 | *V. planifolia* | Mayotte | VATEL | ITS | CR0836 |
| plan0852 | *V. planifolia* | Comores | VATEL | ITS | CR0852 |
| plan0862 | *V. planifolia* | Comores | VATEL | ITS | CR0862 |
| plan0876 | *V. planifolia* | Comores | VATEL | ITS | CR0876 |
| plan0883 | *V. planifolia* | Comores | VATEL | ITS | CR0883 |
| plan-14 | *V. planifolia* | ? | UNAM | ITS | KewPWC |
| plan1563 | *V. planifolia* | Madagascar | VATEL | ITS | CR1563 |
| plan1570 | *V. planifolia* | Madagascar | VATEL | ITS | CR1570 |
| plan-18 | *V. planifolia* | Mexico | UNAM | ITS | Soto8808 |
| plan-22 | *V. planifolia* | ? | UNAM | ITS | clon1324 |
| plan2497 | *V. planifolia* | Mexico | ITT | *mat*K | muestra-03 |
| plan2499 | *V.* cf. *planifolia* | Mexico | ITT | *mat*K | muestra-05 |

| Code | Species | Country of origin | Depository | Locus | Code in collection / voucher |
|---|---|---|---|---|---|
| plan2500 | *V.* cf. *planifolia* | Mexico | ITT | *mat*K | muestra-06 |
| plan2518 | *V. planifolia* | Mexico | ITT | ITS & *mat*K | muestra-24 |
| plan2523 | *V. planifolia* | Mexico | ITT | ITS | muestra-29 |
| plan2530 | *V. planifolia* | Mexico | ITT | ITS | muestra-01 |
| plan2531 | *V. planifolia* | Mexico | ITT | ITS | muestra-02 |
| plan2533 | *V. planifolia* | Mexico | ITT | ITS & *mat*K | muestra-09 |
| plan2534 | *V. planifolia* | Mexico | ITT | ITS | muestra-10 |
| plan2535 | *V. planifolia* | Mexico | ITT | ITS | muestra-11 |
| plan2540 | *V. planifolia* | Mexico | ITT | ITS | muestra-18 |
| plan2541 | *V. planifolia* | Mexico | ITT | ITS | muestra-19 |
| plan2544 | *V. planifolia* | Mexico | ITT | ITS | muestra-22 |
| plan2549 | *V. planifolia* | Mexico | ITT | ITS | muestra-28 |
| plan2551 | *V. planifolia* | Mexico | ITT | ITS | muestra-30 |
| plan2552 | *V. planifolia* | Costa Rica | UNA | ITS | UNA-VAN-0126 |
| plan2555 | *V. planifolia* | Costa Rica | UNA | ITS | UNA-VAN-0198 |
| plan2559 | *V. planifolia* | Costa Rica | UNA | ITS | none |
| plan2582 | *V. planifolia* | Mexico | ITT | ITS | muestra-32 |
| plan2586 | *V. planifolia* | Mexico | ITT | ITS | muestra-36 |
| plan2587 | *V. planifolia* | Mexico | ITT | ITS | muestra-37 |
| plan2588 | *V. planifolia* | Mexico | ITT | ITS & *mat*K | muestra-38 |
| plan2589 | *V. planifolia* | Mexico | ITT | *mat*K | muestra-39 |
| plan2590 | *V. planifolia* | Mexico | ITT | ITS | muestra-40 |
| plan2591 | *V. planifolia* | Mexico | ITT | ITS | muestra-41 |
| plan2592 | *V. planifolia* | Mexico | BUAP | ITS | #001 |
| plan2599 | *V. planifolia* | Mexico | BUAP | ITS | #027 |
| plan2605 | *V. planifolia* | Mexico | BUAP | ITS | #044 |
| plan2608 | *V. planifolia* | Mexico | BUAP | ITS | #056 |
| plan2616 | *V. planifolia* | Mexico | BUAP | ITS | #081 |
| plan2625 | *V. planifolia* | Mexico | BUAP | ITS | #111 |
| plan2632 | *V. planifolia* | Mexico | BUAP | ITS | #128 |
| plan2645 | *V. planifolia* | Mexico | BUAP | ITS | #180 |
| plan2650 | *V. planifolia* | Mexico | BUAP | ITS | #mut |
| plan2678 | *V. planifolia* | Guatemala | Orquideario_Archila | ITS | VG-008 |
| plan2679 | *V. planifolia* | Guatemala | Orquideario_Archila | ITS | VG-009 |
| plan2680 | *V. planifolia* | Guatemala | Orquideario_Archila | ITS | VG-010 |
| plan2681 | *V. planifolia* | Guatemala | Orquideario_Archila | ITS | VG-011 |
| plan2682 | *V. planifolia* | Guatemala | Orquideario_Archila | ITS | VG-012 |
| pomp0018 | *V. pompona* | French Polynesia | VATEL | *mat*K | CR0018 |
| pomp0052 | *V. pompona* | La Réunion | VATEL | *mat*K | CR0052 |
| pomp0064 | *V. pompona* | ? | VATEL | *mat*K | CR0064 |
| pomp0070 | *V. pompona* | Brazil | VATEL | *mat*K | CR0070 |
| pomp0079 | *V. pompona* | Guadelupe | VATEL | *mat*K | CR0079 |
| pomp0096 | *V. pompona* | French Guyana | VATEL | *mat*K | CR0096 |
| pomp-02 | *V. pompona* | ? | UNAM | ITS | Soto_7632 |

| Code | Species | Country of origin | Depository | Locus | Code in collection / voucher |
|---|---|---|---|---|---|
| pomp0691 | *V. pompona* | ? | VATEL | *mat*K | CR0691 |
| pomp1529 | *V.* cf. *pompona* | Madagascar | VATEL | *mat*K | CR1529 |
| pomp1923 | *V. pompona* | Mexico | VATEL | *mat*K | CR1923 |
| pomp2581 | *V. pompona* | Mexico | VATEL | ITS | CR2581 |
| sp.0068 | *V.* sp. | Costa Rica | VATEL | ITS & *mat*K | CR0068 |
| sp.2180 | *V.* sp. | Costa Rica | VATEL | ITS & *mat*K | CR2180 |
| sp.2543 | *V.* cf. *planifolia* | Mexico | ITT | ITS | muestra-21 |
| sp.2552 | *V.* sp. | Costa Rica | VATEL | *mat*K | CR2552 |
| sp.2553 | *V.* sp. | Costa Rica | UNA | ITS & *mat*K | UNA-VAN-0002 |
| sp.2554 | *V.* sp. | Costa Rica | UNA | ITS & *mat*K | UNA-VAN-0047 |
| sp.2557 | *V.* sp. | Costa Rica | UNA | ITS & *mat*K | UNA-VAN-0002 |
| sp.2719 | *V.* sp. | Costa Rica | VATEL | ITS & *mat*K | CR2719 |
| sp.2720 | *V.* sp. | Costa Rica | VATEL | ITS | CR2720 |
| sp.2721 | *V.* sp. | Costa Rica | VATEL | ITS | CR2721 |
| sp.2722 | *V.* sp. | Costa Rica | VATEL | ITS & *mat*K | CR2722 |
| sp.UNA022 | *V.* sp. | Costa Rica | UNA | ITS | UNA-VAN-0022 |
| sp.UNA049 | *V.* sp. | Costa Rica | UNA | ITS | UNA-VAN-0049 |
| sp.UNA059 | *V.* sp. | Costa Rica | UNA | ITS | UNA-VAN-0059 |
| sp.UNA228 | *V.* sp. | Costa Rica | UNA | ITS | UNA-VAN-0228 |
| sp.UNA229 | *V.* sp. | Costa Rica | UNA | ITS | UNA-VAN-0229 |
| sp.UNA230 | *V.* sp. | Costa Rica | UNA | ITS | UNA-VAN-0230 |
| xtah0017 | *V.* × *tahitensis* | French Polynesia | VATEL | ITS & *mat*K | CR0017 |
| xtah0163 | *V.* × *tahitensis* | French polynesia | VATEL | *mat*K | CR0163 |
| xtah0164 | *V.* × *tahitensis* | French Polynesia | VATEL | ITS & *mat*K | CR0164 |
| xtahi-02 | *V.* × *tahitensis* | French Polynesia | UNAM | ITS | Colin_sn |

**DNA extraction and sequencing**

Total DNA was extracted from lyophilized leaf samples using the Qiagen DNA plant minikit (Dusseldorf, Germany) according to manufacturer protocols. Quantity and quality of the DNA were estimated using a Nanodrop spectrophotometer (Wilmington, USA), and DNA extracts were adjusted to 20 ng $\mu l^{-1}$ for polymerase chain reaction (PCR) amplification.

The Internal Transcribed Spacer (ITS) of nuclear ribosomal DNA and part of the plastid maturase K (*mat*K*)* gene were chosen for molecular characterization because they are among the most discriminant loci for orchids (Cameron 2009; Hollingsworth *et al.* 2009; Xu *et al.* 2015) and have distinct inheritances, biparental for ITS and maternal for *mat*K. The DNA samples were PCR amplified using GoTaq kit (Promega, USA) with the two primer pairs AB101/AB102 (Sun *et al*. 1994) for the ITS sequence, and *mat*K-743F (5'-CTTCTGGAGTCTTTCTTGAGC-3') / *mat*K-1520R (5'-CGGATAATGTCCAAATACCAAATA-3') for *mat*K. The 25 µl PCR reaction mixture contained: PCR reaction buffer, 50 nmol $MgCl_2$, 1 U *Taq* polymerase (GoTaq, Promega, USA), 5 nmol dNTPs, 10 nmol of each forward and reverse primer and 40 ng of genomic DNA. Amplification reactions were performed using a 96-well GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA). The annealing temperatures for primers were 60°C for ITS and 56°C for *mat*K. Samples from Costa Rica were also amplified using the Multiplex PCR kit (Qiagen) with the same primers, and the PCR Mix and cycling conditions defined by the provider. Amplicons were sequenced in both directions as part of the

Bibliothèque du Vivant project (Paris, France), and by Genwiz (Takeley, UK) and Genoscreen (Lille, France). Nucleotide sequences were aligned using the ClustalW package included in Bioedit software (Hall 1999) and cleaned manually to generate consensus sequences. The data set was complemented with reference sequences obtained previously (Soto Arenas & Dressler 2010).

**Identification and quantification of aromatic precursors in mature fruits**

Hand-pollinated fruits of VanL (CR0068) and three *V. planifolia* accessions (CR0196, CR0040 and CR0038), cultivated under shade-house in La Réunion, were harvested at 8 months after pollination, in 2013 and 2014, then freeze-dried to minimize possible enzyme degradation. The fruits were ground to a fine powder with a mortar and pestle and stored at minus 20°C until extraction.

Extraction of volatile compounds was performed using a protocol adapted from Palama *et al*. (2009) and Pérez Silva *et al*. (2011). Fifty milligrams of the ground material was suspended in 10 ml of phosphate buffer (0.1 M; pH 5). The mixture was ultra-sonicated (frequency 35 kHz; AXTOR Model CD-4820) for 10 min at ambient temperature. The mixture was rapidly heated to 80°C for 10 min to inactivate endogenous enzymes and, after cooling at 25°C, was centrifuged at 5000 rpm for 3 min and then filtrated on a Whatman no.1 paper (Sigma-Aldrich, USA). The filtrate was complemented with 0.4 ml of a glycosidase rich enzyme preparation (AR2000®, Sigma–Aldrich, Mexico; 70 $mg \cdot ml^{-1}$ in phosphate buffer mentioned above) and adjusted to a volume of 10 ml with the pH 5 buffer solution. The extract was vortexed and incubated for 4 h at 40°C for enzymatic hydrolysis of the glycosylated precursors. One milliliter of hydrolyzed extract was filtered at 0.45 µm prior to HPLC analysis.

