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BRIEF COMMUNICATION

Neotropical roots of a Polynesian spice: the hybrid origin of Tahitian vanilla, *Vanilla tahitensis* (Orchidaceae)[1]

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Absent in the wild, Tahitian vanilla (*V. tahitensis*) is a gourmet spice restricted in distribution to cultivated and feral stands in French Polynesia and Papua New Guinea. Its origins have been elusive. Our objective was to test the purported hybrid derivation and parentage of *V. tahitensis* from aromatic, neotropical progenitors. Nucleotide sequences from *V. tahitensis* and neotropical *Vanilla* were assayed for phylogenetic relatedness in two independently inherited genomic regions, the nuclear ITS region, and the *trnH-psbA* noncoding region of chloroplast DNA. As predicted to occur for early generation hybrids, placement of *V. tahitensis* was nonconcordant. All *V. tahitensis* clustered with *V. planifolia* from analysis of cpDNA sequences, suggesting *V. planifolia* as the maternal genome contributor. Phylogenetic reconstruction of ITS sequences showed that most *V. tahitensis* nested incongruently with *V. odorata*, but others remained sister to *V. planifolia*. Recovery of ITS clones in *V. tahitensis* related to both *V. planifolia* and *V. odorata* also supports its biphyletic origin from these two taxa. We interpret the high percentage (95%) of additive polymorphic sites in *V. tahitensis* relative to its parents as indication of a recent, and probably human-mediated, evolutionary origin.

Key words: crop origins; French Polynesia; hybridization; Mesoamerica; Orchidaceae; Tahitian vanilla; *Vanilla*.

Vanilla Plumier ex Miller and its allies (the "vanilloid orchids" or subfamily Vanilloideae) are the extant descendants of a basal lineage involved in the early diversification of the Orchidaceae (20 000–30 000 species), estimated to have occurred during the Late Cretaceous (Bremer, 2002; Cameron and Soto Arenas, 2003; Ramírez et al., 2007). Vanilloids are notable for having morphological features atypical of most orchids (Dressler, 1993; Cameron and Soto Arenas, 2003). For instance, instead of the characteristically dry orchid capsule with dustlike seeds, certain vanilloids produce fleshy fruits, and close to 20 species (but probably more) of exceptional, neotropical *Vanilla* have fleshy fruits that are also noticeably redolent (a supposedly aromatic species from Borneo, *V. abundiflora*, has yet to be confirmed) (Rolfe, 1896; Portères, 1954; Chan et al., 1994; Weiss, 2002). How these fruit types are adaptive is unknown; scents may attract dispersers such as fragrance-seeking, neotropical endemics like male Euglossine bees (Lubinsky et al., 2006), be selected for a defense response (vanillin, for example, is a phenolic) (Kessler and Baldwin, 2002), or both.

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Commercial vanilla is the naturally redolent, cured fruit of the Mesoamerican orchid *Vanilla planifolia* Jacks. (Orchidaceae). Although vanilla is widely known as a high-value crop, its price is also notably volatile. In 2003–2005, the unit import price of vanilla in the United States, the leading consumer of vanilla, soared to one of the highest unit values of any agricultural import including meats and poultry only to drop more than 80% in the following year (FAO, 2008). World production of vanilla has been dominated by the Indian Ocean, particularly Madagascar, since the mid to late 1800s. It was then that stem cuttings of the succulent, epiphytic vine *V. planifolia* were introduced by colonial authorities and that an artificial method for effecting pollination was developed out of necessity (along with date palm, vanilla is one of the world's few crops that are exclusively hand-pollinated) (Smith et al., 1992). The only other commercial *Vanilla* species currently exploited is the more expensive Tahitian vanilla (*V. tahitensis* J. W. Moore), which has been produced in French Polynesia also since around the end of the 19th century. Unlike the fruits of *V. planifolia*, a raw material widely employed in flavor and fragrance manufacturing, the rich, sweet flavor and abundant oleoresins of Tahitian vanilla beans make them a mainstay of the luxury, gourmet market (Ecott, 2004; Rain, 2004).

The origin and evolution of Tahitian vanilla have been enigmatic subjects because the species, described from feral material found on the island of Raiatea (French Polynesia), does not exist in the wild (Moore, 1933; Portères, 1954; Cameron and Soto Arenas, 2003). Compared with *V. planifolia*, *V. tahitensis* differs morphologically mainly in the leaves, which are much narrower, and in some of its floral traits, such as margins that

are more fimbriate than ungulate (Portères, 1954). In the 1950s, two hypotheses were simultaneously advanced to account for how *V. tahitensis* may have originated, each one positing *V. planifolia* and the process of interspecific hybridization, with either *V. pompona* Schiede or *V. odorata* C. Presl. acting opposite *V. planifolia* as parental donor species (Portères, 1954). Both *V. pompona* and *V. odorata* are native to the neotropics, and possess, or were thought to possess, characteristics making them similar to *V. tahitensis*, i.e., fruit aroma and vegetative morphology, respectively. No molecular study has so far found evidence in the favor of either supposition (Besse et al., 2004; Bory et al., 2007), nor have any novel hypotheses been proposed to substitute in their places. In this respect, attempts at solving the riddled origins of *V. tahitensis* have been left without much of a forward direction.

