Species delimitation in the Caribbean *Gesneria viridiflora* complex (Gesneriaceae) reveals unsuspected endemism

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Abstract Accurate taxonomy and species boundaries are of great importance in biodiversity hotspots with high species endemicity like the Caribbean. Indeed, inaccurate species delimitations can affect biodiversity estimates and influence the decisions taken on conservation issues. The genera *Gesneria* and *Rhytidophyllum* constitute the main representatives of the Caribbean Gesneriaceae and contain a few complexes with unclear taxonomic boundaries that are characterized by a confusing history of taxonomic changes and by the presence of several recognized varieties and subspecies. *Gesneria viridiflora* is a good example of such a complex. Four geographically isolated subspecies are recognized that have similar but variable vegetative and reproductive characters, and numerous taxonomic changes have been suggested in this group over the years. In this study, we used multivariate approaches to delimit distinct clusters of individuals using morphological (qualitative and quantitative characters) and molecular data from four nuclear markers. These groups are then tested using a Bayesian coalescent species delimitation approach and compared using multivariate analyses of bioclimatic variables obtained from occurrence data. The results suggest the presence of four distinct species in this complex according to the unified species concept: *G. quisqueyana*, *G. sintenisii*, *G. sylvicola* and *G. viridiflora*. We also maintain *G. viridiflora* subsp. *acrochordonanthe* that does not fulfill our criteria for a species but that shows morphological variation associated to a specific geographical area. A distribution map, an identification key to the species, and a taxonomic treatment are provided. The new taxonomy proposed in this study shows an unsuspected species endemism in some regions of the Caribbean and underlines the importance of investigating species delimitation in diversified groups containing taxonomically complex taxa with poorly defined boundaries.

Keywords Caribbean; endemicity; *Gesneria viridiflora*; Gesneriaceae; species delimitation; unified species concept

Supplementary Material Electronic Supplement (Tables S1–S3; Figs. S1 & S2) and DNA sequence alignment are available in the Supplementary Data section of the online version of this article at http://ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

Biodiversity is not equally distributed around the globe and is instead clustered in hotspots located in or near the tropics. The Caribbean is one of the top five biodiversity hotspots because of its large proportion of endemic plant and animal species and the recent drastic loss of primary forests (Myers & al., 2000). The West Indies, which consists of the major islands of the Caribbean region from Cuba in the northwest to Grenada in the southeast, harbours an especially high plant endemism (Santiago-Valentin & Olmstead, 2004; Smith & al., 2005; Wege & al., 2009). Approximately 71% of the ca. 12,280 plant taxa and 181 of the 1471 plant genera present in the West Indies are endemic (Acevedo-Rodríguez & Strong, 2012). This great diversity has been favoured by the isolation provided by the multiple islands along with the important geographic, topographic, and bioclimatic diversity that have provided ample evolutionary opportunities (Acevedo-Rodríguez & Strong, 2008). At the same time, the endemism generated by these features also place this diversity in a vulnerable position.

Well delimited species are fundamental to allow adequate conservation and biodiversity management (Campbell & Hammond, 1989). Poorly circumscribed species boundaries could result in extensively subdivided species (Bohonak, 1999), underestimating species diversity and making it difficult to properly address conservation issues (Zink, 2004). Species delimitation is particularly difficult in complexes where characters within species co-vary with geography (Wade & Goodnight, 1998). Such taxonomic problems can only be solved using detailed analyses of different sources of information for large numbers of individuals across the species range.

The genera *Gesneria* L. and *Rhytidophyllum* Mart. (Gesneriaceae) are ideal groups to study plant diversity in the Caribbean biodiversity hotspot. This monophyletic group

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diversified into approximately 75 species (Skog, 2012) from a most recent common ancestor estimated to have lived ca. 8 million years ago (Roalson & al., 2008). Most species are found within the Greater Antilles (Cuba, Hispaniola, Puerto Rico, Jamaica) with only two species found in the Lesser Antilles and two species in South America (Skog & Boggan, 2007). This group has been the source of considerable taxonomic confusion over the years (Morton, 1957; Skog, 1976; Borhidi & Kereszty, 1979; Clark & al., 2013) and contains several closely related species with unclear boundaries as well as species with several recognized subspecies. One example is *Gesneria viridiflora* (Decne.) Kuntze, the only species of sect. *Duchartrea* (Decne.) Fritsch (Skog, 1976). The plants of this species are large resinous shrubs up to 6 m high and grow in wet forests of the Greater Antilles with the exception of Jamaica. They produce long peduncles that exceed the subtending leaves and bear numerous flowers with campanulate corollas. The taxa can be distinguished by the presence or absence of warts on the calyx and corolla, by the shape of calyx lobes (filiform or thick), by the corolla lobe margins (smooth, denticulate or fimbriate) and by the abaxial leaf color (green, pale green, brown or reddish) (Skog, 1976).

In his monograph, Skog (1976) recognized four allopatric subspecies based on morphology: *G. viridiflora* subsp. *acrochordonanthe* L.E.Skog from southwestern Hispaniola, subsp. *quisqueyana* (Alain) L.E.Skog from Dominican Republic, subsp. *sintenisii* (Urb.) L.E.Skog from eastern Puerto Rico and subsp. *viridiflora* from Cuba. Of these taxa, only *G. viridiflora* subsp. *acrochordonanthe* was newly described by Skog. Skog's treatment still corresponds to the accepted taxonomy of the complex, although it is not completely settled yet. Morton (1957) had previously defined three varieties of *G. viridiflora* in Cuba based on leaf shape, which were synonymized to *G. viridiflora* subsp. *viridiflora* by Skog (1976) who considered leaf shape characteristics to be too variable within and between populations. Nevertheless, Borhidi & Kereszty (1979) suggested to elevate one of these varieties to the status of subspecies: *G. viridiflora* subsp. *colorata* (C.V.Morton) Borhidi. Borhidi & Kereszty (1979) also proposed further changes that involved elevating *G. viridiflora* subsp. *acrochordonanthe* and *G. viridiflora* subsp. *sintenisii* to the species level and combining *G. viridiflora* subsp. *quisqueyana* as a subspecies of *G. sintenisii*. In brief, most of these entities have been recognized as varieties, subspecies and species by different authors, creating confusion regarding their evolutionary significance.

The isolation of the taxa composing the *G. viridiflora* complex presents an interesting biogeographic pattern with remarkable geographic barriers such as the sea or mountain ranges. Given the morphological variation in this complex (both within and among subspecies), the allopatric nature of the subspecies, and a confusing history of taxonomic changes, the *G. viridiflora* complex represents a good candidate within the genus *Gesneria* to re-investigate taxonomic boundaries.

A clear species concept is critical to circumscribe species boundaries in a group and to allow a good understanding of the criteria involved in taxonomic decisions. For instance, Skog (1976) did not mention a specific species concept for his taxonomic decisions on the *G. viridiflora* complex, which makes it hard to understand the reasons and motivations behind his treatment such as why he chose to recognize the taxonomic entities in *G. viridiflora* as subspecies instead of distinct species. In this study, species are delimited with the *unified species concept* (de Queiroz, 2007) that defines species as a lineage of metapopulations evolving separately from other such entities. The concept considers that all criteria potentially providing evidence of lineage separation are relevant to species delimitation because discontinuities between species are not expected to evolve at the same rate for all criteria following speciation. Even if a single criterion may be sufficient for species delimitation, multiple lines of evidence are preferred and can demonstrate an accentuated evolutionary independence of the lineages.

In this study, we first define distinct evolutionary groups in the *G. viridiflora* complex using morphological (qualitative and quantitative) and genetic data. We then test these putative species using a coalescent-based species delimitation method and describe the bioclimatic characteristics of the different species. We conclude that four distinct species should be recognized in the complex. We provide a new taxonomic treatment for the group, an identification key and a distribution map, and discuss our findings in terms of evolution and biogeography.

■ MATERIALS AND METHODS

Morphometric analyses. — Seventy-eight herbarium specimens of *Gesneria viridiflora* and synonyms of all its currently recognized subspecies (Skog & Boggan, 2007) were examined for morphological measurements (Electr. Suppl.: Table S1). The specimens considered were those presenting all characters studied with few exceptions (see below). Seventy-three specimens came from herbarium loans (FLAS, NY, S, US) and five specimens (MT) were collected in the field in Haiti and Puerto Rico. According to the current taxonomy, these samples consist of 11 specimens of *G. viridiflora* subsp. *acrochordonanthe*, 30 of *G. viridiflora* subsp. *quisqueyana*, 16 of *G. viridiflora* subsp. *sintenisii* and 21 of *G. viridiflora* subsp. *viridiflora* (5 from central Cuba and 16 from eastern Cuba). These cover the entire geographic range of *G. viridiflora* from central Cuba to eastern Puerto Rico. The type locality of all taxa studied was included, except for *G. viridiflora* subsp. *acrochordonanthe*. The holotype of *G. quisqueyana* Alain was included as well as an isotype of *G. viridiflora* var. *colorata* C.V.Morton. The corolla has a high potential for taxon differentiation in *Gesneria* (Skog, 1976), but few herbarium specimens had corollas. In some instances, specimens with incomplete features (e.g., lacking calyx lobes or capsules) were nevertheless included to ensure a good geographic representation of all taxa. This is true for *G. viridiflora* subsp. *acrochordonanthe* that is poorly collected and *G. viridiflora* subsp. *sintenisii* that is highly endemic.

