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Combining morphology and population genetic analysis uncover species delimitation in the widespread African tree genus *Santiria* (Burseraceae)

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Abstract

Taxonomic classification based on morphology alone can prove difficult. This is the case of the polymorphic forest tree species *Santiria trimera* in Africa, whose classification has remained controversial for over a century. Studies combining chloroplast and nuclear DNA sequences show the existence of several phylogenetic clades in this taxon, with some occurring in sympatry in western Central Africa suggesting the existence of at least two species. By combining genetic and morphological markers, we aim to assess the species delimitation in the *Santiria* species complex. Morphological trait (trunk, leaflet, flower and fruit characteristics) analysis using 223 standing individuals and 103 herbarium samples were combined with genetic analyses using 479 individuals genotyped at eight microsatellite markers. Genetic clusters were identified using Bayesian assignment in order to delimit species following the Biological Species Concept and to identify distinctive characters from morphometric analyses in retrospect. Three genetic clusters were identified and found to occur in sympatry. The type of inflorescence and the colour of unripe fruit were the most discriminant morphological traits among those genetic clusters, while many quantitative traits showed overlapping distributions between genetic clusters and explain the difficulty encountered by previous botanists to resolve the taxonomy of *Santiria*. The combination of genetic and morphological data suggests the presence of three species within the taxon *Santiria trimera* from western Central Africa. This work should guide a taxonomic revision within the genus *Santiria* in Africa.

Key words: Cryptic species, genetic cluster, morphotype, western Central Africa

Introduction

The species concept is central to biology, but it has received various definitions (Le Guyader 2002). For example, the species concept based on typology is defined as a group of individuals that share a set of morphological traits, with particular traits that distinguish them from other species. This concept is therefore based on nomenclatural types that subsequently are associated with a set of specimens with similar morphological characteristics and bear diagnostic traits (Candolle 1813, Le Guyader 2002). This is the concept usually considered by botanists who establish species identification keys based on morphological characters. Another major concept is the biological species concept, which defines species as reproductively isolated units (Mayr 1963). Nowadays, this species concept can be investigated using population genetics tools (genetic markers) that allow determining whether groups of coexisting individuals are interbreeding or not. Indeed, when a sample of individuals genotyped at multiple nuclear genetic markers form distinct yet sympatric genetic clusters, it gives strong clues for the existence of reproductive barriers between these clusters, which could then correspond to distinct species. Overall, species delimitation is an essential prerequisite for many other biological works. When species delimitations based on genetic tools and morphological markers are congruent,

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species delimitation is well supported (Duminil & Di Michele 2009). However, there are many exceptions, and lack of agreement between genetic and morphological markers may result from a lack of taxonomic revision based on representative biological material, but it may also result from a lack of clear morphological differentiation despite the genetic divergence between species, leading to "cryptic species" (Heinrichs *et al.* 2009). In addition, interspecific hybridization is very common in plant species, has important consequences on biological evolution and speciation, further complicating species delimitation (Mallet 2005). Species delimitation based solely on morphological markers is particularly challenging in highly diverse ecosystems, such as tropical forests where many related species with complex evolutionary histories can co-occur and for which good material is seldom available (Duminil *et al.* 2006, Duminil & Di Michele 2009).

The tropical African flora, particularly in Lower Guinea (LG, a phytogeographical area of Central Africa, which extends from southwest Nigeria to north Angola and separated from the Congo Basin by the Sangha River; White 1979), is no exception to the rule as illustrated by several taxonomic revisions resulting in splitting species previously grouped in a single taxon, or in lumping previously recognized species (e.g. Breteler 2011, Walters *et al.* 2011, Hyam *et al.* 2012, Lachenaud & Jongkind 2013, Van der Burgt *et al.* 2015). Several population genetic studies conducted on different species in tropical Africa demonstrated the existence of sympatric genetic groups, sometimes highly differentiated, suggesting that cryptic species could be common and thus that taxonomy based on morphological markers alone may overlook many species (Dauby *et al.* 2010, Koffi 2010, Duminil *et al.* 2012, 2013, Heuertz *et al.* 2014). To clarify taxonomy within species complexes, combining methods based on morphological and genetic markers is highly relevant (Murakam *et al.* 1998, Sei & Porter 2007). The main aim of this study was to clarify species delimitation in the widespread and often abundant tree *Santiria trimera* (Oliver 1868: 441) H.J.Lam ex Aubréville (1948: 344) in the Burseraceae (see also Aubréville 1962, Onana 2009) by using these two approaches.

Santiria trimera was described in 1868 by Daniel Oliver as *Sorindeia ? trimera* Oliver (1868: 441) (Anacardiaceae) collected in the area around 'River Kongui', Gabon. Delimitation of this African tree species has been a recurrent problem despite taxonomic revisions (Engler 1890, 1910, Pierre 1896, Guillaumin 1908, Aubréville 1962, Onana 2009). Indeed, *S. trimera* exhibits considerable morphological variability: the trunk can develop stilt roots, buttresses or show a cylindrical basis, individuals produces large or small leaflets, axillary or terminal inflorescences, and red or green immature fruits. Despite these polymorphic traits, all African species previously recognized in *Santiria* Blume (1850: 209) were grouped as *S. trimera* in the most recent taxonomic revision (Onana 2009). Nevertheless, different morphotypes are often found in sympatry in several areas of central Africa, especially in Gabon, the Republic of Congo and Cameroon (Troupin 1958, Florence & Hladik 1980, Hladik & Blanc 1987).

Recent studies combining phylogenetic data and morphological traits suggest that at least two species could be distinguished in this taxon (Koffi *et al.* 2010, 2011). However, morphological analyses of Koffi *et al.* (2011) were limited to a small area in northern Gabon, including four populations, and based on a reduced set of traits: trunk architecture (presence or absence of stilt roots), leaflet size (small or large) and immature fruits (red or green). Furthermore, this study did not use highly polymorphic genetic markers, such as microsatellite markers (nSSR), to characterize sympatric morphotypes of *S. trimera* and test if they correspond to distinct genetic clusters. The present study combines morphological traits and population genetic data to delimit sympatric morphotypes of *S. trimera*. To this end, we first used nSSR markers to identify the different genetic clusters of *S. trimera* localized in LG. We then compared morphological traits between genetic clusters to delimit species by identifying diagnostic morphological traits of each cluster in retrospect.

Materials and Methods

Taxon of study:—The genus *Santiria* belongs to the subtribe Dacryodiinae Lam (1932) characterized by an axial intrusion in the fruit. As currently circumscribed by Onana (2009), *Santiria trimera* is a medium tree 9–15 m high, ≤ 60 cm diameter at breast height (dbh), except for submontane forest forms in São Tomé Island that go up to 30 m high and 100 cm dbh. Trunk bases either have stilt roots, buttresses or are cylindrical (Aubréville 1948, 1962, Troupin 1958). Exudate is yellowish, smells like turpentine and, in São Tomé Island only, it flows abundantly and is very easily inflammable. Timber from *S. trimera* is fine-grained and greyish to yellowish in colour (Burkill 1985). Leaves (up to 1 m long) are alternate, usually imparipinnate (3–13 leaflets) or sometimes paripinnate (4–8 leaflets). The petiole is canaliculate on the upper side. Leaflets are opposite or subopposite, 3–9 × 9–20 cm, elliptical or oblong-elliptical, shortly pointed or acuminate, and with a rigidly papery to coriaceous blade composed of brochidodromous veins

(Hickey 1973). The species is dioecious (therefore allogamous), with racemose or paniculate inflorescences. Male and female inflorescences are similar and have racemes borne in terminal, subterminal or axillary positions. Flowers are 3-merous or rarely 4-merous, small (≤ 1.5 cm), pubescent or glabrous, and subsessile to pedicellate. Sepals are united at the base, petals are free and valvate (rarely imbricate). Male flowers have six stamens inserted under a staminal disc and a small pistillode. Female flowers have six staminodes (inserted like stamens) and one glabrous or pubescent pistil. The fruit of *S. trimera* are drupaceous with one seed, variable in colour (red, yellowish or green at immaturity; black at maturity) and up to 3.5 × 2.5 cm (Onana 2009, Koffi 2010). Seeds have two laciniated cotyledons with seven segments each that are red or green (G. Joffroy, unpubl. data).

According to the latest taxonomic revision, *S. trimera* has a large distribution in African rain forests and occurs from Sierra Leone to Angola, including São Tomé island (Aubréville 1948, Onana 2009) and Príncipe island (Exell 1944). It is common from 50 to 500 m above sea level in Gabon and in the Republic of the Congo (Gillardin 1959, Doucet 2003, Ngomanda *et al.* 2005). Orophytic forms are observed in Cameroon (Letouzey 1968), Equatorial Guinea (Wilks & Issembé 2000) and Ivory Coast (Aké Assi 2001).