The samples were analyzed on an Agilent 1100 series equipped with a UV–VIS detector (G1314A), degasser (G1379A), column oven (G1316A), quaternary pump unit (G1311A) and autosampler (G1313A) controlled by OpenLAB CDS EZChrom Edition (Agilent Technologies, Inc. 2013). The column used was Zorbax Eclipse plus XDB C18 (150 mm long and 4.6 mm in diameter; 5 µm, Agilent Technologies, Mexico) and the mobile phase was a mixture of two solvents: A (0.1 M $KH_2PO_4$, pH 3.2) and B (MeOH, HPLC grade). Elution was achieved at 30°C with a gradient of 3−7% B for 2 min ($0.8\ ml \cdot min^{-1}$), 7% B for 10 min ($0.8\ ml \cdot min^{-1}$), 7% B for 11 min ($1.5\ ml \cdot min^{-1}$), followed by isocratic elution in 19% B for 25 min ($1.5\ ml \cdot min^{-1}$) and 19% B for 40 min ($2.0\ ml \cdot min^{-1}$). The compounds were monitored at 230 nm, and the injection volume was 10 µl. The compounds were quantified using the external standard technique. Solutions at concentrations ranging from 10 to 100 $mg \cdot ml^{-1}$ in the mobile phase were injected into the HPLC system to build the calibration curve.

All chemicals used were of analytical grade. The standard compounds for HPLC analyses, i.e., vanillin, vanillic acid, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, *p*-hydroxybenzyl alcohol, vanillyl alcohol, anisyl alcohol and anisic acid, were from Sigma–Aldrich (Saint Quentin Fallavier, France). Anisaldehyde was obtained from Chromadex (Irvine, CA, USA) and methanol (HPLC-grade) from J.T. Baker® (Saint Quentin Fallavier, France). The water used was Milli-Q-purified.

For each sample, extraction and HPLC were run in duplicate.

**Data analysis**

All statistical analyses were performed with the R statistical software (R Core Team 2013). Multiple comparisons of means of morphological and aromatic traits were performed using the Multcomp package in R (Hothorn *et al*. 2008).

Phylogenetic trees were inferred using the maximum likelihood method implemented in MEGA7 (Kumar *et al.* 2016) after determining the best substitution model using the online version of JModelTest2 (Guindon *et al.* 2003; Darriba *et al.* 2012). Branch robustness was assessed by bootstrapping 1000 datasets and branches with less than 65% bootstrap support were collapsed.

## Results

### *Field observations*

A total of 131 vanilla plants were collected in the survey carried out throughout Costa Rica in the time period 2013−2016. Among them, 17 were tentatively classified as *V. planifolia* (12) or VanL (5) according to the morphology of the leaves, stem or flower. The geographic origin and code numbers of these plants are provided in Fig. 1 and Table 2.

Morphologically, the VanL plants showed most similarity with *V. planifolia* and *V. ×tahitensis*, a species having a hybrid origin involving *V. planifolia* and *V. odorata* (Lubinsky *et al*. 2008). However, compared to these two species VanL plants had much smaller leaves, flowers and fruits (Fig. 2) and a distinct shape of leaves; elliptic-obovate in the case of VanL, oblong for *V. planifolia* and lanceolate for *V. × tahitensis*. On the other hand, VanL plants differed markedly from the 12 other species of the *V. planifolia* group (namely *V. appendiculata* Rolfe (Rolfe 1895), *V. bahiana* Hoehne (Hoehne 1950), *V. cristagalli* Hoehne (Hoehne 1944), *V. dubia* Hoehne (Hoehne 1944), *V. dungsii* Pabst (Pabst 1975), *V. fimbriata* Rolfe (Rolfe 1899), *V. helleri, V. insignis* Ames (Ames 1934), *V. odorata*, *V. phaeantha* Rchb.f. (Reichenbach

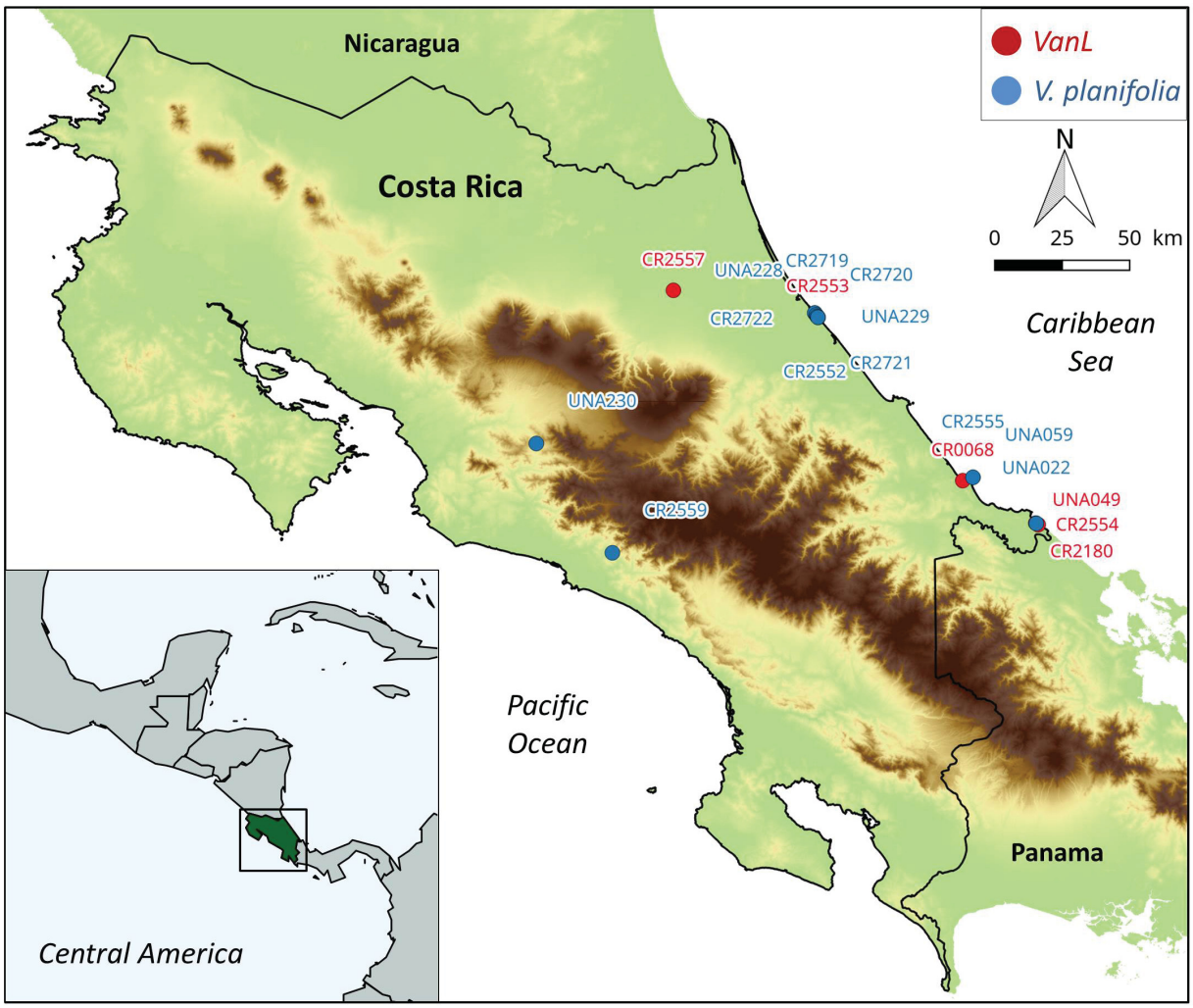


**Fig. 1.** Localization of *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. (VanL) and *V. planifolia* Jacks. ex Andrews samples collected in Costa Rica.

**Table 2.** Geographic origin of the samples collected in Costa Rica.

| Collection date | Municipality (Province) | Site | Latitude (°N) | Longitude (°W) | Elevation (m) | Collectors | *Ex situ* conservation | Code in collection | DNA code | Genotype | Status |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 Jan. 1993 | Cahuita (Limón) | – | – | – | – | *B. Gosselin* | MNHN | CH588 | CR0068 | VanL | ? |
| 8 Feb. 2013 | Siquirres (Limón) | Barra de Parismina | 10.294 | 83.338 | 14 | *B. Azofeifa, JA Garcia, A. Paniagua* | UNA | UNA-VAN-00126 | CR2552 | *planifolia* | wild |
| 8 Feb. 2013 | Siquirres (Limón) | Barra de Parismina | 10.281 | 83.329 | 18 | *B. Azofeifa, JA Garcia, A. Paniagua* | UNA | UNA-VAN-00002 | CR2553 | VanL | wild |
| 14 Nov. 2013 | Talamanca (Limón) | Refugio Nacional Mixto de Vida Silvestre Gandoca-Manzanillo | 9.594 | 82.602 | 14 | *M. Grisoni, B. Azofeifa* | BRC VATEL | CR2180 | CR2180 | VanL | wild |
| 14 Nov. 2013 | Talamanca (Limón) | Refugio Nacional Mixto de Vida Silvestre Gandoca-Manzanillo | 9.594 | 82.601 | 17 | *B. Azofeifa, JA Garcia* | UNA | UNA-VAN-00022 | FSC-22 | *planifolia* | wild |
| 14 Nov. 2013 | Talamanca (Limón) | Refugio Nacional Mixto de Vida Silvestre Gandoca-Manzanillo | 9.595 | 82.603 | 16 | *B. Azofeifa, JA Garcia* | UNA | UNA-VAN-00047 | CR2554 | VanL | wild |
| 14 Nov. 2013 | Talamanca (Limón) | Refugio Nacional Mixto de Vida Silvestre Gandoca-Manzanillo | 9.5895 | 82.595 | 19 | *B. Azofeifa, JA Garcia* | UNA | UNA-VAN-00049 | FSC-08 | VanL | wild |
| 10 Sep. 2014 | Talamanca (Limón) | Parque Nacional Cahuita-Puerto Vargas | 9.747 | 82.812 | 27 | *B. Azofeifa, JA Garcia* | UNA | UNA-VAN-00059 | FSC-07 | *planifolia* | wild |
| 10 Sep. 2014 | Talamanca (Limón) | Parque Nacional Cahuita-Puerto Vargas | 9.747 | 82.812 | 27 | *B. Azofeifa, J.A. Garcia* | UNA | UNA-VAN-00198 | CR2555 | *planifolia* | wild |
| 11 Nov. 2014 | San Isidro (Dota) | – | 9.496 | 84.012 | 847 | *A. Barquero, B. Azofeifa* | Finca Santiago Parra | – | CR2559 | *planifolia* | cultivated |
| 11 Nov. 2014 | Guápiles (Limón) | – | 10.353 | 83.804 | 79 | *A. Barquero, B. Azofeifa* | UNA | UNA-VAN-00216 | CR2557 | VanL | cultivated |
| 18 Nov. 2014 | Siquirres (Limón) | Barra de Parismina | 10.295 | 83.338 | 15 | *B. Azofeifa, JA Garcia* | UNA | UNA-VAN-00228 | FSC-01 | *planifolia* | wild |
| No data | Mora (San José) | Guayabo, Colón | 9.859 | 84.265 | 962 | *A. Paniagua, JA Garcia* | UNA | UNA-VAN-00230 | FSC-02 | *planifolia* | cultivated |
| 23 May 2015 | Siquirres (Limón) | Barra de Parismina | 10.287 | 83.333 | 6 | *B. Azofeifa* | UNA | UNA-VAN-00229 | FSC-06 | *planifolia* | wild |
| 12 Mar. 2016 | Siquirres (Limón) | Barra de Parismina | 10.279 | 83.327 | 6 | *M. Grisoni, B. Azofeifa, A. Paniagua, M. Vieira Nascimiento* | BRC VATEL | CR2719 | CR2719 | *planifolia* | wild |
| 12 Mar. 2016 | Siquirres (Limón) | Barra de Parismina | 10.279 | 83.327 | 8 | *M. Grisoni, B. Azofeifa, A. Paniagua, M. Vieira Nascimiento* | BRC VATEL | CR2720 | CR2720 | *planifolia* | wild |
| 12 Mar. 2016 | Siquirres (Limón) | Barra de Parismina | 10.279 | 83.327 | 8 | *M. Grisoni, B. Azofeifa, A. Paniagua, M. Vieira Nascimiento* | BRC VATEL | CR2721 | CR2721 | *planifolia* | wild |
| 12 Mar. 2016 | Siquirres (Limón) | Barra de Parismina | 10.279 | 83.327 | 8 | *M. Grisoni, B. Azofeifa, A. Paniagua, M. Vieira Nascimiento* | BRC VATEL | CR2722 | CR2722 | *planifolia* | wild |

