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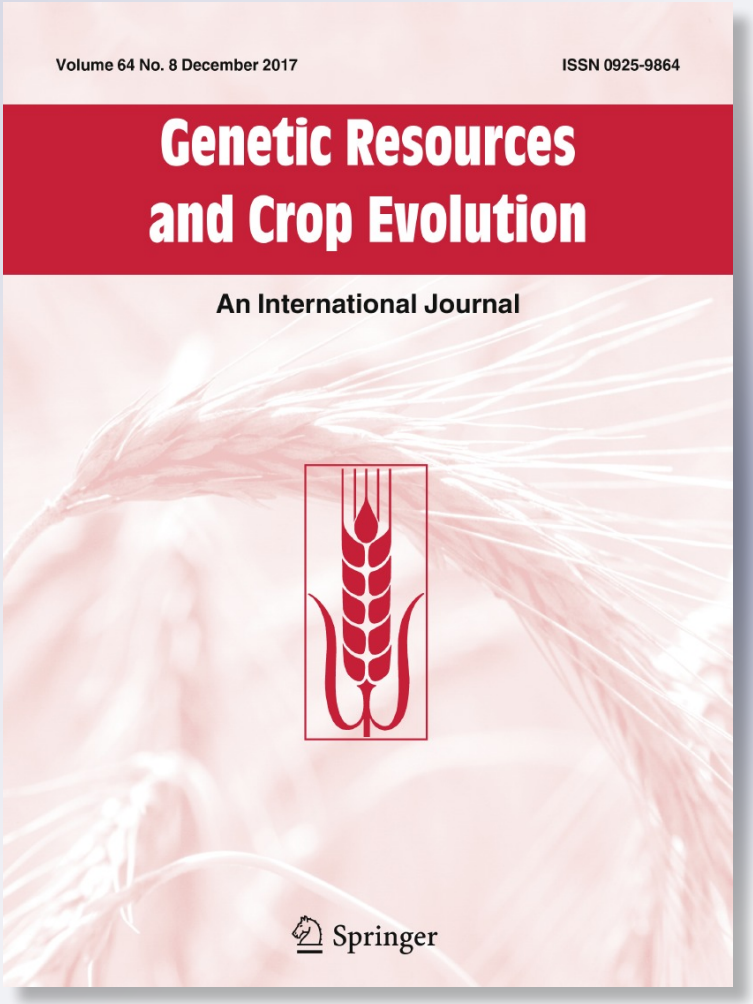

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REVIEW ARTICLE

The Criollo cacao tree (*Theobroma cacao* L.): a review

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Abstract In commercial terms, Criollo cacao trees (*Theobroma cacao* L.) are reputed to be the source of the commercial product (fermented and dried cocoa beans), which sells for the best price on the market. Nevertheless, the term "Criollo" has numerous meanings and interpretations depending on if it is used by commercial users or botanists, growers or breeders. Our review aims to specify which cocoas can justifiably carry the Criollo name. "Criollo" is a botanical subspecies of *Theobroma cacao,* i.e. *Theobroma cacao* subsp. *cacao*; however, the true Criollos form just one of the ten currently accepted genetic groups in the species. We thus provide an overview of genetic studies on the subject (published or not), along with what is currently known about "True Criollo" or "Ancient Criollo" cacao trees. In fact, there are few representatives in collections that are duly acknowledged to be true Criollos, particularly in the two International Cocoa Genebanks, where only seven clones are available. It is nonetheless certain that some true Criollos do exist in other collections but have not been formally identified (by genetic studies) as members of the Criollo genetic group. Likewise, some true Criollos, be they cultivated or subspontaneous, exist in Mexico and Central and South America (Venezuela and Colombia). However, certain clones called "modern Criollos", which are closely related to the true Criollos but arise from hybridization with other genetic groups, are more common.

Keywords Cocoa · Genetic group · Variety · Domestication · Genetic diversity · Population structure · Theobroma cacao

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Introduction

Of all the "flavour cocoas" produced in the world, which only account for 5–6% of the total cocoas produced (Pitipone 2016), cocoa beans labelled "Criollo" are always more desired and fetch higher prices, up to 5 times the price of "bulk" cocoa. However, there is no clear chemical or sensorial characterization of what a Criollo cacao is: Jinap et al. (1995), Afoakwa et al. (2008), Smulders et al. (2012) and Kongor et al. (2016). It is therefore important to define what truly has the right to be considered "Criollo" out of the numerous sources of fermented and dried cocoa beans. This is the aim of this study.

The historical concept of "Criollo"

The Spanish term "criollo" (from the Portuguese "crioulo") is basically an adjective that describes people (according to the *Diccionario de la lengua española* of the Spanish Royal Academy); however, in this case, it may also be secondarily used as a noun (in lower case).

When used by extension to qualify other items, such as the cacao tree, it might be translated to mean "local", "native" or "indigenous".

In its adjectival form, it is very widely used to describe the cacao tree in all American countries where "local" cacao trees are present, whether truly spontaneous or not. Under these conditions, the term does not imply any particular quality regarding the commercial product. The same applies when the adjective is used as a noun, such as Pound's famous "Criollo de la montagne" (Pound 1945; in a mixture of Spanish and French in his text).

The term "Criollo" with a capital letter (first used in Trinidad according to Bartley 2005, or more likely in Venezuela) qualifies a local "variety" (or even a "species" for some) that is phenotypically different from the "foreign" Forastero[1] and Trinitario[2] cacao trees and produces "better-quality" fermented and dried cocoa (with that cocoa sometimes being called "Caracas").

Botany

Morris published the first valid nomenclature of *Theobroma cacao* cultivars in 1882 (Morris 1882; quoted by Cuatrecasas 1964). He divided cacao trees into two major classes: Criollo Cacao and Forastero Cacao (with capital letters[3]), the second being divided into 8 varieties.

Since then, botanists have always separated "Criollo" cacao trees, which are presumed to originate from Mexico and Central America, from other cacao trees.

