P~~hylogenetic~~reliminary placement~~,~~ ~~identity~~ and new records of an overlooked Amazonian tree ~~species,~~ *Christiana mennegae* (~~Brownlowioideae,~~ Malvaceae). ~~in the Amazon~~

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Abstract

~~Considering that collecting and knowledge gaps persist still persist in the Amazon, it is vital to promote access to existing studies and data through the development of all-inclusive online databases, if we are to accelerate the studies of biodiversity in the region.~~ *Christiana mennegae* is a phylogenetically enigmatic taxon and is a case in point of a species whose presence ~~that~~ escaped the radar of the Amazon lists and the Brazilian Flora project, and we expand ~~expanded the~~ its distribution ~~of the species~~ by adding new records from Peru and overlooked ones from Brazil~~ and Peru~~. T~~In~~ o ~~order to~~ investigate its phylogenetic placement in the Brownlowioideae (Malvaceae)~~Browloideae subfamily~~, part of the rbcL ~~region~~ gene of the plastid and the intergenic ITS2 region~~s~~ were sequenced. Macro- and micromorphological investigation of ~~its~~ features of *C. mennegae* using SEM ~~stu~~of~~dies for~~ foliar~~leaf~~, flower, fruit and seed structures are presented. The morphology of trichomes revealed five types of trichomes ranging from glandular to branched and unbranched and we also report stomata on the seed surface for the first time in Brownlowioideae. *Christiana mennegae* and *C. africana* were recovered as sister

Comentado [A1]: Why enigmatic? What is the enigma? The unknown phylogenetic placement is not a enigma.

Comentado [A2]: "investigation are presented" is odd. Also, the verb tenses should be standardized.

Comentado [A3]: Tector trichomes?

species in the phylogenetic analysis, albeit with low to moderate support, and more species of this and closely related genera must be sampled and analyz~~s~~ed in order to obtain a clearer picture of the group's affinities and ~~inter~~relationships. W~~Our efforts bringe an~~ provide an update after 20 years of the conservation status of Vulnerable for Least Concern. We also highlight the need for investment in the digitiz~~s~~ation of biological collections and the availability of online literature to speed the study of Amazonian plant diversity.

Comentado [A4]: I suggest highlighting also the need for investment in training botanists and examine herbarium material as claimed in literature.

Keywords: Barcode, Malvales, rain forest, SEM, vascular plants.

Introduction

The exploration of the Amazon rainforest has sparked ~~sparkled~~ scientific curiosity since the travels of naturalists such as Alexandre Rodrigues Ferreira, La Condamine, Spix and Martius, Schomburgk, Spruce, Wallace and Bates (La Condamine & Godin des Odonais, 1808; Ferreira et al., 2008; Spix & Martius, 2013; Hemming, 2015; Schomburgk & Roth, 2017). The stunning variety of life forms, coupled with the difficulty of accessing ~~to access~~ vast areas of the biome, has led to repeated efforts to quantify its diversity (Black~~, Dobzhansky & Pavan,~~ et al. 1950; Pires~~, Dobzhansky & Black,~~ et al. 1953; Gentry, 1988; Hubbell et al., 2008; Milliken et al., 2010; ter Steege et al., 2016; Cardoso et al., 2017). Recent studies indicate that the lowland Amazon region is home to 14,003 seed plant species (Cardoso et al., 2017). Despite the global importance of the Amazon Forest, the information deficit is still high, and knowledge is fragmentary, with vast areas still poorly known (BFG, 2018; Hopkins, 2019), ~~and~~ with some of them already destroyed (Piontekowski et al., 2019). The number of species based on herbarium data and/or large online datasets has changed little and spatial collecting gaps ~~still~~ persist (Forzza et al., 2012; ~~Brazil Flora Group [~~BFG~~]~~, 2015; Hopkins, 2019) .

Comentado [A5]: Who Spruce?

Formatado: Realce

Formatado: Realce

Attempts to accelerate the ~~taxonomists~~ work to describe ~~in describing~~ the Amazon's ~~underestimated~~ plant diversity include the development of massive online data repositories (Canhos et al., 2014; Morim & Lughadha, 2015; BFG, 2018). Both herbarium collections and literature sources have been included in this endeavor (Ebach~~, Valdecasas & Wheeler~~ et al., 2011; Funk, 2018) and, in the age of big data, a plurality of sites and online libraries help to minimiz~~s~~e this taxonomic impediment (Schonfeld, 2003; Gwinn & Rinaldo, 2009). However, botanical studies are still scarce and do not cover the taxonomic complexity of a megadiverse biome such as the Amazon (Hopkins, 2019). Access to existing studies is essential for the advancement of this knowledge.

