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Cecropia telenitida Cuatrec. (Urticaceae: Cecropieae): Phytochemical diversity, chemophenetic implications and new records from Central America

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ABSTRACT

The Neotropical genus *Cecropia* is the largest genus of Cecropiae in the Urticaceae family with 61 described species. For many years, the taxonomic study of *Cecropia* has been based on morphological and anatomical data. However, recent studies have shown that chemical entities present in *Cecropia* can be used to establish differences between species providing important additional support on its taxonomic classification. The goal of the present study was to contribute to the phytotaxonomic knowledge of this genus to better inform taxonomic decisions. In addition, this is the first time that chemical constituents have been described in the leaves of *Cecropia telenitida* Cuatrec., a species that until now had not been reported in Central America. We characterized and quantified the polyphenolic composition of the methanol leaf extract of *C. telenitida* using UPLC-DAD-MS and HPLC-DAD, respectively. Phytochemical analysis showed that this extract was rich in chlorogenic acid and flavone *C*-glycosides, with isoorientin and isoorientin 2"-O-xyloside as the main compounds. Our data showed a lower chemical diversity and metabolite concentrations than other related species. Morphological, distributional and taxonomic notes, images of the plant and phytochemical comparisons between *C. telenitida* and selected congeners from Panama are also provided.

1. Introduction

Urticaceae is one of the largest Angiosperm families. It comprises more than 2000 species distributed worldwide in 54 genera, mainly in tropical regions (Wu et al., 2013). The Cecropieae tribe (previously Cecropiaceae family) (Conn and Hadiah, 2009) includes three Neotropical genera (*Cecropia, Coussapoa* and *Pourouma*) and two Afrotropic genera (*Musanga* and *Myrianthus*). Recent molecular data indicated that both Cecropieae and its five representative genera are monophyletic (Treiber et al., 2016; Gutiérrez-Valencia et al., 2017). The largest genus in Cecropieae is the Neotropical genus *Cecropia*, with 61 described species (Berg and Franco-Rosselli, 2005). *Cecropia* species have a relevant ecological significance due to their rapid growth rate, which make them primary colonizers of deforested tropical areas (Monro, 2009) and act as invasive species in non-native regions (Conn et al., 2012). Species of this genus are naturally distributed across the tropical and subtropical rainforests from Mexico to Central and South America below 2600 m above sea level (Franco-Rosselli and Berg, 1997). Currently, the flora of Panama includes 11 out of the 12 species of *Cecropia* registered for Central America (Berg, 2015).

Most *Cecropia* spp. are myrmecophytes, by their mutualistic relationship with a colony of symbiotic ants (especially the genus *Azteca*). The myrmecophytism in this genus can vary between species (also within species), and it is influenced by their geographical locations and elevational gradients (Longino, 1989; Berg and Franco-Rosselli, 2005; *Gutiérrez-Valencia* et al., 2017). *Cecropia* species provide specialized structures for housing and feeding these symbiotic ants in exchange for protection against natural enemies as herbivores and encroaching vines (Davidson and McKey, 1993; Davidson, 2005; Oliveira et al., 2015; Marting et al., 2018). *Cecropia* trees can supply two types of food rewards in the form of trichomes: Müllerian bodies and pearl glands (Berg and Franco-Rosselli, 2005). The Müllerian bodies usually occur in

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Received 28 June 2019; Received in revised form 19 August 2019; Accepted 21 August 2019 Available online 28 August 2019 0305-1978/ © 2019 Elsevier Ltd. All rights reserved. patches of dense indumentum (called trichilia) at base of the petiole and is characterized by being rich in lipids and contain proteins and glycogen (Rickson, 1971, 1973, 1976). The pearl bodies are found on the lower surface of leaf blades and contain glycogen plastids, lipids and amino acids (Rickson, 1976; Davidson, 2005).