Vanilla was likely introduced into French Polynesia from the Pacific littoral of Mesoamerica via the Philippines. Although there are no records that have been found to date, regular shipments of vanilla beans and/or stem cuttings could well have been made aboard the 300-yr Manila Galleon trade, a primary vehicle for the introductions of many New World domesticates into SE Asia and Oceania (Schurz, 1939; Merrill, 1954). Whereas today there is no indication that there are natural or remnant populations of aromatic *Vanilla* in the Philippines, M. Blanco's 19th-century botanical treatise, *Flora de Filipinas,* does mention a "vanilla of Guatemala" that had been known (Blanco, 1837). This comment is attention grabbing to the student of historical crop geography: the only region in Mesoamerica documented to have produced vanilla for export is Papantla, Veracruz, on the Gulf side of Mexico (Sauer, 1993; Kourí, 2004). Rather than traverse the Isthmus of Tehuantepec, it would seemingly have been more economical for a Pacific-based vanilla trade to exploit the closest available populations of vanilla to west coast sea traffic, i.e., in southern Chiapas, Guatemala, and El Salvador. Evidence for such trade is not altogether lacking. In the 1630s, the pirate and explorer Thomas Gage made a note of vanilla use among the Maya in tandem with cacao (*Theobroma cacao* L.) and annatto/achiote (*Bixa orellana* L.) cultivation from the coastal Suchitepequez region of Guatemala (Thompson, 1958).

A major disadvantage that has confronted all prior attempts to elucidate the origins of *V. tahitensis* has been an inability to positively identify *V. odorata* from live, reproductively mature specimens. *Vanilla* taxonomy in general is afflicted with a lack of reliable herbarium vouchers, a result of the rarity of the plants themselves, the ephemeral nature of their flowers, and the relatively low rates of natural pollination characterizing the genus (Cameron and Soto Arenas, 2003). With the historic information at hand, we undertook to reexamine the origins of *V. tahitensis* by focusing on correct species determinations of plant material collected directly from wild populations of different *Vanilla* taxa in Mesoamerica, including both *V. pompona* and *V. odorata*. To test for a pattern consistent with a hybrid origin, DNA samples from these individuals and from cultivated individuals of *V. tahitensis* were subjected to molecular phylogenetic comparisons of nuclear and plastid DNA sequence data, which have been sufficient at resolving *Vanilla* at the interspecific level (Cameron et al., 1999).

MATERIALS AND METHODS

Aromatic and nonaromatic *Vanilla* species were collected from natural and cultivated populations of *Vanilla* from southern Mexico to Peru to survey for possible parental contributors to *V. tahitensis*. These consisted of collections of *V. planifolia*, *V. odorata*, *V. insignis*, *V. pompona*, *V. inodora*, *V. mexicana*, *V. hartii*, *V. bicolor*, and several undetermined *Vanilla*. Further samples were obtained as donations of leaf tissue and included taxa from the Caribbean (i.e., *V. claviculata*) and Africa/Madagascar (i.e., *V. polylepis*, *V. madagascariensis*, *Vanilla* sp.). Vouchers of specimens used in this study are on deposit at herbaria and in living collections (Table 1). Eleven samples of *V. tahitensis* were provided via a Material Transfer Agreement between the University of California and the Ministry of Agriculture of Tahiti and consisted of individuals identified through flow cytometry as either diploid ($2n = 32$) or tetraploid ($2n = 64$) (Table 1). Tetraploids were represented by cvs. Tiarei (C09–20), Haapape (the most ubiquitous cultivar, [C10–49, C07–27, C01–85]), Tahiti long (C01–40) and diploids by cvs. Tahiti (T4–41), Potiti (C01–30), Parahurahu (C07–33), Pupa (C02–28), and Paraauti (C09–31). The chromosome number for one accession, cv. Sauvage (C09–57), was not determined. One additional *V. tahitensis* from Papua New Guinea was also collected and included in the analysis. *Vanilla tahitensis* is the only species reported in the genus to be mixoploid (i.e., $2n = 32, 64$) (Cameron and Soto Arenas, 2003). One of its putative parents, *V. planifolia,* is most frequently reported as $2n = 32$, as are most members of the genus including *V. pompona*. The ploidy level for *V. odorata* is yet to be determined.

TABLE 1. Identities and localities of *Vanilla* accessions used in phylogenetic analyses of the hybrid origin of *V. tahitensis*. Ploidy level for *V. tahitensis* indicated in parentheses, n.d. = not determined. Living vouchers and herbarium specimens of accessions from Mexico, Guatemala, Belize, Ecuador, and Trinidad and Tobago are deposited at UC Riverside (UCR), the Quito Herbarium (QCA), the Belize Botanical Garden, and at the INIFAP experimental station in Martinez de la Torre, Veracruz, Mexico. Living vouchers of *V. tahitensis* accessions are maintained at a germplasm center in Uturoa, Raiatea, French Polynesia.