1865), *V. ribeiroi* Hoehne (Hoehne 1910) and *V. schwackeana* Hoehne (Hoehne 1944)) by color of sepal, color of petal, size and shape of tube, label ornamentation, and shape, thickness and texture of leaves.

Five out of the six VanL samples, CR0068, CR2180, CR2553, CR2554 and UNA-049, were collected in natural forests at three localities of the Caribbean province of Limón (Costa Rica), indicated in Table 2. The sixth VanL accession (CR2557) was cultivated in a farm with no information on its origin. During the survey, ten *V. planifolia* plants were found growing wild at two localities of the Limón province. Five of these accessions were collected in Barra Parismina, a human settlement established about 50 years ago that is still not connected by road to the rest of the country and where, according to one of the first settlers, vanilla was present prior to human occupation and no vanilla was ever introduced by man (Gabriel Taylor, Parismina, Costa Rica, pers. comm.). They are therefore considered to be occurring

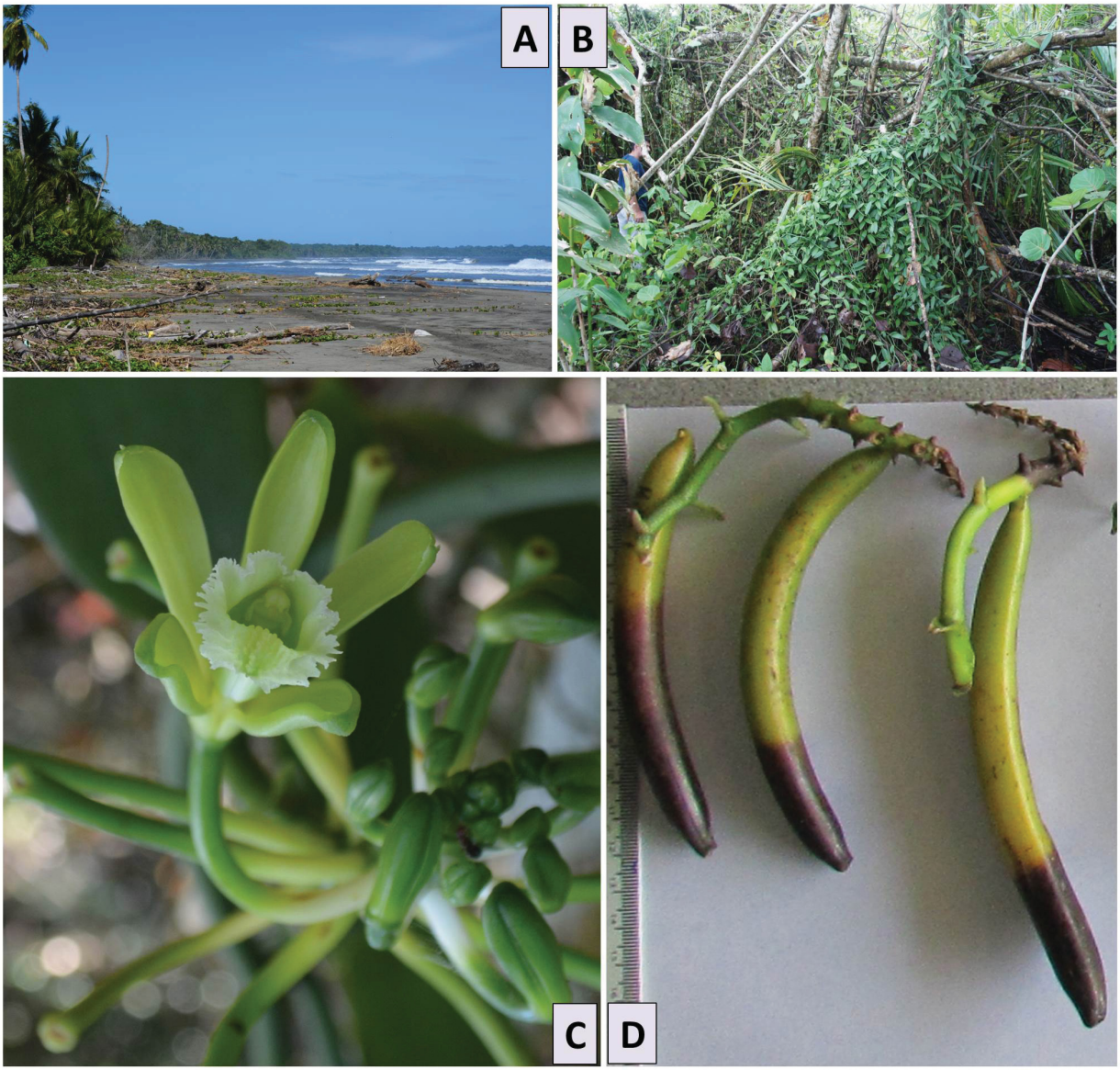


**Fig. 2.** Natural biotope of *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. (VanL) at Refugio Nacional Mixto de Vida Silvestre, Gandonca Manzanillo, Costa Rica. **A**. View of the littoral region of Limón Province harboring VanL populations. **B**. Important development of VanL in the humid littoral forests of Limón. **C**. Flower of VanL. **D**. Naturally pollinated fruits of VanL at maturity.

naturally in this area and not having escaped from local cultivation. They were morphologically indistinguishable from cultivated *V. planifolia* collected at farms in San Isidro (CR2559) and Mora (UNA-0230) or the three *V. planifolia* from Talamanca (UNA-0022, UNA-0059, CR2555).

To date, the wild populations of VanL and *V. planifolia* were both sampled in tropical humid forests of the southern coastal lowlands of Limón Province (Fig. 3). This biotope is characterized by plant species adapted to sandy soils such as *Terminalia catappa* L. (Combretaceae), *Coccoloba uvifera* (L.) L. (Polygonaceae), *Cocos nucifera* L. (Arecaceae), *Costus spicatus* (Jacq.) Sw. (Costaceae), *Hibiscus pernambucensis* Arruda (Malvaceae), *Acrostichum aureum* L. (Pteridaceae), *Chrysobalanus icaco* L. (Chrysobalanaceae), *Amphitecna latifolia* (Mill.) A.H.Gentry (Bignoniaceae), *Rhizophora mangle* L. (Rhizophoraceae) and *Pterocarpus officinalis* Jacq. (Fabaceae).

The VanL plant from Guápiles (CR2557) was sampled in a vanilla plot where vanilla was associated with pepper and cinnamon.

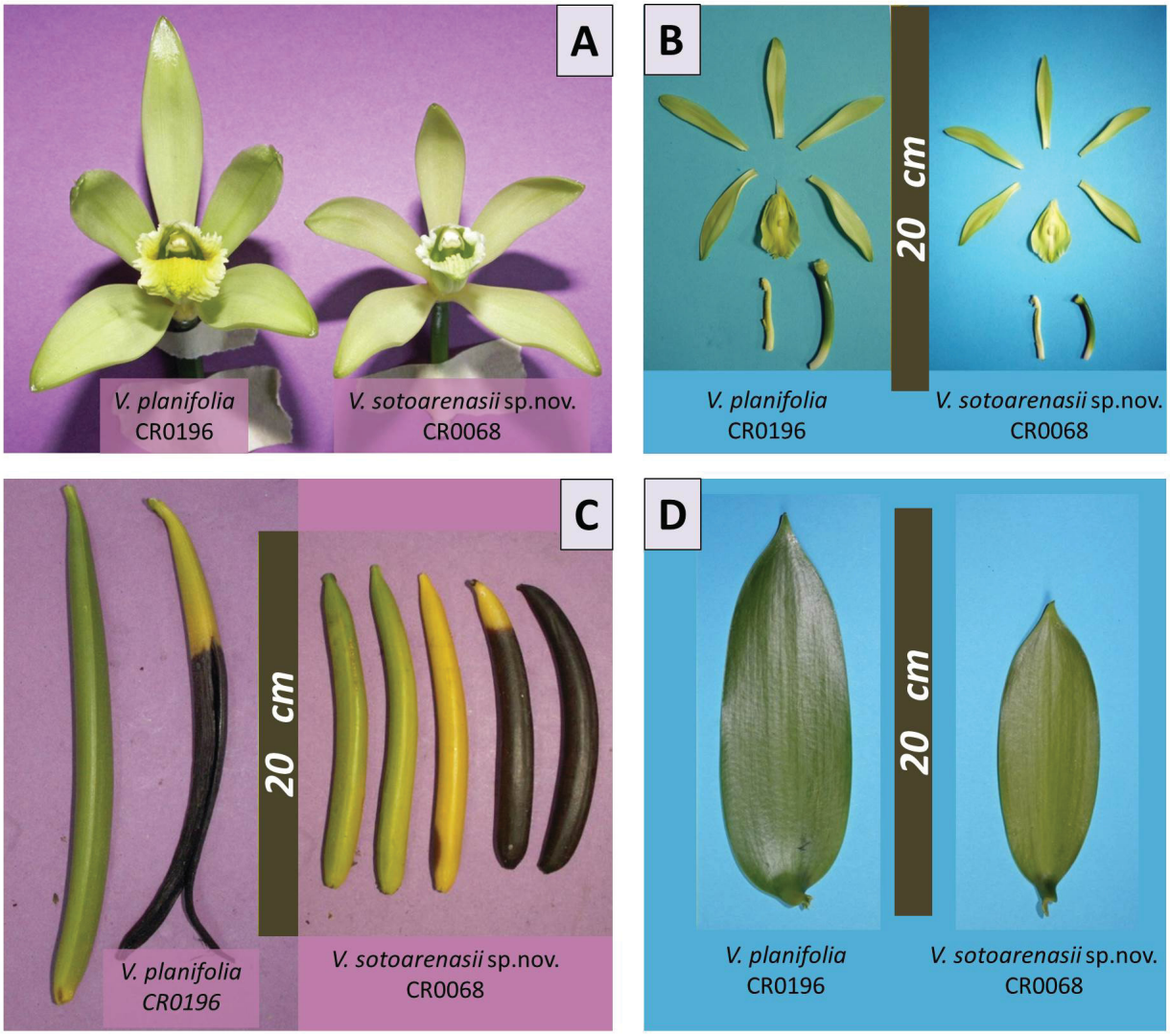


**Fig. 3.** Comparison of morphological traits between *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. (accession CR0068) and *V. planifolia* Jacks. ex Andrews (CR0196) cultivated under shade house in La Réunion. **A**. Front view of entire flowers. **B**. Separated flower parts. **C**. Mature fruits. **D**. Leaves.