Because species of the genus Cecropia are used as traditional medicine and are commercially available as food supplements, studies have been conducted on this genus in order to determine their phytochemical composition (Rivera-Mondragón et al., 2017). A rich phenolic profile from their leaf extract has been characterized, in which chlorogenic acid and glycosylated flavonoids, such as flavone C-glycosides and flavonol O-glycosides have been found as the main constituents. Apigenin, luteolin and diosmetin derivatives have been described to be the most widely reported flavones in Cecropia leaves, whereas quercetin O-glycosides were representative of the flavonols present in this genus (da Silva Mathias and Rodrigues de Oliveira, 2018; Ortmann et al., 2017; Rivera-Mondragón et al., 2019a, Rivera-Mondragón et al., 2019c; Costa et al., 2011). A literature survey revealed that very little is known about the phytochemical composition of C. telenitida Cuatrec. Previous research on the roots of this species collected in Colombia, established the presence of abundant pentacyclic triterpenes (yarumic acid, isoyarumic acid, serjanic acid, spergulagenic acid A, 20-hydroxy-ursolic acid and goreishic acid I) (Montoya Peláez et al., 2013; Mosquera et al., 2018).

Plant chemophenetic studies are define as the characterization and description of an array of natural chemical products of a taxon. The goal of plant chemophenetics is not to replace or supplant phylogenetic studies based on DNA sequence analysis, but its information could be used to support clades by means of a chemo-phenetic characterization (Zidorn, 2019). In this context, chemophenetics (formerly referred to as chemosystematics or chemotaxonomy) can be useful tools for solving challenges that may affect the establishment of natural classifications of plants in general (Fairbrothers et al., 1975; Waterman and Gray, 1987; Reynolds, 2007). Taking into account that current taxonomic studies on Cecropia rely only on morphological and anatomical data (Berg, 1978; Berg and Franco-Rosselli, 2005), this work contributes to the phytotaxonomic knowledge of this genus. As such, there is increasing evidence that the phytochemical profile of Cecropia could be useful to establish differences between species providing important information for a better taxonomic understanding of this genus (Rivera-Mondragón et al., 2019a). Furthermore, this study provides the first chemical composition of leaves of C. telenitida, whose presence in the Central American region was unknown up until now. Additional photographs of the plant, geographical and taxonomic notes have been also provided in this study.

Although a comprehensive discussion of the chemophenetic significance of phytochemicals in *Cecropia* species lies beyond the scope of this study, this paper attempts to compare the chemical composition of *C. telenitida* with other congeners from Panama. This study was based on previous research on four *Cecropia* species (*C. obtusifolia* Bertol., *C. peltata* L., *C. insignis* Liebm. and *C. hispidissima* Cuatrec.) reported by our research group (Rivera-Mondragón et al., 2019a), accompanied by the newly phytochemical description of single collections of *C. telenitida* and *C. angustifolia* Trécul.

2. Materials and methods

2.1. Plant material and identification

Botanical specimens together with material for phytochemical analysis (leaf blades) were collected at different locations on different dates from 2015 to 2018, throughout the Republic of Panama. Collection permits were issued by the "Ministerio de Ambiente" (SC/P-4-19, SC/P-4-16 and SC/P-4-15). Vouchers were deposited at the University of Panama Herbarium (PMA) (Table S1). In order to identify botanical specimens, literature from Berg and Franco-Rosselli (2005) and Berg (2015) were consulted. Plant identifications were confirmed by comparing collected voucher specimens with those identified earlier and housed at the University of Panama Herbarium (PMA). In addition, type specimens of each species were examined by consulting the JSTOR Global Plants database (Gallagher, 2010). Since *C. obtusifolia* had two distinctive morphotypes, these were analyzed independently (see Table S1). Data on species distribution were obtained from Berg and Franco-Rosselli (2005) and TROPICOS (2019).

2.2. Reagents

Methanol (HPLC grade) and acetonitrile (HPLC grade) were acquired from Fisher Chemical UK Ltd. Formic acid (FA) (98+%, analytical grade) was obtained from Acros Organics (Belgium). Ultrapure water with a resistivity of $18.2 \times M\Omega \times cm$ at 25 °C (Milli-Q, Waters) was used as extraction solvent and for mobile phase preparation.

