The new Hispaniolan genus Tainus (Rubiaceae) constitutes an isolated lineage in the Caribbean biodiversity hotspot

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The new Hispaniolan genus *Tainus* (*Rubiaceae*) constitutes an isolated lineage in the Caribbean biodiversity hotspot

Abstract: The Caribbean islands are a well-known biodiversity hotspot of global importance. Hispaniola (Dominican Republic and Haiti) is a highly diverse island with 42% of its species endemic. Here we describe the new monotypic genus *Tainus* (*Rubiaceae*, *Rondeletieae*), which is endemic to the Sierra de Bahoruco-La Selle. This taxon was originally published as *Rondeletia pitreana*, but its taxonomic status has been doubtful. We performed phylogenetic analyses with three chloroplast regions (*petD*, *trnK-matK* and *trnL-F*) in order to test the relationships of *R. pitreana* to other species of *Rondeletia* and allied genera. Our molecular phylogeny underscores that *R. pitreana* does not belong to *Rondeletia*, but has an isolated position within *Rondeletieae*, whereas *Acrosyanthus* is sister to an assemblage of *Mazaea*, *Phyllomelia*, *Rachicallis*, *Roigella* and *Rondeletia*. The recognition of the genus *Tainus* is supported by molecular (simple sequence repeats) and morphological characters, among which the most conspicuous is the densely papillate ornamentation of the corolla mouth and lobes. Our findings reveal another case of endemism at a deeper phylogenetic level in flowering plants on Hispaniola. Furthermore, they emphasize the importance of exploring, collecting and performing phylogenetic analyses integrating samples from larger geographical areas across countries as a basis to support nature conservation.

Resumen: Las islas del Caribe son un conocido hotspot de biodiversidad mundial. Entre ellas, La Española (República Dominicana y Haití) tiene una diversidad relevante y alrededor de 42% de sus especies son endémicas. En este trabajo describimos el nuevo género *Tainus* (*Rubiaceae*, *Rondeletieae*), monotípico y endémico de la Sierra de Bahoruco-La Selle en La Española. Anteriormente, la especie que sirve para reconocer el nuevo género fue clasificada como *Rondeletia pitreana*. El estatus taxonómico de esta especie había sido puesto en duda en filogenias moleculares en las cuales se recuperaba fuera del clado de *Rondeletia*. Nuestros análisis incluyen tres marcadores de cloroplasto (*petD*, *trnK-matK* y *trnL-F*) para poner a prueba la posición filogenética de *R. pitreana* con respecto a *Rondeletia*. Nuestra filogenia molecular corrobora que *R. pitreana* no pertenece a *Rondeletia* y más bien tiene una posición aislada, en tanto que *Acrosyanthus* es hermano de un clado que incluye a *Mazaea*, *Phyllomelia*, *Rachicallis*, *Roigella* y *Rondeletia*. Para ser congruentes con la filogenia, describimos el género *Tainus*, apoyado por varias sinapomorfías, incluyendo caracteres microestructurales de ADN (repeticiones simples de secuencia) y caracteres morfológicos, entre los que destaca la superficie adaxial de los lobos de la corola y la garganta densamente papillosa. La descripción de este nuevo género cambia nuestra percepción del endemismo en La Española revelando otro caso de endemismo a nivel filogenético profundo en las plantas con flor de La Española. Esto resalta la importancia de la exploración, colecta y la realización de trabajos filogenéticos que integren muestreos de regiones geográficas más amplias para generar bases sólidas para la conservación.

Key words: Caribbean, Greater Antilles, Hispaniola, Dominican Republic, Haiti, Sierra de Bahoruco-La Selle, *Rubiaceae*, *Rondeletieae*, *Rondeletia*, *Tainus*, molecular phylogeny, taxonomy, endemism, nature conservation

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Introduction

The Caribbean islands constitute one of the global biodiversity hotspots (Myers & al. 2000) with approximately 12,300 species of vascular plants (Acevedo-Rodríguez & Strong 2012). The affinities of the Caribbean island floras are complex, as shown by the spectrum of hypotheses to explain their biogeographic origin (Santiago-Valentín & Olmstead 2004; Cano & al. 2009). Recently, Cervantes & al. (2016) provided evidence that Mexico and Mesoamerica could have played a more important role than previously thought as a source for ancestors of plants that later diversified on the Caribbean islands from the Miocene onwards.

Within the Antilles, the islands of Cuba and Hispaniola stand out by the number of species and infraspecific taxa that are endemic (51% of 5,778 and 42% of 4,433, respectively). From the 1,210 genera reported from Cuba, 65 are considered as Caribbean endemics, whereas from the 1,102 genera listed for Hispaniola, 34 are Caribbean endemics. Therefore, Cuba and Hispaniola house nearly 24% and 15%, respectively, of the endemism in the Antilles (Acevedo-Rodríguez & Strong 2012). Five of the endemic genera are restricted to Hispaniola: Garcíadelia Jestrow & Jiménez (Euphorbiaceae), Hispanianthus Cornejo & Ilitis (Capparaceae), Hottea Urb. (Myrtaceae), Ignurbia B. Nord. (Asteraceae) and Tortueella Urb. (Rubiaceae) (Nordenstam 2006; Cornejo & Ilitis 2009; Borhidi 2010; Jestrow & al. 2010; Acevedo-Rodríguez & Strong 2012). However, only Garcíadelia has so far been evaluated with phylogenetic methods.