In Gandoca-Manzanillo, Parismina and Cahuita the flowering happened from October to January, as deduced from the many racemes found in mid-November bearing flowers and young fruits. Mature vanilla beans were harvested in December 2015 from the VanL population of Guápiles (CR2557), which indicated a flowering time probably ending in March.

### *Phylogenetic analysis*

To elucidate the taxonomic position of VanL plants, the nuclear ITS DNA and the *mat*K plastid gene were sequenced for 125 and 55 vanilla accessions, respectively. The sample set included VanL specimens, accession representatives of the diversity of the *V. planifolia* group, as well as outgroup species (Table 1).

The phylogenetic tree inferred from the 506 positions of the 125 aligned ITS sequences (465 to 500 nt) revealed a clade separated with 93% bootstrap support, containing the five VanL accessions (CR2180, CR2553, CR2554, CR2557 and UNA049) along with CR0068 from BRC Vatel and two accessions from the AMO, collected in Costa Rica and Honduras respectively, identified as *V. planifolia* cf. plan-02 and plan-03 (Fig. 4). This clade was within the *V. planifolia* accessions and close to the *V. bahiana* / *V. phaeantha* clade. It was more distantly related to the *V. insignis* and the *V. helleri* – *V. odorata* – *V.* × *tahitensis* clades.

The eight partial ITS sequences in the VanL clade differed from all the accessions of the *V. planifolia* clade by two conserved nucleotides at positions 390 and 479 of the alignment (Table 3). Similarly, *V. bahiana* differed from *V. phaeantha* by only two conserved nucleotides (nt 172 and 420). In contrast, the *V. planifolia* clade differed from the *V. bahiana* and *V. phaeantha* group by 13 conserved nucleotides, and from *V. insignis* by 15 conserved nucleotides.

The phylogenetic tree inferred from the 741 positions of the 55 aligned *mat*K sequences (699 to 734 nt) separated *V. planifolia* with high bootstrap support from its most closely related species, including *V. odorata, V. insignis, V. bahiana* and *V. phaeantha* (Fig. 5). The eight VanL accessions sequenced fell within the *V. planifolia* clade, confirming their very close relationship with this species.

Phylogenetic trees inferred using the Neighbor Joining, Parsimony and Bayesian methods were congruent with the ML trees (data not shown).

### *Morphology of vegetative and reproductive organs of VanL*

The morphology of the leaf, stem, and flower of VanL is very similar to that of *V. planifolia* (Fig. 2). However, the former differs from the latter by a highly significant reduction in size of the vegetative and reproductive organs (Table 4). In average, leaf and stem dimensions are 29 to 41% smaller in VanL compared to *V. planifolia*.

The size reduction in VanL is less important in the flower, with tube length only 7% and sepal length 12% lesser than for *V. planifolia*. However, in VanL, the dorsal sepal is 29% narrower and the ovary 19% shorter than in *V. planifolia*. The blooming period of VanL cultivated in La Réunion was approximately two weeks earlier than that of *V. planifolia* accessions, but they overlapped from October to November.

Mature fruits of VanL were 45% shorter and 55% lighter than those of *V. planifolia* (Fig. 2; Table 4). They were also indehiscent and more cylindrical, contrary to most *V. planifolia* fruits, which dehisce at maturity and have a more triangular section.

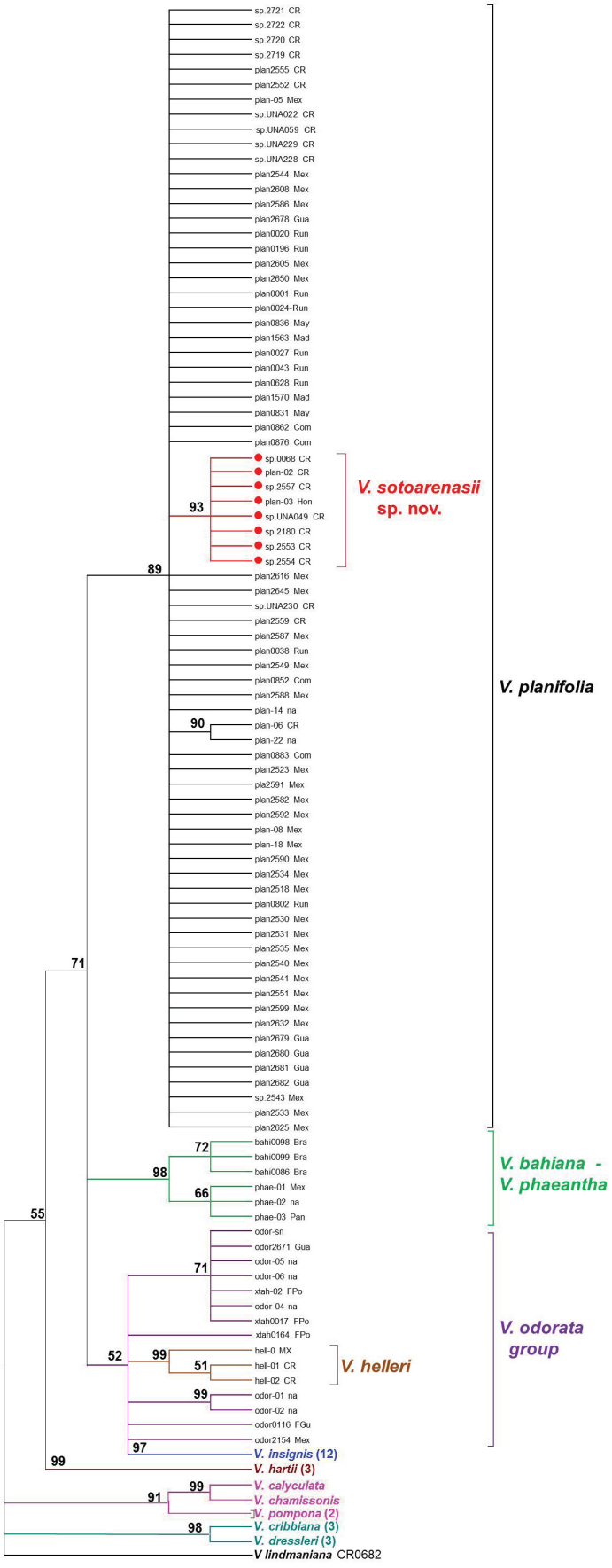


**Fig. 4.** Phylogenetic tree derived from the partial ITS sequences (506 positions) of the 125 accessions listed in Table 1, showing the differentiation of the *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. clade from *V. planifolia* Jacks. ex Andrews and all other related species. The tree was inferred using the Maximum Likelihood method based on the Tamura-Nei model with invariant sites and Gamma distribution of evolutionary rates. The figures indicate the percentage of bootstrap support. Branches with less than 65% support were collapsed. Countries of origin: Bra = Brazil; Com = Comoros: CR = Costa Rica; FGu = French Guiana; FPo = French Polynesia; Gua = Guatemala; Hon = Honduras; Mad = Madagascar; May = Mayotte; Mex = Mexico; Run = La Réunion; na = geographic origin not available. Numbers in brackets indicate the number of similar accessions merged in one branch for outgroup species.

**Table 3.** Inter-clade polymorphisms of within-clade-conserved nucleotides along the partial ITS sequence of *Vanilla planifolia* Jacks. ex Andrews, *V. sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. and their most closely related species. The triangular matrix on the right indicates the number of polymorphic sites between species. Nucleotides diverging from the *V. planifolia* sequence are in bold. Y = C or T, K = G or T.

| Position | 12 | 25 | 33 | 34 | 54 | 58 | 80 | 93 | 112 | 127 | 172 | 178 | 188 | 192 | 204 | 390 | 399 | 420 | 422 | 423 | 424 | 444 | 473 | 479 | 480 | 493 | 494 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *V. planifolia* | C | T | G | C | T | T | Y | A | - | A | T | A | C | Y | C | C | C | T | C | G | G | C | G | A | Y | C | K |
| *V. sotoarenasii* | C | T | G | C | T | T | T | A | - | A | T | A | C | C | C | **G** | C | T | C | G | G | C | G | **G** | C | C | T |
| *V. bahiana* | C | T | **A** | **T** | **C** | **C** | **C** | **C** | **T** | **T** | **C** | A | C | T | C | C | **T** | T | **T** | G | G | **T** | **A** | A | T | **T** | G |
| *V. phaeantha* | C | T | **A** | **T** | **C** | **C** | **C** | **C** | **T** | **T** | T | A | C | T | C | C | **T** | **C** | **T** | G | G | **T** | **A** | A | T | **T** | G |
| *V. insignis* | **T** | **C** | G | C | T | C | T | A | - | A | T | **G** | **T** | T | **A** | C | **T** | Y | **T** | **T** | **C** | C | **A** | A | T | **T** | G |

| | *V. planifolia* | *V. sotoarenasii* | *V. bahiana* | *V. phaeantha* | *V. insignis* |
|---|---|---|---|---|---|
| *V. planifolia* | | 2 | | | |
| *V. sotoarenasii* | 2 | | | | |
| *V. bahiana* | 13 | 19 | | | |
| *V. phaeantha* | 13 | 18 | 2 | | |
| *V. insignis* | 11 | 17 | 16 | 15 | |

**Table 4.** Means of different measurements on vegetative and reproductive organs of two accessions of *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. (CR0068 and CR2180) and two accessions of *V. planifolia* Jacks. ex Andrews (CR0040 and CR0196). Measurements are in mm unless otherwise indicated. In each line, means with different letters are significantly different ($p < 0.005$, Tukey's test). StE = standard error.

| | ***V. sotoarenasii* sp. nov.** | | | | | | ***V. planifolia*** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **CR0068** | | | **CR2180** | | | **CR0040** | | | **CR0196** | | |
| **Characters** | **mean** | **StE** | | **mean** | **StE** | | **mean** | **StE** | | **mean** | **StE** | |
| length of leaf | 76.0 | 2.28 | *a* | 105.8 | 3.85 | *b* | 149.4 | 2.58 | *c* | 146.5 | 3.65 | *c* |
| width of leaf | 32.7 | 0.58 | *a* | 27.4 | 1.29 | *a* | 50.0 | 0.96 | *b* | 47.9 | 1.08 | *b* |
| diameter of stem | 5.8 | 0.09 | *a* | 6.1 | 0.12 | *a* | 10.3 | 0.25 | *b* | 9.9 | 0.26 | *b* |
| length of internode | 71.4 | 3.00 | *a* | 91.0 | 3.03 | *a* | 117.7 | 3.65 | *b* | 122.2 | 4.66 | *b* |
| Number of items | 30 | | | 14 | | | 24 | | | 30 | | |
| length of sepal sup | 50.3 | 0.45 | *a* | 50.6 | 0.64 | *a* | 57.9 | 0.43 | *b* | 56.8 | 0.53 | *b* |
| width of sepal sup | 9.5 | 0.22 | *a* | 8.9 | 0.33 | *a* | 13.5 | 0.29 | *b* | 12.6 | 0.33 | *b* |
| length of tube | 42.6 | 0.32 | *a* | 43.2 | 0.67 | *a* | 47.0 | 0.40 | *b* | 45.7 | 0.48 | *b* |
| width of tube | 10.8 | 0.12 | *a* | 11.3 | 0.18 | *a* | 13.6 | 0.19 | *b* | 13.0 | 0.22 | *b* |
| length of ovary | 37.8 | 0.53 | *a* | 45.9 | 0.93 | *b* | 53.1 | 0.61 | *c* | 49.9 | 0.72 | *c* |
| Number of items | 30 | | | 19 | | | 22 | | | 30 | | |
| weight of mature fruit (g) | 6.6 | 0.17 | *a* | – | – | | 18.1 | 0.84 | *b* | 18.3 | 0.65 | *b* |
| length of mature fruit | 114 | 2.20 | *a* | – | – | | 201 | 3.66 | *b* | 203 | 2.26 | *b* |
| Number of items | 16 | | | – | | | 17 | | | 22 | | |