External standards like chlorogenic acid (99.0%) and rutin (96.9%) were obtained from Sigma-Aldrich (St. Louis, MO), while isovitexin, orientin and isoorientin (all with purity \geq 99%) were from Extrasynthese (Genay, France). Vitexin (99.7%) was purchased from Adipogen (Liestal, Switzerland). Characterized extracts from *C. obtusifolia*, named as flavonoid rich fraction and flavonolignans, were used as reference material.

2.3. General experimental procedures

2.3.1. Plant extraction

Three samples of dried leaves of C. telenitida and C. angustifolia were independently extracted according to Rivera-Mondragón et al., 2019b). An UPLC-DAD-MS system was used for the characterization of the chemical composition of these two plant species. Analysis was done using a TQD mass spectrometer (Waters, Milford, MA, USA) coupled with an ACQUITY LC system equipped with MassLynx version 4.1 software. For analysis, 5 µL of samples were injected on an ACQUITY UPLC BEH C18 column (100 mm \times 2.10 mm, 1.7 μ m, Waters, Milford, MA, USA). The mobile phase solvents consisted of $H_2O + 0.1\%$ FA (A) and ACN + 0.1% FA (B), and the gradient was set as follows (min/B%): 0.0/5.0, 3.0/5.0, 18.0/15, 20.0/15, 28.0/100, 30.0/100, 32.0/5, 37.0/ 5. The flow rate was 0.4 mL/min. During the first analysis, full scan data were recorded in ESI (-) and ESI (+) mode from m/z 120 to 1500. The spray voltage was set at either +3.5 kV and -3.5 kV; cone gas flow and desolvation gas flow at 50.0 L/h and 850.0 L/h, respectively; and source temperature and desolvation temperature at 120 °C and 500 °C, respectively. Data were also recorded using MS2 scan mode in the positive and negative ionization modes (three analysis per mode), and a ramp collision energy from 30 till 40 V was applied to obtain additional structural information. DAD spectra were recorded between 190 and 400 nm.

2.3.2. HPLC-DAD for quantitative analysis of chlorogenic acid, flavonoids and flavonolignans

A validated HPLC-DAD analysis was carried out according to Rivera-Mondragón et al., 2019b. Briefly, an Agilent 1200 series system with degasser, quaternary pump, automatic injection, thermostatic column compartment and a DAD (Agilent Technologies, Santa Clara, CA, USA) was used. For analysis, 20 µL sample extract was injected on an RP-18 Kinetex column (2.10×100 mm, 2.6μ m, Phenomenex, Torrence, CA, USA). Aqueous formic acid (0.1%, v/v) and acetonitrile with 0.1% formic acid were used as mobile phases A and B, respectively. The gradient program was set as follows: 10% B (0-5 min), 10-15% B (5-20 min), 15% B (20-30 min), 15-25% B (30-40 min), 25% B (40-45 min), 25-40% B (45-55 min), 40% B (55-60 min), 40-100% B (60-65 min), 100% B (65-70 min), 100-10% B (70-75 min), 10% B (75-85 min). A flow rate of 0.7 mL/min was used. The column temperature was maintained at 26 °C. The DAD signal was recorded between 190 and 500 nm. TF and CA were monitored at 340 nm, while FL was detected at 390 nm.

2.4. Data analysis

Results were expressed as mean ± standard deviation (SD). Raw data files acquired from the UPLC-DAD-MS analysis were processed with MassLynx (version 4.1, Waters, Milford, MA, USA). Data processing, calculations and graphic plotting were performed using GraphPad Prism for Windows (version 6.01, La Jolla, CA, USA). Multivariate data analysis was performed using JMP Pro 14 (SAS Institute Inc., Cary, NC, USA). Hierarchical clustering algorithm (HCA) using Euclidian distance measurements and Ward's method without a second data standardization was carried out. The number of clusters in HCA was chosen arbitrarily. Chemical diversity was calculated using the specific richness index (S), the Margalef index (Dmg) and the Shannon entropy (H), These last indices are widely used in ecological studies to provide a measure of diversity (Krebs, 1999). In this study, the specific compound richness consisted in making an inventory of the total number of chemical compounds identified in each studied species. The Margalef index was calculated for each Cecropia species as Dmg = (S-1)/log(N), where N was the total abundance of chemical compounds and S the number of chemical compounds. Shannon entropy was calculated as $H = -\Sigma pi \log p$ (pi), where pi is the proportion i.e. abundance of the compound of an individual chromatogram. The calculations of diversity indices were performed with PAST v.3.0 software (Hammer et al., 2001).