According to Cano-Ortiz & al. (2016), Hispaniola has 19 areas of particular high diversity, which are explained by altitudinal gradients and specific soil types. Hispaniola has the highest mountains in all the Antilles (c. 2,400 m) and the greatest richness in endemism is said to occur at medium altitudes (May 2001; Cano & al. 2010). The Sierra de Bahoruco-La Selle stands out by being the most diverse mountain system within Hispaniola with several different ecosystem types and c. 700 species of vascular plants, of which about two thirds are endemic (Cano-Ortiz & al. 2016).

Based on the checklist of Acevedo-Rodríguez & Strong (2012), the Rubiaceae are by far the most speciose family of angiosperms in the Antilles in terms of native species (c. 820) and also have the highest number of endemic species (c. 696). On the other hand, the Asteraceae are considered to have more native and endemic genera (145 native and 41 endemic) than Rubiaceae (89 and 27, respectively), whereas the total number of species in Asteraceae (c. 660) is considerably lower than in Rubiaceae. Among the Antillean islands, Cuba and Hispaniola are the most diverse for Rubiaceae, together housing almost 90% of the species in the Caribbean islands. With 177 species, almost all of them endemic, the tribe Rondeletieae is the most diverse within Rubiaceae (data from Acevedo-Rodríguez & Strong 2012). Nevertheless, this tribe with its nine genera (seven endemic) is not the most diverse in the Caribbean islands with respect to genera. It rather occupies the third position, after Spermacoceae (18 genera, four endemic) and Chiococceae (17, 12).

The circumscription of genera within Rondeletieae has varied among different authors from nine to 15 genera, whereas a diversity of c. 200 species is more commonly accepted (Fernández-Zequeira 1994; Rova & al. 2002; Borhidi & al. 2004; Robbrecht & Manen 2006; Rova & al. 2009; Manns & Bremer 2010; Borhidi & al. 2011; Tange 2013; Delprete & Jardim 2012; Razafimandimbison & al. 2011), mainly restricted to the Caribbean islands. The tribe consists of small trees or shrubs with entire stipules, imbricate corolla lobes, heterosty- lous flowers, tricolporate pollen and dry fruits (Robbrecht 1988). The tribe Rondeletieae belongs in Cinchonoideae according to the subfamily circumscription sensu Robbrecht & Manen (2006) and also sensu Bremer & al. (1995). Whereas molecular phylogenetic studies agree on the composition of Rondeletieae as a clade (Manns & Bremer 2010; Torres-Montúfar & al. 2017) and its position as sister to Guettardeae, a detailed understanding of phylogenetic relationships within Rondeletieae is still lacking.

The characters commonly used to differentiate genera within Rondeletieae (Robbrecht 1988; Delprete 1999a, 1999b; Rova & al. 2002, 2009) are: inflorescence position (axillary vs terminal), flower merosity (four to six), corolla mouth ornamentation (naked or with a fleshy/hairy ring), position of the insertion of the stamens on the corolla (at the base, at the middle or near the throat), and capsule dehiscence (loculicidal vs septoidal). This morphological heterogeneity has resulted in a controversial circumscription of the tribe Rondeletieae and particularly of the genus Rondeletia L. (Robbrecht 1988; Delprete 1999a, 1999b).

Rova & al. (2009), using ITS, rps16 and trnL-F sequences, first noted a position of Rondeletia pitreana Urb. & Ekman, which was described from Haiti (Urban & Ekman 1929), outside a well-supported Rondeletia clade. Furthermore, they were not decisive in the inclusion of this species within Rondeletieae. The authors nevertheless decided to maintain the species as part of Rondeletia as they considered an incomplete trnL-F sequence for R. pitreana compared to other taxa as a shortcoming of their analysis. In fact, the tree published by Rova & al. (2009) depicts R. pitreana as an early diverging branch sister to an assemblage of genera of Guettardeae and Rondeletieae but none of the deeper nodes received any statistical support. Later, Manns & Bremer (2010) used ITS, atpB-rbcL, ndhF, rps16 and trnL-F in a study of the subfamily Cinchonoideae and recovered R. pitreana as sister of a clade comprising Arcosynanthus Urb.—Mazaea Krug & Urb.—Rachicallis DC.—Rondeletia. Despite this, they also maintained the species as part of Rondeletia and suggested that the status of the taxon required further investigation before any change of classification would be
warranted. We here took over the task to revisit the phylogenetic position of *R. pitreana* and assess its taxonomic status. For this investigation we extended our recent data set of combined intron and spacer sequence data, which has already provided a much better resolved and statistically supported phylogenetic framework for the Cinchonoideae (Torres Montúfar & al. 2017) compared to previous publications. We also studied morphological characters of *R. pitreana* in comparison to the other lineages of Rondeletieae.