Qualitative and quantitative characters were selected because of their variation in the complex or based on subspecies descriptions (Skog, 1976). Twenty-four qualitative characters

(Table 1) were coded into binary or multistate variables using a dissecting microscope. Twelve quantitative characters (Table 1) were measured with a 15 cm ruler (0.1 cm precision) or with an electronic caliper of 0.01 cm precision. The characters calyx lobe apex width (CalLobApexWid) and verruca width (VerWid) were measured on a binocular with an ocular micrometer of 0.02 cm precision. The number of flowers (FlwNb) was counted. Whenever possible, the final value of quantitative characters is the mean of the two most extreme values found within an individual. A morphological distance matrix between individual specimens was calculated using the Gower distance method (Gower, 1971), using the "daisy" function of the "cluster" package (Maechler & al., 2016) in R (R Core Team, 2015), that can combine both quantitative and qualitative characters and that is little affected by missing data (Brown & al., 2012). The morphological distance matrix was visualized by means of a principal coordinates analysis (PCoA) using the "pcoa" function from the "ape" R package (Paradis & al., 2004). Ward's minimum variance clustering method computed using the "hclust" R function was also used to look for interpretable objective clusters. The optimal number of groups on this dendrogram was the one that resulted in the highest Mantel correlation between the original distance matrix and the groups using scripts of Borcard & al. (2011). The contribution of the quantitative and qualitative binary and ordinal variables to the PCoA axes was determined with the calculation of the Pearson correlation between the values of the variables and the PCoA axes (Legendre & Legendre, 2012). The contribution of the qualitative nominal variables to PCoA axes was determined by calculating a one-way ANOVA with each qualitative variable as an explanatory variable and the PCoA axis as the response variable. The variables with the best *p*-values after Sidák correction are considered as significantly contributing to the variation along the axes.

Sequence data acquisition. — Material for DNA extractions came from leaf samples collected in the field and conserved in silica gel and from leaf samples removed from herbarium specimens collected since 1980 (Appendix 1; Electr. Suppl.: Table S2). DNA was extracted using the plant DNeasy kit (QIAGEN, Mississauga, Ontario, Canada) following the manufacturer's instructions. Four unlinked single-copy nuclear genes were amplified and sequenced: *CHI*, *CYCLOIDEA*, *F3H* and *UF3GT*. PCR amplification followed Joly & al. (2016), except for recalcitrant samples for which we used the Phire Hot Start II (Thermoscientific, Missisauga, Ontario, Canda) enzyme for amplification following the manufacturer's instructions. Sequencing reactions were performed by the Genome Quebec Innovation Centre and run on a 3730xl DNA Analyzer (Applied Biosystems, Beverly, Massachusetts, U.S.A.). DNA sequences from both primers were assembled into contigs and manually edited in Geneious v.1.8 (Drummond & al., 2014). *Gesneria fruticosa* and *G. ekmanii* were included as outgroups because they were found to be closely affiliated with the studied group in previous analyses (Martén-Rodríguez & al., 2010; Joly & al., 2016). Sequences were aligned using MAFFT v.7 (Katoh & Standley, 2013) and alignments were visually inspected to confirm homology assessments. DNA sequences generated for this study were deposited in GenBank and combined with sequences from previous studies (Appendix 1; Electr. Suppl.: Table S2).

Multivariate analysis of molecular data. — To first delimit groups of genetically distinct individuals, we used ordination and cluster analyses similar to those used for the morphological data. We estimated genetic distances between individuals using the genpofad distance (Joly & al., 2015) using the "pofadinr" R package (Joly, 2016) from a concatenated alignment of all markers. This distance has the advantage to use the information present in polymorphic nucleotides. To avoid undefined distances, only individuals sequenced for at least two markers were included in these multivariate analyses. A Ward phenogram was obtained and the optimal number of clusters was assessed as described above. A PCoA was also performed to graphically represent the genetic distances between individuals.

Species delimitation analysis. — The groups delimited by the multivariate analyses (morphological and molecular) were tested and their phylogeny jointly estimated from the full molecular dataset using the unguided Bayesian species delimitation method implemented in Bayesian Phylogenetics and Phylogeography (BPP) v.3.1 (Yang & Rannala, 2014). This coalescent-based method infers species delimitation from genetic data using a priori groupings by trying to merge the groups and estimating if these merged species are better supported by the data. The program also simultaneously estimates the species tree for the species during the analysis. Because the program cannot split groups given a priori, we used as a priori grouping all groups identified by either the morphological or genetic multivariate analyses, which is also consistent with the unified species concept. We also tested currently recognized infraspecific taxa in a separate analysis. The analyses with BPP were performed using the following parameters: speciesdelimitation = 1, algorithm = 1, finetune (a) = 2, finetune (m) = 1, speciesmodelprior = 1, usedata = 1 and cleandata = 0. The Markov Chain Monte Carlo (MCMC) analysis was run for 100,000 generations with a sampling frequency of 1 and a burn-in of 8000. We present the phylogenetic tree representing the a priori species given to the program, which was the highest sum of clade credibility tree amongst the trees from the posterior distribution that contained these species. This tree was selected and annotated in TreeAnnotator v.1.8.2 (Drummond & Rambaut, 2007).

Bioclimatic niche analyses. — We investigated whether the species circumscribed with the morphometric and genetic analyses also had different bioclimatic preferences. Presence data were collected from GPS points taken in the field in Cuba and Haiti and from georeferenced localities gathered from herbarium specimens (FLAS, NY, S, US). Georeferencing of herbarium specimen without GPS coordinates was done with the help of Geolocate (Rios & Bart, 2010). This resulted in a total of 95 presence points. Bioclimatic variables were extracted from the WORLDCLIM database (Hijmans & al., 2005) using a resolution of 30 arc-seconds. Several bioclimatic variables from the WORLDCLIM database are autocorrelated with each other, which can have undesirable consequences for statistical analyses with prediction objectives. However, because the objective

Table 1. Code, description and type of quantitative and qualitative characters measured in herbarium specimens used for morphological analyses.

Code	Character	Type
Quantitative characters		
PetLen	Petiole length [mm][a]	Continuous
LeavLen	Leaf length (from petiole base to apex) [cm][a]	Continuous
LeavMTooS	Distance between teeth on leaf margins [mm][b]	Continuous
LeavWidPt	Leaf length from petiole base to widest point [cm][a]	Continuous
LeavWid	Leaf width at widest point [cm][a]	Continuous
PedLen	Peduncle length from peduncle base to first cyme division [cm][a]	Continuous
PedDiam	Peduncle diameter [mm][b]	Continuous
PediLen	Pedicel length from base of pedicel to base of floral tube [mm][b]	Continuous
FlwNb	Flower number per inflorescence	Discrete
CalLobLen	Calyx lobe length from base to apex [mm][b]	Continuous
CalLobApexWid	Calyx lobe apex width [mm][c]	Continuous
CalLobBaseWid	Calyx lobe base width [mm][b]	Continuous
VerWid	Verruca width on calyx lobes and capsule [mm][c]	Continuous
CapLen	Capsule length from pedicel apex to base of calyx lobe [mm][b]	Continuous
CapWid	Capsule width at widest point/apex [mm][b]	Continuous
Qualitative characters		
BarkCol	Bark colour (brown-red; dark brown-red)	Nominal
ApexRes	Apex resinous (0 = low; 1 = high)	Binary
LeavMarg	Leaf margin (denticulate; serrate; serrulate)[d]	Nominal
CorLeav	Leaf texture (0 = not coriaceous; 0.5 = subcoriaceous; 1 = coriaceous)	Ordinal
LeavAbaCol	Leaf abaxial colour (copper; green; light brown; light green)[d]	Discrete
LeavMainVein	Leaf main vein prominence (0 = faint; 1 = present; 2 = prominent)	Ordinal
LeavMinVein	Leaf minor vein prominence (0 = faint; 1 = present; 2 = prominent)	Ordinal
CenVeinRes	Central vein resin abundance (0 = low; 1 = high)	Binary
CenVeinVer	Central vein verruca abundance (0 = low; 1 = high)	Binary
LeavBase	Leaf base shape (cuneate; round)[d]	Nominal
LeavApex	Leaf apex shape (acuminate; acute; retuse)[d]	Nominal
LeavShape	Leaf shape (oblanceolate; obovate)[d]	Nominal
PetRes	Petiole resin abundance (0 = low; 1 = high)	Binary
PetCol	Petiole colour (brown-red; dark brown-red)	Nominal
PetVer	Petiole verruca abundance (0 = low; 1 = high)	Binary
PedCol	Peduncle colour (brown-red; dark brown-red)	Nominal
PedVer	Peduncle verruca abundance (0 = low; 1 = high)	Binary
PedRes	Peduncle resin abundance (0 = low; 1 = high)	Binary
CalLobApexThick	Calyx lobe apex thickness (0 = thin; 1 = thick)	Binary
CalLobApex	Calyx lobe apex shape (acuminate; acute; rounded)	Nominal
CalVer	Calyx verruca abundance (0 = low; 1 = high)	Binary
CalLobVer	Calyx lobe verruca abundance (0 = low; 1 = high)	Binary
CapRes	Capsule resin abundance (0 = low; 1 = high)	Binary
CarVer	Capsule verruca abundance (0 = low; 1 = high)	Binary