For instance, Pittier (1930) mentioned three species of cacao trees (*Theobroma cacao*, *T. leiocarpa*, *T. pentagonum*), the first two being explicitly the Criollo and the Calabacillo (or the Forastero[4]), respectively. Cheesman (1944), who conversely judged it pointless to recognize more than one species of cacao, whether cultivated or not, considered two origins for Criollo, Central America and South America, while questioning their spontaneous nature in Central America. In 1960, León mentioned three cultivated sub-species of *Theobroma cacao*, *T. c.* subsp. *sativa* (Lam.) Lign. et Le Bey, corresponding "more or less" to Criollo from Mexico and Panama, *T. c.* subsp. *leiocarpa* Bern. from South America (Colombia and Brazil) and *T. c.* subsp. *pentagona* Bern. from Guatemala to Panama (León 1960).

The last revision of the genus *Theobroma* dates back to 1964 and was conducted by Cuatrecasas (1964). He recognized two sub-species of cacao trees: *Theobroma cacao* subsp. *cacao* and *T. cacao* subsp. *sphaerocarpum.*

The first, for which he acknowledged three "forms" (forma *lacandonense* Cuatr., forma nov; forma *pentagonum* (Bernouilli) Cuatr., comb. nov.; and forma *leiocarpum* (Bernouilli) Ducke), corresponded to the criollo (which he wrote in lowercase) and the second to the forastero.

In classic botany (i.e., without considering recent molecular analysis), the current situation is as follows (Cuatrecasas 1964):

- *Theobroma cacao* subsp. *cacao* (cacao criollo, or criollo), originally thought to originate from Mexico and Central America, with three pod shapes:
- *Theobroma cacao* subsp. *cacao* forma *pentagonum* (= Cacao lagarto, alligator) is only known in crops of Central American origin. Very low vigor but one of the most sought cacao trees for its alleged quality, although its "uniqueness" may just reflect its rarity.
- *Theobroma cacao* subsp. *cacao* forma *leiocarpum* (= cumacaco) contains trees that are especially found on the Atlantic coast of Guatemala, only known in cultivation and reputed to provide high-quality dried cocoa.

[1] Forastero = foreign (but from another region, not another country).

[2] Trinitario = hybrid between Criollo and Amelonado, from Trinidad.

[3] In the rest of the text, the spelling of the term "criollo" is that adopted by the respective authors (with or without capitals, with or without S in the plural).

[4] Term which should not be used because it is too vague (Motamayor et al. 2010).

- *Theobroma cacao* subsp. *cacao* forma *lacandonense* is found mostly in the Lacandona forest in Mexico and was claimed by Cuatrecasas to be spontaneous and "semi liana" (Miranda 1962).
- *Theobroma cacao* subsp. *sphaerocarpum* (Chevalier) Cuatr., comb. nov. (= Calabacillo, amelonado, Amazonian forastero, forastero, etc.) spontaneously occurs in the middle Amazonia and the Guyanas; this cacao tree is widely cultivated for its robustness and hardiness, but its commercial product varies in quality.

Classic botany therefore considers two sub-species that are morphologically quite dissimilar and would seem to originate from different regions. However, the "forms" of the "Criollo" sub-species are not particularly valid, as admitted by Cuatrecasas himself, who indeed accepted that *leiocarpum* and *pentagonum* are probably cultivated mutants (Cuatrecasas 1964).

In accordance with the International Code of Nomenclature for cultivated plants (ICNCP), the term Criollo refers to a genetic complex corresponding to "variety". The Criollo cacao trees mentioned in our work, which are varieties (= genetic complex) or clones (= cultivars) do not generally have individual names that comply with the ICNCP, as ICNCP does not regulate "trademarks" for plants, nor regulate the naming of plant varieties. However, as explained by Soria (1970b), in ICNCP, the term "variety" is the same as "cultivar" when it is applied to "a group of individuals which show genetic differences but which have one or more characteristics by which they may be differentiated from other cultivars (varieties)". Following this definition, only the common name applied to "populations" of *Theobroma cacao* will be used for "varieties". Criollo varieties are "varieties" within the species *Theobroma cacao* L. Criollo clones, or cacao genetic groups, names mentioned in the text or tables are only usual names, given by breeders, curators or growers.

In cacao cultivation

When referring to cacao cultivation or curing, researchers reflect a somewhat different situation.

Soria (1962, 1966, 1970a, b) considered "Criollo" to be a "genetic complex", i.e., a variety, (Soria 1970b) whose main, if not discriminant, characteristics are a high-quality commercial product, pods with a thin cortex, reduced lignified zone of the mesocarp, small and dark green leaves, and small flowers with pink staminodes. The seeds are large, rounded, white or slightly pigmented. He considered three main types:

- Mexican Criollos
- Nicaraguan Criollos (or "Cacao Real")
- "Porcelanas"

Other types are mentioned: Cacao "lagarto" (Mexico and Guatemala), "Criollo from Colombia" (similar to the Mexican type), and "Criollo from Venezuela" (or "Criollo morado", "Criollo colorado"), similar to the Nicaraguan type.

Braudeau (1969) noted that criollo trees are virtually no longer cultivated, despite their very great qualities, and cannot resist the introduction of the hardier and more productive Forastero-based varieties. Based on the work by Soria quoted above, he listed the same cultivars, to which he added "Criollo from Madagascar and the Comoro Islands", with small pods of the Angoleta shape, red, with small, plump beans.

He quoted Pittier (1930), Cheesman (1944) and Cuatrecasas (1964) and stated that it seemed even difficult to adopt Cuatrecasas' proposal, which differentiated between only two sub-species, corresponding to Morris's two major groups.

In 1979, Lockwood and Gyamfi confirmed that the term "Criollo" applied to a "clearly defined group of cacao tree types" but that the definition was not very clear, with many exceptions to the accepted traits (deeply furrowed fruits, warty, pointed, pale beans). They cited several Criollos among the ICS clones.[5] However, Lockwood now considers that it was a mistake (Personal communication 2014).

In their "Cacao International Catalog", Soria and Enríquez (1981) cited only 6 true Criollo clones (one from Madagascar, a "Nicaraguan" and 4 Porcelanas). They also mentioned some Criollo "hybrids" (in fact, Trinitarios).