### *Aromatic content of mature fruits*

Strongly contrasted aromatic profiles were observed between VanL and *V. planifolia* by comparing the contents of nine volatile precursors detected in mature fruits by HPLC (Table 5). The fruits of VanL had a much lower content of vanillyl compounds, particularly vanillin which did not exceed 0.26% of dry matter, while it was over 2.28% in *V. planifolia* beans. Conversely, VanL fruits had significant amounts of anisyl compounds which were not detected by HPLC in *V. planifolia* fruits, and *p*-hydroxybenzyl (PHB) alcohol was present at a much higher titer in VanL compared to *V. planifolia.* The contents of the two other *p*-hydroxyl compounds were not significantly different between VanL and *V. planifolia*.

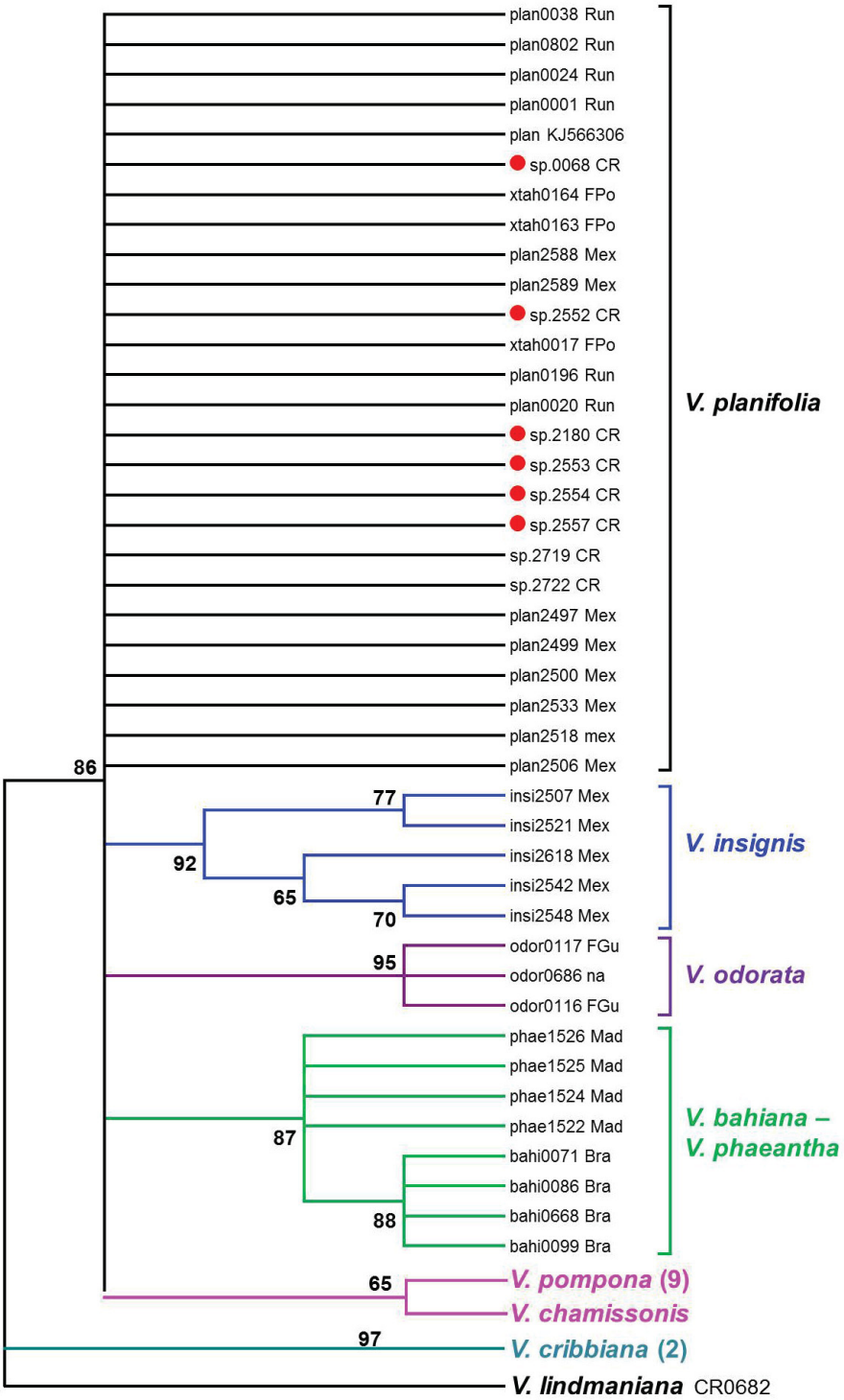


**Fig. 5.** Phylogenetic tree derived from the partial *mat*K sequences (725 positions) of the 55 accessions listed in Table 1, showing the *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. accessions (red dots) within the *V. planifolia* clade but distinct from other related species. The tree was inferred using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model and Gamma distribution of evolutionary rates. The figures indicate the percentage of bootstrap support. Countries of origin: Bra = Brazil; CR = Costa Rica; FGu = French Guiana; FPo = French Polynesia; Mad = Madagascar; Mex = Mexico; Run = La Réunion; na = geographic origin not available. Numbers in brackets indicate the number of similar accessions merged in one branch for outgroup species.

As a whole, the VanL fruit had a significantly lower (about 30%) level of total aromatic content (all nine molecules) and a very distinct aromatic profile compared to *V. planifolia*. Indeed, the volatiles of *V. planifolia* fruits were strongly dominated by vanillyl compounds (83.1% of all volatiles, particularly vanillin which represented 68%). By contrast, the VanL fruits contained a more equilibrated profile with a slight dominance of vanillyl compounds (41.9% of all volatiles, in which vanillyl-alcohol represented 31%), *p*-hydroxybenzyl and anisyl compounds (35.9% and 22.2%, respectively).

The above results indicate than VanL and *V. planifolia* are very closely related but significantly distinct in morphological and biochemical traits, as well as in nuclear nucleotide sequences. We therefore propose to place VanL in a specific taxon distinct from *V. planifolia*, and to name it *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov.

## *Taxonomy*

Class Equisetopsida C.Agardh (Agardh *et al.* 1825)
Subclass Magnoliidae Novák ex Takht. (Takhtajan 1967)
Superorder Lilianae Takht. (Takhtajan 1967)
Order: Asparagales Link (Link 1829)
Family Orchidaceae Juss. (de Jussieu 1789)
Subfamily Vanilloideae (Lindl.) Szlach. (Szlachetko 1995)
Tribe Vanilleae (Blume 1835)

Genus ***Vanilla*** Plum. ex Mill. (Miller 1754)
Subgenus ***Xanata*** Soto Arenas & Cribb (Soto Arenas & Cribb 2010)
Section ***Xanata*** Soto Arenas & Cribb (Soto Arenas & Cribb 2010)

***Vanilla sotoarenasii*** M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov.
urn:lsid:ipni.org:names:77160155-1
Figs 2, 3, 6

### Diagnosis

*A Vanilla planifolia similis, sed folia caulesque breviores (folium: 7.6−10.6 × 2.7−3.3 cm versus 14.7−14.9 × 4.8−5 cm), laminae breviores, ellipticae-oblongaeque, flores albiores, tubus floris, sepala petalaque breviores, sepalum dorsale angustius, labellum angustiius cum papillis salientioribus, ovarium brevitius (3.8−4.6 cm versus 5−5.3 cm), fructus brevitior (11.4 cm versus 20.1−20.3 cm) indehiscensque, sectio fructi cylindrica (versus trigona). Moleculae aromaticae fructi absimilis.*

### Etymology

This species is dedicated to Dr. Miguel Angel Soto Arenas (1963−2009), authority in orchid floristics and ecology, particularly in the *Vanilla* genus.

### Type material

#### Holotype

COSTA RICA: Cahuita (ex hort. parc E. Liais, from plants collected by B. Gosselin in 1993), 8 Oct. 1996, *Pignal 396 b* (holo-: P: P00075132).

#### Paratypes

COSTA RICA: Limón Province: Talamanca, Refugio Nacional Mixto de Vida Silvestre Gandoca-Manzanillo, 9.594° N, 82.602° W, altitude 2 m, 14 Nov. 2013 (originally collected), cultivated in BRC Vatel, Saint Pierre, La Réunion, France CRV2180, 28 Feb. 2016, *M. Grisoni & J.B. Azofeifa-Bolaños*

**Table 5.** Means of volatile compounds quantified by HPLC in mature beans of *V. sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. (CR0068; 3 samples) and *V. planifolia* Jacks. ex Andrews (CR0196, CR0038, CR0040; 9 samples). Contents are expressed as grams of compound per 100 g dry matter. nd = compound not detected. Averages with different letters within rows are significantly different ($p < 0.05$).

| | ***V. sotoarenasii* sp. nov.** | | ***V. planifolia*** | |
|---|---|---|---|---|
| **Compounds** | **g / 100g dw** | **%** | **g / 100g dw** | **%** |
| Vanillin | 0.26 a | 10.9 | 2.28 b | 68.0 |
| Vanillic acid | nd a | 0.0 | 0.19 b | 5.5 |
| Vanillyl alcohol | 0.73 a | 31.0 | 0.32 b | 9.6 |
| Total vanillyl compounds | 0.99 a | 41.9 | 2.79 b | 83.1 |
| *p*-hydroxybenzaldehyde | 0.20 a | 8.5 | 0.18 a | 5.4 |
| *p*-hydroxybenzoic acid | 0.37 a | 15.8 | 0.37 a | 11.2 |
| *p*-hydroxybenzyl alcohol | 0.27 a | 11.6 | 0.01 b | 0.3 |
| Total *p*-hydroxybenzyl compounds | 0.85 a | 35.9 | 0.56 b | 16.9 |
| Anisyl alcohol | 0.24 a | 10.2 | nd b | 0.0 |
| Anisaldehyde | 0.03 b | 1.4 | nd b | 0.0 |
| Anisic acid | 0.25 a | 10.6 | nd b | 0.0 |
| Total anisyl compounds | 0.52 a | 22.2 | nd b | 0.0 |
| **Total** | **2.36 a** | **100** | **3.35 b** | **100** |

*JJMM01* (REU: REU13363); Canton of Talamanca, Refugio de Vida Silvestre Gandoca-Manzanillo (originally collected), cultivated in INISEFOR, Heredia, Costa Rica, 14 Nov. 2013, *M. Grisoni, B. Azofeifa and J. García 2180* (CR 281507), cultivated at the same locality, *B. Azofeifa and J. García 0047* (CR 281508); Canton of Siquirres, Barra de Parismina (originally collected), cultivated in INISEFOR, Heredia, Costa Rica, 8 Feb. 2013, *B. Azofeifa, J. García and A. Paniagua 0002* (CR 281509).