**Material and methods**

**Taxon sampling** — The taxon set for Rondeletieae included in Torres-Montúfar & al. (2017) was extended and now includes all the genera undoubtedly classified within Rondeletieae by Manns & Bremer (2010) and corroborated by further studies that, although focusing on other tribes, included genera classified in Rondeletieae sensu Robbrecht (1988) or Delprete (1999a) (Tange 2013; Delprete & Jardim 2012; Razafimanambison & al. 2011). Several genera representing other tribes of Cinchonoideae (Chicoceae, Condamineae, Guettardeae and Sabiceae) and Ixoroideae (Gardenieae) were used as outgroups. Taxon names with authors, vouchers and GenBank accession numbers are presented in Appendix 1.

**Morphology** — Information on morphological characters traditionally used to distinguish genera of Rondeletieae was obtained from the literature (Standley 1918; Liogier 1962, 1995; Aiello 1979; Delprete 1999a, 1999b; Lorence & Taylor 2012) and through our own examination of the voucher specimens. The phylogenetic distribution of taxonomically important floral characters is shown next to the consensus tree obtained from the plastid data (Fig. 1).

The air-dried corolla of *Rondeletia pitreana* (from herbarium material) was coated with gold and observed using a scanning electron microscope (Hitachi, model SU1510) at the Instituto de Biología, UNAM.

**DNA extraction, amplification and sequencing** — Silicagel-dried leaves were used for DNA extraction when available, complemented with herbarium material. DNA was extracted using NucleoSpin Plant II kit (Macherey Nagel, Düren, Germany) following the manufacturer’s protocol or a three-fraction cetyltrimethylammonium bromide (CTAB) method (Borsch & al. 2003). Three plastid markers (*trnK-matK, trnL-F* and *petD*) were amplified and sequenced in this study. The amplification of each marker was performed in reaction volumes of 50 μL, containing 2 μL of extracted DNA (with a concentration of 10–20 ng/μL), 14.7 μL of H2O, 5 μL of 10x peqLab Taq buffer S containing MgCl2, 3 μL of MgCl2 (25 mM), 10 μL of betaine monohydrate (5 M), 1 μL of BSA (10 μg/μL), 2 μL of forward primer (20 pm/μL), 2 μL of reverse primer (20 pm/μL), 10 μL dNTPs (each 0.25 mM) and 0.3 μL Taq polymerase 5 units/μL (PeqLab, Erlangen Germany).

For *trnl-F* the amplification was performed in two halves using the primers trnPbryo (Wicke & Quandt 2009) and COmatK670R (Tesfaye & al. 2007) plus NYmatK480F (Hilu & al. 2003) and psbA5R (Steele & Vilgalys 1994). PCR conditions were: 1 cycle of denaturation (90 seconds at 96 °C, 60 seconds at 50 °C, 120 seconds at 68 °C), 35 cycles of annealing (30 seconds at 95 °C, 60 seconds at 48 °C, 120 seconds at 68 °C), extension (20 minutes at 68 °C). The *trnL-F* region was amplified using the primers trnTc and trnTf (Taberlet & al. 1991). PCR conditions were: 30 cycles of denaturation (60 seconds at 96 °C), annealing (60 seconds at 50 °C), and extension (120 seconds at 72 °C). The *petD* intron including the *petB-petD* spacer was amplified using the primers PlpetB1411F or PlpetB1365F and PlpetD738R or PlpetD346R (Löhne & Borsch 2005). PCR conditions were: 35 cycles of denaturation (60 seconds at 97 °C), annealing (60 seconds at 48 °C), extension (45 seconds at 72 °C) and a final extension step (7 min at 72 °C).

All amplification products were purified by QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany). Sequencing was performed by Macrogen Inc., South Korea (http://www.macrogen.com).

Because of the availability of only older herbarium material for some species and technical difficulties to obtain PCR products, sequences were not obtained for all genomic regions for each taxon. Therefore, there are 26 terminals that only have two of the three markers (the corresponding lacking marker was coded with missing values).

**Sequence assembly, alignment and indel coding** — The matrix comprises 88 taxa and 217 accessions, 25 of which were generated for this study. Another 179 sequences generated for a previous study (Torres-Montúfar & al. 2017) and 13 published by Rova & al. (2002, 2009) were downloaded from GenBank. New pherograms were edited and assembled using PhyDE v.0.995 (Müller & al. 2005). Sequences were then added to the multiple sequence alignment of Torres-Montúfar & al. (2017) following the criteria proposed by Löhne & Borsch (2005). Gaps were coded manually following the simple gap criterion of Simmons & Ochoterena (2000); Simple Sequence Repeats (SSR) and inversions were manually coded following Ochoterena (2009). Regions of uncertain homology (mutational hotspots) were removed from the matrices prior to phylogenetic analyses.