Wood and Lass (1985) in their reference book ("Cocoa") provided few new elements on "Criollo" confirming that the beans ferment rapidly and mentioning the poor vigor and the non-existent or incomplete jorquette (3 main branches rather than 5). They cited the types described by Soria (though excluding

[5] ICS = Imperial College Selection.

Porcelana) and mentioned the small average number of beans per pod (20–30).

Reyes (1992, 1993) stated that Criollo was disappearing from Venezuela and was cultivated in only few regions and in others was present only as relics. He mentioned the following main types:

- "Porcelanas"
- "criollos del Guasare"
- "criollos from the central region"
- "criollos andinos"

A rescue program led by Reyes was under way in Venezuela at the time and led to the collection of 231 criollos (and hybrid) selections, mainly in the states of Aragua (Chuao, Choroni, Cata, Cumboto), Zulia (Río Escalante and Guasare) and Mérida (Zea and Bocadillos). However, the selections were not cited.

These texts, often inspired by those of Soria (1962, 1966, 1970a, b), show that "Criollo" is confused with many poorly defined "types", often local "cultivars", sometimes displaying great variability. Such is the case, for example, with the "Porcelana" cacao tree from Venezuela, perhaps the most easily recognizable of the "Criollos". The shape, appearance and color of the pods are nonetheless very variable, as are the seeds. For other types, such as "Lagarto" (or "pentágona", or "4 filos"), one of us (P. L.) encountered this typical pod shape (Cuatrecasas 1964, p. 500), undoubtedly resulting from a mutation as assumed by Cuatrecasas in cultivated hybrids in Cameroon and in French Guiana on a cacao tree of the "Guiana" group.

The true geographical origin of the Criollos is not known with certainty, even though a South American origin seems most likely (Motamayor et al. 1998; Motamayor 2001; Motamayor et al. 2008; Zhang et al. 2009; Motilal et al. 2010; Thomas et al. 2012). Cuatrecasas (1964) and de la Cruz et al. (1995) suggested a Central American origin, but what they considered to be "wild" were probably only very old, sub-spontaneous cacao trees or relics of ancient cacao stands of genetic material cultivated by the Mayas.

"There is no universally accepted criterion for distinguishing between fine cocoas and the bulk (or ordinary or basic cocoas)" (Fowler 1994), and this seems the same for Criollo cocoa (Lockwood and Eskes 1995).

Clones considered as "Criollo"

In the literature, many clones are considered to be "Criollo", but often without any true genetic evidence, and caution is therefore called for.

The clones called "Criollo" include the following:

- In Lockwood and Gyamfi (1979), quoting Reyes et al. (1973):
- VLA 7, 1, 2, 3, 4, 5, 6 = 7 Ocumare clones (OC 60, 61, 63, 66, 67, 73, 77)
- VLA 8–19 = 12 Choroni clones (Cho 7, 18, 24, 28, 31, 36, 41, 42, 44, 45, 163, 174)
- VLA 20–23 = 4 Chuao clones (Chu 2, 116, 120, 135)
- VLA 101, 102, 103, 110–119, 121 = 14 Porcelana clones (POR 1, 2, 3, 10–19 and PV-R-21).
- In Soria and Enríquez (1981): 234-5 AM2 (Criollo from Madagascar), ICS 100, VLA020, VLA101, VLA102, VLA117.
- In the ICGD[6] (Turnbull and Hadley 2014):
- clones from Colombia: SCS 27, 56
- from Costa Rica: Criollo [CRI], Criollo Lolita, Criollo/A-Ang, Criollo/B-Ang, Criollo 1–8, 10–30, 33–68, 79, 122, 215 and 216;
- from Honduras: Criollo [HDN]
- from Indonesia: G8
- from Venezuela: Porcelana 1–7, 10–19, 21
- from Madagascar: IFM 3, 6, 15
- Criollo [SLV]

In the ICGD database, when searching in the genetic groups, 41 clones are obtained for "Criollo" (those cited by Motamayor et al. 2008, plus IFM 3, 6, 15).

- In the internal database of ICG, T (International Cocoa Genebank, Trinidad) at C.R.C.,[7] in November 2016, the following clones were classed as "Criollo":
- Belize 97-61 B2
- IB 9 (from Belize)
- Belize S1 (may now be Belize 1)

[6] ICGD = International Cacao Germplasm Database (http://www.icgd.reading.ac.uk/index.php).

[7] CRC = "Cocoa Research Centre (University of the West Indies, St Augustine, Trinidad and Tobago)".

Recent developments: genetic studies

In 2005, Bartley (2005) criticized Cuatrecasas (1964), highlighting the fact that his classification was based only on fruit traits. For him, the variability encountered in all groups was considerable, obviously making phenotypic classifications difficult. He wrote that the main phenotypic characteristic of the varieties included in the "Criollo" concept is the existence of anthocyanin pigment in the fruits, while nonetheless admitting that fruit pigmentation is sometimes found in the populations of the Amazon region.

Given this unsatisfactory situation, both botanically and practically, including for breeding by hybridization, for which knowledge of the true genetic groups is paramount, some works have been undertaken using molecular biology tools. The first were those of de la Cruz et al. (1995) using RAPD[8] markers, Laurent (1993)[9] using RFLP markers, and Motamayor (2001) using AFLP[10] markers.

Motamayor (2001) divided Criollos into two groups: "ancient" (= true = criollo antiguos) and "modern" (introgressed with foreign alleles, i.e., equivalent to Trinitario). The division adopted is based more on location criteria than on morphology. The ancient Criollos were collected from zones where "pollution" by Forastero or Trinitario pollen was impossible or unlikely, whereas the "moderns" were polluted in plantations or came from international collections. He showed that the "traditional variety" called "Criollo" is not a cacao sub-species but rather a population among many others. He also showed that the genetic variability of the ancient Criollos is very small.

Motamayor et al. (2002) used 16 microsatellite markers to identify 41 ancient Criollo clones with low diversity and virtually complete homozygosity, which they considered to be typical of the group (which was confirmed by Argout et al. 2011). They also made a slight distinction between "modern Criollos" and Trinitarios but nonetheless stated that the former are hybrids (similar to the latter).