Notes: a – Measured with a ruler; b – Measured with an electronic calliper; c – Measured on a binocular with an ocular micrometer; d – Coded as discrete nominal factors

of the present study was descriptive, all variables were included in the analysis. A principal component analysis (PCA) of the correlation matrix was used to represent the samples in a reduced space. Bioclimatic analyses were performed using the R software (R Core Team, 2015) with packages dismo (Hijmans & al., 2015) and ade4 (Dray & Dufour, 2007).

Biogeographic analyses. — A formal biogeographic analysis is not appropriate in this groups for two reasons. First, the small number of species does not provide sufficient information to robustly estimate parameters of likelihood-based method (e.g., Ree & Smith, 2008; Matzke, 2014). Second, the typical biogeographic models in which speciation is independent of geographic range evolution are not appropriate for insular or highly endemic taxa such as the species studied here (see below). Therefore, to estimate which island is more likely to represent the ancestral area for this complex, we used Fitch parsimony optimization (Ronquist, 1994) and inferred the ancestral area for 10,000 random trees sampled from the posterior distribution of the species delimitation search.

■ RESULTS

For sake of simplicity, the figures present the taxonomy proposed in this study and not the currently accepted taxonomy. This does not reflect any a priori taxonomic pre-conceptions and is only used to avoid possible confusion between the old and the new taxonomy. The relation between what we conclude to be the true species and the previously recognized taxonomy can be found in the Taxonomic Treatment section below.

Determination of distinct evolutionary clusters. — A PCoA of the morphological dataset explained 27.1% of the total morphological variation on the first three axes (Fig. 1A, B). The other axes were not further considered because they do not allow to identify distinct groups of individuals and they represent less than 5% of the total variation. The number of clusters that best represents the original Gower's distance is four (Fig. 1C); these clusters are highlighted on the PCoA (Fig. 1A, B) and the dendrogram (Fig. 1D). Axis 1 of the PCoA mainly distinguishes the group represented by circles from the diamonds and all triangles (pointing both up and down) groups. This axis is significantly correlated with abaxial leaf color, the abundance of verruca on the calyx lobes, the width of the verruca on calyx and capsule, the number of flowers, the length of pedicel, peduncles and capsules and the width of the capsule, among others (Electr. Suppl.: Table S3). PCoA axis 2 mainly distinguishes the group of individuals represented by squares from the others and is significantly correlated with the shape of the leaf margin, the coriaceous leaves, prominence of the main leaf vein, and shape of the leaf apex, among others (Electr. Suppl.: Table S3). Finally, PCoA axis 3 mainly distinguishes the group represented by diamonds from the others and is correlated with abaxial leaf color, the prominence of the minor leaf veins, the distance between leaf teeth, and resinous petioles and peduncles (Electr. Suppl.: Table S3).

The PCoA and Ward's dendrogram of the genetic distances showed distinct clusters of individuals (Fig. 2A, B). The optimal number of clusters in the dataset was five (Fig. 2C). Three of these were congruent with the morphological data (squares, circles and diamonds; Figs. 1, 2). The others comprised the individuals represented by triangles (pointing both up and down). Because one of these two clusters consisted of a single individual, it was considered to belong to its closest group for the identification of a priori groupings for the species delimitation analysis.

Genetic species delimitation. — To avoid unnecessary restrictions in the species delimitation analyses and to be consistent with the unified species concept, all groups identified by either the morphological or genetic multivariate analyses were included in the species delimitation analysis. The species delimitation analysis suggested the presence of three distinct species, as this is the scenario that received the highest posterior probability (0.57 PP; Fig. 3A). However, scenarios with four species also received considerable support with 0.43 PP. The groups represented by diamonds and all triangles (pointing both up and down) received highest support (>0.99 PP; Fig. 3B). A group that combined individuals represented by circles and squares received slightly more support (PP = 0.57) than separate groups for circles and squares (0.43 PP for both). No other species delimitation received a posterior probability above 0.001. We also performed a separate analysis, considering a priori the downward triangle group, which includes individuals currently recognized as *G. viridiflora* subsp. *acrochordonanthe* and that was found to be slightly distinct morphologically from the upward triangles group (Fig. 1B). The analysis gave higher support for considering this downward triangle individual as a separate species (0.55 PP) compared to a combined group that would consist of all triangles (0.39 PP; Electr. Suppl.: Fig. S1B). Finally, we found that two morphologically distinct species, *G. nipensis* and *G. bracteosa*, were found to be genetically affiliated with the *G. viridiflora* complex (Joly & al., 2016). A species delimitation analysis that included these species showed that although these species are likely to be nested within the complex, they do not affect the results from the species delimitation (Electr. Suppl.: Fig. S2).

Phylogeny and biogeography. — The phylogenetic tree for a scenario with four distinct species (excluding the outgroup) showed moderate support for a monophyletic *G. viridiflora* complex (0.87 PP; Fig. 3C), although the inclusion of *G. nipensis* and *G. bracteosa* (Electr. Suppl.: Fig. S2) suggests they should potentially also be included in this group despite their morphological distinctiveness. Relationships within the *G. viridiflora* complex are not strongly supported, apart for the groups represented by squares and circles.

Fitch optimization on 10,000 random trees from the posterior distribution of trees with four species showed that 70% of the trees supported an ancestor on Hispaniola for the group, while 30% of the trees reported an ambiguous optimization between Hispaniola and Cuba. This suggests that a Cuban ancestor of the group cannot be completely rejected. Although Fitch optimization is a rather simple approach for ancestral area estimation, parsimony reconstructions have been demonstrated to be accurate when trait shifts are spaced out on the phylogeny (Steel, 2001), which appears to correspond with our data.