Species	Collection no.	Locality
V. insignis	PL753	Mexico, Oaxaca, S.J.B. Valle Nacional
V. insignis	PL803	Mexico, Quintana Roo, Lázaro Cárdenas
V. insignis	PL1038	Guatemala, Alta Verapaz, Coban
V. odorata	PL614	Ecuador, Imbabura Prov., Lita
V. odorata	PL716	Mexico, Oaxaca, Ayotzintepec
V. odorata	PL1008	Belize, Toledo, Blue Creek
V. odorata	PL1018	Belize, Toledo, Blue Creek
V. odorata	PL1043	Guatemala, Alta Verapaz, Coban
V. planifolia	PL613	Ecuador, Napo Prov., INIAP
V. planifolia	PL797	Mexico, Oaxaca, Tuxtepec
V. planifolia	PL958	Trinidad/Tobago
V. planifolia	PL1000	Belize, Cayo Dist., El Pilar
V. planifolia	PL1003	Belize Cayo Dist., El Pilar
V. planifolia	PL1047	Guatemala, Alta Verapaz, Coban
V. pompona	PL822	Mexico Veracruz, Ma. de la Torre
Vanilla sp.	PL956	Trinidad/Tobago
Vanilla sp.	PL752	Mexico, Oaxaca, S.J.B. Valle Nacional
Vanilla sp.	PL1026	Belize, Cayo Dist., San Ignacio
Vanilla sp.	PL1046	Guatemala, Alta Verapaz, Coban
V. claviculata	PL94	Puerto Rico
V. tahitensis	C02–28 cv. Pupa (2×)	French Polynesia
V. tahitensis	C09–31 cv. Paraauti (2×)	French Polynesia
V. tahitensis	C09–20 cv. Tiarei (4×)	French Polynesia
V. tahitensis	C10–49 cv. Haapape (4×)	French Polynesia
V. tahitensis	C07–27 cv. Haapape (4×)	French Polynesia
V. tahitensis	C01–85 cv. Haapape (4×)	French Polynesia
V. tahitensis	C01–40 cv. Tahiti long (4×)	French Polynesia
V. tahitensis	C09–57 cv. Sauvage (n.d.)	French Polynesia
V. tahitensis	C01–30 cv. Potiti (2×)	French Polynesia
V. tahitensis	C07–33 cv. Parahurahu (2×)	French Polynesia
V. tahitensis	T4–41 cv. Tahiti (2×)	French Polynesia
V. tahitensis	PNG (n.d.)	Papua New Guinea

Total genomic DNA was isolated from dry and fresh leaf tissue using a DNeasy plant mini kit (Qiagen, Valencia, California). PCR-amplification of the ITS region was performed as follows: 25-μL reactions (1 μL total genomic DNA, 13.5 μL ddH_2O, 2.5 μL DMSO, 6 μL mix of Tricine Taq buffer + dNTPs, 1 μL ITS4, 1 μL ITS5, and 0.25 μL *Taq* polymerase) were initially denatured for 5 min at 95°C, followed by primer annealing for 1 min at 50°C, and then by a 1 min extension for 72°C. These three settings were repeated for 40 additional cycles, but with only 1 min allotted for denaturing. A final extension was performed for 10 min at 72°C. Reaction conditions were the same for amplification of *trnH-psbA* except no DMSO was used and ddH_2O volume was adjusted to 16 μL. PCR products of at least 40 μL/sample were purified with QiaQuick PCR Purification kit (Qiagen, Valencia, California, USA). Direct sequencing of PCR products was performed at the Core Instrumentation Facility at the UCR Genomics Institute and was based on 6-μL reactions (1μL primer + 4 μL ddH_2O + 1 μL DNA template). For sequencing of ITS, two additional internal primers, ITS3 and ITS2 (White et al., 1990), were used. Base calling and sequence editing were performed with the program Sequencher version 4.1 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were manually entered and visually aligned in the program MacClade (version 4.0; Madison and Madison, 2000).

Phylogenetic analyses using Fitch parsimony were performed with the program PAUP* (version 4.0b10; Swofford, 2002) with the heuristic search option set to tree-bisection-reconnection (TBR) branch swapping and with MULPARS on. Support for groups was examined by 1000 bootstrap replicates (Felsenstein 1985) using the heuristic search option from a simple addition sequence with TBR branch swapping. Pairwise sequence divergence was calculated using the Kimura 2-parameter method (Kimura, 1980) and a neighbor-joining (NJ) tree (Saito and Nei, 1987) was constructed using PAUP*. For maximum likelihood analysis, optimal models of molecular evolution were chosen using the likelihood ratio test (Goldman, 1993) implemented in the program ModelTest, version 3.7 (Posada and Crandall, 1998). Model parameters were then imported into PAUP*, and heuristic searches were executed.