Then, in an extensive study, the use of 96 microsatellite markers on a sample of 1241 clones (but 735 kept after discarding duplicates or off-types) provided a picture of the genetic structuring of the species *T. cacao* for the first time (Motamayor et al. 2008). It turned out that this species is structured in 10 genetic groups: Amelonado, Contamana, Criollo, Curaray, Guiana, Iquitos, Marañon, Nacional, Nanay and Purús (Fig. 1).

Thirty-nine genotypes were assigned to the Criollo group and therefore corresponded to the "ancient Criollos". Three sub-groups were acknowledged, Belize, Lacandona and Santa Marta, which may correspond to the ancient Criollos of Central America, Mexico and the Andes, respectively (Table 1). Most of these genotypes came from surveys in 5 countries (Mexico, Belize, Nicaragua, Venezuela and Colombia) and do not exist in collections.

The only known clone (accessible in International Germplasm Collections), which must therefore serve as a reference for the group, is Criollo 13.

Since that broad study in 2008, Criollo genetic material has been studied on several occasions.

- In 2009, Zhang et al. published a study on the CATIE[11] collection using 15 "standard" microsatellite markers for cacao, but they presented their results by geographical origin groups (12 groups, 548 accessions). Clone Criollo 13, one of the 3 controls used, seemed very distinct from the central America, Mexico and Trinidad accession groups

As the SSR[12] profiles of the accessions are available in the ICGD, it is possible to study them.

We therefore undertook a study (unpublished) on 194 genotypes of the CATIE collection, including some called "Criollo", along with some from the PMCT (= "Programa de Mejoramiento de Cultivos Tropicales"), ARF (central American collections), and RIM (= "Rosario Izapa Mexico") groups, and some others from much less known groups, with clone Criollo 13 as the control for the ancient Criollo group. Our study showed that the PMCT clones (except PMCT 26 and 46), along with the MEX and ARF clones, were highly distinct from the ancient Criollos.

The final result, with 68 conserved clones, is shown in Fig. 2. It shows that, among the clones of the

[8] RAPD = Random Amplified Polymorphic DNA.

[9] RFLP = Restriction Fragment-Length Polymorphism.

[10] AFLP = Amplified Fragment-Length Polymorphism.

[11] Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica.

[12] SSR = Simple Sequence Repeats.

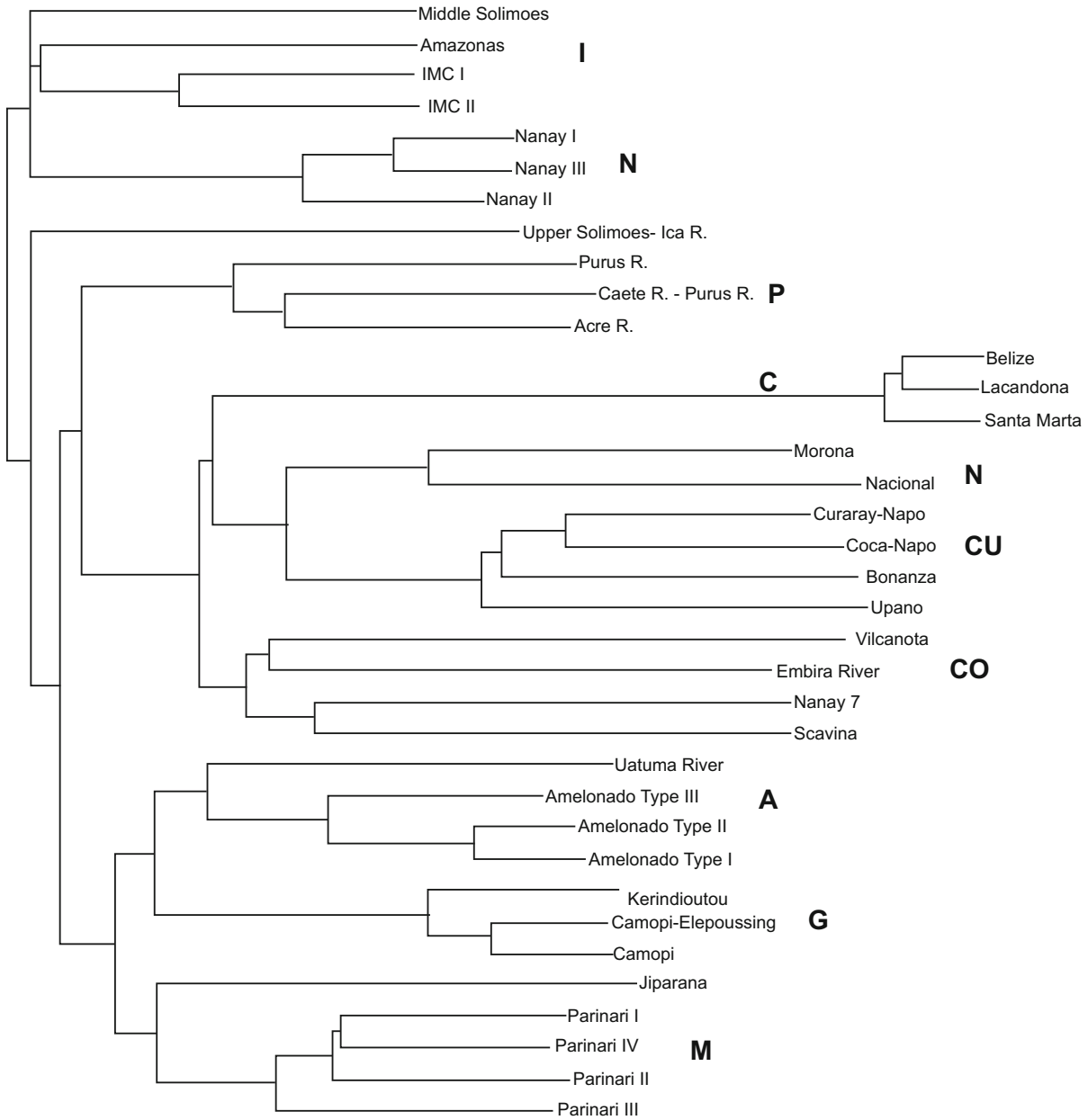

Fig. 1 Dendrogram illustrating the genetic structuring of the species *Theobroma cacao*. *I* Iquitos, *N* Nanay, *P* Purús, *C* Criollo, *N* Nacional, *CU* Curaray, *CO* Contamana, *A* Amelonado, *G* Guiana and *M* Marañon (Data from Motamayor et al. 2008)