3. Results

3.1. First report of Cecropia telenitida Cuatrec. in Central America

Cecropia telenitida Cuatrec., Revista Acad. Colomb. Ci. Exact. 6: 295 (Cuatrecasas, 1945).

Type: Colombia, Departamento Norte de Santander: Hoya de Samaria (municipio de Toledo), 2000–2100 m, 30 Oct 1941, *J. Cuatrecasas, R.E. Schultes & E. Smith 12781* [holotype: COL (image seen!); isotype: F]. Fig. 1.

3.1.1. Geographical distribution

The specimens collected in this study represent the first records of *C. telenitida* in the Central American region. This species has been now reported for Panama (western Darién), the Andean region of Venezuela to southern Colombia, in the central and eastern cordilleras, and from southern Ecuador to northern Peru (Fig. S1).

3.1.2. Habitat and ecology

Cecropia telenitida grows mainly in Andean cloud forests at 1200–2600 m. In Central America, this species was found only in eastern Panama, specifically in cloud forests of Chucantí Nature Reserve (Darién Province), between 1300 and 1450 m in *Premontane rain forest* and *Tropical wet forest* life zones (Holdridge et al., 1971), in association with other montane tree genera like *Oreomunnea* and *Quercus*. According to the ecoregion classification system, this location is part of the *Eastern Panamanian montane forests* ecoregion, which is characterized by precipitation that ranges between 3000 and 4000 mm annually and elevations between 500 and 1800 m (WWF, 2018). *C. telenitida*, like other species of montane habitats, lack myrmecophytism (Gutiérrez-Valencia et al., 2017).

3.1.3. Material examined (new records)

Panama, Darién: Serranía de Majé, Reserva Privada Chucantí, Sendero Los Helicópteros, in close proximity to the top of Cerro Chucantí, 8°47′45″N, 78°27′47″W, 1325 m, 4 April 2018 (\bigcirc flowers and fruits), *O. Ortiz* et al. 3144 (PMA, MO, FT, SCZ); Reserva Privada Chucantí, camino hacia la cima del Cerro Chucantí, 8°47′31″ N, 78°26′51″ W, 699 m, 21 September 2018 (fruits), *O. Ortiz* et al. 2962 (PMA); Serranía de Majé, Reserva Privada Chucantí, cima del Cerro Chucantí, cerca de la lámina, 8°48′7.93″ N, 78°27′9.36″ W, 1300 m, 13 April 2019 (\bigcirc and \bigcirc flowers), *O. Ortiz 3536* (MO, PMA, UCH).

3.2. Identification and quantification of chemical constituents of C. telenitida

Qualitative characterization of phenolic compounds in the methanol extract of C. telenitida was carried out by UPLC-DAD-MS in negative and positive ionization modes. Multiple approaches, including authentic compounds, UV absorption spectra and ESI-MS data analysis were used for structural identification. Information regarding the phenolic constituents, such as retention time (min), UV absorption bands (nm), molecular formula, observed m/z values and concentration ($\mu g/g$ dried weight) are summarized in Table 1. Among these compounds, four (1, **3. 4** and **7**) constituents were unambiguously identified by comparison with reference standards and seven (2, 5, 6, 8–11) were determined in comparison to previous characterized samples from C. obtusifolia (Rivera-Mondragón et al., 2019a). The UPLC-DAD profile and chemical structures (Fig. 2) showed 11 main peaks corresponding to chlorogenic acid (1) and ten flavone glycosides (2-11). Luteolin, apigenin and diosmetin in the form of mono C-glycosides and di-C,O-glycosides were found to be the main flavones observed in C. telenitida. As shown in Table 1, chlorogenic acid (325.9 \pm 2.1 µg/g) and luteolin *C*-glycosides, such as isoorientin (1265.4 \pm 17.7 µg/g) and isoorientin 2"-O-xyloside $(613.1 \pm 4.7 \,\mu\text{g/g})$ were found to be the major compounds in C. telenitida. In contrast to our earlier findings, malonyl C-glycosides, O-glycosides and flavonolignans were not present in this plant.