The ~~pantropical~~ genus *Christiana* DC. (Malvaceae, Brownlowioideae), which comprises~~,~~ ~~which has~~ five species worldwide ~~in the world, with four of them~~ mostly in tropical regions~~, species~~ is an example of this situation. Described by De Candolle (1824), the type of the genus, *Christiana africana* DC., is a ~~pan~~tropical tree with similar distribution to the kapok tree (*Ceiba pentandra* (L.) Gaertn.). A second species, *C. macrodon* Toledo (1952), was described from the Atlantic Rainforest of the state of São Paulo. Kubitzki (1995)~~,~~ widened ~~widening~~ the circumscription of the genus to include two other Neotropical species (*C. mennegae* (Jans-Jac. & Westra) Kubitzki and *C. eburnea* (Sprague) Kubitzki, ~~— formerly *Asterophorum* Sprague)~~ as well as the~~a~~ Polynesian ~~species~~~~taxon,~~ *C. vescoana* (Baill) Kubitzki~~) — formerly *Berrya* Roxb.)~~.

Despite the fact that Kubitzki (~~Kubitzki,~~ 1995) mentioned the occurrence of *Christiana* ~~*C.*~~ *mennegae* in Brazil, this ~~fact~~ was overlooked ~~not picked up~~ by successive editions of the Catalog of Brazili~~l~~an Plants and Fungi (Forzza et al., 2010), List of Species of the Brazilian Flora (Lista de Espécies da Flora do Brasil, 2008–2015), BFG 2015 (Brazil Flora Group [BFG], 2015) and ~~during the construction preparation of the~~ Brazilian Flora 2020 (Flora do Brasil, 2020~~under construction~~). In addition, ~~Inadvertently,~~ Secco (~~Secco,~~ 2000) recorded *Asterophorum mennegae* Jans.-Jac. & Westra for the state of Pará, although ~~this~~ his publication also was ~~sadly~~ overlooked. ~~Also,~~ *Christiana*~~.~~ *mennegae* is not listed ~~on~~ in the Amazon tree list (ter Steege et al., 2019), nor was it recorded in the list of Amazonian seed plant species (Cardoso et al., 2017). All ~~this~~abovementioned factors ~~makes~~ collaborated to the presence of this species ~~remain~~ in the Red List since 1998 (World Conservation Monitoring Centre. 2017).

Morphological studies with other ~~genus~~genera? of Brownlowioideae have explored characteristics such as seed coat and the type of trichome on the leaf surface for the delimitation of species (Chung et al. 2012), which is still unknown in *Christiana*. In addition, phylogenetic sampling of the Brownlowioideae is still limited to only one species per genus, with the genus *Christiana* ~~being~~ represented ~~sampled~~ only by *Christiana africana* DC. (Hernández-Gutiérrez & Magallón 2019). Thus, *Christiana mennegae* is another rare ~~and~~, phylogenetically enigmatic~~,~~ and poorly understood tree species from~~unexplored in~~ the Amazon (Cardoso et al. 2015). During fieldwork linked to a project to study the natural capital of the Amazon forest in the region of Carajás,

Comentado [A6]: What does literature indicate the placement of Christiana in Malvaceae and Brownlowioideae??
What are the morphological evidence for that??

Comentado [A7]: This is clearly related to the historical lack of specialists in Malvaceae from Brazil. I cannot see much importance in this taxon being overlooked by general floristic lists (e.g., ter Steege et al. or Cardoso et al.) because this lack would be expected.
I would consider more helpful to highlight/argue for the importance of greater investment in training botanists (see, for example, Ahrends et al., 2011 in Biological Conservation) and examine extant herbarium collections (e.g., Bebber et al. 2010 in PNAS) to disclose diversity of plants.

Comentado [A8]: Please provide correct citation.

Comentado [A9]: The citation format (e.g., use of commas) needs to be standardized throughout the mss.

Comentado [A10]: It would be fair to cite former phylogenetic papers on Malvaceae (e.g., Alverson et al., 1999; Bayer et al., 1999; Nyffeler et al., 2005) especially that produced sequences for Brownlowioideae taxa.