**Phylogenetic analyses** — Parsimony (P) analyses were performed using the combined matrix with nucleotides, gaps, inversions and SSRs, only including parsimony-informative characters. A heuristic search with 10000 replicates of Wagner trees constructed with random taxon addition followed by TBR branch swapping was performed in TNT version 1.1 (Goloboff & al. 2003); ten trees were saved on each replicate and a further TBR was conducted to completion saving up to 10000 trees.
These trees were submitted to further analysis using the “new technology” option, alternating ratchet parsimony (Nixon 1999a), sectorial, drift and tree fusion (Goloboff 1999) options. One hundred initial sequences were used until the consensus was stabilized ten times using a 100% factor. All the most parsimonious trees were collected and opened in WinClada (Nixon 1999b) and summarized in a strict consensus tree. Additionally, a Jackknife analysis (JK) was executed in TNT version 1.1 (Goloboff & al. 2003), re-sampling the matrix 10 000 times with the same parameters used for the new technology searches.

For Bayesian Inference (BI) and Maximum Likelihood (ML) analyses, the optimal models of sequence evolution were estimated using ModelTest 3.6 (Posada 1998) using the following parameters: 203 substitution schemes, +F base frequencies, +I and +G rate variation, nCat=4 and SPR tree search. BI analyses of the concatenated nucleotide matrix were conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). Four independent Markov Chain Monte Carlo (MCMC) runs were carried out, each with four parallel chains. Each chain was performed for 1 000 000 generations, saving one random tree every 1000 generations. The burn-in was set to 1000 (10%) when the stability of the chain was reached. Phylogenetic trees were visualized using Figtree (Rambaut & Drummond 2010). ML topologies and Bootstrap values (BS) from 1000 replicates were inferred using the Randomized Accelerated Maximum Likelihood graphic user interface RAxMLGUI version 1.2 (Silvestro & Michalak 2011).

The P and BI consensus as well as the ML trees (Appendix 2, see Supplementary Materials online) were graphically summarized rather than presenting any of them as a preferred topology, to be conservative trusting only nodes supported by all three inference methods. This was achieved in WinClada by manually collapsing nodes that were insufficiently supported in at least one of the trees obtained by alternative methods. We considered cutoff values as follows: 64% for JK (Farris & al. 1996), 0.95 for posterior probabilities (PP; Alfaro & Holder 2006) and ≥70% for ML BS (Felsenstein & Kishino 1993; Hillis & Bull 1993).

### Results

The aligned matrix with all regions consisted of 5030 characters (without mutational hotspots), of which 771 (15%) were potentially parsimony informative. The coding of microstructural mutations resulted in 115 characters (Appendix 3, see Supplementary Materials online), of which 96 (83%) were potentially parsimony informative. The general time-reversible model including parameters for invariant sites and rate variation (GTR+I+G) best fit all data sets and was used to conduct the probabilistic analyses. The alignments, microstructural matrices, the combined matrix including microstructural characters, and the matrix used for the analyses are presented as Supplementary Materials online. Detailed matrix statistics regarding each marker contribution are given in Table 1.

**Table 1. Contribution of the different genomic regions to the total matrix.**

| Character Type            | trnK-matK | trnL-F | petD | Total |
|---------------------------|-----------|--------|------|-------|
| Sequences                 | 62        | 83     | 68   | 217   |
| Aligned positions         | 2934      | 1046   | 1050 | 5030  |
| Number of nucleotide characters (nts) without hotspots | 2839 | 1017 | 1007 | 4863 |
| Microstructural characters (mch) with I & G rates | 47     | 28     | 40   | 115   |
| Potentially informative characters (nts/mch) | 471/39 | 162/23 | 138/34 | 771/96 |

**Discussion**

**Phylogenetic position of Rondeletia pitreana** — The phylogenetic position of *R. pitreana* is well supported as nested inside *Rondeletieae* but outside of the clade...
constituted by the Rondeletia assemblage plus Acrosynanthus. Our much better-resolved and -supported trees clarify the earlier statement of Rova & al. (2009), who could not assign the species to either Guettardeae or Rondeletieae. Two factors contributed to the better understanding of the phylogenetic position of *R. pitreana*: an improved marker selection and the use of a sufficiently dense taxon sampling. The two SSRs in *trnL-F* are also present in the original but incomplete sequence generated by Rova (2002). This observation, combined with the fact that the incomplete sequence groups with our sequences in phylogenetic analyses of *trnL-F* sequences (pers. obs.), suggests that the lack of about 40 nt does not create a spurious signal and the sequence published by Rova (2002) is a correct sequence of *R. pitreana*. However, their trees were insufficiently resolved and supported because of the limited sampling of molecular characters. Whereas Manns & Bremer (2010) improved the resolution and support within Rondeletieae within their tree by using several DNA markers, they sampled just four other species of *Rondeletia*, thus limiting the possibility to test the monophyly of the genus. Our taxon sampling of *Rondeletia* covers the range of the genus throughout Mesoamerica and the Antilles and also its morphological diversity.