3.3. Phytochemical diversity in C. telenitida and comparison between selected congeners from Panama

Ten chemical categories were considered in order to achieve a better understanding of differences and similarities between *C. telenitida* and related species (See Supplementary Fig. S3). Our results showed that out of the six species studied, *C. telenitida* has the lowest content of chlorogenic acid ($325.9 \,\mu g/g$) and of total flavonoids ($2682.0 \,\mu g/g$). Similar to *C. hispidissima*, flavonolignans were not detected in this species. In addition, the amount of luteolin and apigenin *C*-glycosides (1915.8 and $634.4 \,\mu g/g$, respectively) from the leaves of *C. telenitida* were below the average content of the leaves of *C. obtusifolia*, *C. peltata*, *C. insignis* and *C. angustifolia*, but higher to those reported for *C. hispidissima*. On the other hand, the concentration of diosmetin *C*-glycosides in the leaves of *C. telenitida* (131.9 $\mu g/g$) was relatively higher than the content in the leaves of *C. obtusifolia*, *C. peltata*, *C. angustifolia* and *C. hispidissima*, and similar to the average reported for *C. insignis* (137.3 $\mu g/g$).

According to multivariate analysis, three main clusters were observed (Fig. 3). *Cecropia telenitida* was located in Cluster 1 together with *C. obtusifolia* (CO-M1), *C. insignis* and *C. angustifolia*, while *C. peltata* and *C. obtusifolia* (CO-M2) where located in Cluster 2. As reported before, *C. hispidissima* was observed as belonging to Cluster 3 as the most distant species (Rivera-Mondragón et al., 2019a). Inspection of Cluster 1 revealed that *C. telenitida* is more related to *C. insignis* in term of its chemical profile: relatively low concentration or absence of mono *C*glycosides (such as 7 and 12), *O*-malonyl glycosides (20–25) and quercetin *O*-glycosides (26–28 and 31–32), and concentrations in a similar range of isoorientin (6), isoorientin 2″-O-xyloside (3) and apigenin *C*-hexoside-O-pentoside (13).

The average number of chemical constituents detected ranged from 15 in *C. obtusifolia* (CO-M1) to eight in *C. telenitida*. The highest richness of chemical compounds was observed in *C. obtusifolia* (CO-M1), *C. angustifolia* and *C. insignis*; however, *C. telenitida* had the lowest number of constituents (Fig. S4). These results coincide with those obtained using the Margalef index (Fig. S4). According to the Shannon entropy index, the species with the greatest diversity of compounds turned out to be *C. obtusifolia* (CO-M1). Almost all the studied species showed relatively higher diversity values than *C. telenitida* (Fig. S4).



Fig. 1. Cecropia telenitida Cuatrec. (voucher specimen: O. Ortiz et al. 3144). (A) Leaf blade (upper surface). (B) Leaf blade (lower surface). (C) Stipule and pistillate spike. (D) Branches with the staminate inflorescence. (E) Petiole without trichilia. (F) Infructescence. (G) Internode pith. Photo credit: Orlando O. Ortiz.