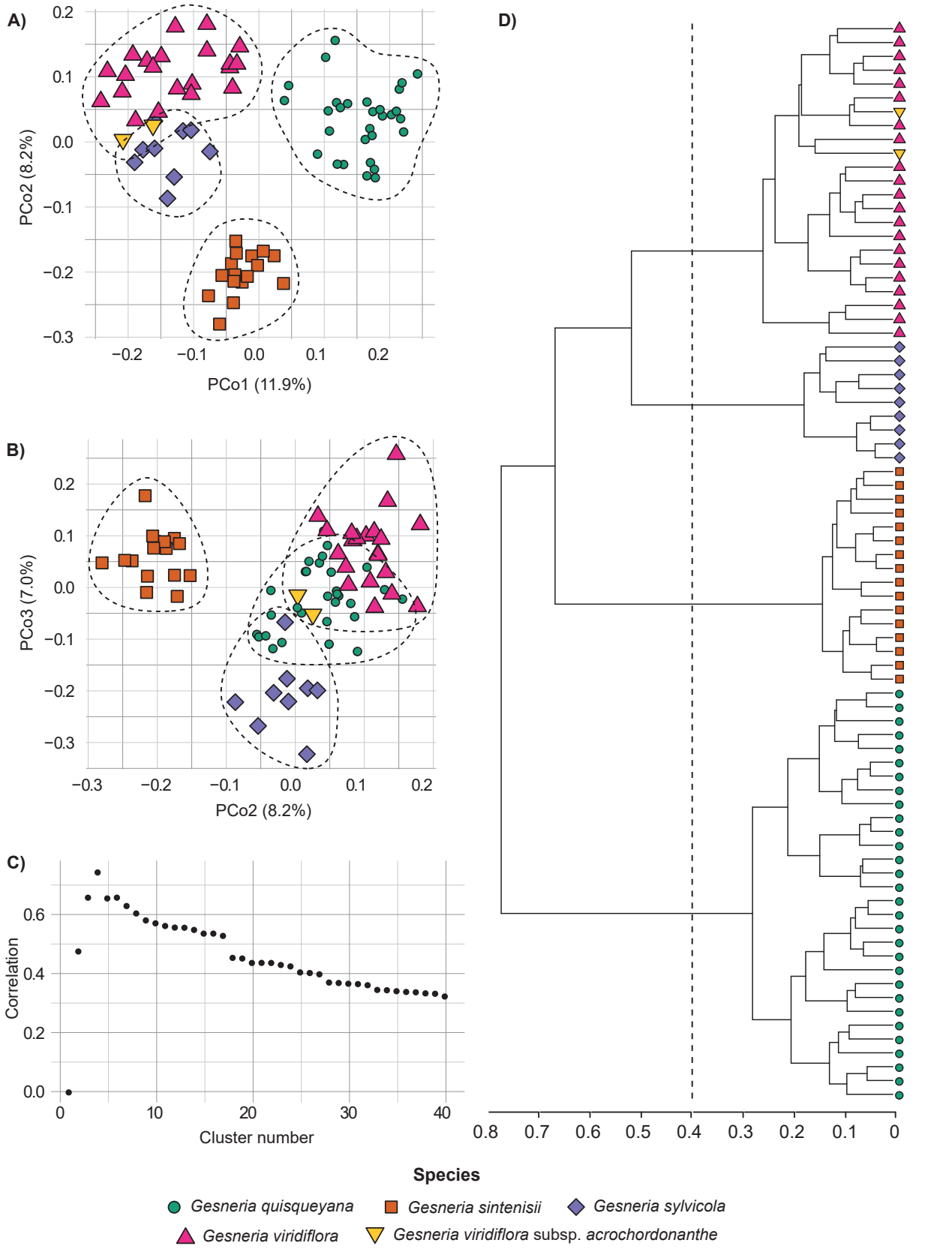

Fig. 1. A & B, Principal coordinates analysis (PCoA) of Gower's distance matrix for qualitative and quantitative morphological traits. The groups circumscribed by the dotted lines represent the optimal number of clusters as determined by the Mantel correlation between Gower's distance and the clusters obtained from a Ward clustering algorithm. **C,** Correlations for different numbers of clusters. **D,** Ward's dendrogram.

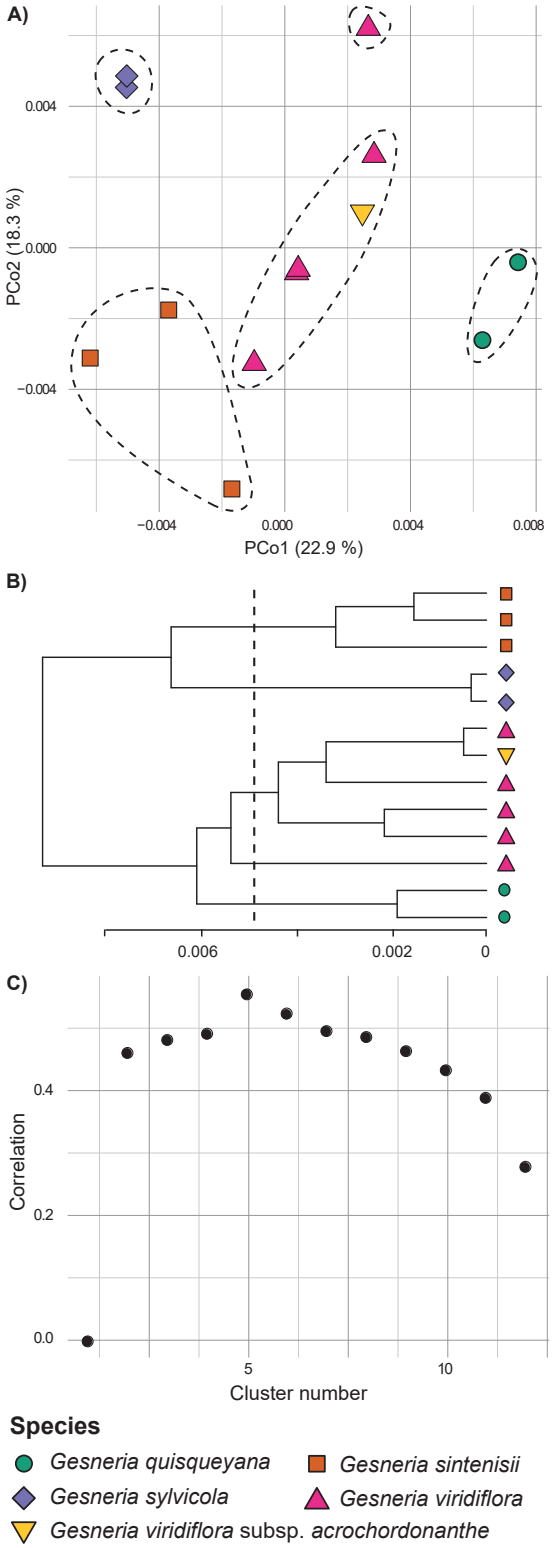

Fig. 2. A, Principal coordinates analysis (PCoA) of the genpofad genetic distances from four nuclear markers; **B,** Ward's dendrogram of the genpofad distances between individuals; **C,** Plot showing the correlation between the genpofad distance matrix and the cluster structure for different numbers of clusters obtained from the Ward dendrogram. — The five optimal clusters are shown with dotted lines in the PCoA (A) and the dendrogram (B).

Bioclimatic results. — The recognized species (see below) were also compared for their bioclimatic characteristics. The first three principal components (PC) of the PCA (Fig. 4) accounted for 91.20% of the total variance and showed that the groups delimited above have relatively different bioclimatic characteristics, although they do not form clearly distinct clusters. Along the first axis, the group represented by circles has lower scores whereas the diamonds and squares have higher scores. On PC1, higher scores are correlated with higher max. temperature of the warmest month, higher mean temperature of the wettest and warmest quarters, lower annual

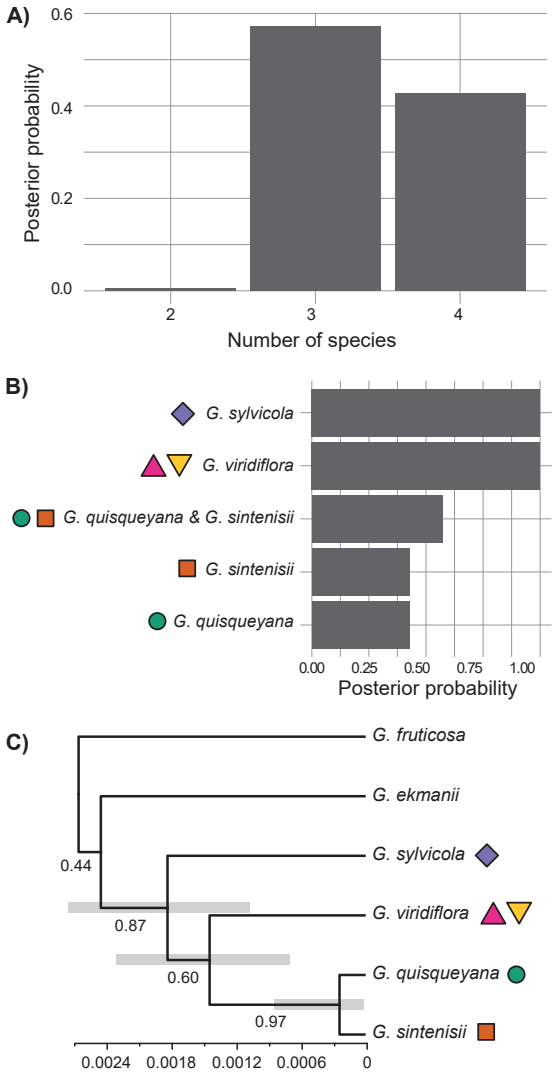

Fig. 3. Species delimitation results obtained with BPP using the genetic data. **A,** Posterior probability for the total number of species according to the data (excluding outgroups); **B,** Posterior probability for all group delimitations (either single a priori defined groups or merged groups) that received more than 0.001 PP in the analysis; **C,** Maximum clade credibility species phylogeny for the scenario with four species plus outgroups. Node bars indicate 95% credibility intervals for node heights and number below branches indicate clade posterior probability.

mean temperature, lower mean diurnal range, lower isothermality and precipitation seasonality, and lower precipitation of the wettest, warmest and coldest quarters. PC2 mainly distinguishes the diamonds group (with higher scores) and the squares group (with lower scores). Higher scores on PC2 are associated with a higher annual range in temperature, a higher annual precipitation and a higher precipitation in the driest quarter, a lower min. temperature of the coldest month, and a lower mean temperature of the driest and coldest quarters. Finally, PC3 mainly distinguishes the triangles groups (upward and downward) that has lower scores than the others. These lower scores on PC3 are correlated with a higher precipitation in the driest month, a lower annual precipitation, and lower precipitation in the wettest month.

■ DISCUSSION

Four groups of individuals were found to be distinct according to either the morphological or the genetic data and were considered to be evolving independently from each other according to the unified species concept. The genetic species delimitation further supported these boundaries, with one exception that was equivocal and that is discussed below. The species are introduced sequentially using headings that correspond to the new taxonomy, and a discussion follows.