RESULTS

The aligned matrix for ITS consisted of 649 total characters. Base calling for all accessions of *V. planifolia* and for some individuals of *V. tahitensis* could not be deciphered beyond 520 bp (ITS2). Otherwise, complete ITS1, 5.8S, and ITS2 sequences were obtained for all taxa. Two indels were detected: a 5-bp insertion in ITS1 unique to *V. claviculata* and a 7-bp indel present in some individuals from all species (the indel could not be determined to occur in *V. planifolia* because reliable chromatogram peaks could not be obtained). Tandem repeats with one extra repeat were recovered in *V. insignis* (TC) and *V. claviculata* (CC), both from the ITS2 region. No polymorphisms were found in the 5.8S region, with one exception of a species-specific single nucleotide polymorphism (SNP) in *V. planifolia*. The overall number of SNPs was generally low (i.e., <2/taxa), except in the cases of *V. planifolia* and *V. tahitensis*, which each had more than 10. A high percentage of additive polymorphic sites (APSs) were observed in *V. tahitensis* relative to sequences generated for *V. planifolia* and *V. odorata* (Table 2). Parsimony analysis was performed twice using the heuristic search option on both the total amount of characters and on a trimmed, 520-character matrix (55 of which were parsimony informative) and yielded identical relationships with high bootstrap support, low levels of homoplasy, and sufficient resolution to interpret interspecific relationships. Indels were treated as missing data. The aligned matrix of *trnH-psbA* consisted of 637 characters, 23 of which were parsimony informative. Maximum parsimony analysis similarly resulted in

TABLE 2. Additive polymorphic sites (APS) in *Vanilla tahitensis* relative to its parental species, *V. planifolia* and *V. odorata*. Only bp37 (shown in bold) is not additive when compared to its parental alleles. Base calls include all variation found among individuals that were surveyed.

Taxon	1. bp15	2. bp36	**3. bp37**	4. bp55	5. bp62	6. bp90	7. bp95
V. odorata	T	A	**T**	C	C	C/T	T/C
V. tahitensis[§]	Y	R	**C**	Y	Y	Y	Y
V. tahitensis[†]	C	G	**C**	T	T	C	T
V. planifolia	C	R	**Y**	Y/T	T	Y/T	T

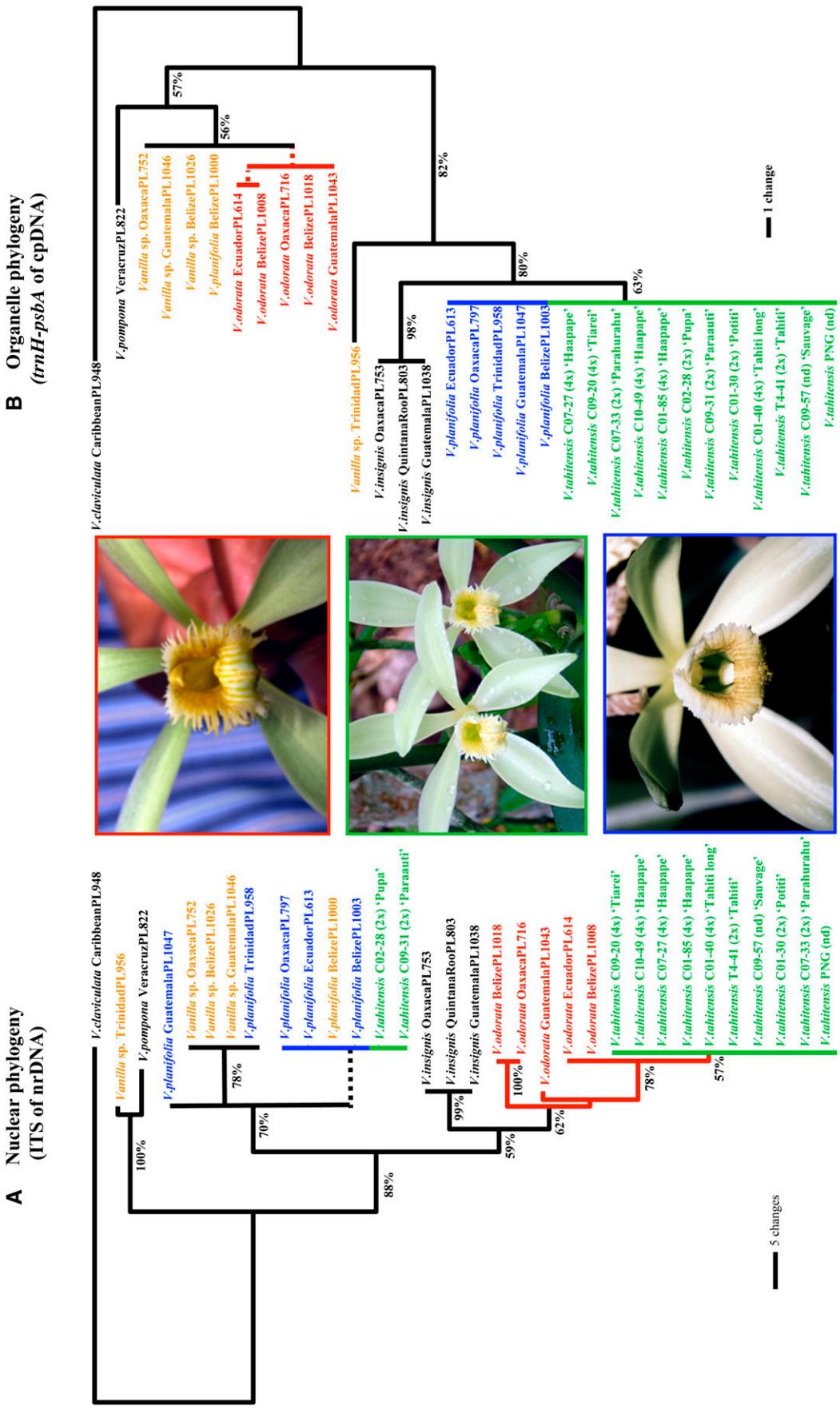
	8. bp146	9. bp172	10. bp181	11. bp184	12. bp191	13. bp407	14. bp418
V. odorata	T	A	G	A/G	T	T	C
V. tahitensis[a]	Y	R	R	R	Y	Y	Y
V. tahitensis[b]	C	G	A	A	C	C	T
V. planifolia	C	R/A/G	A	A	C	C	T