CATIE collection for which genetic profiles obtained with microsatellite markers were available (e.g., 26 clones called Criollo, out of 42), only clones Criollo 12, 13 and 65 are true Criollos. All the others, especially Criollo 3, 4, 8, 15, 17, 21, 27, 63, 64, and 216; Pentágona 7, 8, 10, 17, ICS 29, 39, and 100; and the ML clones (Mata Larga from Santo Domingo) were very distinct. Criollo 10, 19, 28, 33, 34, 43, 48, 54, 55, 56, 60 and 62 were considered "modern Criollos". By providing more precision, these results confirmed those of Zhang et al. (2009).

- In 2010, Motilal et al. (2010) presented the genetic diversity of 77 accessions collected among Criollo relics in Belize (Mooleedhar et al. 1995) and studied with 30 microsatellite markers. Criollo 13

Table 1 The genotypes (= individuals) of the Criollo genetic group (Ancient Criollo) of Motamayor et al. (2008) and their sub-group (the asterisk indicates an undefined sub-group, which have under 5 members)

Genotype	Origin	Country	Sub-group
B3		Belize	Lacandona
B48		Belize	Belize
BEN1	Zea (Merida)	Venezuela	Santa Marta
BEN5	Zea (Merida)	Venezuela	Santa Marta
CAS5	Lacandona Forest	Mexico	Lacandona
CHA13	Lacandona Forest	Mexico	Lacandona
CHA18	Lacandona Forest	Mexico	Belize
CHA20	Lacandona Forest	Mexico	Lacandona
CHA5	Lacandona Forest	Mexico	Lacandona
COL10	Santa Marta	Colombia	Santa Marta
COL11	Santa Marta	Colombia	Santa Marta
COL2	Santa Marta	Colombia	Santa Marta
COL3	Santa Marta	Colombia	Santa Marta
COL4	Santa Marta	Colombia	Santa Marta
COL5	Santa Marta	Colombia	Santa Marta
COL8	Santa Marta	Colombia	Santa Marta
CRIOLLO 13		Costa Rica	*
LAN21	Lacandona Forest	Mexico	Lacandona
LAN22	Lacandona Forest	Mexico	Lacandona
LAN23	Lacandona Forest	Mexico	Belize
LAN26	Lacandona Forest	Mexico	Belize
LAN27	Lacandona Forest	Mexico	Lacandona
LAN28	Lacandona Forest	Mexico	Lacandona
LAN29	Lacandona Forest	Mexico	Lacandona
LAN30	Lacandona Forest	Mexico	Lacandona
LIB1	Libertad de Chontales	Nicaragua	Belize
LIB2	Libertad de Chontales	Nicaragua	Belize
PER2	Parque Tayrona	Colombia	*
RANCHITO1	Michoacan	Mexico	Lacandona
SAUCITO1	Michoacan	Mexico	Lacandona
SJU1	San Juan de Lagunillas	Venezuela	*
SJU3	San Juan de Lagunillas	Venezuela	*
SJU6	San Juan de Lagunillas	Venezuela	*
STA MARIA2	Michoacan	Mexico	Lacandona
TC1		Panama	*
TC3		Panama	*
TC9		Panama	*
THCA	Lacandona Forest	Mexico	Lacandona
WILD	Esmeraldas	Ecuador	*

was among the control clones.

The diversity found was low, and only 11 distinct ancient Criollo genotypes were identified. The authors also showed that the ICS clones claimed to be "Criollo from Nicaragua", ICS 39, ICS 40, and ICS 100 were Trinitarios, as were clones OC61, Pentagona and Stahel, Motilal et al. (2010).

- The diversity of the germplasm used by growers in Honduras and Nicaragua was studied by Ji et al. (2013) using 70 SNP ("Single Nucleotide