***Gesneria sylvicola*. —** The group that is represented by diamonds corresponds to individuals that were ambiguously defined as either *G. viridiflora* subsp. *acrochordonanthe* or *G. viridiflora* subsp. *quisqueyana*. This group was found to

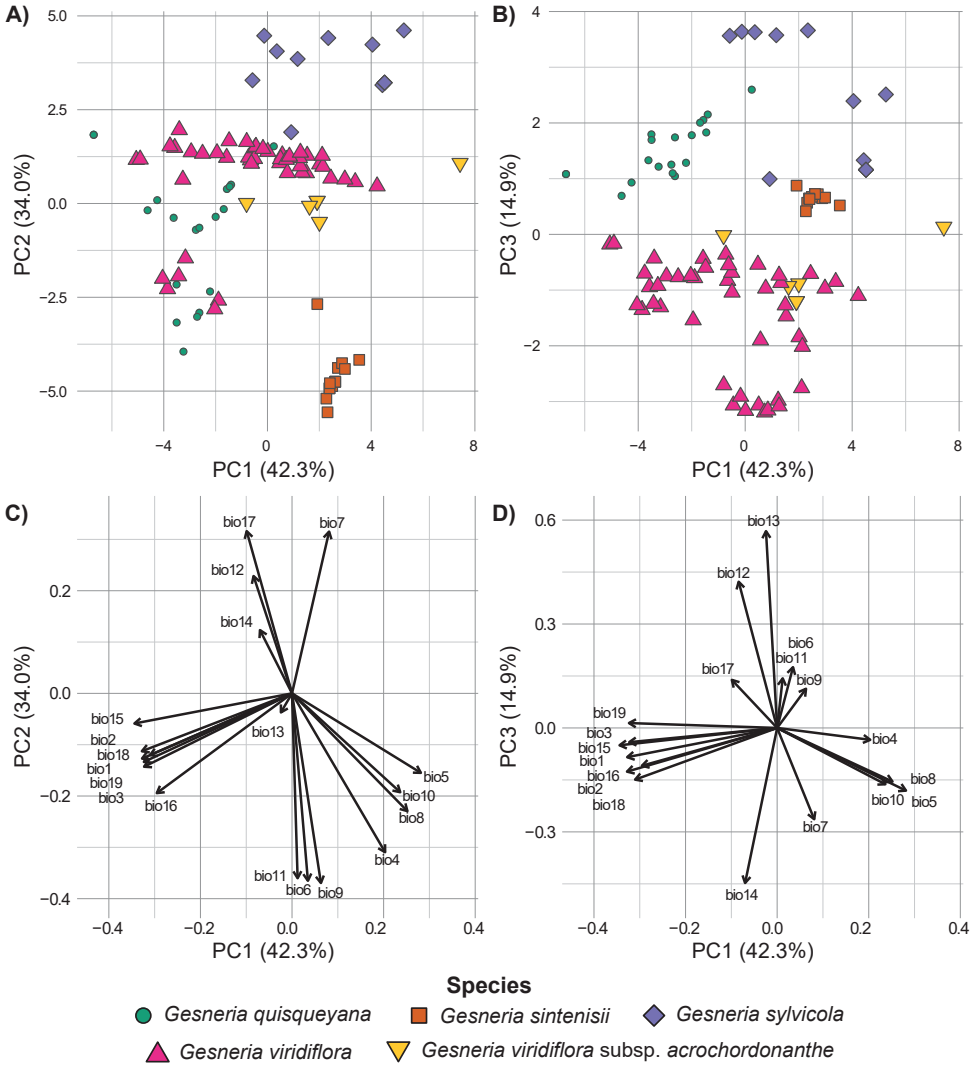

Fig. 4. Principal components analysis (PCA) of the correlation matrix of the bioclimatic variables for occurrence data. Upper panels **(A, B)** show the positions of collections in the reduced space and lower panels **(C, D)** show the variable scores along the principal axes. Bioclimatic variables description: bio1 = annual mean T°, bio2 = mean diurnal range, bio3 = isothermality ((bio2 / bio7) * 100), bio4 = T° seasonality (stand. dev. *100), bio5 = max T° of warmest month, bio6 = min T° of coldest month, bio7 = T° annual range (bio5–bio6), bio8 = mean T° of wettest qtr, bio9 = mean T° of driest qtr, bio10 = mean T° of warmest qtr, bio11 = mean T° of coldest qtr, bio12 = annual precipitation, bio13 = precipitation in wettest month, bio14 = precipitation in driest month, bio15 = precipitation seasonality (coefficient of variation), bio16 = precipitation in wettest qtr, bio17 = precipitation in driest qtr, bio18 = precipitation in warmest qtr, bio19 = precipitation in coldest qtr.

be clearly distinct according to morphological, genetic and bioclimatic data and had strong support in the genetic species delimitation analysis. It clearly satisfies the criteria of a distinct species. Importantly, this group does not include the populations from the Massif de la Hotte in southwest Haiti, which is the type locality of *G. acrochordonanthe* (Skog) Borhidi (downward triangles in our analysis). Therefore, we cannot name this species *G. acrochordonanthe*. The correct name for this group is *G. sylvicola* Alain (Liogier, 1973), which is currently considered a synonym of *G. viridiflora* subsp. *quisqueyana* (Skog, 1976). However, *G. sylvicola* has a morphology and geographic range that corresponds to the group delimited here and not to subsp. *quisqueyana* (Liogier, 1973). It is also important to point out that *G. denticulata* Alain (Liogier, 1976) should be considered a synonym of *G. sylvicola* as its morphology is very similar to that of *G. sylvicola*. Morphologically, *G. sylvicola* is mainly characterised by a brown abaxial leaf surface and calyx lobes that are curved outwards apically.

***Gesneria quisqueyana* and *Gesneria sintenisii*. —** The group represented by circles corresponds to specimens previously identified as *G. viridiflora* subsp. *quisqueyana* from the northern Dominican Republic. This group is morphologically distinct and also has a relatively distinct bioclimatic signature. The same conclusions are true for the group represented by squares. These latter individuals are all located in Puerto Rico and were all formerly identified as *G. viridiflora* subsp. *sintenisii*. In contrast, the genetic species delimitation analysis was equivocal regarding the respective distinctiveness of these two groups, giving a slightly higher probability for one species combining these two groups. However, because they have distinct morphologies, different bioclimatic niches, different pollination strategies (subsp. *sintenisii* is pollinated by bats and hummingbirds whereas subsp. *quisqueyana* is strictly pollinated by bats; Martén-Rodríguez & al., 2009), and gene flow is clearly restricted because the two groups are located on different islands, we believe that it is reasonable to recognize these entities as two distinct species under the unified species concept (de Queiroz, 2007). Because current gene flow is strongly restricted between these groups, the genetic affinities observed with the species delimitation study suggest a recent origin. The proper name for the species endemic to northern Dominican Republic that corresponds to previous *G. viridiflora* subsp. *quisqueyana* is *Gesneria quisqueyana* Alain (Liogier, 1971), and *G. viridiflora* subsp. *quisqueyana* thus becomes a synonym. For the individuals from Puerto Rico currently known as *G. viridiflora* subsp. *sintenisii*, the proper species name is *Gesneria sintenisii* Urb. (Urban, 1901). Morphologically, *G. quisqueyana* is mostly characterised by a reddish abaxial leaf surface and denticulate corolla lobe margins, whereas *G. sintenisii* is characterised by a pale green abaxial leaf surface, smooth corolla lobe margins and denticulate leaf margins.

***Gesneria viridiflora*. —** The group represented by upward and downward triangles consists of individuals previously identified as *G. viridiflora* subsp. *viridiflora* and *G. viridiflora* subsp. *acrochordonanthe*, respectively. This group is morphologically distinct and also received high support by the genetic species delimitation analysis. It is also bioclimatically distinct from all other groups. According to these results, it should be considered a distinct species. Interestingly, the downward triangle individuals showed some morphological distinction in the multivariate analyses even if it was not significant. Furthermore, a species delimitation analysis where the individuals of this subgroup was recognized a priori gave greater support to the hypothesis that it forms a distinct species. The downward triangles group consists of individuals currently recognized as *G. viridiflora* subsp. *acrochordonanthe* (Skog, 1976). Because this group did not form clearly distinct clusters in the multivariate analyses and because only a single individual was included in the species delimitation analysis, it seems premature to recognize it as a distinct species. However, the slight morphological differentiation from the upward triangle individuals and its geographic isolation (these individuals are found in southwest Haiti whereas the upward triangles individuals are all found in Cuba) justifies a taxonomic recognition at the infraspecific level. In conclusion, all specimens represented by triangles (pointing both up and down) form a distinct evolving lineage that belong, to a single species, *Gesneria viridiflora* (Decne.) Kuntze (Kuntze, 1891). We also chose to maintain the current taxonomy for the specimens from southwest Haiti (downward triangles), which is *G. viridiflora* subsp. *acrochordonanthe*. This subspecies is mostly characterised by fimbriate upper corolla lobe margins in contrast to fimbriations on all corolla lobes that are common in other species of *Gesneria*. The remaining individuals, found on Cuba, thus below to *G. viridiflora* subsp. *viridiflora* and are mainly characterised by fimbriate corolla lobe margins and thickened calyx lobes apexes.