Santiria trimera is used for various purposes. The bark (and leaflets) are composed of more than 60% terpenes with antimicrobial properties (Da Silva *et al.* 1990, Martins *et al.* 2003, Bikanga *et al.* 2010, Samy & Gopalakrishnakone 2010) and is therefore used for medicinal purposes (Burkill 1985). In Gabon, Masango people (Bantu speaking people) eat the pounded bark with banana (*Musa ×paradisiaca* Linnaeus (1753: 1043), Musaceae) to treat diarrhoea (Raponda-Walker & Sillans 1961, Akendengue & Louis 1994). The bark is also used to treat whooping cough, and eczema and is considered to be vermifugal (Burkill 1985). This species is occasionally planted in Sierra Leone (Hawthorne & Jongkind 2006) for its edible fruits that are usually sold (Burkill 1985). According to Haurez *et al.* (2015), the fruits are consumed by gorillas (*Gorilla gorilla*) and chimpanzees (*Pan troglodytes*). The seeds are eaten by local people in Liberia (Burkill 1985).

Sampling:—Our analysis combines observations and measurements of living material (field-collected specimens), dried and spirit-preserved herbarium specimens for morphological analyses, and DNA from cambium or silica-dried leaf material and from herbarium specimens (without silica-dried material) for the population genetic data.

Field surveys:—From 2012 to 2015, we observed and measured in the field morphological traits of 231 individuals of *Santiria* in nine localities in Gabon (Table 1). For all sampled individuals, cambium or leaf material was collected and dehydrated using silica gel. Cotyledons from 59 seeds of 18 female trees from these individuals were also collected. Phenology was recorded for 129 individuals from January 2014 to January 2015 in two areas in eastern Gabon: Ipassa (N = 114) and Franceville (N = 15). Herbarium specimens (N = 1–7 of each individual) were collected and processed for 88 individuals (N = 1–29 per locality) and deposited at BR, BRLU, LBV, MO, P and WAG (actually grouped in L) (herbarium acronyms follow Holmgren *et al*. 1990). Reproductive material (either flowering or fruiting) from 46 adults were preserved in spirit (55% ethanol and 5% glycerol). Information linked to herbarium specimens is available in the Missouri Botanical Garden database (www.tropicos.org).

Additional samples:—To extend our analyses to the central African region, we added DNA from 55 individuals previously extracted and used by Koffi *et al.* (2011) and from 31 herbarium specimens without silica-dried material. Forty six of these individuals have herbarium specimens deposited at BR, BRLU, LBV and P and their morphological traits were also recorded.

TABLE 1. Geographic coordinates and number of individuals of *Santiria* sampled in nine localities in Gabon.

Locality	Latitude	Longitude	Total collected for DNA analyses	Total collected with herbarium specimens
Agricole	00°02’12’’N	10°14’44’’E	8	0
CEB	00°49’55’’S	13°13’25’’E	119	16
Franceville	01°39’00’’S	13°35’00’’E	11	8
Ipassa	00°30’25’’N	12°47’46’’E	115	26
Lac Azingo	00°29’39’’S	10°02’09’’E	20	5
Lac Onangué	00°57’24’’S	10°02’56’’E	3	3
Mabounié	00°43’52’’S	10°35’36’’E	47	29
Mandji-Pové	01°13’54’’S	12°23’02’’E	4	0
Onal	01°09’46’’S	10°20’14’’E	1	1

DNA extraction, amplification and genotyping:—Total genomic DNA was extracted from 15–25 mg dry plant material, using the NucleoSpin 96 Plant II kit (Macherey-Nagel, Düren, Germany) for silica-dried material or the Ancient Seeds aDNA extraction protocol 1.15 (Cappellini 2011) after grinding with a Retsch MM 301 (Germany) instrument for herbarium specimens without silica-dried material. Eight polymorphic nuclear microsatellite markers originally developed for *Dacryodes* Vahl (1810: 115) (*St03, St05*) (Born 2007) and *Santiria* (*Santri07, Santri10, Santri15, Santri20, Santri26* and *Santri27*) (Koffi *et al.* 2012), were used for genotyping 479 georeferenced individuals and 59 seeds. We included seeds to test hybridization between morphotypes observed in sympatry.

The forward primers of markers *Santri07*, *Santri15* and *Santri20* were directly labelled with a fluorescent dye (6-FAM). A unique 20bp linker sequence (Q1, Q2, Q3 or Q4) was added to the 5′ end of the forward primers of markers *St03*, *St05*, *Santri10*, *Santri26*, *Santri27* to allow genotyping them following the protocol of Micheneau *et al.* (2011) using fluorescently labelled linker sequences (Q1 with 6-FAM, Q2 with NED, Q3 with VIC and Q4 with PET). Three primer mixes were designed based on the compatibility of dye colours and expected size ranges: Mix1 (*Santri07, Santri10_Q4* and *Santri15*), Mix2 (*Santri26_Q3* and *Santri27_Q2*) and Mix3 (*St03_Q1, St05_Q4* and *Santri20*). Polymerase chain reaction (PCR) was carried out in PTC-100 or PTC-200 thermocyclers (MJ Research) in a 15 µl reaction volume using the QIAGEN Multiplex PCR kit (QIAGEN, Venlo, Netherlands). Each PCR reaction mix contained 0.1 µl of directly labelled forward primers (0.07 µM) with 0.3 µl each of the tailed forward and reverse primers, and the labelled linkers (0.2 µM); 1.5 µl of template DNA (10–100 ng), 7.5 µl of QIAGEN Multiplex solution and 3.8–4.3 µl of sterile water to complete the mix. The cycling profiles for PCR included an initial step of 15 min at 95°C followed by 22 cycles of 30 s at 94°C, 90 s at 57°C and 60 s at 72°C. A third step of 10 cycles of 30 s at 94°C, 45 s at 53°C and 60 s at 72°C, was followed by a 30 min final elongation step at 60°C. Electrophoretic separation of PCR products was carried out on the 3730 Genetic Analyzer (Applied Biosystems, Lennik, the Netherlands) using 12 µl of Hi-Di Formamide with 0.3 µl of the GeneScan 500 Liz size standard (Applied Biosystems, Warrington, United Kingdom) and 1.2 µl of PCR product. Peak Scanner 1.0 software (Applied Biosystems) and visual inspection were employed in scoring fragment sizes.

Both *Santri10* and *Santri20* amplified two loci each (*Santri10a* and *Santri10b*, *Santri20a* and *Santri20b* respectively). The alleles observed at these co-amplifying loci could be distinguished by their size ranges and/or electrophoretic profiles, hence, the eight original nSSR markers were used to study 10 loci. However, null alleles occurred in some loci, leading to missing data despite repeated DNA extraction and PCR amplification.

Identification of genetic clusters:—Bayesian clustering analyses were performed to identify distinct genetic clusters using STRUCTURE 2.3.4 (Pritchard *et al.* 2000). This software simulates hypothetic *K* genetic clusters of individuals, which approach, as far as possible, both Hardy-Weinberg and linkage equilibria. To this end, we selected the admixture model with correlated allele frequencies, from which the fraction of ancestry from each genetic cluster was estimated per individual. We declared the potential presence of null alleles for all loci. We performed five runs at each K ranging from 1 to 10 over 10^5 generations (burn-in period of 2×10^4 generations). The results were treated using the online application STRUCTURE HARVESTER (Earl & Von Holdt 2012) to plot the mean and variance of the log-likelihood [ln P(D)] of the data as a function of K. The optimum number of genetic clusters (K) was reached when the ln P(D) plateaued as K increased. When individuals obtained ancestry of $p \geq 0.7$ from a genetic cluster, they were considered as belonging to that cluster. The frequency of null alleles per locus from each genetic cluster was estimated with INEst 2.0 (Chybicki & Burczyk 2009). *Quantum GIS* 2.8.1 (Quantum GIS Development Team 2014) was used to map the genetic clusters. Bayesian clustering analyses were first performed within two different localities (CEB and Ipassa) using 119 and 115 individuals, and then on the whole dataset covering Lower Guinea to compare the genetic clusters identified at local and large geographical scales.

Morphological traits and analyses:—We observed and measured morphological traits of 231 individuals (103 individuals with herbarium specimens associated). Three kinds of trait were characterized: (i) trunk base characteristics (231 individuals with $2 \leq$ dbh ≤ 75 cm); (ii) end of twig, and leaf and leaflet characteristics (103 individuals); and (iii) reproductive organ (flowers and fruits) characteristics from 15 male and 76 female mature trees. Overall, 19 quantitative (Table 2) and 11 qualitative variables (Table 3) were observed and/or measured.