In addition, the comparative analysis of morphological features clarified the morphological differences of *Rondeletia pitreana* compared to allied taxa. We found the papillate corolla to constitute a morphological apomorphy that differentiates *R. pitreana* from the rest of the *Rondeletia* species (Fig. 1B, Fig. 2, Fig. 3). Furthermore, the combination of a 5-merous corolla, the presence of domatia, and stamens inserted at the corolla mouth is unique for *R. pitreana*.

The morphological and molecular characters differentiating *Rondeletia pitreana* from the rest of the *Rondeletia* species grants the recognition of a new monotypic genus that we call *Tainus*. The alternative option would be to include *R. pitreana* as well as *Acrosynanthus* within *Rondeletia* (as well as all the other currently accepted genera of the *Rondeletia* assemblage; Fig. 1). The expansion of the concept of *Rondeletia* to include all the species in the clade would result in the lack of diagnostic characters for the group and would create confusion over the well-defined monophyletic
genus *Acrosynanthus*. The monophyly of *Rondeletia* (without *Tainus*) is another issue, which will require obtaining phylogenetic resolution at species level within the *Rondeletia* assemblage. As such, this will require further investigation with additional taxon sampling and considerably extended character sampling.

A new genus for Hispaniola in the Caribbean hotspot — Although *Rondeletia pitreana* was already recognized as an endemic species to Hispaniola, the description of the new genus *Tainus* adds value in our view to the endemism in Hispaniola, highlighting the importance of this island for lineage diversification. The genus *Tainus* joins the recent discoveries and description of new genera for Hispaniola of different plant lineages including now six endemic genera, but in this case phylogenetic evidence provides stronger support for the naturalness of *Tainus*.

The monotypic genus *Tainus* appears to come from one diversification event within the Antilles (Fig. 1), but it is not possible to establish further detail on its biogeographic origin in the Antilles due to bias sampling within the sister clade: in our study *Acrosynanthus* was only sampled from Cuba, but it also occurs in Jamaica; and further sampling of *Rondeletiae* is obviously needed.

The recent findings stress the need to explore, collect and carry out phylogenetic studies to increase the floristic knowledge of Hispaniola and the Antilles. Within Hispaniola, a site of particular interest is the Sierra de Bahoruco-La Selle, for which the genus *Hottea* is endemic in addition to *Tainus*, underscoring its importance as an area of high diversity in Hispaniola, as stated by Cano-Ortiz & al. (2016), and definitely highlighting the need to protect this area of Hispaniola.

**Taxonomic treatment**

*Tainus* Torr.-Montúfar, H. Ochot. & Borsch, *gen. nov.* — Fig. 3.  
Type: *Tainus pitreanus* (Urb. & Ekman) Torr.-Montúfar, H. Ochot. & Borsch.

**Morphological diagnosis** — *Tainus* is clearly distinguished...
from all other genera of Rondeletiae by the densely papillate surface of the corolla lobes and mouth. It is distinguishable from Rondeletia by the lack of a conspicuous fleshy ring in the corolla mouth. Tainus is distinguished from Acrosynanthus by the stamens inserted at the distal portion of the corolla tube (vs at the basal portion of the corolla tube) and the 5-merous flower parts (vs mainly 6-merous, with only one species 5- or 6-merous).

**Molecular diagnosis** — Tainus is uniquely characterized by two trnL-F simple sequence repeats: a “TGAT” in positions 389–392 and a “TTTTC” in positions 426–430.

**Eponymy** — The name Tainus is dedicated to the indigenous people of the Caribbean islands. The Taino people are closely related to Mesoamerican ancient civilization, with many migrations and high cultural exchange between both regions. This resembles the biogeographic patterns in Rondeletiae, with several independent events of migration and diversification among the Caribbean islands and Mesoamerica.

**Tainus pitreanus** (Urb. & Ekman) Torr.-Montúfar, H. Ochot. & Borsch, **comb. nov.** = Rondeletia pitreana Urb. & Ekman in Ark. Bot. 22A(10): 83. 1929. — **Lectotype (designated here)**: Haiti, Massif de La Selle, group Morne des Commissaires, Anses-à-Pitres, between Banane and Tête de l’Eau, steep riverside, 26 Aug 1926, **E. L. Ekman H 6753** [online image]; islectotype: A00095861 [online image], G00436657!, GH00095862!, K000174024 [online image], LL00373191!, S 007-14924! [online image], US00137618!, US00794308!).