Historically, many varieties and subspecies have been described for *G. viridiflora* in Cuba (Morton, 1957; Borhidi & Kereszty, 1979). As noted above, Skog (1976) did not recognize the varieties identified by Morton (1957), described according to leaf shape variation that is strongly affected by environmental conditions. Clark & al. (2013) reached similar conclusions, reporting extensive leaf shape variation within and among populations. Similarly, our analyses did not highlight further division within this group and these varieties are considered synonyms of *G. viridiflora* subsp. *viridiflora*.

Overall, the species delimitation we propose generally agrees with previous taxonomic treatments of this complex. Indeed, most species recognized here had been identified in the past as either species or subspecies (e.g., Skog, 1976; Borhidi & Kereszty, 1979). However, there are two important novelties associated with this treatment. First, it is the first time that individuals from southwest and central Hispaniola have been allocated to distinct species. Indeed, our results clearly showed that *G. viridiflora* subsp. *acrochordonanthe* has closer affinities to Cuban individuals (*G. viridiflora* subsp. *viridiflora*) than to individuals from the same island (*G. sylvicola*). Second, the current study identified more entities as distinct species. Given that the proposed species clearly represent independently evolving units, this taxonomy provides a more accurate picture of the diversity of this complex and will help to better inform future decisions regarding the conservation of this region.

Biogeography. — Reconstruction of ancestral areas identified Hispaniola as the most likely ancestral area for the *G. viridiflora* complex, although a Cuban ancestor cannot be completely discarded. According to the maximum clade credibility tree (Fig. 3C), an origin on Hispaniola would imply allopatric speciation within Hispaniola as well as migration towards Cuba and more recently to Puerto Rico given the recent divergence between *G. quisqueyana* and *G. sintenisii*. Affinities between Hispaniola and both Cuba and Puerto Rico have been frequently observed in many plant groups (Santiago-Valentin & Olmstead, 2004). Interestingly, Borhidi (1991) suggested that the species that constitute the Cuban mountain rainforest probably originated from Hispaniola, a proposition that could find support in our results given that the individuals from southwest Hispaniola and Cuba belong to the same species.

Species of the *G. viridiflora* complex are all allopatric, being isolated from each other by the sea or by valleys. Endemism is often very localised, such as with *G. sintenisii* from the Sierra de Luquillo in Puerto Rico, or at the infraspecific level with *G. viridiflora* subsp. *acrochordonanthe* from the Massif de la Hotte in Haiti (Fig. 5). The fact that this latter group belongs to another species than other individuals from Hispaniola is particularly interesting as it supports the floristic distinctiveness of the Massif de la Hotte from the remaining of Hispaniola, a pattern emphasized by numerous studies (Ekman, 1926, 1928; Dod, 1984; Judd, 1987; Majure & al., 2014). Although the biogeographic history of the West Indies is complex and not completely resolved, the southwest of current Hispaniola remained separated for a long time and only connected with the main island in the Miocene or Pleistocene (reviewed in Santiago-Valentin & Olmstead, 2004). This could explain the floristic similarities between the region of the Massif de la Hotte and eastern Cuba (Ekman, 1926, 1928; Dod, 1984; Judd, 1987; Majure & al., 2014), which is supported here as individuals from the Massif de la Hotte are conspecific with the Cuban individuals.

The high local endemism observed here clearly highlights the importance of protecting local habitats within the Caribbean to protect the biodiversity in this hotspot. For instance, the populations of *G. viridiflora* subsp. *acrochordonanthe* are currently under high threat in the Massif de la Hotte mountain range where the habitat has been severely degraded due to massive deforestation in the last few decades. The last remnants of primary forests are found in Pic Macaya National Park and surrounding mountains west of the park that are all threatened by deforestation for pastures, cultivation of crops and charcoal production, even on the steepest slopes. Despite the presence of a national park, the socio-economic conditions prevailing in this region make the future of this endemic species very uncertain. During our last botanical expeditions to this region in 2014–2015, a single population was found over four days of exploration. Clearly, this subspecies is presently at risk of extinction if deforestation of its habitat is not halted.

Our results suggest that there may be more species endemism than previously thought in Caribbean Gesneriaceae. The study also shows that, given appropriate approaches, it is possible to obtain convincing species delimitations even in groups known for their taxonomic complexity. This is critical to accurately monitor and protect biodiversity.

Identification key

1. Corolla lobes margins smooth or denticulate. Peduncles (9–)12–22 cm long, pedicels 3–9(–11) mm long, flowers (2–)6–13(–18) per inflorescence. Calyx lobes filiform, 0.25–0.75 mm wide at apex. Calyx slightly verrucose; verrucae 0.15–0.3 mm wide …… 2
1. All corolla lobes or only upper corolla lobe margins fimbriate. Peduncles (4–)8–12(–25) cm long, pedicels (2–)8–12(–22) mm long, flowers 2–5 per inflorescence. Calyx lobes thick, 0.75–1.5 mm wide at apex. Calyx markedly verrucose; verrucae 0.2–0.8 mm wide …… 3
2. Abaxial leaf surface pale green, leaf margins denticulate, leaves subcoriaceous, corolla lobe margins smooth, corolla greenish yellow. Eastern Puerto Rico …… ***Gesneria sintenisii***

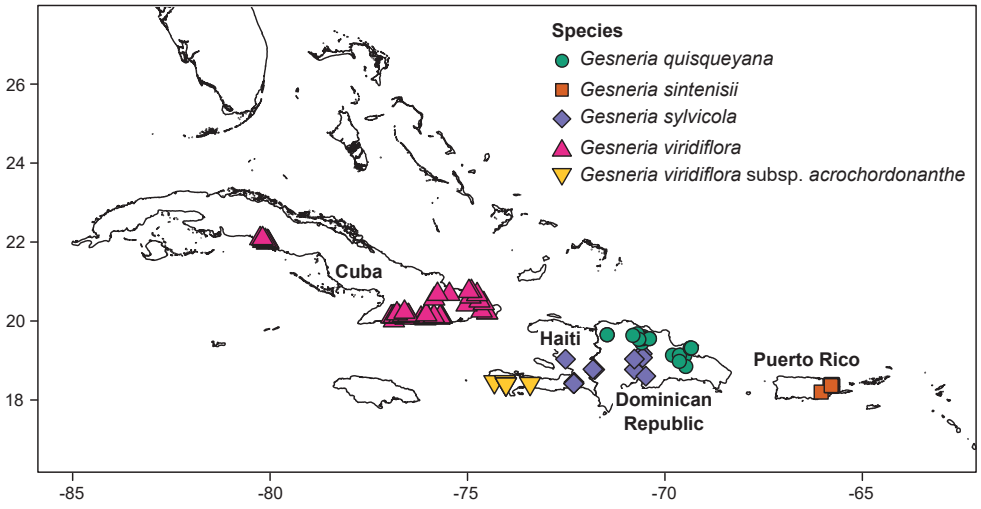

Fig. 5. Distribution map of the species of *Gesneria* described in this study.

2. Abaxial leaf surface reddish, leaf margins serrate, leaves coriaceous, corolla lobe margins denticulate, corolla white to reddish. Northern Dominican Republic …… ……. ***Gesneria quisqueyana***
3. Abaxial leaf surface brown, abundantly resinous, leaf margins serrulate; corolla slightly resinous, brownish to yellow-green outside, upper corolla lobes slightly frimbriate; calyx lobes curved outwards apically, apex not swollen; central Hispaniola (southeast and central Haiti to central Dominican Republic) …… ***Gesneria sylvicola***
3. Abaxial leaf surface pale green, slightly resinous, leaf margins serrate to serrulate; corolla distinctly resinous, dark reddish to yellow with reddish spots outside, upper or all corolla lobes fimbriate; calyx lobes straight or curved inwards apically, apex thickened or not; western Hispaniola or Cuba …… ***Gesneria viridiflora*** → 4
4. All corolla lobe margins frimbriate, leaf margins serrate, corolla yellow with reddish spots to dark red, calyx lobes apex thickened. Central and eastern Cuba …… …… ***Gesneria viridiflora*** subsp. ***viridiflora***
4. Upper corolla lobe margin fimbriate, leaf margins serrulate, corolla dark red, calyx lobes apex not thickened. Massif de la Hotte, Haiti …… …… ***Gesneria viridiflora*** subsp. ***acrochordonanthe***

Taxonomic treatment

Gesneria quisqueyana Alain in Mem. New York Bot. Gard. 21(2): 147. 1971 ≡ *G. sintenisii* subsp. *quisqueyana* (Alain) Borhidi in Acta Bot. Acad. Sci. Hung. 25: 35. 1979 ≡ *G. viridiflora* subsp. *quisqueyana* (Alain) L.E.Skog in Smithsonian Contr. Bot. 29: 140. 1976 – Holotype: Dominican Republic, La Cumbre, Jamao, Moca, 700–800 m, 27 May 1969, *A. Liogier 15386* (NY barcodes 00111455! & 00111456! [mounted on two sheets]; isotypes: GH barcode 00092131!, NY barcode 00111457!, US barcode 00136325!).