	15. bp430	16. bp431	17. bp454	18. bp482	19. bp486	20. bp489	21. bp503
V. odorata	T	T	C/T	A	T	T	G
V. tahitensis[a]	Y	K	Y	R	K	Y	K
V. tahitensis[b]	C	G	C	G	G	C	T
V. planifolia	C	G	C	G	G	Y	T/K

[a] *V. tahitensis* accessions C09-20, C10-49, C07-27, C01-85, C01-40, T4-41, C01-30, and C07-33.
[b] *V. tahitensis* accessions C02-28 and C09-31. Y = T+C, R = A+G, K = G+T.

→

Fig. 1. Phylograms based on maximum parsimony analysis showing nonconcordant placement of *V. tahitensis* relative to its parental species *V. planifolia* and *V. odorata* in (A) nuclear (ITS) and (B) cpDNA (*trnH-psbA*) phylogenetic reconstructions. Other taxa with nonconcordant placement are in orange. Photos (top to bottom): *V. odorata*, *V. tahitensis*, and *V. planifolia*. Bootstrap values based on 1000 replicates are given below branches and represent relationships found in >50% of replicates. Ploidy level for *V. tahitensis* is indicated in parentheses, n.d.= not determined. Dashed lines in ITS represent branches collapsed in a strict consensus tree [(A) ITS, one of 5000 equally parsimonious trees; T. L. = 147, C. I. = 0.8707, H. I. = 0.1293, R. I. = 0.9148), (B) *trnH-psbA*, one of six equally parsimonious trees; T. L. = 53, C. I. = 0.8302, H. I. = 0.1698, R. I. = 0.9379)]. No interspecific rearrangements occurred in other trees with equal parsimony.


A Nuclear phylogeny (ITS of nrDNA)
V.claviculata CaribbeanPL948
Vanilla sp. TrinidadPL956
V.pompona VeracruzPL822
V.planifolia GuatemalaPL1047
Vanilla sp. OaxacaPL752
Vanilla sp. BelizePL1026
Vanilla sp. GuatemalaPL1046
V.planifolia TrinidadPL958
V.planifolia OaxacaPL797
V.planifolia EcuadorPL613
V.planifolia BelizePL1000
V.planifolia BelizePL1003
V.tahitensis C02-28 (2x) 'Pupa'
V.tahitensis C09-31 (2x) 'Paraauti'
V.insignis OaxacaPL753
V.insignis QuintanaRooPL803
V.insignis GuatemalaPL1038
V.odorata BelizePL1018
V.odorata OaxacaPL716
V.odorata GuatemalaPL1043
V.odorata EcuadorPL614
V.odorata BelizePL1008
V.tahitensis C09-20 (4x) 'Tiarei'
V.tahitensis C10-49 (4x) 'Haapape'
V.tahitensis C07-27 (4x) 'Haapape'
V.tahitensis C01-85 (4x) 'Haapape'
V.tahitensis C01-40 (4x) 'Tahiti long'
V.tahitensis T4-41 (2x) 'Tahiti'
V.tahitensis C09-57 (nd) 'Sauvage'
V.tahitensis C01-30 (2x) 'Potiti'
V.tahitensis C07-33 (2x) 'Parahurahu'
V.tahitensis PNG (nd)
100%
78%
70%
88%
99%
59%
100%
62%
78%
57%
5 changes
B Organelle phylogeny (trnH-psbA of cpDNA)
V.claviculata CaribbeanPL948
V.pompona VeracruzPL822
Vanilla sp. OaxacaPL752
Vanilla sp. GuatemalaPL1046
Vanilla sp. BelizePL1026
V.planifolia BelizePL1000
V.odorata EcuadorPL614
V.odorata BelizePL1008
V.odorata OaxacaPL716
V.odorata BelizePL1018
V.odorata GuatemalaPL1043
Vanilla sp. TrinidadPL956
V.insignis OaxacaPL753
V.insignis QuintanaRooPL803
V.insignis GuatemalaPL1038
V.planifolia EcuadorPL613
V.planifolia OaxacaPL797
V.planifolia TrinidadPL958
V.planifolia GuatemalaPL1047
V.planifolia BelizePL1003
V.tahitensis C07-27 (4x) 'Haapape'
V.tahitensis C09-20 (4x) 'Tiarei'
V.tahitensis C07-33 (2x) 'Parahurahu'
V.tahitensis C10-49 (4x) 'Haapape'
V.tahitensis C01-85 (4x) 'Haapape'
V.tahitensis C02-28 (2x) 'Pupa'
V.tahitensis C09-31 (2x) 'Paraauti'
V.tahitensis C01-30 (2x) 'Potiti'
V.tahitensis C01-40 (4x) 'Tahiti long'
V.tahitensis T4-41 (2x) 'Tahiti'
V.tahitensis C09-57 (nd) 'Sauvage'
V.tahitensis PNG (nd)
57%
56%
82%
98%
80%
63%
1 change