**Description** — Treelets 7–10 m tall. **Stems** striate; **twigs** terete to slightly angulate, sparsely hispid with minute trichomes, glabrescent. **Stipules** erect, triangular, 2–3 × 2–3 mm, deciduous, abaxially glabrous or sparsely pubescent at base, adaxially with a row of small reddish colleters and white trichomes, margin ciliate. **Leaves** opposite, petiolate; **petiole** 3–5 mm long, slightly winged, glabrate; **leaf blade** elliptic, 4.2–9.1 × 2–3.4 cm, leathery, abaxially glabrate, adaxially lustrous and glabrate, base attenuate, apex acute; primary and secondary veins abaxially prominent, adaxially depressed, secondary veins 5–7 pairs; domatia present. **Inflorescence** axillary, pedunculate, paniculate, exceeding subtending leaf, 8.1–9.4 × c. 2.5 cm including flowers, with 4–6 pairs of secondary axes; **peduncle** 2–3 cm long, hispid to strigose; **secondary axes** 1–2 cm long, 1- or 2-flowered, strigose, each subtended by a triangular bract 2–3 mm long. **Flowers** pedicellate; each subtended by a triangular bracteole 2–3 mm long; **hypanthium** spheroid to ovoid, c. 1 × 1 mm, densely strigose; **calyx** 5-merous; **calyx lobes** erect in flower and fruit, triangular, equal, abaxially sparsely strigose toward base, margin ciliate; **corolla** 5-merous, white, salver-shaped at anthesis; **corolla tube** 6–7 mm long, externally strigose, hairs denser at tube base, sparser at apex, internally sparsely strigose on basal ½ and with a villosulous ring at base; **corolla mouth** papillate; **corolla lobes** ovate, 3–4 mm long, usually one lobe larger than others, margin slightly crisped, especially on larger lobe, abaxially strigose at base and densely papillose at margin and apex, adaxially densely papillose; **heterostyly unknown**; **anthers** adnate c. 1.5 mm below corolla mouth; **style** bilobed, c. 7 mm long, glabrate. **Capsule** loculicidal, ovoid to globose, 3–4 × c. 3 mm, woody, sparsely strigose. **Seeds** flat, winged, c. 1 × 0.5 mm, reticulate.

**Fig. 3. Tainus pitreanus** — A: stipules; B: inflorescence; C: longestylous flowers; D: SEM micrograph showing papillate corolla. — A–C: Dominican Republic, Sierra de Bahoruco, Apr 2013, S. Fuentes & al. 1110, photographs by A. Torres-Montúfar. — D: Dominican Republic, Sierra de Bahoruco, Apr 2003, T. Clase & al. 3493, photograph by B. García-Mendoza.
Phenology — The species flowers from March to July and fruits from July to August.

Distribution and ecology — The genus *Tainus* is restricted to Hispaniola, in the central southern region near the border of the Dominican Republic and Haiti (Fig. 4). The species was known only from the type collection by Ekman (1926), from La Selle in Haiti. Almost eighty years later it was collected again by botanists at the Jardín Botánico Nacional de Santo Domingo (JBN), who found another locality in the Dominican Republic, and again more recently by a joint expedition with the Botanic Garden and Botanical Museum Berlin. The species is endemic to the mainly calcareous mountain system of Sierra de Bahoruco-La Selle. It grows in riparian vegetation of tropical rain forest associated with *Fuertesia domingensis* Urb., *Guarea* sp., *Ocotea* sp. and *Piper* sp.

Conservation status — Although *Tainus pitreanus* has been collected in Haiti and the Dominican Republic, the collecting sites are nearby and share ecological conditions, so they could represent maybe one or two populations. Therefore, the species meets the criteria B1ab(iii) for the category of Critically Endangered (CR) according to the IUCN (2012). Additional exploration across this region and surrounding areas is needed to assess the density of individuals and the population status. Since there is about 50 km distance from the populations in the Dominican Republic to the type locality in Haiti, further exploration should evaluate to what extent plants have persisted in the mountain system.

Despite the Sierra de Bahoruco National Park having been founded as a conservation area (Hoppe 1989), due to its high biodiversity it is necessary to implement efforts for its conservation. Cano & al. (2016) recommended the establishment of a Biosphere Reserve, including the Sierra de Bahoruco and La Selle (Dominican Republic and Republic of Haiti, respectively) to preserve and manage unique ecosystems in the Antilles.

Additional specimens examined — Dominican Republic: Sierra de Bahoruco, Provincia de Pedernales, Carretera Internacional, en las márgenes del Río Mulito, próximo al balneario, 18°09’N, 71°46’W, 200–300 m, fl., fr., 27 Jul 2006, *T. Clase* & al. 4228 (JBSD, MO); entre Río Mulito y Cabo Duarte, 18°09’N, 71°45’W, 300 m, fl. bud, fl., 3 Apr 2003, *T. Clase* & al. 3493 (JBSD); Cabo Duarte a 500 m antes del cuartel, 18°13’38.2”N, 71°47’51.2”W, 1440 m, fl., 16 Apr 2013, *S. Fuentes* & al. 1110 (B, JBSD).

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References

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Appendix 1

List of taxa used in the phylogenetic analyses with voucher information: geographic origin, collector(s) and collecting number, herbarium, project code at Botanischer Garten und Botanisches Museum Berlin (e.g. RUB000), and GenBank accession numbers per marker (trnK-matK, trnL-F and petD; markers for which no sequences were obtained are indicated by a dash “–”). New sequences with respect to Torres-Montúfar & al. (2017) are marked with an asterisk (*); sequences used by Rova & al. (2002, 2009) are marked with two asterisks (**).