Comentado [A11]: I cannot see the reasonament for “enigmatic”.

our attention was drawn to the existence of an unidentified malvaceous tree by DS, one of the authors of this paper, at the Parque Zoobotânico in the Floresta Nacional de Carajás (FLONA Carajás) that turned out to be *C. mennegae*.

~~In order to~~With the purpose of clarifying and complementing the knowledge regarding ~~*C.*~~ *Christiana mennegae*, we provide additional data on its morphology and phylogenetic affinities to~~for~~ ~~,~~ establish~~ing~~ with certainty its presence in ~~the Brazilian and Peruvian territories~~Brazil and Peru.~~3~~

Comentado [A12]: It is not clear from introduction what morphological evidence lead authors to identify material as the belonging to subfamily Brownlowioideae of the Malvaceae. It would be informative and helpful to have (diagnostic?) morphological characterization of this poorly known subfamily in Brazil.

Comentado [A13]: Based on combination of characters X, Y, Z...(fused sepals? Presence of petals? Free stamens? staminal thecae divergent at the base? Presence of staminodes? etc.)

No characterization of the genus was provided above so I wonder what characters allowed authors recognize it. Providing that would contribute to understanding of the subfamily which is poorly known in Brazil.

Material and methods

Taxonomic treatment. The m~~M~~orphological description~~s~~ was~~ere~~ mostly based on herbarium material supplemented by measurements from living plants collected in recent expeditions to the Floresta Nacional de Carajás (FLONA Carajás/63324-1), Pará state, Brazil. Herbarium material ~~has been~~was rehydrated to dissect and illustrate reproductive structures. We analyzed specimens from the HCJS and MG and searched the web ~~web search~~ for records available ~~for in the~~in REFLORA (~~REFLORA,~~ 2020), Tropicos (~~Tropicos,~~ 2020) and~~,~~ Virtual Herbarium of Flora and Fungi of Brazil (INCT, 2020). We found ~~recovered~~ specimens deposited in ~~from~~ F, IAN, INPA, K, MG, MO, P, RB, U and VEN herbaria (acronyms according to Thiers 2020, continuously updated). A total of~~Totaling~~ three new physical specimens and ten digitally imaged~~s~~ specimens were located. Morphological terminology follows Harris & Harris (~~Harris & Harris,~~ 2001) and Radford et al. (~~Radford et al.,~~ 1974).

Distribution map and Conservation status. The distribution map was prepared using QGIS (QGIS Development Team, 2016) with coordinates obtained based on information available from herbarium labels. All studied specimens~~ examined~~ were plotted on the map. The IUCN conservation status (IUCN, 2012) was ascertained using the GeoCat Tool (Bachman et al., 2011).

SEM. Petioles, abaxial surface of mature~~the~~ leaf-blade~~s~~ ~~leaf of leaves mature~~, external surface of the calyx, epicarp and seed ~~Vegetative and reproductive structures~~ were ~~as~~ dried~~y~~ and extracted from voucher specimens deposited at MG. ~~–~~These structures were~~were~~ mounted on stubs using press-on, double-sided tape tabs, ~~subjected to analysis under~~examined with a Scanning Electron Microscope (SEM) and imaged in Zeiss Sigma VP microscope under full vacuum at the Laboratório de Microanálises of the Universidade Federal do Pará (IG - UFPA). It was not necessary to ~~The~~ critical-point dry them ~~dried was not needed~~. The trichomes were described according to Theobald et al. (~~Theobald, Krahulik & Rollins,~~ 1979). The images were ~~adjusted~~ falsely colored using Photoshop CS4 (Adobe).

Comentado [A14]: These are results, not methods.

Comentado [A15]: Voucher information should be provided. It would be nice if the same specimen(s) sampled for the molecular study.

Comentado [A16]: How many specimens? How many structures from each specimen?