relationships with sufficient resolution and strong statistical support. Of all neotropical taxa included in the survey, the closest relatives to *V. tahitensis* were revealed in both genome analyses to be: *V. planifolia*, *V. odorata*, *V. insignis*, and *V. pompona,* as well as four unidentified accessions of *Vanilla*. All other species and accessions were subsequently excluded in phylogenetic analyses. These relationships were confirmed using both NJ and UPGMA genetic distances and maximum likelihood analyses. Phylograms based on maximum parsimony analysis depicting relationships among these individuals, using *V. claviculata* as an outgroup species, are shown in Fig. 1.

An inconsistent genetic pattern with regard to *V. tahitensis*, involving most but not every accession, is clear. All *V. tahitensis* accessions share a chloroplast haplotype identical to *V. planifolia*. In contrast, in the nuclear gene tree, all but two accessions of *V. tahitensis*, including the most popular cultivar, Haapape, were embedded among samples of *V. odorata*. The two accessions that did not fit this pattern, C02–28 (cv. Pupa) and C09–31 (cv. Paraauti), remained most closely related to *V. planifolia*. To test whether ITS-based polyphyly of *V. tahitensis* was the manifestation of "PCR artifacts" or a rendering of real phylogenetic differences, three accessions of *V. tahitensis* (C07–33[2×], cv. Parahurahu; C07–27[4×], cv. Haapape; C02–28[2×], cv. Pupa) were cloned so as to assess placement of ribotypes at the intraindividual level. Cloning and sequencing of PCR products was conducted using a TOPO TA kit (Invitrogen, Carlsbad, California, USA) following the manufacturer's protocols. Clones related to both *V. planifolia* and *V. odorata* were subsequently recovered for two accessions, C07–33 (eight clones) and C07–27 (nine clones), corroborating the hypothesis of biparental inheritance established from the results of direct sequencing. However, *V. odorata*-like ribotypes were not encountered in C02–28 after surveying 10 clones, an indication that perhaps *V. odorata* made no genetic contribution to this accession (Fig. 2). With the exception of some occasional, unique singletons, which are most likely due to PCR error (Nieto Feliner et al., 2004), only two ribotypes, one related to *V. planifolia* and the other to *V. odorata*, were recovered from cloning.

DISCUSSION

Phylogenetic nonconcordance is either the result of the incomplete lineage sorting of ancestral polymorphisms or an indication of an interspecific, mongrel origin (Rieseberg et al., 1996; Arnold, 1997; Sang and Zhong, 2000). In the case of *V. tahitensis*, there are three reasons that convincingly support nonconcordance due to hybridization as opposed to the outcome of lineage sorting. First, the relatively high amounts of average pairwise sequence divergence between *V. planifolia* and *V. odorata* in both ITS (3.2% ± 0.0032, calculated by the Kimura 2-parameter method) and *trnH-psbA* (1.923% ± 0.0071) markers are a strong indication of their relatively distant genetic relationship as completely differentiated, nonsibling species. Second, the high number (20 of 21) of additive polymorphic sites (APS) in most *V. tahitensis* in relation to its parental species is detectable when analyzing a matrix of aligned DNA sequences from the biparentally inherited ITS region (Table 2), a pattern most consistent with recent, or, when complete, F_1 hybrid formation (Rieseberg and Ellstrand, 1993; Nieto Feliner et al., 2004). Because at one site (bp37, Table 2) we did not observe an additive pattern in any accessions of *V. tahitensis*, a hypothesis for an F_1 hybrid origin cannot be supported. However, this nonadditive site may be the result of sampling error or, alternatively, the forced conversion of ITS ribotypes during hybrid formation (Weeks and Simpson, 2004). A final reason for favoring hybridization over lineage sorting is that hybridization is the most parsimonious explanation; lineage sorting requires the assumption that *V. tahitensis* existed or exists naturally.