Distribution. – Hispaniola, mountains in the north of Dominican Republic.

Additional specimens examined. – See Electr. Suppl.: Table S1.

Gesneria sintenisii Urb., Symb. Antill. 2: 375. 1901 ≡ *Duchartrea sintenisii* (Urban) Britton in Sci. Surv. Porto Rico & Virgin Islands 6: 206. 1925 ≡ *G. viridiflora* subsp. *sintenisii* (Urb.) L.E.Skog in Smithsonian Contr. Bot. 29: 142. 1976 – Holotype: Puerto Rico, Sierra de Naguabo "in sylva prim. montis los Rabanos", 5 Feb 1886, *P.E.E. Sintenis 5332* (M barcode M-0185854!; isotypes: E barcode E00259327!, F barcode V0060577F!, G barcodes G00365447!, G00365491! & G00365499!, GH barcode 00338172!, ISC barcode ISC-V-0000409!, HBG barcode HBG-517402!, MO barcode MO-716378!, NY barcode 01113836, P barcodes P00587313!, P00587314! & P00587315!, S No. S-05-174!, US barcode 00403567!, WU No. 043578!, Z barcode Z-000017849!)

Distribution. – East Puerto Rico.

Additional specimens examined. – See Electr. Suppl.: Table S1.

Gesneria sylvicola Alain in Phytologia 25: 276. 1973 – Holotype: Dominican Republic, La Descubierta, Constanza, 1–2 May 1971, *A.H. Liogier 18024* (NY barcode 00111464!; isotype: US barcode 00136335!).

= *Gesneria denticulata Alain* in Moscosoa 1: 41–42, fig. 11. 1976 – Holotype: Dominican Republic, Sierra de Neiba, 1500 m, 24–26 May 1975, *A.H. Liogier 22661* (SD?; isotypes: NY barcodes 00111400! & 00111401!).

Distribution. – Hispaniola, southeast Haiti and southwest to central Dominican Republic.

Additional specimens examined. – See Electr. Suppl.: Table S1.

Gesneria viridiflora (Decne.) Kuntze subsp. ***viridiflora***, Revis. Gen. Pl. 2: 473. 1891 ≡ *Duchartrea viridiflora* Decne. in Ann. Sci. Nat., Bot., sér. 3, 6: 109, t. 8. 1846 ≡ *Pentaraphia viridiflora* (Decne.) Hanst. in Linnaea 34: 306. 1865 – Holotype: Cuba, prov. Santiago de Cuba, "St. Yago de Cuba, Sierra Maestra", 3000–4000 ft, 1843–1844, *J.J. Linden 1702* (P barcode P00587318!; isotypes: BM barcodes BM000992252! & BM000992253!, G barcodes G00365594! & G00365599!, GENT barcode [BR] 000090031731!, K barcode K000450120!, P barcodes P00587316! & P00587317!).

= *Gesneria viridiflora* var. *acutifolia* C.V.Morton in Brittonia 9: 21. 1957 – Holotype: Cuba, prov. Santiago de Cuba, "Nimanima", 1856, *C. Wright 354* (GH barcode 00092135!; isotypes: S No. S14-11682!).

= *Gesneria viridiflora* var. *colorata* C.V.Morton in Brittonia 9: 21. 1957. ≡ *Gesneria viridiflora* subsp. *colorata* (C.V. Morton) Borhidi in Bot. Közlem. 62: 27. 1975 – Holotype: Cuba, prov. Sancti Spíritus, "Santa Clara, Naranjo, Buenas Aires, Trinidad Hills", 2500–3500 ft, 24 Jul 1930, *J.G. Jack 8111* (US barcode 00136338!; isotype: NY barcode 00073860!).

= *Gesneria viridiflora* var. *obovata* C.V.Morton in Brittonia 9: 21. 1957– Holotype: Cuba, prov. Santiago de Cuba, "Cañizo, S of Loma del Gato, Sierra Maestra", Jul 1921, *Br. León 9821* (US barcode 00136339!; isotype: NY barcode 01401770).

Distribution. – Cuba.

Additional specimens examined. – See Electr. Suppl.: Table S1.

Gesneria viridiflora subsp. ***acrochordonanthe*** L.E.Skog in Smithsonian Contr. Bot. 29: 139. 1976 ≡ *G. acrochordonanthe* (L.E.Skog) Borhidi in Acta Bot. Acad. Sci. Hung. 25: 35. 1979 – Holotype: Haiti, prov. Sud, St. Louis du Sud, Bonnet-Carré, 1200 m, 2 Nov 1927, *E. Ekman H9236* (S No. S07-5652!; isotypes: US barcode 00136337!).

Distribution.– Haiti, Tiburon Peninsula, Massif de la Hotte.

Additional specimens examined. – See Electr. Suppl.: Table S1.

■ AUTHORS' CONTRIBUTIONS

François Lambert: Project conception, data collection, data analysis, results interpretation and manuscript writing. John L. Clark: Project conception, data collection and manuscript review. Simon Joly: Project conception, data collection and manuscript review.

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■ LITERATURE CITED

Acevedo-Rodríguez, P. & Strong, M.T. 2008. Floristic richness and affinities in the West Indies. *Bot. Rev. (Lancaster)* 74: 5–36. https://doi.org/10.1007/s12229-008-9000-1

Acevedo-Rodríguez, P. & Strong, M.T. 2012. *Catalogue of seed plants of the West Indies*. Smithsonian Contributions to Botany 98. Washington, D.C.: Smithsonian Institution Scholarly Press.

Bohonak, A.J. 1999. Dispersal, gene flow, and population structure. *Quart. Rev. Biol.* 74: 21–45. https://doi.org/10.1086/392950

Borcard, D., Gillet, F. & Legendre, P. 2011. *Numerical ecology with R.* New York: Springer. https://doi.org/10.1007/978-1-4419-7976-6

Borhidi, A. 1991. *Phytogeography and vegetation ecology of Cuba*. Budapest: Akadémiai Kiadó.

Borhidi, A. & Kereszty, Z. 1979. New names and new species in the flora of Cuba resp. Antilles. *Acta Bot. Acad. Sci. Hung.* 25: 1–37.

Brown, C.M., Arbour, J.H. & Jackson, D.A. 2012. Testing of the effect of missing data estimation and distribution in morphometric multivariate data analyses. *Syst. Biol.* 61: 941–954. https://doi.org/10.1093/sysbio/sys047

Campbell, D.G. & Hammond, H.D. 1989. *Floristic inventory of tropical countries: The status of plant systematics, collections, and vegetation, plus recommendations for the future.* New York: New York Botanical Garden.

Clark, J.L., Matos, J., Suárez, T.S., Ginzbarg, S. & Skog, L.E. 2013. An annotated species list for the Gesneriaceae of Cuba. *Selbyana* 31: 186–227.

De Queiroz, K. 2007. Species concepts and species delimitation. *Syst. Biol.* 56: 879–886. https://doi.org/10.1080/10635150701701083

Dod, D.D. 1984. Massif de la Hotte, isla peculiar: Orquídeas nuevas iluminan su historia. *Moscosoa* 3: 91–99.

Dray, S. & Dufour, A.-B. 2007. The ade4 package: Implementing the duality diagram for ecologists. *J. Statist. Softw.* 22: 1–20. https://doi.org/10.18637/jss.v022.i04

Drummond, A.J. & Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *B. M. C. Evol. Biol.* 7: 214. https://doi.org/10.1186/1471-2148-7-214

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. & Wilson, A. 2014. Geneious, version 1.8.2. Auckland: Biomatters.

Ekman, E.L. 1926. Botanizing in Haiti. *U.S. Naval Med. Bull.* 24: 461–497.

Ekman, E.L. 1928. A botanical excursion in La Hotte, Haiti. *Svensk. Bot Tidskr.* 22: 200–219.