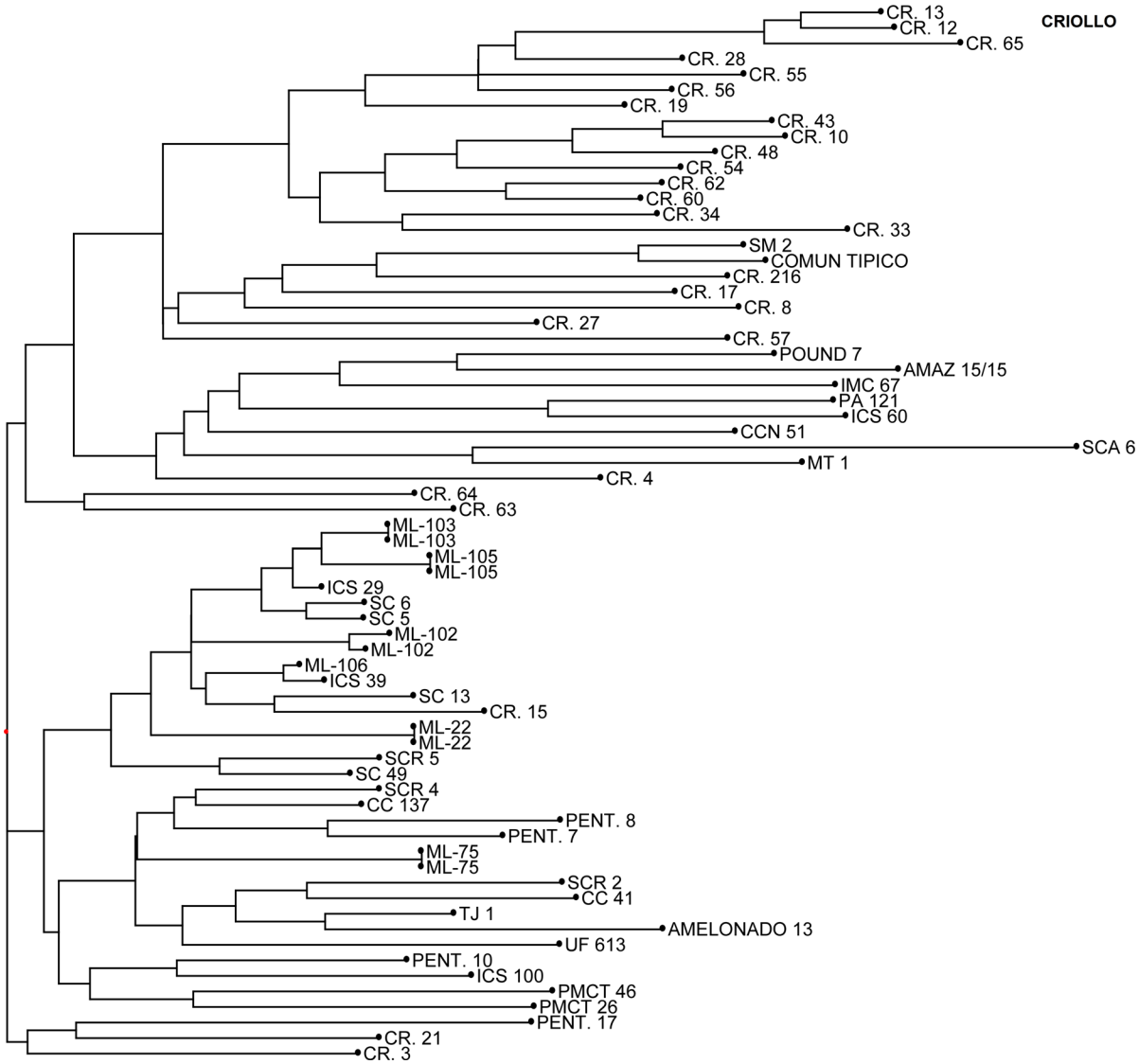

Fig. 2 Dendrogram showing the genetic structuring of a set of 68 clones from the CATIE collection, with clone Criollo 13 as control for the Criollo genetic group (Unweighted Neighbor-joining method, *UPGMA* Unweighted Pair Group Method with Arithmetic Mean, with 100 bootstraps)

Polymorphism") markers. Their sample comprised 84 local origins and 31 controls, including the Criollo 13 reference. They identified 22 "pure Criollos" (= ancient Criollos).

- In a study associated with the previous one (unpublished) focusing mainly on some genotypes from Nicaragua (mostly the Matagalpa region) and some controls (including 5 Criollos from Venezuela), we used 15 microsatellite markers to confirm that clones Matagalpa 13, 16 and 20 were true ancient Criollos, close to Criollo 13, and that clones ICS 40 and 60, OC 77 and 81 (claimed to be from Ocumare in Venezuela), Chuao 120 (claimed to be from Chuao in Venezuela) and PV5 and PV6 (claimed to be Porcelana) were all Trinitarios (cf. Fig. 3).
- In 2016, a study on IFM[13] clones in the FOFIFA[14] collection at Ambanja (Madagascar) with 3000

[13] "Institut Français de Madagascar".

[14] "Foibem-pirenena momba ny Fikarohana ampiharina amin'ny Fampandrosoana ny eny Ambanivohitra" = "National Centre for Research applied to Rural Development".