We performed all morphological data analyses using R 3.2.3 software (R Development Core Team 2015). We used mean values per individual as units of analysis for quantitative variables with repeated measures per individual. Kruskal-Wallis (*H*) tests were used to assess which quantitative variables differed significantly between genetic clusters. Chi-square (χ^2) tests were performed on qualitative variables to assess if their distribution differed significantly between genetic clusters. The morphological variation of twig and leaflet traits from the 103 individuals was assessed using a Hill and Smith ordination (Hill & Smith 1976), which is an extended principal component analysis (PCA), for both quantitative and qualitative variables.

TABLE 2. Quantitative traits measured on trunk, leaf and fruit of *Santiria* trees.

Part of plant	Trait	Notes
Base of trunk	Height of aerial roots (if any)	Stilt roots
	Diameter	At 1.3m or 0.3 m above the highest stilt root
Leaf	Number of leaflets per leaf (NL)	Mean of 2–10 leaves per individual
	Length of leaves (LL)	Mean of 2–5 leaves for each individual
	Length of petiole (LP)	Mean of 2–5 leaves for each individual
	Width of petiole (WP)	Measured at 1.3 cm from petiole base
	Ratio of LP to WP (LP:WP)	To characterize the thickness of petiole
	Terminal petiolule length (TPeL)	Mean of 2–5 leaves for each individual
	Terminal leaflet length (TLL)	Mean of 2–5 leaves for each individual
	Terminal leaflet width (TLW)	Mean of 2–5 leaves for each individual
	Ratio of TLL to TLW (TLL:TLW)	To characterize the shape of leaflets
	Terminal leaflet dry weight per unit area (TLWe)	We weighed 2 discs of 1.9 cm diameter cut from the limb of terminal leaflet (avoiding prominent midrib and secondary vein)
	Apex length (AL) of terminal leaflet	Mean of 4–10 leaflets for each individual
Fruit	Pedicel length (PiL) of mature fruit	Mean of 5–10 fruits for each individual
	Pedicel width (PiW) of mature fruit	Mean of 5–10 fruits for each individual
	Fruit length (FtL) of mature fruit	Mean of 5–10 fruits for each individual
	Fruit width (FtW) of mature fruit	Mean of 5–10 fruits for each individual
	Fruit height (FtH) of mature fruit	Mean of 3–5 fruits for each individual
	Pericarp thickness (Pat) of mature fruit	Mean of 3–5 fruits for each individual

TABLE 3. Qualitative morphological traits recorded.

Variable	States	Notes
Shape of trunk base	Buttresses; Cylindrical; Thickened; Stilt roots	Stilt roots present (SR) or absent (NSR). NSR individuals can present cylindrical (NSRc), thickened (NSRt), or buttresses (NSRb) trunk base.
Shape of stilt roots	Curved; Linear	
Twig lenticels (Le)	Not visible; Few visible; Visible; Clearly visible	Few visible, visible and clearly visible defined increasing density of lenticels, respectively <20, from 20 to 50, and >50 lenticels per cm^2.
Glandular dots (GD) on the underside of leaflet	Few visible; Clearly visible	Observed at G×25. Few visible and clearly visible were noted as 1 and 2 respectively.
Inflorescences	Terminal; Subterminal; Axillary	Subterminal: short inflorescence (10–20 cm length). Axillary: more than 50 cm length.
Flower colour	Green; Yellow	
Coating of flowers	Pubescent; Glabrous	Observed at G×25.
Sex individuals	Male; Female	
Infructescences	Grouped in glomerulus; Isolated	
Immature fruit colour	Green; Yellowish to Red	We grouped yellowish, pink and red immature fruits because we observed a continuous colour gradient.
Colour of cotyledons integument	Reddish; Red; Red-purple	

Results

Genetic clusters and allelic diversity:—All ten loci were polymorphic. Of the 538 genotyped samples, 59 individuals (all 31 herbarium specimens without silica-dried material, six individuals previously extracted and 22 individuals with silica-dried material) were removed, because microsatellite amplification was too weak in most loci to reach a reliable

interpretation of their electropherograms. Bayesian clustering analyses performed on the 479 successfully genotyped individuals revealed an important increase of the log-likelihood values from K = 1 up to K = 3 followed by a plateau (Fig. 1A). The most likely number of genetic clusters was K = 3, using Evanno's delta K method (Evanno *et al.* 2005). Using a cut-off of $p \geq 0.7$, only 9 individuals (six adults and three seeds) were unassigned to a cluster and the three genetic clusters were clearly delimited (Fig. 1B) and contained 185, 116 and 169 samples for GC1, GC2 and GC3, respectively. At the local geographical scale, all 119 and 115 individuals were assigned and the three genetic clusters were found in CEB, but only two genetic clusters (GC1 and GC3) were found at Ipassa.

These three clusters occur in sympatry in Gabon (Fig. 1C), particularly in four localities (southern Monts de Cristal, Mabounié, Kongou, and CEB). While GC1 is mostly distributed in Gabon (some individuals are also recorded in Democratic Republic of the Congo and Equatorial Guinea), GC2 and GC3 are more widespread and occur in Gabon, Cameroon, and the Republic of the Congo. Only GC3 occurs in Central African Republic.

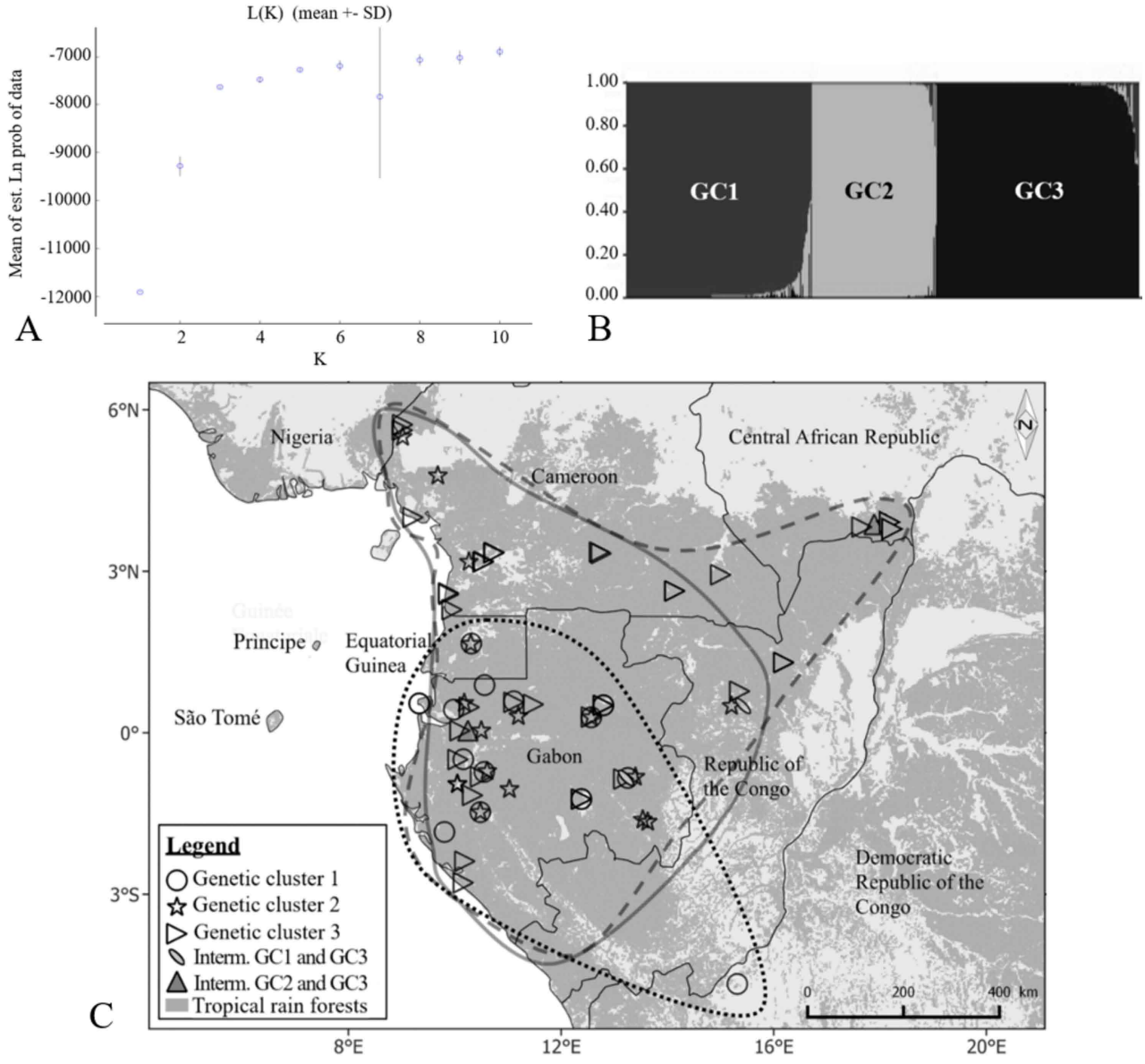

FIGURE 1. Genetic clusters (GC) detected in *Santiria* samples from western Central Africa. Bayesian clustering analyses were performed on 479 individuals genotyped at 10 microsatellites loci. A. Variation in means of Ln (likelihood) of the data as a function of the number of hypothetical genetic clusters (K), showing a plateau at K=3. B. Histogram of genetic assignment of the 481 individuals at K = 3. C. Distribution of the three genetic clusters in western Central Africa, and delimitation of the distribution of each genetic cluster (dotted line: GC1, solid line: GC2, dashed line: GC3). We extended the distribution ranges of GC2 and GC3 because morphotypes of both genetic clusters were observed in the south of the Republic of the Congo.