The lone two accessions of *V. tahitensis* that do not fit into the pattern of nonconcordance (C02–28 [cv. Pupa], and C09–31 [cv. Paraauti]) and whose identical ITS sequences are void entirely of any kind of polymorphic sites may either share an evolutionary history discrete from other *V. tahitensis* individuals, or they may have erroneously resulted from the biased PCR amplification of nonhomologous ITS ribotypes (Baldwin et al., 1995; Álvarez and Wendel, 2003). After generating 10 clones for C02–28, we did not find evidence of a *V. odorata*-like ribotype. This absence may be due to the homogenization of ITS ribotypes via the process of concerted evolution (Álvarez and Wendel, 2003), which has been demonstrated to result in "missing sequences" in multilocus, rDNA arrays like ITS, even in second-generation hybrids (Fuertes Aguilar et al., 1999). It may also be that C02–28 is the progeny of backcrossing to *V. planifolia*, a process that could similarly result in the fixation of a *V. planifolia* ribotype. The words "pupa" and "paraauti" in Tahitian both connote "to reproduce", which suggests that these cultivars have resulted from sexual reproduction. Whatever the pathway for their origin, it is probably not of much agronomic significance because both cv. Pupa and cv. Paraauti are highly unpopular among growers and not currently cultivated.

This study is the first to document the hybrid origins from neotropical progenitors of the important French Polynesian spice crop, *V. tahitensis*. The uniparental, maternal inheritance of cpDNA in angiosperm lineages like the Orchidaceae (Corriveau and Coleman, 1988; Harris and Ingram, 1991; Barkman and Simpson, 2002) suggests that *V. planifolia* and *V. odorata* served as respective maternal and paternal genomic contributors. While the high percentage of APS conserved in most individuals of *V. tahitensis* indicates very little evolutionary divergence from *V. planifolia* and *V. odorata*, in keeping almost with an F_1 hybrid, the mixed ploidy (i.e., 2×, 4×) of the accessions and their ITS-based polyphyly allow at least for episodes of both polyploidy and sexual regeneration to have elapsed. Because *V. tahitensis* is not found in the wild and is evolutionarily recent, a plausible scenario for its historic origin is that cuttings of *V. planifolia* and *V. odorata* were brought together and cultivated in a shared agroforest, where hybridization ensued via artificial or, if it occurred in the New World, natural means (similar to the "dump heap" model, Anderson, 1967; Hughes et al., 2007). This intentional or inadvertent hybridization could have happened during the Late Postclassic

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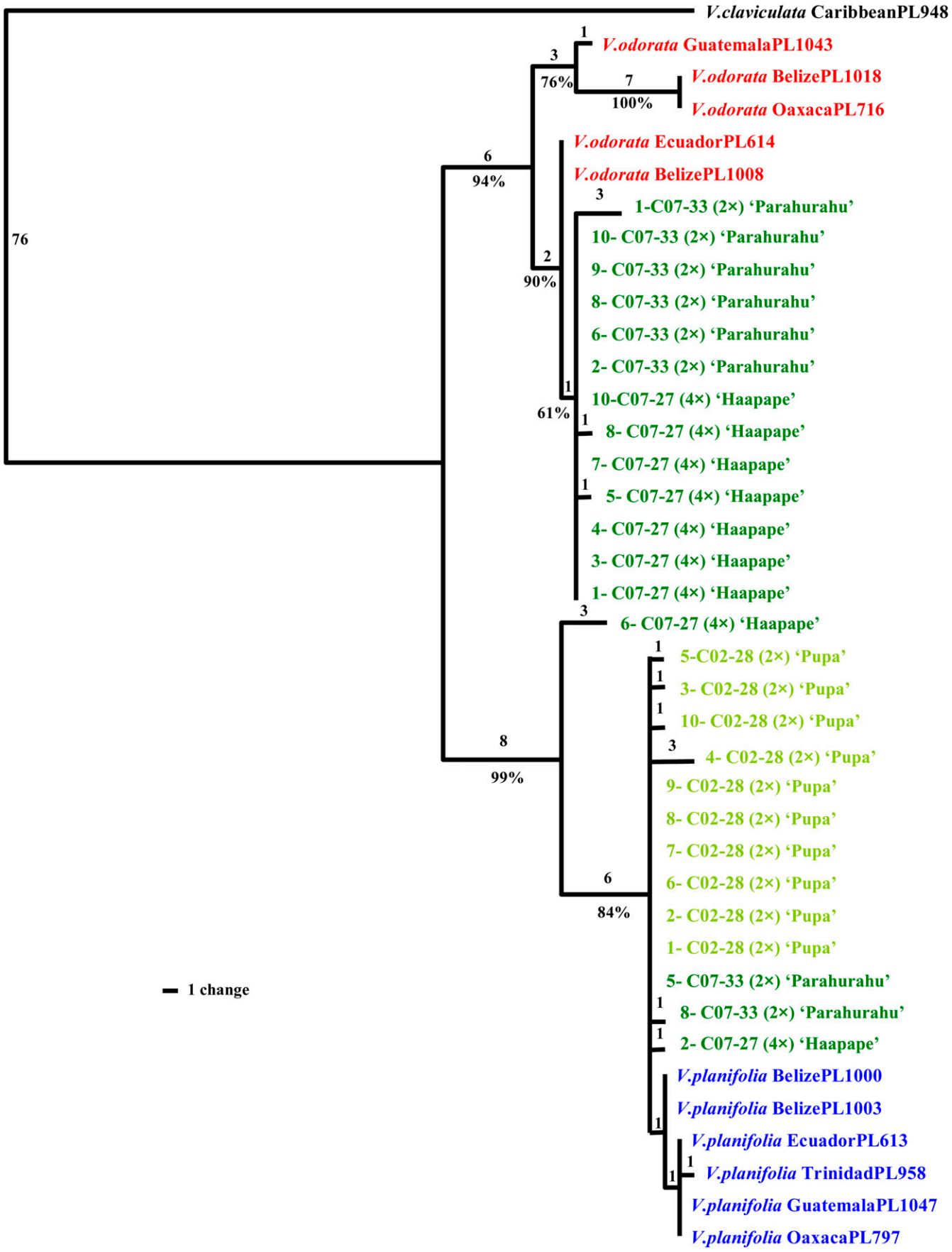
Fig. 2. Phylogram showing placement of cloned ribotypes of *V. tahitensis* relative to *V. planifolia* and *V. odorata* (one of 30 000 equally parsimonious trees; T. L. = 129, C. I. = 0.9225, H. I. = 0.0775, R. I. = 0.9716). Whereas C07–33 ([2×], cv. Parahurahu) and C07–27 ([4×], cv. Haapape) have ribotypes related to both parental species, C02–28 ([2×], cv. Pupa) shows no definite genetic contribution from *V. odorata*. The number of changes are indicated above the branches and bootstrap support based on a full-heuristic search of 1000 replicates are given below branches representing 50% majority rule values.