Sampling, DNA Extraction, PCR Amplification, Sequencing and Phylogenetic analysis. For this study, the subfamilies Bombacoideae, Brownlowioideae, Byttnerioideae, Dombeyoideae, Grewioideae, Helicterioideae, Malvoideae and~~,~~ Sterculioideae ~~and Tilioideae~~ ~~are~~were represented by sequences ~~of 239 species belonging to Malvaceae *s.l.*,~~ published by Hernández-Gutiérrez & Magallón (2019). *Christiana mennegae* was newly sequenced for this study and included in the aligned matrix (Zappi et al. 4562 - MG). The outgroup included species of Bixaceae, Cistaceae, ~~Dipterocarpaceae,~~ Neuradaceae~~,~~ ~~Sarcolaenaceae, Sphaerosepalaceae~~ and Thymelaeaceae. The voucher specimen information and the GenBank accession numbers of the sequences of *C. mennegae* used and generated in this study are listed in the supplementary material.

Comentado [A17]: They are provided just below.

We reconstructed a Malvaceae *s.l.* phylogenetic tree in order to ~~establish~~ infer the placement of *Christiana~~.~~ mennegae* using ~~eight~~ five molecular regions, ~~six~~ four from the plastid genome (*rbc*L, *atp*B, *trn*K-*mat*K, ~~*trn*H-*psb*A, *trn*L-F,~~ *ndh*F), ~~one from the mitochondrial genome (*mat*R)~~ and the nuclear ITS2 from the 35S rRNA ~~ITS2~~. For *C. mennegae*, sequences of ITS2 and *rbc*L were newly obtained ~~anew~~ and deposited in the GenBank database under the accession numbers MT741784 and MT742086, respectively. For a detailed description? of automated DNA extraction protocol see electronic supplementary material.

Comentado [A18]: Delete text? See previous comment.

~~Young leaf tissues of *C. mennegae* were collected in 2% CTAB-NaCl saturated buffer (Rogstad, 1992), then the automated DNA extraction protocol with QIAcube HT (Qiagen) was followed using the 'Q protocol V1' of the QIAamp 96 DNA Kit (Qiagen), according to manufacturer's instructions. The PCR conditions followed Ramalho et al. (Ramalho et al., 2018) and the bidirectional sequencing reactions were performed in an ABI 3730 DNA Analyzer (Applied Biosystems), with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher). Forward and reverse reads obtained for each marker were assembled in a consensus sequence with PIPEBAR (Oliveira et al., 2018), using the recommended settings for coding (*rbc*L) and intergenic (ITS2) regions.~~

The alignment of *Christiana~~.~~ mennegae* DNA sequences was performed manually in the aligned matrix by Hernández-Gutiérrez & Magallón (2019). Bayesian Inference (BI) and Maximum Likelihood (ML) methods were used for the phylogenetic reconstruction. Partitioned BI analyses were performed using MrBayes version 3.2.3 (Ronquist et al., 2012), with DNA substitution models selected by Hernández-Gutiérrez

& Magallón (2019) (GTR + I + G). Further details on phylogenetic analysis can be also found in electronic supplementary material. ~~Markov Chain Monte Carlo (MCMC) analyses were run on MrBayes for 1050,000,000 generations, starting with a random tree and sampling every 15,000 generations. The first 25% of the trees were discarded as the burn-in, and the remaining trees were used to generate a majority-rule consensus tree. Clades with posterior probability values (PP) ≥ 0.95 were considered as well supported (Eriksson, 2001; Alfaro, 2003; Kolaczkowski & Thornton, 2007). ML tree searches and bootstrap estimates of clade support were conducted with RAxML (Stamatakis, Ludwig & Meier, 2005), using the GTR substitution model with gamma-distributed rate heterogeneity among sites and the proportions of invariable sites estimated from the data. For the ML analyses, clades presenting bootstrap support values (BS) ≥ 75% were considered as strongly supported (Hillis & Bull, 1993). Both BI and ML analyses were performed at the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010).~~

Results

Taxonomic treatment

Christiana mennegae (Jans.-Jac. & Westra) Kubitzki, Bot. Jahrb. Syst. 116~~-~~(4): 541. 1995. ≡ *Asterophorum mennegae* Jans.-Jac. & Westra, Proc. Kon. Ned. Akad. Wetensch., B 86(3): 377. 1983.

Type. Suriname, ~~Sipaliwini savanna area on the Brazilian frontier,~~ Sipaliwini, “Morro Grande” camp~~-~~ – forest island, 6 km W of “Morro Grande” dome, Sipaliwini savanna area on Brazilian frontier, 04 nov 1968 (lf, fl, fr), *F.H.F. Oldenburger, R. Norde & J.P. Schulz ON415* (holotype: U [U0006902; digital image!], isotypes: ~~U [U0006903; digital image!],~~ K [K000381142; digital image!], MO [not seen], NY [NY00415374; digital image!], P [P02143012; digital image!], U [U0006903; digital image!], VEN [VEN409940; digital image!]). (Figs. 1,2,~~.~~3,4 and 5).