**Description**

Hemiepiphytic vine up to 15 m high. Stems flexuous, terete, smooth, green, 5−6 mm thick; internodes, sometimes slightly curved apically, ca 10 cm long. Terrestrial roots pubescent, ramified, ca 2 mm thick; both attaching and free aerial roots terete, pubescent, ca 2 mm thick at base, ca 3.5 mm at middle. Leaves regularly alternate. Blade elliptic to obovate, slightly fleshy (ca 27 veins visible on dry specimens); base rounded to cuneate, shortly pseudopetiolate; apex shortly acuminate (acumen sharp, 12 mm long, 5 mm at base), slightly recurved; margin thinned; pseudopetiole canaliculate, 16 × 5 mm. Inflorescence: raceme, ca 10−20-flowered, 7−13 cm, sometimes located on short axillary branches, sometimes lying on leafy stems. 3−4 inflorescence bracts, foliaceous, basal bract pseudopetiolate (40 × 20 mm), upper bract sessile and shorter (20 × 8 mm and 12 × 3 mm). Flowers at anthesis successively, ephemeral, 1−2 simultaneously, white green, sepals forming an angle of approximately 45° with axis of column, petals more or less parallel to this axis. Ovary terete, smooth, slightly arcuate, white at base and green on upper ⅔, 30 mm long and 4 mm in diameter. Parts of perianth with whitish inclusions (visible on dry specimens), longitudinally oriented, ca 0.1−2 mm long, more numerous on petals, dorsal sepal narrow elliptic to oblanceolate, ca 11-veined, 40 × 10 mm, apex acute, rounded, base attenuate-clawed, slightly concave, canaliculate. Lateral sepals, elliptic asymmetric, slightly falciform, ca 11-veined, 38 × 10.5 mm, apex acute, slightly cupuliform, base attenuate, canaliculate, 4 mm wide. Petals elliptic asymmetric, slightly falciform, ca 12-veined, carinate dorsally (carena 1.2 mm wide), 38 × 9 mm, apex rounded, base attenuate, 3 mm wide (Fig. 6). Labellum attached to column along margins of ⅐ (ca 20

mm), funnel-shaped, spread apically (opening about 10 mm), trilobed, ca 30-veined ramified in distal third, margin crenulate. With 5−6 fimbriated scales, ca 1.5 × 1.5 mm, at about middle of labellum. Lateral lobes, obliquely triangular, ca 11-veined, 10 mm high and 7 mm wide, margins widely undulate. Midlobe quadrate, 20 mm wide and 6 mm high, about 12-veined, converging at apex. Papillae on four central veins, on apical half. Column trigonous-semicylindrical, 28 mm long, 2.5 mm wide, apically with 2 lateral auricles, crenulate, 2.5 mm high and 4 mm wide. Rostellum quadrangular, ca 3 × 3 mm. Stigma bilobed. Anther articulated, connective keel-shape, 2 × 2 mm, bicarinate on top. Operculum helmet-shaped, 2 × 3 mm. Pollinarium, 2. Fruit arcuate, banana-shaped, green, turning yellowish and then brown, 11−16 × 1.5 cm, with cylindrical section.

**Phenology**

The flowering period occurs from October to March in Costa Rica.

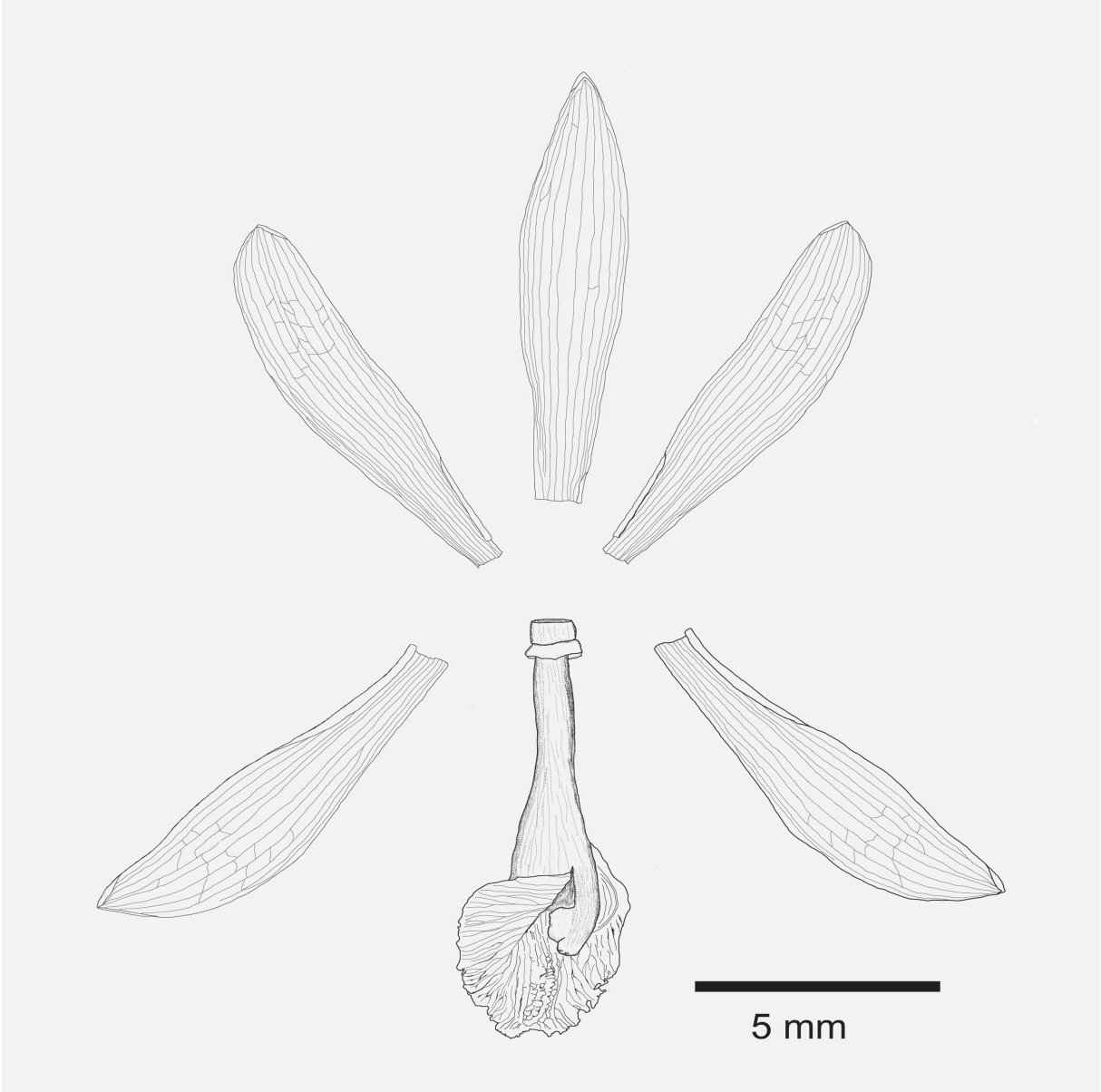


**Fig. 6.** Croquis drawing of a flower of *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov.

**IUCN status –Vulnerable**

The populations of *V. sotoarenasii* sp. nov. observed in the province of Limón showed strong vegetative development of vines and natural seed set was frequently observed. However, a small number of populations have so far been observed and only over a very limited area of Limón Province (10 populations over a coastal strip of about 50 km²), and its habitat is periodically submerged by the ocean. This makes us inclined to tentatively classify *V. sotoarenasii* sp. nov. as vulnerable D2 (IUCN 2012) until further data on distribution and population dynamics have been obtained.

## Discussion

Using complementary approaches involving morphology of the reproductive and vegetative systems, molecular barcoding and the accumulation of secondary metabolites in fruits, we highlighted specific traits for the vanilla populations sampled on the Caribbean coast of Costa Rica which revealed a new taxon, *V. sotoarenasii* sp. nov. The morphology of the flowers and leaves clearly assigned *V. sotoarenasii* sp. nov. within the *V. planifolia* group (Soto Arenas & Dressler 2010). Based on flower morphology, *V. sotoarenasii* sp. nov. was more similar to *V. planifolia* and *V.* × *tahitensis* than to any other vanilla species of this group. However, *V. sotoarenasii* sp. nov. differs from the other two species by several characteristics (Table 6).

Firstly, our data and previous data by Costantin & Bois (1915) and Portères (1953) showed that *V. sotoarenasii* sp. nov. has a significantly smaller size for all organs measured (stem, leaf, flower and fruit) compared to *V. planifolia* and *V.* × *tahitensis*, and had a distinct shape of the leaves: elliptic to obovate in the case of *V. sotoarenasii* sp. nov., elliptic to oblong for *V. planifolia* and narrowly oblong to lanceolate for *V.* × *tahitensis* (Table 6). In addition, the flowers of *V. sotoarenasii* sp. nov. are more whitish, with a narrower label showing marked papillae, compared to those of *V. planifolia*, which are more greenish, with a wider label and smooth papillae.

On the basis of HPLC quantification of hydrolyzed volatile compounds in mature fruits, the aromatic precursors of *V. sotoarenasii* sp. nov. are very distinct from those of *V. planifolia*. In particular, fruits of *V. sotoarenasii* sp. nov. were characterized by less predominant vanillin content and the presence of anisyl compounds. They should develop, after over-maturation or curing, flavors extremely different from those of *V. planifolia* and more similar to those of *V. pompona* or *V.* × *tahitensis* (Pérez-Silva *et al.* 2006; Brunschwig *et al.* 2009; Maruenda *et al.* 2013).

On the other hand, molecular analysis of nuclear DNA sequences (ITS) unambiguously separated the *V. sotoarenasii* sp. nov. group of plants from the *V. planifolia* group including 68 accessions originating from seven countries (Comoros, Costa Rica, Guatemala, Madagascar, Mayotte, Mexico, and La Réunion). This result is corroborated by amplified fragment length polymorphism (AFLP) analyses by Bory *et al*. (2008) that clearly separated *V. sotoarenasii* sp. nov. CR0068 from 303 *V. planifolia* genotypes of diverse origins with an average distance comparable to the distance between *V. planifolia* and *V.* × *tahitensis.* The facts that i) at the plastid DNA level (partial *mat*K sequence) *V. sotoarenasii* sp. nov. and *V. planifolia* share the same clade, and ii) in the ITS phylogeny *V. sotoarenasii* sp. nov. is in an internal position within the *V. planifolia* clade (like *V. helleri* within the *V. odorata* cluster), suggest the recent radiation of *V. sotoarenasii* sp. nov. from *V. planifolia* populations.

So far, *V. sotoarenasii* sp. nov. has only been observed in the Limón Province of Costa Rica where it is sympatric with *V. planifolia*, which has been reported as native to Costa Rica (Soto Arenas & Dressler 2010). However, recent introductions in the country of *V. planifolia* cultivars and hybrids have also been documented (Soto Arenas & Dressler 2010; Belanger & Havkin-Frenkel 2011; Varela Quirós 2011). Historical, genetic and phylogenetic data are insufficient to decide whether *V. sotoarenasii* sp. nov. derived from natural or introduced populations of *V. planifolia*, nor how and when the radiation occurred.