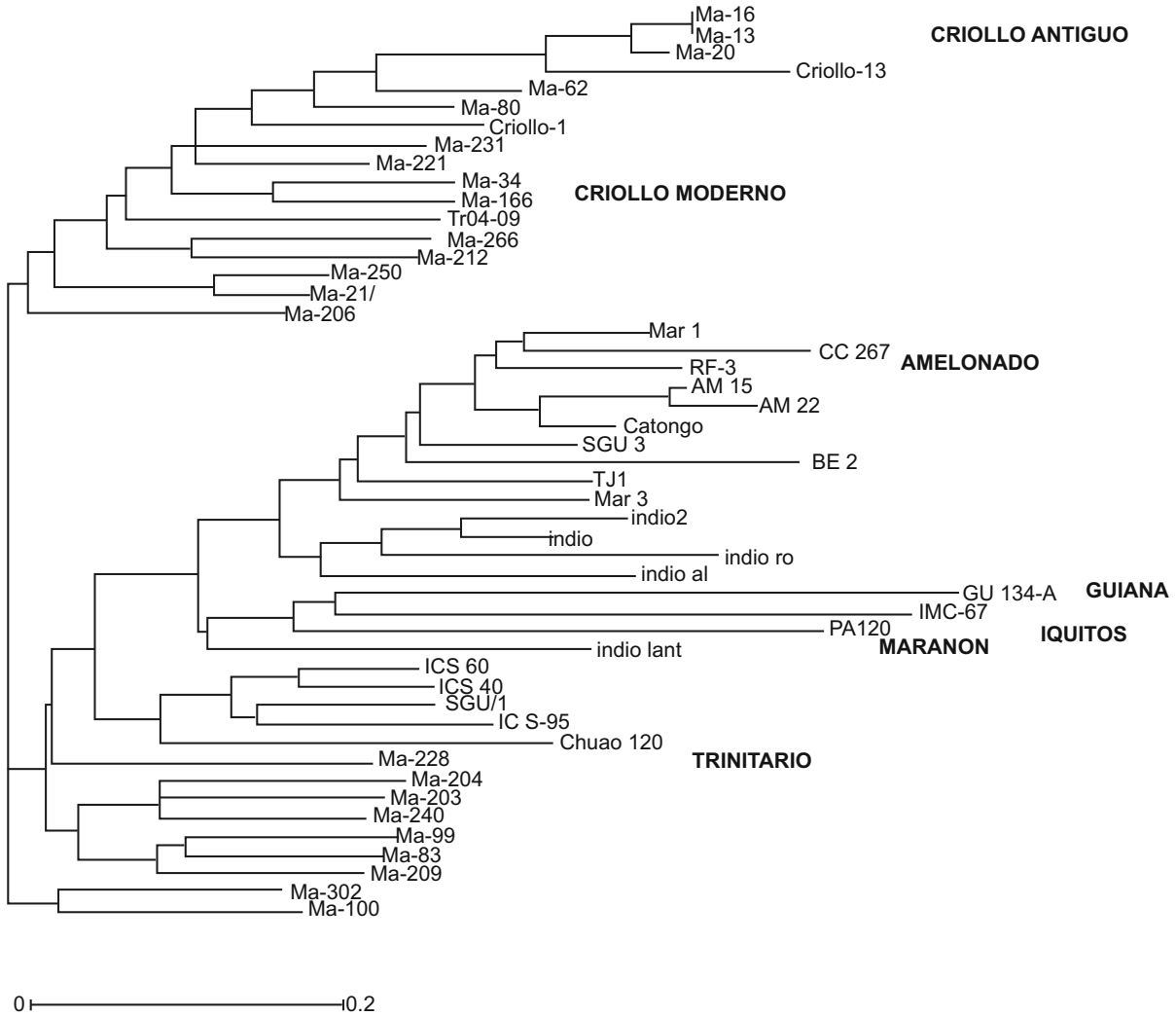

Fig. 3 Dendrogram showing the genetic structuring of a sample of clones from Nicaragua (Ma) and Honduras (Indio), along with various controls (15 microsatellite markers, profiles established by USDA, Unweighted Neighbor-joining method, UPGMA, with 50 bootstraps)

SNP markers spread over the 10 chromosomes (Argout et al., in prep.) showed that 3 clones were true Criollos: IFM 3, 6 and 15. This study was made possible by the sequencing of the cacao genome (Argout et al. 2011), represented by the ancient Criollo clone B97-61-B2 (Table 2).

The true Criollos

The recent studies mentioned above show that only few of the cacao trees called "Criollo" are actually "true Criollos", i.e., "ancient Criollos" similar to the reference Criollo 13. However, they are often genotypes collected in leaf form from growers or in their "wild" uncloned state and do not figure in collections.

Therefore, the only proven true Criollos (= ancient Criollos) are those listed in Tables 1 and 2

The current list of true Criollos

Table 1 shows the 39 members of the "Criollo" genetic group originally defined by Motamayor et al. (2008), with the sub-group to which they belong.

Table 2 The true ancient Criollos mentioned in studies following the one by Motamayor et al. (2008), possible reference to them in the ICGD and their possible location in international collections, where *Mot. 2009* Motilal et al. (2009), *Mot. 2010* Motilal et al. (2010), *Mot.* Motilal due out, *Ji. 2013* Ji et al. (2013), *Argout* Argout et al. (2011) due out; *1* CATIE, *2* CRC Trinidad (ICG, T), *3* Reading Quarantine Centre (UK) and *D* disappeared

Genotype	Studies	ICGD	Location
AC 2 (T1)	Mot. 2010	No	
AC 2/2	Mot.	No	
AC 2/4	Mot.	No	
AC 2/5	Mot.	No	
Aguacarte 3	Mot. 2010	No	D
B97-61 B2	Mot. 2010	No	2
B97-61 B6	Mot. 2010	No	D
Banana creek 2	Mot. 2010	Yes	
Belize S1	Mot.	No	2
C 61	Mot. 2010	No	D
CC 7	Mot. 2010	No	D
CC 8	Mot. 2010	No	D
Criollo 65	(This study)	Yes	1
Criollo 12	Mot. 2010	Yes	1
Criollo 13	Mot. 2009, Ji. 2013	Yes	1
Criollo 22	Mot. 2010	Yes	1, 3
Francisco	Ji. 2013	No	
HF 31	Mot. 2010	No	D
HF 8	Mot. 2010	No	D
Honduras 10	Mot.2010, Ji. 2013	No	
Honduras 11	Mot. 20110	No	
Honduras 11	Ji. 2013	No	
Honduras 12	Ji. 2013	No	
Honduras 13	Ji. 2013	No	
Honduras 15	Ji. 2013	No	
Honduras 16	Ji. 2013	No	
Honduras 17	Ji. 2013	No	
Honduras 18	Mot. 2010	No	
Honduras 19	Ji. 2013	No	
Honduras 20	Ji. 2013	No	
Honduras 6	Mot. 2010	No	
Honduras 9	Mot. 2010	No	
IB 9	Mot. 2009–2010	No	2
IFM 3	Argout	Yes	
IFM 6	Argout	Yes	
IFM 15	Argout	Yes	
Matagalpa 13	Ji. 2013	No	
Matagalpa 16	(This study)	No	
Matagalpa 20	Ji. 2013	No	
Mercedes	Ji. 2013	No	
Oscar	Ji. 2013	No	
ST 41	Mot. 2010	No	D
Tiburcio 1	Ji. 2013	No	
Tiburcio 3	Ji. 2013	No	
Tiburcio 4	Ji. 2013	No	
Tiburcio 5	Ji. 2013	No	
Tiburcio 2	Ji. 2013	No	

Criollo 22 is also present in two other collections in Ghana and Indonesia, and clones IFM 3, 6 and 15 exist in the Ambanja collection in Madagascar

Table 2 shows the genotypes closely related to the Criollo 13 reference in the studies since 2008.

Phenotypic characteristics of the true Criollos

Phenotypic traits cannot be used to determine whether a cacao plant (tree or clone) is a Criollo (Bartley 2005), and the same applies for the "true Criollos". Nevertheless, the simultaneous occurrence of several "Criollo" traits may enable an initial provisional identification.

The phenotypic characteristics of the ancient Criollos are those described by Cheesman (1944) and, to a certain degree, by Cuatrecasas (1964) and Bartley (2005).

Vegetative system

One "discriminant" criterion seems to be the pilosity of young leaves and stems (Soria 1962; Marcano et al. 2009) Young leaves (flushes) are green (and not dark red). The absence of a jorquette seems to be a debatable criterion (Cheesman 1944).

Reproductive system

The pod shape, appearance and color criteria, which are often cited, are not very useful; only the thin cortex and reduced lignified zone of the mesocarp may suggest a true Criollo.

Pod shape cannot be adopted unless it is the true "Porcelana" shape (Motamayor 2001; Bartley 2005) or similar shapes (there are some in Central America and Mexico). Some true Criollos have pod shapes that are frequently found in the Trinitarios.