Subfamily Cinchonoideae, tribe Condamineae: Calycophyllum candidissimum (Vahl) DC., Cuba, T. Borsch & al. 5125 (B; HAJB; PAL), RUB336, KY785298, KY614094. — Tribe Chiococceae: Cethocarpus involucratus (Wernham) Aiello, Cuba, T. Borsch & al. 4995 (B; HAJB; PAL), RUB233, –, KY785213, KY614097; Chiococcia cubensis Urb., Cuba, S. Fuentes & al. 535 (B; HAJB; PAL), RUB264, –, KY785214, KY614098; Erithalis fruticosa L., Dominican Republic, S. Fuentes & al. 221 (B; JBS), RUB183, –, KY785218, KY614101; Erithalis vacciniifolia (Gris.) Wright ex Sauv., Dominican Republic, S. Fuentes & al. 1044 (B;
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746 (MEXU), RUB119, KY785215, KY614099; Gonzalagunia killipii Standl., Ecuador, M. Zak 3566 (MEXU), RUB049, KY785304, –, KY614105; Gonzalagunia panamensis (Cav.) K. Schum., Cuba, N. Köster & al. 2006 (B; HAJB; PAL), RUB418, KY785274, KY785222, –; Gonzalagunia radis (Standl.) Standl., Costa Rica, R. Forero 7419 (MEXU), RUB095, KY785305, KY785223, KY614106; Gonzalagunia thyroidae (Donn. Sm.) B. L. Rob., Guatemala, M. Gonzalez 1455 (MEXU), RUB047, KY785275, KY785224, KY614107; Guettarda camagueyensis Britton, Cuba, T. Borsch & al. 4028 (B; HAJB; PAL), RUB017, Cuba, —, KY785231, KY614114; Guettarda elliptica Sw., Mexico, H. Ochoterena & al. 894 (MEXU), RUB125, KY785306, KY785225, KY614108; Guettarda ferruginea C. Wright ex Griseb., Cuba, T. Borsch & al. 5007 (B; HAJB; PAL), RUB152, KY785278, KY614112; Guettarda lamprophylla Urb., Dominican Republic, S. Fuentes & al. 1202 (B; JBS), RUB382, KY785276, KY614109; Guettarda unica Urb., Cuba, T. Borsch & al. 4429 (B; HAJB; PAL), RUB153, KY785307, KY785227, KY614110; Guettarda praenleopii Urb., Dominican Republic, S. Fuentes & al. 1205 (B; JBS), RUB292, KY785279, KY785230, KY614113; Guettarda pungens Urb., Dominican Republic, S. Fuentes & al. 238 (B; JBS), RUB184, KY785277, KY614111; Javorkacea hondurensis (Donn. Sm.) Borhidi & Járai-Koml., Honduras, J. Linares 6241 (MEXU), RUBmx055, KY785281, KY614117; Machaoncia paciflora Urb., Cuba, T. Borsch & al. 5218 (B; HAJB; PAL), RUB164, KY785282, KY785235, KY614118; Rogiera amoena Planch., Mexico, H. Ochoterena & al. 805 (MEXU), RUB131, KY785284, KY785240, KY614123; Rogiera cordata (Benth.) Planch., Mexico, H. Ochoterena & al. 732 (MEXU), RUB072, KY785285, KY614124; Rogiera ligustroides (Hems.) Borhidi, Mexico, H. Ochoterena & al. 781 (MEXU), RUB068, KY785286, KY614125; Rogiera macdougallii Lorence, Mexico, H. Ochoterena & al. 841 (MEXU), RUB073, KY785288, KY614127; Rogiera nicaraguensis (Oert.) Borhidi, Honduras, J. Linares 3520 (MEXU), RUB064, KY785287, KY614126; Rogiera stenosiphon (Hems.) Borhidi, Mexico, H. Ochoterena & al. 749 (MEXU), RUB075, KY785289, KY614128. — Tribe Rondeletiae: — Acrosy nanthus revolutus Urb., Cuba, T. Borsch & al. 4156 (B; HAJB; PAL), RUB224, MF460511, MF460689, MF460590; Acrosy nanthus trachyphyllus Standl., Cuba, T. Borsch & al. 4444 (B; HAJB; PAL), RUB154, KY785256, KY614076; Acrosy nanthus trachyphyllus Standl., Cuba, S. Fuentes & al. 602 (B; HAJB; PAL), RUB203, (MF460512, MF460690, MF460591); Acacanaetites tinifolius (Griseb.) Borhidi, Cuba, Stahl & al. s.n. (S), –, GQQ52451, –; Blepharidium guatemalense Standl., Guatemala, Gustafsson & al. 211 (GB), –, AF152735, –; Donnellyanthus deami