**Table 6.** Characters differentiating *Vanilla sotoarenasii* M.Pignal, Azofeifa-Bolaños & Grisoni sp. nov. from related species.

| | ***V. planifolia*** | ***V.* × *tahitensis*** | ***V. sotoarenasii* sp. nov.** |
|---|---|---|---|
| **Leaf shape** | elliptic to oblong | narrowly oblong to lanceolate | elliptic to obovate |
| **Leaf length** | more than 14 cm long | more than 14 cm long | less than 12 cm long |
| **Leaf length/width ratio** | less than 4 times as long as wide | more than 4 times as long as wide | about 3 times as long as wide |
| **Sepal size (length × width)** | more than 55 × 10 mm | more than 55 × 10 mm | no more than 53 × 8 mm |
| **Fruit section** | triangular, generally dehiscent | triangular, generally indehiscent | rounded, indehiscent |

Orchid species are often interfertile, which allows them to create interspecific and even intergeneric fertile hybrids. Many interspecific hybrids between species of *Vanilla* have been produced in the last decades by botanists and agronomists (Knudson 1950; Theis & Jiménez 1957; Divakaran *et al.* 2006). In nature, however, interspecific hybrids are prevented by reproductive barriers that result primarily from the inability of pollinators to transfer pollen from one species to another. Within the limits of our sampling, we have never observed intermediary types, at the genetic or morphological level, in the Limón area, which suggests that a reproductive barrier may exist between *V. planifolia* and *V. sotoarenasii* sp. nov. Indeed, in the case of sympatric populations of the two very closely related species *V. barbellata* Rchb.f. and *V. dilloniana* Correll, having synchronous flowering and the same pollinator in western Puerto Rico, intermediary types were observed, demonstrating natural interspecific hybridizations (Nielsen 2000). We hypothesize that the differences in flower morphology observed between *V. planifolia* and *V. sotoarenasii* sp. nov. impede gene flow from one species to another, which enhanced the radiation of the population in Limón. Vanilla pollination is bee-dependent (Ackerman 1986; Gigant *et al.* 2011) and flower size difference is likely to constitute a reproductive barrier between species by selecting compatible pollinators. This has been observed for instance in Peru where, due to their small size, the *Mellipona* bees did not remove pollen when visiting *V. grandiflora* Lindl. flowers, and therefore did not contribute to its pollination (Lubinsky *et al.* 2006). Given the significant size reduction of the flowers of *V. sotoarenasii* sp. nov. compared to those of *V. planifolia*, it is unlikely that a bee capable of pollinating one of the two species would be able to pollinate the other. In addition to size compatibility, the probability of pollinator visits is frequently increased by the aromatic metabolites emitted by orchid flowers, which act as bee attractants (Ackerman 1986; Pansarin & Pansarin 2014). This is particularly important for vanilla flowers that are open a single day, which reduces the chances of pollen-pollinator encounter. Analysis of the volatile compounds emitted by flowers from various *Vanilla* species (unpublished data) has shown in particular that in *V. planifolia* (n = 4) the major compounds are a hydrocarbon monoterpene, (E)-β-ocimene (representing 17.10 ± 14.08% of all detected compounds), and an aromatic compound, 2,6-bis (1,1-dimethylethyl)-4-(1-oxopropyl) phenol (11.59 ± 8, 04%), while in *V. sotoarenasii* sp. nov. (n = 1), only (E)-β-ocimene was predominantly found (63.26%). This difference in the nature of volatile emissions between the two species could also contribute to the specificity of the pollinators visiting the flowers. Furthermore, the earlier flowering of *V. sotoarenasii* sp. nov. compared to *V. planifolia* may also contribute to favor self or geitonogamous pollination as well as provide greater reproductive success to *V. sotoarenasii* sp. nov., as pointed out for non-rewarding orchids (Jersáková *et al.* 2006; Sun *et al.* 2009).

All these factors hindering gene flow between populations of *V. sotoarenasii* sp. nov. and *V. planifolia* may account for the radiation that led to the emergence of *V. sotoarenasii* sp. nov., a species that is genetically, morphologically, and biochemically distinct from *V. planifolia*.

Finally, given its original aromatic content combining vanilyl and anisic notes, its high level of resistance (at least for CR0068) to the *Fusarium* root rot of vanilla (Koyyappurath *et al.* 2015), and its early and abundant flowering in culture, *V. sotoarenasii* sp. nov. could be a promising genitor for breeding programs aiming to produce new vanilla varieties for the agroindustry.

## Acknowledgements

The authors are grateful to the National Council for Scientific and Technological Research of Costa Rica (CONICIT) for the financial support to conduct the collecting missions in Costa Rica, and to the Distance Education University of Costa Rica (UNED) for supporting the MSc work of the first author. The molecular genotyping and phylogenetic analyses were partly funded by the Vanitax (ANR Bibliothèque du Vivant) and the VaBiome (EraNet-NetBiome) projects. The authors would like to warmly thank M. Alain Rongier, Pierre Poulain, Jean-Paul Brixtel and the municipality of Cherbourg-en-Cotentin (France) for allowing access to the material conserved in the Parc Emmanuel Liais, Ana Barquero of INISEFOR-UNA for providing plant material, Pr. Thomas Petit and colleagues from the University Institute of Technology of Saint Pierre (La Réunion) for facilitating HPLC analyses in their lab, and Marc Jeanson from P, Muséum national d'Histoire naturelle (Paris).

## References

Ackerman J.D. 1986. Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* 1: 108–113.

Agardh C.A., Holmberg L.P. & Lundstrom P.M. 1825. *Classes Plantarum.* Literis Berlingianis, Lundae [Lund, Sweden].

Ames O. 1934. An addition to the genus *Vanilla*. *Harvard University Botanical Museum Leaflet* 2: 101–102.

Andrews H.C. 1808. *Vanilla planifolia*. *Botanist's Repository* 8: 538.

Archila F. & Chiron G.R. 2012. Addition à la flore du Guatemala: *Vanilla esquipulensis* (Orchidaceae), espèce des forêts xérophytes. *Richardiana* 13: 3–12.

Azofeifa-Bolaños J.B., Paniagua-Vásquez A. & Garcia-Garcia J.A. 2014. Importancia y desafíos de la conservación de *Vanilla* spp. (Orchidaceae) en Costa Rica. *Agronomía Mesoamericana* 25: 189–202. http://dx.doi.org/10.15517/am.v25i1.14220

Belanger F.C. & Havkin-Frenkel D. 2011. Molecular analysis of a *Vanilla* hybrid cultivated in Costa Rica. *In:* Havkin-Frenkel D. & Belanger F.C. (eds) *Handbook of Vanilla Science and Technology*: 256–263. Wiley-Blackwell, Chichester, United Kingdom.

Besse P. (ed.) 2014. *Molecular Plant Taxonomy – Methods and Protocols.* Humana Press (Springer), New York.

Blume C.L.V. 1835. *Rumphia, sive, Commentationes botanicæ imprimis de plantis Indiæ Orientalis: tum penitus incognitis tum quæ in libris Rheedii, Rumphi, Roxburghii, Wallichii aliorum recensentur / scripsit C.L. Blume cognomine Rumphius*. Vol. 1: 196. Leiden. Available from http://biodiversitylibrary.org/page/41039881 [accessed 18 Jan. 2017].

Bory S., Lubinsky P., Risterucci A.-M., Noyer J.-L., Grisoni M., Duval M.-F. & Besse P. 2008. Patterns of introduction and diversification of *Vanilla planifolia* (Orchidaceae) in Reunion Island (Indian Ocean). *American Journal of Botany* 95: 805–815. http://dx.doi.org/10.3732/ajb.2007332

Bouétard A., Lefeuvre P., Gigant R., Bory S., Pignal M., Besse P. & Grisoni M. 2010. Evidence of transoceanic dispersion of the genus *Vanilla* based on plastid DNA phylogenetic analysis. *Molecular Phylogenetics and Evolution* 55: 621–630. http://dx.doi.org/10.1016/j.ympev.2010.01.021

Brunschwig C., Collard F.X., Bianchini J.P. & Raharivelomanana P. 2009. Evaluation of chemical variability of cured vanilla beans (*Vanilla tahitensis* and *Vanilla planifolia*). *Natural Products Communications* 4: 1393–1400.

Cameron K.M. 2009. On the value of nuclear and mitochondrial gene sequences for reconstructing the phylogeny of vanilloid orchids (Vanilloideae, Orchidaceae). *Annals of Botany* 104: 377–385. http://dx.doi.org/10.1093/aob/mcp024

Cameron K.M. 2010. Vanilloid orchids. *In:* Odoux E. & Grisoni M. (eds) *Vanilla*: 1–13. CRC Press, Boca Raton, FL.

Cameron K.M. 2011. *Vanilla* phylogeny and classification. *In:* Havkin-Frenkel D. & Belanger F.C. (eds) *Handbook of Vanilla Science and Technology*: 241–255. Wiley-Blackwell, Chichester, United Kingdom.

Costantin D. & Bois J. 1915. Sur trois types de vanilles commerciales de Tahiti. *Comptes Rendus de l'Académie des Sciences de Paris* 161: 196–202.

Darriba D., Taboada G., Doallo R. & Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. http://dx.doi.org/10.1038/nmeth.2109

Divakaran M., Babu K.N. & Peter K.V. 2006. Conservation of *Vanilla* species *in vitro*. *Scientia Horticulturae* 110: 175–180. http://dx.doi.org/10.1016/j.scienta.2006.07.003

Gigant R., Bory S., Grisoni M. & Besse P. 2011. Biodiversity and evolution in the *Vanilla* genus. *In*: Grillo O. & Venora G. (eds) *The Dynamical Processes of Biodiversity: Case Studies of Evolution and Spatial Distribution*. InTech, Rijeka, Croatia.

Guindon S., Gascuel O. & Rannala B. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696–704. http://dx.doi.org/10.1080/10635150390235520

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Heller A.H. & Hawkes A.D. 1966. Nicaraguan orchid studies. *Phytologia* 14: 19–20.

Hoehne F.C. 1910. Botânica: I. Bromeliaceas, Pontederiaceas, Liliaceas, Amaryllidaceas, Iridaceas, Orchidaceas, Aristolochiaceas, Droseraceas e Passifloraceas. *Commissão das Linhas Telegraficas Estratégicas, Matto Grosso e Amazonas* 5: 1–71.

Hoehne F.C. 1944. *Vanilla trigonocarpa*. *Arquivos de Botânica do Estado de São Paulo* 1 (6): 126–127.

Hoehne F.C. 1950. Algunas novidades da flora do Brasil austro-oriental de entre Orchidaceas e Convolvulaceas. *Arquivos de Botânica do Estado de São Paulo* 2 (5): 105–110.