Gower, J.C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857–871. https://doi.org/10.2307/2528823

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25: 1965–1978. https://doi.org/10.1002/joc.1276

Hijmans, R.J., Phillips, S.J., Leathwick, J.R. & Elith, J. 2015. dismo: Species distribution modeling. R package version 1.1-4. http://CRAN.R-project.org/package=dismo

Joly, S. 2016. pofadinr: Distance methods from SNP data. Program distributed by the author. Montreal, Canada: Montreal Botanical Garden. http://github.com/simjoly/pofadinr

Joly, S., Bryant, D. & Lockhart, P.J. 2015. Flexible methods for estimating genetic distances from single nucleotide polymorphisms. *Meth. Ecol. Evol.* 6: 938–948. https://doi.org/10.1111/2041-210X.12343

Joly, S., Lambert, F., Alexandre, H., Léveillé-Bourret, É. & Clark, J.L. 2016. Pollinator specialization imposes stronger evolutionary constraints on flower shape. *bioRxiv*: 41533. https://doi.org/10.1101/041533

Judd, W.S. 1987. Floristic study of Morne la Visite and Pic Macaya national parks, Haiti. *Bull. Florida State Mus., Biol. Sci.* 32: 1–136.

Katoh, K. & Standley, D.M. 2013. MAFFT: Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. *Molec. Biol. Evol.* 30: 772–780. https://doi.org/10.1093/molbev/mst010

Kuntze, O. 1891. *Revisio generum plantarum*, vol. 2. Leipzig: Arthur Felix; etc. https://doi.org/10.5962/bhl.title.327

Legendre, P. & Legendre, L. 2012. *Numerical ecology.* Amsterdam: Elsevier.

Liogier, A.H. 1971. Novitates Antillanae IV. *Mem. New York Bot. Gard.* 21: 107–152.

Liogier, A.H. 1973. Novitates Antillanae VI. *Phytologia* 25: 265–280.

Liogier, A.H. 1976. Novitates Antillanae VII. Plantas nuevas de la Española. *Moscosoa* 1: 16–54.

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M. & Hornik, K. 2016. cluster: Cluster analysis basics and extensions. R package version 2.0.5. http://CRAN.R-project.org/package=cluster

Majure, L.C., Judd, W.S., Ionta, G.M., Skean, J.D., Bécquer, E.R. & Neubig, K.M. 2014. *Miconia cineana* (Melastomataceae: Miconieae), a new species from the Massif de la Hotte, Haiti, based on morphological and molecular evidence. *Syst. Bot.* 39: 906–914. https://doi.org/10.1600/036364414X682247

Martén-Rodríguez, S., Almarales-Castro, A. & Fenster, C.B. 2009. Evaluation of pollination syndromes in Antillean Gesneriaceae: evidence for bat, hummingbird and generalized flowers. *J. Ecol.* 97: 348–359. https://doi.org/10.1111/j.1365-2745.2008.01465.x

Martén-Rodríguez, S., Fenster, C.B., Agnarsson, I., Skog, L.E. & Zimmer, E.A. 2010. Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytol.* 188: 403–417. https://doi.org/10.1111/j.1469-8137.2010.03330.x

Matzke, N.J. 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Syst. Biol.* 63: 951–970. https://doi.org/10.1093/sysbio/syu056

Morton, C.V. 1957. Some West Indian Gesneriaceae. *Brittonia* 9: 18–24. https://doi.org/10.2307/2804845

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858. https://doi.org/10.1038/35002501

Paradis, E., Claude, J. & Strimmer, K. 2004. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290. https://doi.org/10.1093/bioinformatics/btg412

R Core Team 2015. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/

Ree, R. & Smith, S. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57: 4–14. https://doi.org/10.1080/10635150701883881

Rios, N.E. & Bart, H.L. 2010. GEOLocate. Belle Chasse, LA: Tulane University Museum of Natural History. http://www.museum.tulane.edu/geolocate/

Roalson, E.H., Skog, L.E. & Zimmer, E.A. 2008. Untangling Gloxinieae (Gesneriaceae). II. Reconstructing biogeographic patterns and estimating divergence times among New World continental and island lineages. *Syst. Bot.* 33: 159–175. https://doi.org/10.1600/036364408783887429

Ronquist, F. 1994. Ancestral areas and parsimony. *Syst. Biol.* 43: 267–274. https://doi.org/10.1093/sysbio/43.2.267

Santiago-Valentin, E. & Olmstead, R.G. 2004. Historical biogeography of Caribbean plants: Introduction to current knowledge and possibilities from a phylogenetic perspective. *Taxon* 53: 299–319. https://doi.org/10.2307/4135610

Skog, L.E. 1976. *A study of the tribe Gesnerieae, with a revision of* Gesneria *(Gesneriaceae: Gesnerioideae).* Smithsonian Contributions to Botany 29. Washington, D.C.: Smithsonian Institution Press.

Skog, L.E. 2012. Gesneriaceae. Pp. 350–364 in: Acevedo-Rodríguez, P. & Strong, M.T. (eds.), *Catalogue of seed plants of the West Indies.* Smithsonian Contributions to Botany 98. Washington, D.C.: Smithsonian Institution Scholarly Press.

Skog, L.E. & Boggan, J.K. 2007 [last updated 21 Mar 2007]. World checklist of Gesneriaceae. Washington, D.C.: Department of Botany, Smithsonian Institution. http://botany.si.edu/Gesneriaceae/Checklist

Smith, M.L., Hedges, S.B., Buck, W., Hemphill, A., Inchaustegui, S., Ivie, M.A., Martina, D., Maunder, M. & Ortega, J.F. 2005. *Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions.* Mexico City: CEMEX.

Steel, M. 2001. Sufficient conditions for two tree reconstruction techniques to succeed on sufficiently long sequences. *SIAM J. Discrete Math.* 14: 36–48. https://doi.org/10.1137/S0895480198343571

Urban, I. 1901 ("1900–1901"). *Symbolae Antillanae*, vol. 2. Lipsiae [Leipzig]: Fratres Borntraeger. https://doi.org/10.5962/bhl.title.144

Wade, M.J. & Goodnight, C.J. 1998. The theories of Fisher and Wright in the context of metapopulations: When nature does many small experiments. *Evolution* 52: 1537–1553. https://doi.org/10.1111/j.1558-5646.1998.tb02235.x

Wege, D.C., Ryan, D., Varty, N., Anadón-Irizarry, V. & Pérez-Leroux, A. 2009. *Ecosystem profile: The Caribbean islands biodiversity hotspot.* Airlington: Critical Ecosystems Partnership Fund.

Yang, Z. & Rannala, B. 2014. Unguided species delimitation using DNA sequence data from multiple loci. *Molec. Biol. Evol.* 31: 3125–3135. https://doi.org/10.1093/molbev/msu279

Zink, R.M. 2004. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 271: 561–564. https://doi.org/10.1098/rspb.2003.2617

Appendix 1. Voucher information and GenBank accession numbers of DNA sequences (*CHI, F3H, GCYC, UF3GT*) included in the genetic analyses. Accession numbers beginning with MF represent new sequences. For a tabular version, see Table S2 in the Electr. Suppl.

Gesneria acrochordonanthe (L.E.Skog) Borhidi, Haiti, Sud, *Clark & al. 14467* (UNA; MT), MF318846, MF318702, MF318763, –; ***Gesneria quisqueyana*** Alain, Dominican Republic, Hermanas Mirabal, *Jestrow & al. 2013-DR-73* (FTBG), –, –, MF318752, –; Dominican Republic, Hermanas Mirabal, *Jestrow & al. 2013-DR-73* (FTBG), –, MF352014, MF318753, –; Dominican Republic, Hermanas Mirabal, *Jestrow & al. 2013-DR-73* (FTBG), –, MF318707, MF318754, MF318606; Dominican Republic, Monte Plata, *Hahn & al. 454* (SRP), –, MF318704, –, –; ***Gesneria sintenisii*** Urb., Puerto Rico, Rio Grande, *Clark 13757* (UNA; MT), MF318841, MF318708, MF318759, MF318611; Puerto Rico, Luquillo, *Martén- Rodríguez 1252* (US), –, –, GU323250, MF352012; Puerto Rico, Caguas, *Monsegur-Rivera & Sanchez 863* (US), –, –, MF318760, MF318607; ***Gesneria sylvicola*** Alain, Haiti, Ouest, *Lambert & Joly 2014-027* (MT), MF318842, MF352013, MF318764, MF352011; Haiti, Ouest, *Lambert & Joly 2014-028* (MT), MF318843, MF318722, MF318765, MF318585; Dominican Republic, Independencia, *Hahn & al. 447* (US), –, –, MF352015, MF318608; Dominican Republic, Independencia, *Hahn & al. 440* (SRP), –, MF318703, AY626227, –; ***Gesneria viridiflora*** (Decne.) Kuntze, Cuba, Sancti-Spíritus, *Clark & al. 10041* (UNA), MF318845, –, MF318766, MF318610; Cuba, Granma, *Clark & al. 10509* (UNA), MF318854, MF318726, MF318767, –; Cuba, Granma, *Clark & al. 10524* (UNA), –, MF318706, MF318768, MF318584; Cuba, Granma, *Clark & al. 10540* (UNA), –, MF318709, MF318769, MF318609; Cuba, Guantánamo, *Clark & al. 10561* (UNA), –, MF318725, MF318770, MF318586