Table 1

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List of phenolic compounds identified and quantified in the UPLC-DAD-MS and HPLC-DAD profiles, respectively, of the methanol extract of Cecropia telenitida.
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No.	Compound	Retention time (min)	λ_{\max} (nm)	Molecular Formula	ESI negative full MS: [M-H] m/z	ESI positive full MS: $[M-H]^+ m/z$	Concentration (µg/g DW)
Phenolic acids							
1	Chlorogenic acid ^a	5.18	220, 324	$C_{16}H_{18}O_9$	353.0	355.0	325.9 ± 2.1
Flavonoids (Flavones)							
Luteolin glycosides							
2	Isoorientin 2"-O-xyloside ^b	12.55	270, 348	C ₂₆ H ₂₈ O ₁₅	579.1	581.1	613.1 ± 4.7
3	Isoorientin ^a	12.83	270, 348	$C_{21}H_{20}O_{11}$	447.1	449.1	1265 ± 18
4	Orientin ^a	13.04	270, 348	$C_{21}H_{20}O_{11}$	447.1	449.1	37.3 ± 0.6
5	Luteolin C-hexoside-O- pentoside ^b	13.56	275, 335	$C_{26}H_{28}O_{15}$	579.1	581.1	< LOD
Apigenin glycosydes							
6	Apigenin C-hexoside-O- pentoside ^b	15.10	271, 338	$C_{26}H_{28}O_{14}$	563.1	565.0	241.3 ± 4.0
7	Isovitexin ^a	15.47	269, 335	C ₂₁ H ₂₀ O ₁₀	431.0	433.0	393.1 ± 9.5
8	Isovitexin 2″-O-rhamnoside ^b	15.98	273, 338	C27H30O14	577.2	579.1	< LOD
9	Apigenin C-hexoside-O- pentoside ^b	15.98	273, 338	$C_{26}H_{28}O_{14}$	563.1	565.0	< LOD
Diosmetin glycosides							
10	Diosmetin <i>C</i> -hexoside- <i>O</i> -pentoside ^b	16.47	271, 345	$C_{27}H_{30}O_{15}$	593.0	595.0	49.3 ± 0.6
11	Diosmetin <i>C</i> -hexoside ^b	16.99	271, 345	$C_{22}H_{22}O_{11}$	461.1	463.1	82.6 ± 1.5

^a Identification by comparison with analytical standards.

^b Identification by comparison with characterized extracts from *C. obtusifolia* (previouslty described by Rivera-Mondragón et al., 2019. Contents of analytes are reported as mean \pm standard deviation (n = 3). Content below the limit of quantification: < LOQ. Dried weight: DW.



4. Discussion

4.1. Morphological comparison between C. telenitida and its relatives

Cecropia telenitida is characterized mainly by having leaf blades with arachnoid indumentum on the lower surfaces (in two distinct layers) and more or less dense arachnoid indumentum above, 7-10 blade segments and 7-16 lateral veins in the free part of the mid-segment of the blade, absent or present trichilia and large stipules (20–55 cm long). For more descriptive details of this species, see Berg and Franco-Rosselli (2005). According to Berg and Franco-Rosselli (2005), C. telenitida is part of the "Cecropia telenitida-group" that comprises many representatives with the upper leaf surface covered with more or less dense arachnoid indumentum. In this group of species, trichilia can be always absent, occur only in more or less reduced states (mostly without Müllerian bodies), or occur in states of being absent through being more or less reduced to occurring in two patches to well-developed and fused (Berg and Franco-Rosselli, 2005). Due to the presence of arachnoid indumentum on the upper blade surfaces, C. telenitida can be confused with C. telealba Cuatrec. However, the latter differs from C. telenitida in a number of important morphologic aspects: consistent well-developed trichilia and lower leaf blades covered with arachnoid indumentum in the areoles or extending to the smaller veins or also to the main veins (not distinctly in two layers as in C. telenitida) (Berg and Franco-Rosselli, 2005).