Note: Interm. GCx and GCy = intermediate individuals between GCx and GCy.

Diagnostic alleles with a frequency $p > 0.3$ were found for each genetic cluster. For example, *Santri07-201, Santri10a-213* and *Santri27-192* were characteristic of GC1; *St03-195*, *Santri10a-218*, *Santri10a-236*, *Santri20b-383*, *Santri20b-385* and *Santri27-214* were characteristic of GC2; and *St03-201, Santri07-195, Santri10a-224, Santri26-156* and *Santri27-202* characterizing GC3 (Table 4).

TABLE 4. Allele size ranges per locus for the three genetic clusters (GC) from western Central Africa (N = 470). (*) indicates high frequencies of null allele.

Loci	GC1 (N = 185)	GC2 (N = 116)	GC3 (N = 169)
St03	191–201	195	173–209
St05	146–196	146–161	142–196
Santri07	187–203	191–201	187–203
Santri10a	213	218–238	222–232
Santri10b	240*	240–247	240
Santri15	263–287	271–298	263–307
Santri20a	371–405	379–385	371–405
Santri20b	381–407	383–388*	381–407*
Santri26	156–162	158–164	156–158
Santri27	192–218	195–227	199–218

Correspondence between genetic clusters and morphological traits:—The most discriminant morphological traits between the three genetic clusters were the architecture of the trunk base, characters of the twigs and leaves, and reproductive traits.

Architecture of the trunk base:—All three genetic clusters presented morphotypes with stilt roots (SR) and without stilt roots (NSR), with, in the latter case, different variants displaying buttresses (NSRb), a thickened trunk base (NSRt) or a cylindrical trunk (NSRc). Stilt roots were observed for 115 individuals ($2 \leq$ dbh ≤ 68 cm) with varying shapes ranging from curved or linear in transverse section, to circular or elliptic in the three genetic clusters. GC2 and GC3 showed similar distributions of trunk shapes (Table 5). NSRc morphotype was observed only in GC1 (Table 5) while SR morphotype dominated in GC2 (81% of individuals) and GC3 (95% of individuals) with stilt roots reaching up to 3 m high. Only 20% of GC1 individuals had stilt roots that were of smaller size (up to 1 m high). It should be noted that differentiation between SR and NSR can sometimes be ambiguous on sloping ground or when stilt roots are attached to the base of the trunk (up to 1.5 m).

Twigs and leaves:—Quantitative and qualitative traits were observed and measured only on herbarium samples (Table 5). The first two axes of the Hill-Smith ordination explained 62% of the variation among the 103 herbarium specimens (Fig. 2) and the first axis separated well specimens assigned to GC3 from those assigned to GC1 and GC2. GC1 differed from GC2 by only one leaf trait: terminal leaflet dry weight per unit area ($H = 8.887$, df = 1, $P = 0.003$). GC3 specimens were distinguished from GC1 and GC2 by small leaves (mean length ± SD = 32 ± 8 cm) and leaflets (length ± SD = 12 ± 4 cm), glandular dots and few or no visible lenticels on twigs ($P < 0.001$ for all eight cases) (Table 5). However, one specimen, *Ikabanga et al. 407ST* (see Appendix) assigned to GC3 had a morphotype similar to the second group, characterized by large leaves (length ≥ 38.7 cm), glandular dots and visible lenticels. Despite this exception in leaflet size and shape, specimens assigned to GC3 can be easily differentiated from the other two. No glandular dots were observed on leaves of seedlings.

Reproductive traits:—Male and female individuals were assigned to each genetic cluster. Females were more often sampled (81%) due to their higher visibility during fruiting season. Individuals assigned to GC3 presented axillary inflorescences while GC1 and GC2 both presented subterminal or terminal inflorescences (Table 6). Flower colour (pale green, yellow, light green and yellow-green) did not distinguish the three genetic clusters. However, females assigned to GC1 were distinguished by having beige to red ovaries before their flowers lost the perianth. Furthermore, male and female flowers of individuals from GC1 (19 individuals) were pubescent while individuals from both GC2 and GC3 (10 individuals) had glabrous flowers. Immature fruits were red to orange in GC1 (24 individuals) and green in both GC2 and GC3 (17 individuals). Yet, one green fruit collected from an individual assigned to GC1 (*Ikabanga et al. 406ST*) displayed characteristics between GC2 and GC3. Mature fruits are always black. However, they differ in size, being larger in GC1 and GC2 than in GC3 (Table 6). Fruits in infructescences were clustered and numerous in both GC1 and GC2 and were isolated or not numerous in GC3. The integument of cotyledons was red in GC1, red-purple in GC2 and reddish in GC3.

Considering the most discriminant traits assessed using statistical tests, the three genetic clusters can be distinguished morphologically by combining (i) immature fruit colour (green immature fruit denoted as GIF and red to orange immature fruit denoted as RIF) or external structure of flowers (pubescent for GIF and glabrous for RIF), with (ii) the presence (p) or absence (a) of glandular dots on the lower surface of leaflets. In fact, RIF, GIFp and GIFa morphotypes corresponded with GC1, GC2 and GC3, respectively. At Ipassa, only RIF and GIFa morphotypes were observed, which is coherent with the identification of only two genetic clusters (GC1 and GC3) when we performed Bayesian clustering analyses.

TABLE 5. Vegetative traits of *Santiria* observed on the base of trunk (2 ≤ dbh ≤ 75 cm, N = 231) and measured on herbarium specimens (N = 103) for each of the three genetic clusters (GC). NL = number of leaflets per leaf; LL = length of leaves; LP = length of petiole; WP = width of petiole; LP/WP = ratio of LP to WP; TPeL = terminal petiolule length; TLL = terminal leaflet length; TLW = terminal leaflet width; TLL/TLW = ratio of TLL to TLW; TLWe = terminal leaflet dry weight per unit area; AL = apex length; GD = glandular dots; Le = lenticels; (N) = (number of individuals); M (X-Y) ± Z / β = mean (minimum-maximum) ± standard deviation; a, b, c: results of statistical tests where genetic clusters that do not differ significantly share a same letter.

Part of plant	Variables	GC1 (N = 111)	GC2 (N = 33)	GC3 (N = 87)
Base of trunk	Buttresses	43	1	3
	Thickened	29	5	1
	Cylindrical	18	0	0
	Stilt roots	22	27	83
		GC1(N = 46)	**GC2 (N = 21)**	**GC3 (N = 36)**
Leaves and twigs from herbarium specimens	NL	9.40 (5.00–13.00) ± 1.64/ a	6.76 (5.00–9.00) ± 1.00 / b	9.94 (7.00–13.00) ± 1.71 / a
	LL (cm)	43.75 (17.00–85.00) ± 13.17 / a	38.66 (23.50–62.00) ± 8.23 / a	32.40 (21.85–56.00) ± 8.03 / b
	LP (cm)	7.50 (2.10–16.50) ± 3.74 / a	4.16 (1.60–8.50) ± 1.49 / b	5.12 (1.30–14.00) ± 2.40 /c
	WP (cm)	0.37 (0.25–0.60) ± 0.08 / a	0.30 (0.10–0.50) ± 0.11 / a	0.17 (0.10–0.40) ± 0.07 / b
	LP/WP	20.32 (7.00–55.00) ± 10.16 / a	18.03 (5.00–70.83) ± 15.91 / a	36.24 (8.67–93.33) ± 22.18 / b
	TPeL (cm)	4.00 (1.80–7.00) ± 1.22 / a	3.91 (1.50–6.00) ± 1.28 / a	2.68 (0.50–8.00) ± 1.19 / b
	TLL (cm)	20.31 (11.50–31.50) ± 4.63 / a	21.14 (15.70–26.50) ± 3.29 / a	12.05 (7.50–24.00) ± 3.88 / b
	TLW (cm)	10.10 (4.80–18.00) ± 2.24 / a	10.20 (6.00–17.00) ± 2.50 / a	5.21 (3.20–10.50) ± 1.70 / b
	TLL/TLW	2.04 (1.36–2.80) ± 0.34 / a	2.14 (1.53–3.33) ± 0.38 / ab	2.51 (1.50–4.10) ± 0.49 / b
	TLWe (mg/cm^2)	0.09 (0.06–0.19) ± 0.03 / a	0.12 (0.06–0.19) ± 0.04 / b	0.14 (0.07–0.26) ± 0.06/ b
	AL (cm)	1.06 (0.60–2.10) ± 0.29 / a	1.23 (0.60–1.70) ± 0.30 / b	1.38 (0.50–2.80) ± 0.57 / b
	GD	Clearly visible (45); Few visible (1) / a	Clearly visible (20); Few visible (1) / a	Clearly visible (1); Few visible (35) / b
	Le	Clearly visible (33); Visible (12), Few visible (1) / a	Clearly visible (16); Visible (5) / a	Clearly visible (1); Visible (1), Few visible (31); Not visible (3) / b