V.claviculata CaribbeanPL948
V.odorata GuatemalaPL1043
V.odorata BelizePL1018
V.odorata OaxacaPL716
V.odorata EcuadorPL614
V.odorata BelizePL1008
1-C07-33 (2×) 'Parahurahu'
10- C07-33 (2×) 'Parahurahu'
9- C07-33 (2×) 'Parahurahu'
8- C07-33 (2×) 'Parahurahu'
6- C07-33 (2×) 'Parahurahu'
2- C07-33 (2×) 'Parahurahu'
10-C07-27 (4×) 'Haapape'
8- C07-27 (4×) 'Haapape'
7- C07-27 (4×) 'Haapape'
5- C07-27 (4×) 'Haapape'
4- C07-27 (4×) 'Haapape'
3- C07-27 (4×) 'Haapape'
1- C07-27 (4×) 'Haapape'
6- C07-27 (4×) 'Haapape'
5-C02-28 (2×) 'Pupa'
3- C02-28 (2×) 'Pupa'
10- C02-28 (2×) 'Pupa'
4- C02-28 (2×) 'Pupa'
9- C02-28 (2×) 'Pupa'
8- C02-28 (2×) 'Pupa'
7- C02-28 (2×) 'Pupa'
6- C02-28 (2×) 'Pupa'
2- C02-28 (2×) 'Pupa'
1- C02-28 (2×) 'Pupa'
5- C07-33 (2×) 'Parahurahu'
8- C07-33 (2×) 'Parahurahu'
2- C07-27 (4×) 'Haapape'
V.planifolia BelizePL1000
V.planifolia BelizePL1003
V.planifolia EcuadorPL613
V.planifolia TrinidadPL958
V.planifolia GuatemalaPL1047
V.planifolia OaxacaPL797
76
1
3
76%
7
100%
6
94%
2
90%
1
61%
3
1
1
1
3
1
1
1
3
6
84%
8
99%
1
1
1
3
1
1
1
1
1
1 change

(1350–1500) in Mesoamerica, when populations of *Vanilla* were exploited by Maya silviculturalists along with *T. cacao* and *B. orellana* to supply transregional demand for cacao-based beverages (Caso Barrera and Fernández, 2006; McNeil, 2006).

This study demonstrates the Mesoamerican origins of a gourmet Polynesian spice crop. It identifies novel crop wild relatives (CWRs) for vanilla, namely *V. odorata*, which can be used in the improvement of a new generation of vanilla cultivars. This study also provides an example of a complicated situation in which two or more countries may justifiably lay claim to ownership or patrimony over the same genetic resources (see Maxted and Kell, 2008). The highly desirable flavor and fragrance of *V. tahitensis* illustrates the promise of breeding new commercial varieties of vanilla through hybridization. Species incompatibility due to reproductive isolation is not likely to pose a challenge to future improvement efforts. For example, successful artificial crosses of *Vanilla* have already been made between taxa native to different continents (Minoo et al., 2007). Natural hybridization has likewise been documented in Caribbean *Vanilla* (Nielsen, 2000), and possible evidence for geographically widespread natural hybridization in Mesoamerica was serendipitously detected in the nonconcordant positions of five accessions in this study (shown in orange in Fig. 1). Such taxa are worthy of more research because they can both provide insight into the role that hybridization has played in the evolution of *Vanilla* and because of their potential value as vehicles for sustainable development projects in Mexico and Central America.

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