(Donn. Sm.) Borhidi, Honduras, S. Duery 172 (MEXU), RUB056, KY785271, KY785216; *Mazaea shaferi* (Standl.) Delprete, Cuba, T. Borsch & al. 4075 (B; HAJB; PAL), RUB144, (MF460567, MF460749, MF460657); Phyllomelis coronata Griseb., Cuba, T. Borsch & al. 4620 (B; HAJB; PAL), RUB158, KY785303, KY785210, KY614093; Rachialis americana (Jacq.) Hitchc., Cuba, N. Köster 2465 (B; HAJB; PAL), RUB170, KY785283, KY785238, KY614121; *Rachialis americana* (Jacq.) Hitchc., Cuba, T. Borsch & al. 5625 (B; HAJB; PAL), RUB217, (MF460568, MF460750, MF460658); **Roe-gella correifolia** (Griseb.) Borhidi & M. Fernández, Cuba, Rova & al. 2262 (GB), –, AF152746, –; **Rondele-tia alaternoides** Rich, Cuba, Rova & al. 2228 (GB), –, AF152740, –; **Rondeletia barahonensis** Urb., Dominican Republic, S. Fuentes & al. 315 (B; JBS), RUB187, KY785290, KY785246, KY614129; *Rondeletia berton- Martinelli 1017 (B; JBS), RUB409, KY785297, KY785237, KY614132; Rondeletia microphylla Griseb., Cuba, T. Borsch & al. 4169 (B; HAJB; PAL), RUB018, KY785308, KY785250, KY614133; **Rondeletia nipensis** Urb., Dominican Republic, Delprete & al. 8651 (UPS), –, GQ852547, –; **Rondeletia portoricensis** Krug & Urb., Puerto Rico, C. Taylor 11687 (MO), –, AF152743, –; *Roveaen-thus strigosus* (Benth.) Borhidi, Guatemala, M. Veliz 6539 (MEXU), RUB063, KY785295, KY785252, KY614135; *Roveaenanthus strigosus* (Benth.) Borhidi, Guatemala, D. Lorence 8920 (PTGB), –, GQ852550, –; **Roveaenanthus suffrutescens** (Brandegee) Borhidi, Mexico, B. Bremer 2712 (S), –, AF152738, –; **Suberanthus brachycarpus** (Griseb.) Borhidi & M. Fernández, Cuba, McDowell 4824 (DUKE), –, HMO45004, –; **Suber-anthus stellatus** (Griseb.) Borhidi & M. Fernández, Cuba, Rova & al. 2219 (GB), –, AF152736, –; *Tainus pitreatus* (Urb. & Ekman) T. Montúfar, H. Ochot. & Borsch, Dominican Republic, S. Fuentes & al. 1110 (B; JBS), RUB311, (MF460587, MF460773, MF460686); **Tainus iner-mis** (Spreng.) Krug & Urb., Puerto Rico, Acevedo-Rodríguez & al. 7691 (NY), –, AF152745, –; **Rondeletia intermixta** Britton, Cuba, Rova & al. 2245 (GB), –, AF152742, –; **Rondeletia merilloana** Urb., Dominican Republic, S. Fuentes & al. 1136 (B; JBS), RUB353, KY785293, KY785249, KY614132; Rondeletia microphylla Griseb., Cuba, T. Borsch & al. 4169 (B; HAJB; PAL), RUB018, KY785308, KY785250, KY614133; **Rondeletia nipensis** Urb., Dominican Republic, Delprete & al. 8651 (UPS), –, GQ852547, –; **Rondeletia portoricensis** Krug & Urb., Puerto Rico, C. Taylor 11687 (MO), –, AF152743, –; *Roveaen-thus strigosus* (Benth.) Borhidi, Guatemala, M. Veliz 6539 (MEXU), RUB063, KY785295, KY785252, KY614135; *Roveaenanthus strigosus* (Benth.) Borhidi, Guatemala, D. Lorence 8920 (PTGB), –, GQ852550, –; **Roveaenanthus suffrutescens** (Brandegee) Borhidi, Mexico, B. Bremer 2712 (S), –, AF152738, –; **Suberanthus brachycarpus** (Griseb.) Borhidi & M. Fernández, Cuba, McDowell 4824 (DUKE), –, HMO45004, –; **Suber-anthus stellatus** (Griseb.) Borhidi & M. Fernández, Cuba, Rova & al. 2219 (GB), –, AF152736, –; *Tainus pitreatus* (Urb. & Ekman) T. Montúfar, H. Ochot. & Borsch, Dominican Republic, S. Fuentes & al. 1110 (B; JBS), RUB311, (MF460587, MF460773, MF460686); **Tainus iner-mis** (Spreng.) Krug & Urb., Puerto Rico, Acevedo-Rodríguez & al. 7691 (NY), –, AF152745, –; **Rondeletia intermixta** Britton, Cuba, Rova & al. 2245 (GB), –, AF152742, –; **Rondeletia merilloana** Urb., Dominican Republic, S. Fuentes & al.

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