TABLE 6. Qualitative and quantitative traits of reproductive organs of individuals (10 ≤ dbh ≤ 68 cm) assigned to each genetic cluster (GC). Three to ten fruits per tree were measured for each variable respectively in herbarium samples preserved in spirit and in the field. Colour of teg. of cot. = Colour of integument of cotyledons; FtL = fruit length; FtW = fruit width; FtH = fruit height; PiL = pedicel length; PiW = pedicel width; Pat = pericarp thickness; N = number of individuals; M (X–Y) ± Z / β = mean (minimum–maximum) ± standard deviation / result of Kruskal-Wallis tests.

Qualitative variables	GC1 (N = 48)	GC2 (N = 10)	GC3 (N = 33)
Sex	Male (8), Female (40)	Male (3), Female (7)	Male (4), Female (29)
Flower colour	Yellow (19)	Light green (5), yellow-green (1)	Pale green (4)
Coating of flowers	Pubescent	Glabrous	Glabrous
Immature fruit colour	Red to orange (24)	Green (6)	Green (10)
Colour of teg. of cot.	Red (16)	Red-purple (6)	Reddish (5)
Inflorescences	Terminal or subterminal (21)	Terminal or subterminal (7)	Axillary (5)
Infructescences	Grouped in glomerules	Grouped in glomerules	Isolated or few grouped
Quantitative variables	**GC1 (N = 14)**	**GC2 (N = 8)**	**GC3 (N = 8)**
FtL (cm)	3.30 (2.20–4.00) ± 0.54 / a	3.25 (3.00–3.80) ± 0.28 / a	2.09 (1.50–2.60) ± 0.36 / b
FtW (cm)	2.51 (1.70–3.10) ± 0.40 / a	2.43 (2.20–2.90) ± 0.23 / a	1.59 (1.00–2.30) ± 0.43 / b
FtH (cm)	2.06 (1.60–4.10) ± 0.69 / a	1.76 (1.60–2.10) ± 0.19 / a	1.19 (0.80–1.80) ± 0.32 / b
PiL (cm)	0.42 (0.20–0.73) ± 0.15 / a	0.49 (0.20–0.82) ± 0.19 / b	0.83 (0.60–1.00) ± 0.15 / b
PiW (cm)	0.28 (0.13–0.40) ± 0.08 / a	0.27 (0.12–0.41) ± 0.11 / a	0.12 (0.10–0.16) ± 0.02 / b
Pat (cm)	0.34 (0.23–0.50) ± 0.07 / a	0.34 (0.30–0.40) ± 0.05 / a	0.16 (0.10–0.20) ± 0.05 / b

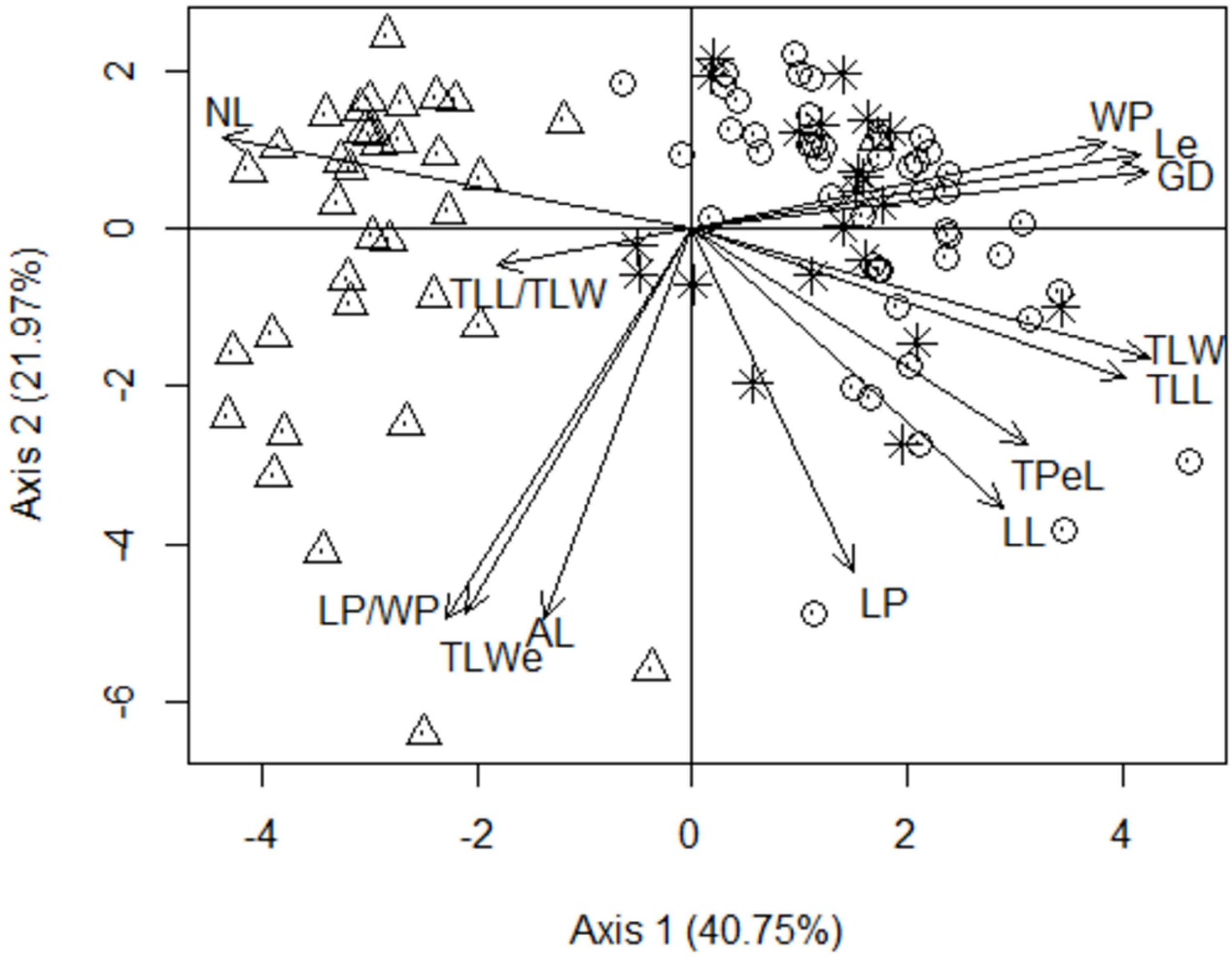

FIGURE 2. Extended Principal Component Analysis (the Hill-Smith ordination) of quantitative and qualitative traits assessed in 103 *Santiria* herbarium samples assigned to GC1 (N = 46, open circles), GC2 (N = 21, stars) and GC3 (N = 36, open triangles).
Note: NL = number of leaflets per leaf; LL = length of leaves; LP = length of petiole; WP = width of petiole; LP/WP = ratio between LP and WP; TPeL = terminal petiolule length; TLL = terminal leaflet length; TLW = terminal leaflet width; TLL/TLW = ratio between TLL and TLW; TLWe = terminal leaflet weight dry portion; AL = apex length; GD = glandular dots; Le = lenticels.

Discussion

The genus *Santiria* has been revised in Africa by Onana (2009) who recognized only one species, *Santiria trimera*, with substantial morphological variability. In spite of this, recent studies combining morphological traits and phylogenetic analyses, suggested the existence of at least two species (Koffi *et al.* 2010, 2011). The current study combines microsatellite genotyping and morphometric data on a large sample (N = 479) to revisit species delimitation in the genus *Santiria* in Lower Guinea (without São Tomé island). We identified three sympatric genetic clusters, corresponding to three species, following both the biological and the typological species concepts, with more reliable morphological diagnostic traits than those proposed by Koffi *et al.* (2010, 2011).