The same applies for floral criteria (size and red or pink staminodes), apart from the possible exception of the number of ovules per ovary, which is small (under 40, Bartley 2005). The particular color of Criollo flower staminodes, which is red or pink (never violet nor white), is a characteristic that is shared with certain Amelonados (e.g., certain Indio trees from Honduras).

The main "discriminant" criterion would therefore seem to be the bean color when cut, which is white or very slightly colored (pink or pale violet), but combined with the large size of the beans, their small number and their "rounded" or "plump" shape.

However, the "bean color" trait is additive (incomplete dominance), and when pollinated by a neighboring Forastero with violet beans, a Criollo with fundamentally white beans (e.g., in self-fertilization) will have colored beans (pale violet), so this criterion must be used with care.

The white bean color is not specific to the Criollos nor is it rare. Indeed, in the 22 species of *Theobroma* acknowledged by Cuatrecasas (1964), all but one, cacao tree (*T. cacao*), have white beans, and within the cacao species, some trees, clones, populations or "varieties" have white beans. For instance, this color is found in the Criollos and certain upper and lower-Amazon Forasteros (not to mention Trinitario hybrids). The best known are possibly Catongo and Almeida cacao trees originating from Brazil, which arise from a mutation within the Amelonados.

In relation to the reproductive system, true Criollos are self–compatible.

Commercial product

One characteristic of the Criollos would seem to be the rapid fermentation of the beans, from 2 to 4 days rather than the 5–7 for the other origins, due to their low polyphenol content (Wood and Lass 1985). The bean color of the fermented and dried cocoa produced, which is pale and rather yellow, may be an indication but never a certainty. In this field, if a difference between ancient and modern Criollos exists, it remains unknown.

To conclude, no morphological criterion taken alone can be considered "discriminant" for separating ancient Criollos from modern Criollos.

Access to the true Criollos

There are virtually no ancient Criollo clones in the major collections, especially in the so-called international ones (that of CATIE and that of CRC, ICG, T). Table 2 shows that only 8 are mentioned (hence known) in the ICGD and that 7 may be available in the 2 International Cocoa Genebanks and the Reading Quarantine (B97-61 B2, BS 1, Criollo 12, 13, 22, 65, and IB9).

Nevertheless, some other clones exist, but in the absence of genetic studies, they cannot be formally included in the ancient Criollos. We refer to the "Criollo" clones existing in Venezuela (in the Caucagua, Ocumare, Chama collections; Motamayor 2001), i.e., CHU, CHO, OC, CATA, HE, POR, Porcelana, CUM, JS, and ZEA, along with certain clones from Honduras.

In Central America, many Criollo trees can still be found in orchards as relics (Motamayor et al. 1998, 2008; Motilal et al. 2010).

Conclusion

The term "Criollo" has many meanings and interpretations depending on whether it is by commercial users or botanists, growers or breeders. In commercial terms, Criollo cacao trees are the source of the commercial product that fetches the best price on the market, which suggests better "quality"; however, this can sometimes be subjective or "not transparent" (Lockwood and Eskes 1995). The higher price is more a result of its rareness than its subjective flavor. Criollo is associated with a higher nutty/caramel flavor, low astringency and bitterness but relatively lower cocoa flavor. Moreover, it is likely that even the most reputable origins partly come from cacao trees that are fairly distant from the true Criollos. However, we know that what matters is the *terroir,* of which genetic origin is only one component.

According to the classic botanical classification, summarized by Cronquist (1981), Criollo is a subspecies of the cacao tree (*Theobroma cacao* L.): *Theobroma cacao* subsp. *cacao*. However, the genetic

studies described in our article showed that this subspecies concept is outmoded and that Criollo, according to the new phylogenetic classification ("Angiosperms Phylogeny Group IV" 2016), is merely one of the 10 genetic groups currently known in the species. Indeed, as shown in our previous publications (Motamayor et al. 2001, 2008) the genetic distances between Criollo and the genetic group Curaray are lesser than the genetic distances between Curaray and other genetic groups.

Furthermore, *Theobroma cacao* subsp. *cacao* is "not accepted" in the new phylogenetic classification (retrieved [07/20/2017] from the Integrated Taxonomic Information System on-line database http://www.itis.gov).

However, the duly acknowledged members of this group are very poorly represented in the international collections, particularly in the two International Cocoa Genebanks of the ICG, T. and CATIE, where only 4 clones are available. This may be due to several reasons: i) they have long been gradually abandoned in crops and/or hybridized with some more productive varieties resistant to the various pests and diseases; ii) recent genetic studies have only rarely resulted in the studied genotypes being preserved by cloning; iii) phenotypic characteristics do not enable definite identification; and iv) true Criollos (such as Criollo 13 in CATIE) show very low vigor and are very difficult to propagate (Wilbert Phillips, pers. comm.).

However, many true Criollos still exist in the collections of Venezuela (Caucagua, Ocumare, San Juan de Lagunillas, Chama), but not all of them have been formally identified (by genetic studies) as members of the Criollo genetic group in the sense of Motamayor et al. (2008). In addition, the true Criollo clones of Venezuela do not exist anywhere other than Venezuela, as all the clones claimed to be such (such as the OC, POR clones) have proven to be false. They would appear in fact to be open progenies of the cited clones (hence mostly Trinitario).

Some so-called "modern Criollo" clones (Motamayor 2001), i.e., quite close to the ancient Criollos but arising from hybridization with other groups (though to a lesser degree than the common Trinitarios), are more numerous and therefore more readily available. They display a certain number of morphological characteristics of the ancient Criollos, but they are in fact Trinitarios that are relatively close to the Criollos. For instance, at CATIE, many clones that are called "Criollo" are "modern Criollos", such as clones Criollo 10, 19, 28, 33, 34, 43, 48, 54, 55, 56, 60 and 62.

There is no doubt that the commercial product of "modern Criollos" is known on the market as "Criollo".

The ICS clones called "Criollo from Nicaragua", such as ICS 39, 40, 45, 60 and 100, are not Criollos but Trinitarios. The same applies for the Mexican RIM clones.

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Compliance with ethical standards

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