Hollingsworth P.M., Forrest L.L., Spouge J.L., Hajibabaei M., Ratnasingham S., van der Bank M., Chase M.W., Cowan R.S., Erickson D.L., Fazekas A.J., Graham S.W., James K.E., Kim K.-J., Kress W.J., Schneider H., Van AlphenStahl J., Barrett S.C.H., van den Berg C., Bogarin D., Burgess K.S., Cameron K.M., Carine M., Chacón J., Clark A., Clarkson J.J., Conrad F., Devey D.S., Ford C.S., Hedderson T.A.J., Hollingsworth M.L., Husband B.C., Kelly L.J., Kesanakurti P.R., Kim J.S., Kim Y.-D., Lahaye R., Lee H.-L., Long D.G., Madriñán S., Maurin O., Meusnier I., Newmaster S.G., Park C.-W., Percy D.M., Petersen G., Richardson J.E., Salazar G.A., Savolainen V., Seberg O., Wilkinson M.J., Yi D.-K. & Little D.P. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences* 106: 12794–12797. http://dx.doi.org/10.1073/pnas.0905845106

Hothorn T., Bretz F. & Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50: 346–336. http://dx.doi.org/10.1002/bimj.200810425

IUCN 2012. *IUCN Red List Categories and Criteria: Version 3.1*. Second edition. IUCN, Gland, Switzerland and Cambridge, United Kingdom.

Jersáková J., Johnson S. & Kindlmann P. 2006. Mechanisms and evolution of deceptive pollination in orchids. *Biological Reviews of the Cambridge Philosophical Society* 81: 219–235. http://dx.doi.org/10.1017/S1464793105006986

Jussieu A.-L. de 1789. *Genera Plantarum, Secundum Ordines Naturales Disposita, juxta Methodum in Horto Regio Parisiensi Exaratam*. Herissant, Paris. Available from http://biodiversitylibrary.org/page/5437140 [accessed 12 Jan. 2017].

Knudson L. 1950. Germination of seeds of vanilla. *American Journal of Botany* 37: 241–247.

Koyyappurath S., Conéjéro G., Dijoux J.B., Lapeyre Montes F., Jade K., Chiroleu F., Gatineau F., Verdeil J.L., Besse P. & Grisoni M. 2015. Differential responses of vanilla accessions to root rot and colonization by *Fusarium oxysporum* f. sp. *radicis-vanillae*. *Frontiers in Plant Science* 6. http://dx.doi.org/10.3389/fpls.2015.01125

Kumar S., Stecher G. & Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33 (7): 1870–1874. http://dx.doi.org/10.1093/molbev/msw054

Link H.F. 1829. *Handbuch zur Erkennung der nutzbarsten und am häufigsten vorkommenden Gewächse*. Haude und Spener, Berlin.

Lubinsky P., Van Dam M. & Van Dam A. 2006. Pollination of *Vanilla* and evolution in Orchidaceae. *Lindleyana* 75: 926–929.

Lubinsky P., Cameron K.M., Molina M.C., Wong M., Lepers-Andrzejewski S., Gómez-Pompa A. & Kim S.-C. 2008. Neotropical roots of a Polynesian spice: the hybrid origin of Tahitian vanilla, *Vanilla tahitensis* (Orchidaceae). *American Journal of Botany* 95: 1040–1047. http://dx.doi.org/10.3732/ajb.0800067

Maruenda H., Vico M.D.L., Householder J.E., Janovec J.P., Cañari C., Naka A. & Gonzalez A.E. 2013. Exploration of *Vanilla pompona* from the Peruvian Amazon as a potential source of vanilla essence: quantification of phenolics by HPLC-DAD. *Food Chemistry* 138: 161–167. http://dx.doi.org/10.1016/j.foodchem.2012.10.037

Miller P. 1754. *The Gardeners Dictionary: Containing the Methods of Cultivating and Improving all Sorts of Trees, Plants, and Flowers for the Kitchen, Fruit and Pleasure Gardens and also those which are used in Medecine*. Fourth edition. J. & J. Rivington, London.

Molineros-Hurtado F., González-Mina R.T., Flanagan N.S. & Otero J.T. 2014. *Vanilla rivasii* (Orchidaceae), a new species from the Colombian Pacific region. *Lankesteriana* 13: 353–357.

Moore J.W. 1933. New and critical plants from Raiatea. *Bulletin of the Bernice P. Bishop Museum* 102: 1–53.

Nielsen L.R. 2000. Natural hybridization between *Vanilla claviculata* (W.Wright) Sw. and *V. barbellata* Rchb.f. (Orchidaceae): genetic, morphological, and pollination experimental data. *Botanical Journal of the Linnean Society* 133: 285–302. http://dx.doi.org/10.1111/j.1095-8339.2000.tb01547.x

Pabst G.F.J. 1975. *Vanilla dungsii*. *Bradea* 2: 49.

Palama T.L., Khatib A., Choi Y.H., Payet B., Fock I., Verpoorte R. & Kodja H. 2009. Metabolic changes in different developmental stages of *Vanilla planifolia* pods. *Journal of Agricultural and Food Chemistry* 57: 7651–7658. http://dx.doi.org/10.1021/jf901508f

Pansarin E.R. & Pansarin L.M. 2014. Floral biology of two Vanilloideae (Orchidaceae) primarily adapted to pollination by euglossine bees. *Plant Biology* 16: 1104–1113. http://dx.doi.org/10.1111/plb.12160

Pansarin E.R., Salatino A., Pansarin L.M. & Sazima M. 2012. Pollination systems in Pogonieae (Orchidaceae: Vanilloideae): A hypothesis of evolution among reward and rewardless flowers. *Flora – Morphology, Distribution, Functional Ecology of Plants* 207: 849–861. http://dx.doi.org/10.1016/j.flora.2012.09.011

Pérez-Silva A., Odoux E., Brat P., Ribeyre F., Rodriguez-Jimenes G., Robles-Olvera V., García-Alvarado M.A. & Günata Z. 2006. GC–MS and GC–olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*Vanilla planifolia* G. Jackson) beans. *Food Chemistry* 99: 728–735. http://dx.doi.org/10.1016/j.foodchem.2005.08.050

Pérez Silva A., Gunata Z., Lepoutre J.-P. & Odoux E. 2011. New insight on the genesis and fate of odor-active compounds in vanilla beans (*Vanilla planifolia* G. Jackson) during traditional curing. *Food Research International* 44: 2930–2937. http://dx.doi.org/10.1016/j.foodres.2011.06.048

Portères R. 1953. Le potentiel de variation clonale de *Vanilla tahitensis* J. W. Moore. *L'agronomie Tropicale* 8: 639–640.

Portères R. 1954. Le genre *Vanilla* et ses espèces. *In:* G. Bouriquet (ed.) *Le Vanillier et la Vanille dans le Monde*: 94–290. Editions Paul Lechevalier, Paris.

Presl C.B. von 1827. *Reliquiae Haenkeanae.* J.G. Calve, Prague, Czech Republic.

R Core Team 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Ramírez S.R., Gravendeel B., Singer R.B., Marshall C.R. & Pierce N.E. 2007. Dating the origin of the Orchidaceae from a fossil orchid with its pollinator. *Nature* 448: 1042–1045. http://dx.doi.org/10.1038/nature06039

Reichenbach F.H.G. 1865. *Vanilla phaeantha*. *Flora* 48: 274.

Reyes-López D., Flores-Jiménez A., Huerta-Lara M., Kelso-Bucio H.A., Avendaño-Arrazate C.H., Lobato-Ortiz R., Aragón-García A. & López-Olguín J.F. 2014. Variación morfométrica de fruto y semilla en cuatro especies del género *Vanilla*. *Ecosistemas y Recursos Agropecuarios* 1: 205–218.

Rolfe R.A. 1895. Vanillas of commerce. *Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew* 1895: 169–178. Available from http://biodiversitylibrary.org/page/40984721 [accessed 12 Jan. 2017].

Rolfe R.A. 1899. New Orchids. – Decades 23 and 24. *Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew* 1899: 126–133. Available from http://biodiversitylibrary.org/page/40848484 [accessed 12 Jan. 2017].

Roux-Cuvelier M. & Grisoni M. 2010. Conservation and movement of *Vanilla* germplasm. *In*: Odoux E. & Grisoni M. (eds) *Vanilla*: 31–41. CRC Press, Boca Raton, FL.

Sambin A. & Chiron G.R. 2015. Deux nouvelles espèces de *Vanilla* (Orchidaceae) de Guyane française. *Richardiana* 15: 306–316.

Schiede C.J.W. 1829. Botanische Berichte aus Mexico. *Linnaea* 4: 574–604.

Soto Arenas M.A. & Cribb P. 2010. A new infrageneric classification and synopsis of the genus *Vanilla* Plum. ex Mill. (Orchidaceae: Vanillinae). *Lankesteriana* 9: 355–398.

Soto Arenas M.A. & Dressler R.L. 2010. A revision of the Mexican and Central American species of *Vanilla* Plumier ex Miller with a characterization of their ITS region of the nuclear ribosomal DNA. *Lankesteriana* 9: 285–354.

Sun H.-Q., Cheng J., Zhang F.-M., Luo Y.-B. & Ge S. 2009. Reproductive success of non-rewarding *Cypripedium japonicum* benefits from low spatial dispersion pattern and asynchronous flowering. *Annals of Botany* 103: 1227–1237. http://dx.doi.org/10.1093/aob/mcp066

Sun Y., Skinner D.Z., Liang G.H. & Hulbert S.H. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26–32. http://dx.doi.org/10.1007/BF00226978

Szlachetko D.L. 1995. *Systema Orchidalium.* W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Takhtajan A.L. 1967. *Sistema i Filogeniia Tsvetkovykh Rastenii (Systema et Phylogenia Magnoliophytorum).* Soviet Science Press, Moscow.

Theis T. & Jiménez T.A. 1957. A *Vanilla* hybrid resistant to *Fusarium* root rot. *Phytopathology* 47: 578–581.

Thiers B. Continuously updated. *Index Herbariorum, a Global Directory of Public Herbaria and Associated Staff.* Available from http://sweetgum.nybg.org/ih/ [accessed Aug. 2016].

Varela Quirós E. 2011. Vanilla production in Costa Rica. *In:* Havkin-Frenkel D. & Belanger F.C. (eds) *Handbook of Vanilla Science and Technology*: 40–49. Wiley-Blackwell, Chichester, United Kingdom.

Xu S., Li D., Li J., Xiang X., Jin W., Huang W., Jin X. & Huang L. 2015. Evaluation of the DNA barcodes in *Dendrobium* (Orchidaceae) from mainland Asia. *PLoS ONE* 10: e0115168. http://dx.doi.org/10.1371/journal.pone.0115168

*Manuscript received: 18 March 2016*
*Manuscript accepted: 2 September 2016*
*Published on: 22 February 2017*
*Guest editors: Line Le Gall, Frédéric Delsuc, Stéphane Hourdez, Guillaume Lecointre and Jean-Yves Rasplus*
*Desk editor: Danny Eibye-Jacobsen*

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'Histoire naturelle, Paris, France; Botanic Garden Meise, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Natural History Museum, London, United Kingdom; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands.

# ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: European Journal of Taxonomy

Jahr/Year: 2017

Band/Volume: 0284

Autor(en)/Author(s): Azofeifa-Bolanos Jose B., Gigant Rodolphe, Nicolas-Garcia Mayra, Pignal Marc, Tavares-Gonzalez Fabiola B., Hagsater Eric, Salazar-Chavez Gerardo A., Reyes-Lopez Delfino, Archila-Morales Fredy L., Garcia-Garcia Jose A., Silva Denis da, Allibert Agathe, Solano-Campos Frank, Rodriguez-Jomenes Guadalupe del Carmen, Paniagua-Vasquez Amelia, Besse Pascale, Perez-Silva Araceli, Grisoni Michel

Artikel/Article: A new vanilla species from Costa Rica closely related to V. planifolia (Orchidaceae) 1-26