Diagnostic morphological traits for species delimitation in *Santiria*:—Based on stilt roots, leaflet size, colour and coating of reproductive organs, individuals from each genetic cluster composed a particular morphogroup.

Stilt roots and leaflet size:—Three morphotypes were defined by Koffi *et al.* (2010, 2011, 2012) based on the absence of stilt roots (NSR), or the presence of stilt roots combined with small leaflets (SRsl) or with large leaflets (SRll). The SRsl and SRll morphotypes were clearly delimited using nuclear and chloroplast DNA sequences from samples collected in northern Gabon (Koffi 2010). Compared to the present analysis, NSR, SRll and SRsl morphotypes largely corresponded respectively to RIF (GC1), GIFp (GC2) and GIFa (GC3). However, our analyses reveal that the shape of the base of the trunk (with or without stilt roots) and the leaflet size (small or large) were not reliable

diagnostic characters. Indeed, we observed individuals with stilt roots in all three genetic clusters, and the distinction between stilt roots and buttresses was not always obvious in the field. Moreover, although mean leaflet size differs between some genetic clusters, there is much overlap in their respective distributions, so that this character can also remain ambiguous for species identification.

There was no relationship between the presence of stilt roots and stem diameter or the sex of individuals. Morphotypes with and without stilt roots were often found next to each other (within 5–10 m) so that the development of stilt roots does not seem to be related to soil and hydrology. However, Hladik & Blanc (1987) noted an exceptional growth of stilt roots when light becomes available in the understorey. As in *Socratea exorrhiza* (Martius 1824: 36) Wendland (1860: 103) (Arecaceae), where stilt roots develop according to vertical growth (Goldsmith & Zahawi 2007), stilt roots in *Santiria* could contribute to rapid growth towards the canopy. Chevalier (1948) suggested that stilt roots originally developed on alluvium and expansion of stilt roots is recent on dry soils, e.g. *Musanga cecropioides* R.Br. ex Tedlie in Bowdich (1819: 372) (Urticaceae). However, *M. cecropioides* does not develop stilt roots in Uganda (Schnell 1970). The morphotype without stilt roots in the African genus *Santiria* was recognised by Troupin (1958). The identification of males and females in all three genetic clusters of the current study refutes the hypothesis that sexual dimorphism could explain the differences between the SRll and NSR morphotypes (Koffi 2010). The functions provided by stilt roots should, however, be examined in the genus *Santiria*.

Leaflets were usually larger on saplings than adults, and on lateral stolon branches from which herbarium samples were often collected. Thus, variations of leaf and leaflet sizes using only herbarium samples are not reliable.

Colour and coating of reproductive organs:—In situ, observations revealed the importance of recording some traits on fresh material for morphological species delimitation. Indeed, if collectors had not noted the colour of reproductive organs, flower and fruit colours could not be easily distinguished when using dried and spirit-preserved specimens. In addition, inflorescences occurring along twigs were not commonly observed on herbarium samples.

Interestingly, RIF and GIFp morphotypes observed at Lastourville, Franceville, Ipassa and Mabounié showed similar inflorescences (terminal or subterminal) and produced flowers at the same period. By contrast, GIFa morphotype presented axillary inflorescences and produced flowers a month later than the RIF and GIFp morphotypes. This variation in phenology between RIF (≈NSR) and GIFa (≈SRsl) was also reported by (Koffi 2010) and can contribute to the reproductive isolation of GIFa (GC3), but other mechanisms of reproductive isolation must occur between RIF (GC1) and GIFp (GC2).

Some characters based on the colour of reproductive organs were confounding. Collectors described flowers of *Santiria* as yellowish, pale green, light green, olive green or reddish. However, reddish flowers characterized females assigned to GC1 (RIF) due to the colour change of their ovaries (from pale yellow to red) before flowers lost the perianth. Males assigned to GC1 could not be clearly distinguished from those in the GIFa and GIFp morphotypes using only flower colour. Immature fruit colour was also confounding. Wilks & Issembé (2000) reported green, red and then black fruits in *Santiria trimera*. However, fruits were green or orange to yellow during their development and they turn black at maturity. We observed reddish tints around pedicel insertions on fruits of the GIFa and GIFp morphotypes.

We found another diagnostic morphological character of the RIF morphotype: the pubescent coating of inflorescences. Indeed, males and females assigned to GC1 had pubescent rachilla and flowers (pedicels, perianth, pistil and pistillode), while both the GIFa and GIFp morphotypes presented a glabrous coating. Onana (2009) reported pubescence, but did not consider it a diagnostic trait as the hair density was variable in the studied specimens. These variations could be due to hairs that were abraded on older specimens (Webber & Woodrow 2006) and strong ontogenetic variation observed in the RIF morphotype: rachilla and other reproductive parts lost hairs and became glabrous during fructification. However, pubescence can be used to distinguish younger vegetative organs (Aubréville 1962) and as demonstrated in the current study, RIF morphotype was clearly distinguished from both GIFa and GIFp morphotypes based on observations of pubescence in younger reproductive organs of *Santiria*.

Species delimitation supervised by population genetics tools:—Based only on visual inspection (De Vogel 1987) of herbarium specimens, species delimitation in the African tree genus *Santiria* provided many difficulties and eventually lead to the recognition of a single species according to the latest revision (Onana 2009). In the present work, our ability to identify diagnostic traits of three species of *Santiria* in Lower Guinea results from our strategy to first identify genetic clusters with highly polymorphic nuclear markers, then to consider a large range of traits, including those only visible in the field, to search for differences between clusters. It appears that the species identified within *Santiria* are not cryptic species as they bear diagnostic traits, but some of the most important traits are often missing from herbarium samples, for instance the colour of immature fruits and inflorescence type.

Molecular data are now frequently incorporated in species delimitation research where it often supports morphological and/or ecological data (Bickford *et al.* 2007). Microsatellite markers are helpful for such studies due to their high polymorphism and ability to establish the level of gene flow between individuals and populations (Duminil & Di Michele 2009). Here, Bayesian clustering analyses performed on 479 samples (individuals and seeds) identified three largely sympatric genetic clusters in the LG region (Fig. 1C). By contrast, several studies on the spatial genetic structure of African tropical tree species also revealed different genetic clusters, but displaying allopatric or parapatric distribution (Dick 2008, Ley & Hardy 2010, Duminil *et al.* 2012, 2013, Heuertz *et al.* 2014). These studies suggested that the inferred clusters resulted from populations previously disconnected by historically fragmented habitats; hence they do not represent new species. In our study, the three clusters identified occur in sympatry, generally without gene flow, which is a strong indication of the existence of different biological species. Within each of these main clusters, additional secondary clusters could be observed by running the clustering algorithm again, but these "secondary clusters" were always occurring in parapatry, showed many intermediate individuals, and were little differentiated genetically (results not shown). Thus they corresponded to differentiated populations of the same *Santiria* species due to (past) isolation, as commonly observed in other African tree species.

Despite the clear correspondence between genetic clusters and morphotypes, morphological analyses identified two individuals (an adult and a seedling) out of 470 where the assignment to genetic clusters did not correspond to their morphotype. Moreover, 2.1% of individuals were intermediates between two clusters according to genetic clustering. Two hypotheses could be formulated to explain these observations: (i) occasional hybridization between species, (ii) shared allelic variants between species/genetic clusters. Indeed, without hybridization, alleles that co-occur in different genetic clusters could cause some errors of assignment to clusters. Hence, additional investigations to test these two hypotheses are needed to determine if gene flow sometimes occurs between these genetic clusters.

Conclusion

In Lower Guinea (LG), *Santiria* is comprised of three genetic clusters interpreted here as biological species. *Santiria* also occurs in São Tomé island, Upper Guinea and the Congo basin. Koffi (2010) demonstrated genetic differentiation between populations from São Tomé island, Upper Guinea and LG. The limited number of samples available from Upper Guinea and São Tomé island did not allow us to extend our study beyond LG. However, diagnostic morphological traits found in LG allow the realization of a new taxonomic revision of African *Santiria*.

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References

Aké Assi, L. (2001) Flore de la Côte-d'Ivoire: catalogue systématique, biogéographie et écologie. I. *Boissiera* 57: 169–170.