The individuals of the Central American population of *C. telenitida* registered in this study usually lack a developed trichilia, but

sometimes they can present villous petioles (in all parts), with long white hairs, arachnoid indumentum and brown pluricellular trichomes at the base (which may suggest the occurrence of trichilia) (see Fig. 4), but never present Müllerian bodies. In Central America, *C. telenitida* is the only species to have blades with dense arachnoid indumentum above, as well as, arachnoid indumentum in two distinct layers on the lower surfaces (Supplemental Data S1). Because this species may lack well-developed trichilia (Fig. 1), it can be confused with *C. pittieri* B.L. Rob. from Cocos Island (Costa Rica). Nonetheless, the latter species differs from *C. telenitida* in having shorter stipules (8–17 cm vs. 20–55 cm in *C. telenitida*) and leaf blades without arachnoid indumentum above, with lamina incisions down to 2/10–3/10 from the margin (vs. 4/10–9/10 in *C. telenitida*).

4.2. Phytochemical constituents in C. telenitida

This is the first collecting evidence of this species in Central America, and first phytochemical characterization and quantitative analysis of leaf extracts for this species. Our results indicated that the content of polyphenols in *C. telenitida* was relatively lower than those reported for other *Cecropia* species from Panama. In a preliminary study (Rivera-Mondragón et al., 2019a), chlorogenic acid and *C*-glycosyl flavones were identified as the main constituents in leaves of *Cecropia* species from Panama. Despite their reduced phytochemical profile, low relative metabolite concentration and complete absence of *O*-glycosides and flavonolignans in the methanol leaf extract of *C. telenitida*, our results indicated other similarities between this species and other



Fig. 3. Multivariate analysis of the phytochemical composition of *Cecropia* species. (A) Hierarchical cluster analysis (HCA) for authentic plant species of *Cecropia* shown as a heatmap. Colors represent the concentration ($\mu g/g$) in the samples from minimum (green) to maximum (red). Numbers are referred to compounds name at supplementary Table S2. (B) Constellation plot. (C) Principal component analysis with K-means clustering.



Fig. 4. Trichilia types in some species of Cecropia from Panama. (A) C. angustifolia. (B) C. heterochroma. (C) C. hispidissima. (D) C. insignis. (E) C. membranacea. (F) C. obtusifolia (Morphotype 1). (G) C. obtusifolia (Morphotype 2). (H) C. peltata. (I) C. telenitida. Photo credit: Orlando O. Ortiz.

relatives such as *C. obtusifolia, C. peltata, C. insignis* and *C. angustifolia* in terms of chlorogenic acid and *C*-glycosyl flavones profiles. These results are similar to those reported for *C. obtusifolia, C. peltata, C. insignis, C. pachystachya* Trécul and *C. hololeuca* Miq. (da Silva Mathias and Rodrigues de Oliveira, 2018; Ortmann et al., 2017; Rivera-Mondragón et al., 2019a).

4.3. Implication of the chemodiversity in chemoecological and chemophenetic aspects

According to these chemodiverse results, the richness in chemical constituents of *C. telenitida* was low and due to the predominant abundance of some chemical compounds (such as luteolin and diosmetin *C*-glycosides), heterogeneity was also found to be low. Consequently, all diversity indexes showed that when comparing the chemical profile of the leaf of *C. obtusifolia* (CO-M1), this was clearly more diverse than the other *Cecropia* species (Fig. S4). In general, the hypothesis about chemical diversity in plants has been associated mainly with the diversity of defensive compounds and as well as the

degree of specialization of herbivores (Richards et al., 2015; Salazar et al., 2016). Latteman et al. (2014), found significant differences in the levels of chemical defenses (tannins or phenolic compounds) between young and mature leaves of C. sciadophylla Mart. (suggesting the possible importance of chemical defenses on herbivory, as compensation by the absence biotic defenses). By contrast, no significant differences in the levels of chemical defenses between young and mature leaves of C. tacuna C.C. Berg & P. Franco (non-myrmecophytic Andean species) and C. membranacea (myrmecophytic lowland species) were found. Taking into account that most Cecropia species (ca. 80%) have a mutualistic relationship with ants that can protect the plant from predators (Davidson, 2005; Marting et al., 2018), perhaps one hypothesis would be that the evolution of mutualism had some impact on the chemical diversity among Cecropia species. Another explanation would be that the low chemical diversity found in C. telenitida may be related mainly to the lack of production of the Müllerian bodies and/or pearl bodies (pearl glands) and not so much to the presence or absence of mutualistic strategies with ants (myrmecophytism). For example, C. angustifolia (with higher chemical diversity than C. telenitida), is a species that

lacks myrmecophytism but produces Müllerian bodies. Perhaps the high chemical diversity in *Cecropia* is related to the production of Müllerian bodies and/or pearl bodies, but to confirm this, additional chemical studies must be done, particularly fractions from these structures, and from other *Cecropia* species.