Akéndéngué, B. & Louis, A.M. (1994) Medicinal plants used by the Masango people in Gabon. *Journal of Ethnopharmacology* 41: 193–200.
https://doi.org/10.1016/0378-8741(94)90032-9

Aubréville, A. (1948) Les *Dacryodes* (*Pachylobus*) et les *Santiria* de l'ouest africain. *Bois et Forêts des Tropiques* 8: 342–348.

Aubréville, A. (1962) Burséracées. *In:* Aubréville, A. (Ed.) *Flore du Gabon: Irvingiacées, Simaroubacées, Burseracées, vol. 3*. Muséum National d'Histoire Naturelle Laboratoire de Phanérogamie, Paris, pp. 53–101.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. & Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 22: 148–155.
https://doi.org/10.1016/j.tree.2006.11.004

Bikanga, R., Makani, T., Agnaniet, H., Obame, L.C., Abdoul-Latif, F.M., Lebibi, J. & Menut, C. (2010) Chemical composition and biological activities of *Santiria trimera* (Burseraceae) essential oils from Gabon. *Natural Product Communications* 5: 961–964.

Blume, C.L. von (1850) *Museum Botanicum Vol. 1*. Lugduni-Batavorum, Leiden, 396 pp.

Born, C. (2007) *Diversité génétique et dynamique des forêts d'Afrique centrale: une étude multi-échelle de la structure de la diversité génétique d'un arbre pionnier, Aucoumea klaineana*. Ph.D. thesis. Université Montpellier 2, Montpellier.

Bowdich, T.E. (1819) *Mission from Cape Coast Castle to Ashantee, with a statistical account of that kingdom, and geographical notices of other parts of the interior of Africa*. J. Murray, London, 512 pp.

Breteler, F.J. (2011) Novitates Gabonenses 78. Deux espèces nouvelles du Gabon dans les Bridelieae (Phyllanthaceae, autrefois Euphorbiaceae) avec clés des espèces gabonaises des genres *Bridelia* et *Cleistanthus*. *Adansonia* 33: 233–242.
https://doi.org/10.5252/a2011n2a8

Burkill, H.M. (1985) *The useful plants of West Africa. Ed. 1*. Royal Botanical Gardens, Kew.

Candolle, A. de (1813) *Théorie élémentaire de la botanique, ou exposition des principes de la classification naturelle et de l'art de décrire et d'étudier les végétaux*. Déterville, Paris.

Cappellini, E. (2011) *Ancient seeds aDNA extraction protocol*. Geogenetics, University of Copenhagen, Denmark.

Chevalier, A. (1948) Biogéographie et Ecologie de la forêt dense ombrophile de la Côte d'Ivoire. *Revue Internationale de Botanique Appliquée et d'Agriculture Tropicale* 28: 101–115.
https://doi.org/10.3406/jatba.1948.6138

Chybicki, I.J. & Burczyk, J. (2009) Simultaneous estimation of null alleles and inbreeding coefficients. *Journal of Heredity* 100: 106–113.
https://doi.org/10.1093/jhered/esn088

Da Silva, M.F.D.G., Francisco, R.H., Gray, A.I., Lechat, J.R. & Waterman, P.G. (1990) Lanost-7-en triterpenes from stem bark of *Santiria trimera*. *Phytochemistry* 29: 1629–1632.
https://doi.org/10.1016/0031-9422(90)80135-4

Dauby, G., Duminil, J., Heuertz, M. & Hardy, O.J. (2010) Chloroplast DNA polymorphism and phylogeography of a Central African tree species widespread in mature rainforests: *Greenwayodendron suaveolens* (Annonaceae). *Tropical Plant Biology* 3: 4–13.
https://doi.org/10.1007/s12042-010-9041-6

De Vogel, E.F. (1987) Guidelines for the preparation of revisions. *In:* De Vogel, E.F. (Ed.) *Manual of herbarium taxonomy theory and practice,* UNESCO, Jakarta, 76 pp.

Dick, C.W. (2008) New interpretations of fine-scale spatial genetic structure. *Molecular Ecology* 17: 1873–1874.
https://doi.org/10.1111/j.1365-294X.2008.03728.x

Doucet, J.-L. (2003) *L'alliance délicate de la gestion forestière et de la biodiversité dans les forêts du centre du Gabon*. Ph.D. thesis. Faculté universitaire des sciences agronomiques de Gembloux, Gembloux.

Duminil, J., Caron, H., Scotti, I., Cazal, S.-O. & Petit, R.J. (2006) Blind population genetics survey of tropical rainforest trees. *Molecular Ecology* 15: 3505–3513.
https://doi.org/10.1111/j.1365-294X.2006.03040.x

Duminil, J. & Di Michele, M. (2009) Plant species delimitation: a comparison of morphological and molecular markers. *Plant Biosystems* 143: 528–542.
https://doi.org/10.1080/11263500902722964

Duminil, J., Kenfack, D., Viscosi, V., Grumiau, L. & Hardy, O.J. (2012) Testing species delimitation in sympatric species complexes: the case of an African tropical tree, *Carapa* spp. (Meliaceae). *Molecular Phylogenetics and Evolution* 62: 275–285.
https://doi.org/10.1016/j.ympev.2011.09.020

Duminil, J., Brown, R.P., Ewédjè, E.-E.B., Mardulyn, P., Doucet, J.-L. & Hardy, O.J. (2013) Large-scale pattern of genetic differentiation within African rainforest trees: insights on the roles of ecological gradients and past climate changes on the evolution of *Erythrophleum* spp. (Fabaceae). *BMC Evolutionary Biology* 13: 1–13.
https://doi.org/10.1186/1471-2148-13-195

Earl, D.A. & Von Holdt, M.B. (2012) Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359–361.
https://doi.org/10.1007/s12686-011-9548-7

Engler, A. (1890) Drei neue Burseraceae aus Westafrika africanae. *In* Engler, A. (Ed.) *Beiblatt zu den Botanischen Jahrbüchern*. *Botanische Jahrbücher fur Systematik* 12 (26): 6.

Engler, A. (1910) Burseraceae africanae IV. *Botanische Jahrbücher fur Systematik* 44: 137–155.

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology* 14: 2611–2620.
https://doi.org/10.1111/j.1365-294X.2005.02553.x

Exell, A.W. (1944) *Catalogue of the vascular plants of S. Tomé (with Príncipe and Annobón)*. British Museum (Natural History), London, 135 pp.

Florence, J. & Hladik, A. (1980) Catalogue des Phanerogames et des Pteridophytes du nord est du Gabon. *Adansonia* 20: 235–253.

Gillardin, J. (1959) *Les essences forestières du Congo et du Rwanda-Urundi. Leurs dénominations indigènes, leur distribution et leur habitat*. DAFE, Bruxelles.

Goldsmith, G.R. & Zahawi, R.A. (2007) The function of stilt roots in the growth strategy of *Socratea exorrhiza* (Arecaceae) at two neotropical sites. *Revista de Biologia Tropial* 55: 787–793.
https://doi.org/10.15517/rbt.v55i3-4.5955

Guillaumin, M.A. (1908) Révision des Burséracées du Gabon et du Congo français. *Bulletin de la Société botanique de France* 55: 261–269.
https://doi.org/10.1080/00378941.1908.10831966

Haurez, B., Daïnou, K., Tagg, N., Petre, C.-A. & Doucet, J.-L. (2015) The role of great apes in seed dispersal of the tropical forest tree species *Dacryodes normandii* (Burseraceae) in Gabon. *Journal of Tropical Ecology* 31: 395–402.
https://doi.org/10.1017/S0266467415000322

Hawthorne, W. & Jongkind, C. (2006) *Woody plants of western African forests: a guide to the forest trees, shrubs and lianes from Senegal to Ghana.* Royal Botanic Gardens (Kew), London, 73 pp.

Heinrichs, J., Klugmann, F., Hentschel, J. & Schneider, H. (2009) DNA taxonomy, cryptic speciation and diversification of the Neotropical-African liverwort*, Marchesinia brachiata* (Lejeuneaceae, Porellales). *Molecular Phylogenetics and Evolution* 53: 113–121.
https://doi.org/10.1016/j.ympev.2009.05.032

Heuertz, M., Duminil, J., Dauby, G., Savolainen, V. & Hardy, O.J. (2014) Comparative phylogeography in rainforest trees from lower Guinea, Africa. *PLoS ONE* 9: 1–14.
https://doi.org/10.1371/journal.pone.0084307

Hickey, L.J. (1973) Classification of the Architecture of Dicotyledonous Leaves. *American Journal of Botany* 60: 17–33.
https://doi.org/10.2307/2441319

Hill, M.O. & Smith, A.J.E. (1976) Principal Component Analysis of Taxonomic Data with Multi-State Discrete Characters. *Taxon* 25: 249–255.
https://doi.org/10.2307/1219449

Hladik, A. & Blanc, P. (1987) Croissance des plantes en sous-bois de forêt dense humide (Makokou, Gabon). *Revue d'écologie (Terre et vie)* 42: 209–234.