The results obtained through the multivariate analysis indicated that C. telenitida is more related to C. insignis than the other Cecropia species. These results agree in part with those obtained in the molecular phylogenetic inference made by Gutiérrez-Valencia et al. (2017), where C. telenitida is located in a differentiated clade together with a few myrmecophytes like C. insignis and several non-myrmecophytes Andean Cecropia species (Clade II). It is well known that evolutionary factors related to the colonization of montane habitats could be implicated in the absence of myrmecophytism in Cecropia (Janzen, 1973; Gutiérrez-Valencia et al., 2017). One of the characteristics of this group of nonmyrmecophytes Andean species is that they may lack trichilia and Müllerian bodies (Gutiérrez-Valencia et al., 2017). It is important to mention that the absence of myrmecophytism in Cecropia is not always linked to the absence of these characteristics, since there are nonmyrmecophytism montane species such as C. angustifolia and C. tacuna that usually present trichilia and Müllerian bodies (Janzen, 1973; Latteman et al., 2014).

According to the cluster analysis, C. obtusifolia morphotype 1 (CO-M1) and morphotype 2 (CO-M2) were located at different clusters. These results suggest the existence of inaccurate taxonomic classification in this species. According to Berg and Franco-Rosselli (2005), C. obtusifolia it is a highly variable species, mainly in the number of segments of the leaf blade, as well as in the number of lateral veins in the free part of the mid-segment and in the arachnoid indumentum on the lower leaf surfaces. The differences in the arachnoid indumentum has led to the distinction of the "obtusifolia-type" which is characterized by comprising material with arachnoid indumentum and the "burriadatype" with comprising individuals without arachnoid indumentum (Berg and Franco-Rosselli, 2005). The individuals studied here as C. obtusifolia (CO-M1) share characteristics with the "obtusifolia-type" and C. obtusifolia (CO-M2) lack arachnoid indumentum as the "burriadatype". Other distinguishing characteristics observed in the field is that the "obtusifolia-type" presented thin discolor blades (whitish beneath) and reddish to yellowish veins on lower surfaces, while those of "burriada-type" have sub-coriaceous concolor blades (greenish on both surfaces) and prominent purple to red-purple veins on lower surfaces. The morphological data and the phytochemical evidence presented in this study could provide substantial differences between these two morphotypes described as C. obtusifolia by Berg and Franco-Rosselli (2005). Perhaps these two groups deserve a different taxonomic recognition, but to obtain a more accurate conclusion, it is necessary to analyze individuals from other populations throughout their range of geographical distribution.

5. Conclusions

Based on the morphological characteristics, *C. telenitida* is distinguished from all its Central American relatives in having blades with dense arachnoid indumentum above and arachnoid indumentum in two distinct layers on the lower surfaces. Although the current study is based on a small number of specimens, the phytochemical findings suggest that *C. telenitida* is distinguished from the other congeners analyzed in this work, by lacking flavone *O*-malonyl-*C*-glycosides, flavonol *O*-glycosides and flavonolignans, as well as, by its lower chemical diversity and metabolite concentrations. Due to practical constraints, this paper cannot provide extensive chemophenetic implications. Therefore, the developing of a systematic sampling plan should be further considered in order to obtain better insights and conclusions on the chemophenetic significance for the genus *Cecropia*. Future phytochemical research including multiple plant specimens (e.g. additional *C. telenitida* and *C. angustifolia*), multiple time points (e.g. dry and rainy season), plant age (young and mature leaves), different locations and consideration of their lineage as dioecious trees should be investigated.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2019.103935.

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