Holmgren, P.K., Holmgren, N.H. & Barnett, L.C. (1990) *Index Herbariorum Part I: The Herbaria of the World (Regnum Veg. 120)*. New York Botanical Garden, New York.

Hyam, R., Drinkwater, R.E. & Harris, D.J. (2012) Stable citations for herbarium specimens on the internet: an illustration from a taxonomic revision of *Duboscia* (Malvaceae). *Phytotaxa* 73: 17–30.
https://doi.org/10.11646/phytotaxa.73.1.4

Koffi, K.G. (2010) *Étude de la variabilité et de la phylogéographie de Santiria trimera (Burseraceae). Implications pour une conservation durable des forêts humides d'Afrique*. Ph.D. thesis. Université libre de Bruxelles, Brussels.

Koffi, K.G., Heuertz, M., Doumenge, C., Onana, J.M., Gavory, F. & Hardy, O.J. (2010) A combined analysis of morphological traits, chloroplast and nuclear DNA sequences within *Santiria trimera* (Burseraceae) suggests several species following the Biological Species Concept. *Plant Ecology & Evolution* 143: 160–169.
https://doi.org/10.5091/plecevo.2010.433

Koffi, K.G., Hardy, O.J., Doumenge, C., Cruaud, C. & Heuertz, M. (2011) Diversity gradients and phylogeographic patterns in *Santiria*

trimera (Burseraceae), a widespread African tree typical of mature rainforests. *American Journal of Botany* 98: 254–264. https://doi.org/10.3732/ajb.1000220

Koffi, K.G., Heuertz, M., Jans, R., Hardy, O.J., Vendramin, G.G. & Duminil, J. (2012) Characterization of new microsatellite loci isolated from *Santiria trimera* (Burseraceae). *American Journal of Botany* 99: 334–336. https://doi.org/10.3732/ajb.1200041

Lachenaud, O. & Jongkind, C. (2013) New and little-known *Psychotria* (Rubiaceae) from West Africa, and notes on litter-gathering angiosperms. *Plant Ecology & Evolution* 146: 219–233. https://doi.org/10.5091/plecevo.2013.765

Lam, H.J. (1932) Beitrage zur Morphologie der Burseraceae, inbesondere der Canarieae. *Annales du Jardin botanique de Buitenzorg* 42: 97–226.

Le Guyader, H. (2002) Doit-on abandonner le concept d'espèce? *Le courrier de l'environnement de l'INRA* 46: 51–64.

Letouzey, R. (1968) *Etude phytogéographique du Cameroun*. Paul Chevallier, Paris.

Ley, A.C. & Hardy, O.J. (2010) Species delimitation in the Central African herbs *Haumania* (Marantaceae) using georeferenced nuclear and chloroplastic DNA sequences. *Molecular Phylogenetics and Evolution* 57: 859–867. https://doi.org/10.1016/j.ympev.2010.08.027

Linnaeus, C. (1753) *Species Plantarum, Vol. 2.* Impensis Laurentii Salvii, Stockholm, 1200 pp.

Mallet, J. (2005) Hybridization as an invasion of the genome. *Trends in Ecology and Evolution* 20: 229–237. https://doi.org/10.1016/j.tree.2005.02.010

Martins, A.P., Salgueiro, L.R., Goncalves, M.J., Da Cunha, A.P., Vila, R. & Canigueral, S. (2003) Essential oil composition and antimicrobial activity of *Santiria trimera* bark. *Planta medica* 69: 77–79. https://doi.org/10.1055/s-2003-37025

Martius, C.F.P von (1824) *Historia Naturalis Palmarum vol. 2.* T.O. Weigel, Lipsiae, 152 pp.

Mayr, E. (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge, Massachusetts, 797 pp.

Micheneau, C., Dauby, G., Bourland, N., Doucet, J.-L. & Hardy, O.J. (2011) Development and characterization of microsatellite loci in *Pericopsis elata* (Fabaceae) using a cost-efficient approach. *American Journal of Botany* 98: 268–270. https://doi.org/10.3732/ajb.1100070

Murakam, N., Yokoyama, J., Cheng, X., Iwasaki, H., Imaichi, R. & Iwatsuki, K. (1998) Molecular α-taxonomy of *Hymenasplenium obliquissimum* Complex (Aspleniaceae) Based on rbcL Sequence Comparisons. *Plant Species Biology* 13: 51–56. https://doi.org/10.1111/j.1442-1984.1998.tb00247.x

Ngomanda, A., Chepstow-Lusty, A., Makaya, M., Schevin, P., Maley, J., Fontugne, M., Oslisly, R., Rabenkogo, N. & Jolly, D. (2005) Vegetation changes during the past 1300 years in western equatorial Africa: a high-resolution pollen record from Lake Kamalété, Lopé Reserve, Central Gabon. *The Holocene* 15: 1021–1031. https://doi.org/10.1191/0959683605hl875ra

Oliver, D. (1868) Anacardiaceae. *In:* Oliver, D. (Ed.) *Flora of tropical Africa vol. 1.* Thiselton-Dyer, London, pp. 435–450.

Onana, J.M. (2009) Le genre *Santiria* (Burseraceae) en Afrique: redéfinition de *Santiria trimera*. *Systematics and Geography of Plants* 79: 215–224.

Samy, P.R. & Gopalakrishnakone, P. (2010) Therapeutic Potential of Plants as Anti-Microbials for Drug Discovery. *Evidence-Based Complementary and Alternative Medicine* 7: 283–294. https://doi.org/10.1093/ecam/nen036

Pierre, L. (1896) Plantes du Gabon. *Bulletin mensuel de la Socieìteì linneèenne de Paris* 162: 1280–1282.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959. Available from: http://www.genetics.org/content/155/2/945 (accessed 1 September 2017)

Quantum GIS Development Team (2014) *Quantum GIS*. Vienna: Geospatial Foundation. Available from: http://www.qgis.org/fr/site (accessed 12 June 2016)

R Development Core Team (2015) *A language and environment for statistical computing*. University of Aalborg, Aalborg.

Raponda-Walker, A. & Sillans, R. (1961) Les plantes utiles du Gabon. *Quarterly Journal of Crude Drug Research* 1: 1–115. https://doi.org/10.3109/13880206109066644

Schnell, R. (1970) *Introduction à la phytogéographie des pays tropicaux: les problèmes généraux*. Gauthier-Villars, Paris, 234 pp.

Sei, M. & Porter, A.H. (2007) Delimiting species boundaries and the conservation genetics of the endangered maritime ringlet butterfly (*Coenonympha nipisiquit* McDunnough). *Molecular Ecology* 16: 3313–3325. https://doi.org/10.1111/j.1365-294X.2007.03384.x

Troupin, G. (1958) Burseraceae. *In:* Institut National pour l'Etude Agronomique du Congo Belge (Ed.) *Flore du Congo Belge et du Ruanda-Urundi vol. 7.* Jardin Botanique de l'Etat, Bruxelles, pp. 132–146.

Vahl, M.H. (1810) Beskrifelse over nye planteslægter. *Skrivter af Naturhistorie-Selskabet* 6: 84–128.

Van der Burgt, X.M., Mackinder, B.A., Wieringa, J.J. & De la Estrella, M. (2015) The *Gilbertiodendron ogoouense* species complex (Leguminosae: Caesalpinioideae), Central Africa. *Kew Bulletin* 70: 1–42.
https://doi.org/10.1007/s12225-015-9579-4

Walters, G., Dauby, G., Stevart, T., Dessein, S., Niangadouma, R. & Lachenaud, O. (2011) Novitates Gabonenses 80. Additions and corrections to the flora of Gabon. *Edinburgh Journal of Botany* 68: 423–442.
https://doi.org/10.1017/S0960428611000266

Webber, B.L. & Woodrow, I.E. (2006) Morphological analysis and a resolution of the *Ryparosa javanica* species complex (Achariaceae) from Malesian and Australian tropical rainforests. *Australian Systematic Botany* 19: 541–569.
https://doi.org/10.1071/SB06001

Wendland, H. (1860) Bemerkungen über einige Palmengattungen Amerika's. *Bonplandia* 8: 100–106.

White, F. (1979) The Guineo-Congolian Region and Its Relationships to Other *Phytochoria*. *Bulletin du Jardin botanique national de Belgique* 49: 11–55.
https://doi.org/10.2307/3667815

Wilks, C. & Issembé, Y. (2000) *Guide pratique d'identification: les arbres de la Guinée Equatoriale: région continentale*. Projet CUREF, Paris, pp. 146–147.