Description. *Trees* ~~*Dioecious trees*~~ 5–17 m tall, *functionally dioecious*; external bark in male plants shedding as thin scales, female plants with conspicuous and raised lenticels. *Branches* cylindrical, lenticellate, with sparse stellate trichomes. *Stipules* 2.8–3.8 mm long, linear, apex acute, with stellate trichomes. *Leaves* alternate, spirally arranged at branch apices?; petioles 1.8–5.6 cm long, with scattered minute stellate, sessile trichomes???, pulvinate at both ends; leaf-blades chartaceous, 12.3–22~~-~~(–29) × 5–8(–9) cm, narrowly elliptic, lanceolate or oblanceolate, apex long acuminate, caudate or

Comentado [A19]: Need to designate a lectotype, see below.

Comentado [A20]: It is a political division (district) of Suriname and should be here just after Suriname.

Comentado [A21]: Line drawing shows a morphologically bissexual flower.

Formatado: Fonte: Itálico

Formatado: Fonte: Itálico

cuspidate, base rounded or obtuse, margin entire or rarely slightly erose, adaxial surface shiny when fresh/dried?, glabrescent except for scattered stellate, sessile trichomes on the veins, abaxial surface glabrescent or with scattered glandular trichomes~~,~~ and stellate, sessile??? trichomes ~~stellate~~ on the veins; venation eucamptodromous, 5–6 pairs of secondary veins, veins abruptly spaced out distally. *Inflorescence* axillar, cymose, ~~paniculate, axillary,~~ congested, 3.6–4(–5.5) cm long, 3–8 flowers per inflorescence; stellate trichomes on peduncle???, bracts and bracteoles 3–1.2 mm long, triangular, with lateral linear projections, stellate trichomes on both surfaces, floral bracts 2–2.7 × 0.3–4 mm, entire, linear~~, stellate trichomes~~. Flowers functionally unisexual, pedicellate; calyx gamossepalous, fused by half?? of its length", calyx lobes three??, externally green, with stellate trichomes, internally glabrous, ~~green,~~ subglobose to campanulate; corolla dialypetalous, white, petals obovate to oblanceolate or elliptic, glabrous on both ~~sides~~surfaces. *Staminate flowers:* pedicels 2.5–4 mm long; flowers ca. 8 mm long; ~~pedicels 2.5–4 mm long;~~ calyx (3–)4–merous, c. 2.2 × 3 mm; corolla 5–7-merous, petals 5.5–7 × 1.8–3.5 mm; stamens 63–67, ~~2.5–3 mm long,~~ the inner ones longer than the outer ones, glabrous, filaments 2.5–3 mm long, connate by 1 mm long~~eae~~. ~~1 mm long~~, anthers 0.5–0.8 mm?? long. *Pistillate flowers*: pedicels c. 4 mm long; flowers c. 6(–7) mm long; ~~pedicels c. 4 mm long;~~ calyx (3–)4–5-merous, 1.5–2.8 × 1.5–2(–4) mm; corolla 5–7-merous, petals 5–7.5 × 2–2.8 mm; staminodes 2–2.7 mm long, anthers c. 0.3 mm long; gynoecium syncarpous??, c. 4.2 mm long, ovary c. 2 × 2 mm, shape??, number of carpels?, trichomes stellate, style c. 1.8 mm long, trichomes stellate, stigma 5, c. 0.6 mm long, ovules c. 0.5 mm long. ~~*Capsule* 5-locular,~~ *Fruit* woody, apocarpous, 1.2–1.8 cm long, c. 3.8 cm diam., ~~transversely ellipsoid,~~ externally with stellate trichomes, composed of 5 loculicidal, one-seeded folicles~~dehiscence loculicidal, locules one-seeded~~; seed obovate, 8–9 × 6–7 mm, glabrous, medium brown with darker irregular marks.

Nomenclatural notes: *Christiana mennegae* was described ~~based upon~~from a single collection;~~,~~ however, there are two separate sheets at herbarium U. Although Jansen-Jacobs & Westra (1983) did not specify which sheet should ~~would~~ be the type, they published a photo of the specimen that nowadays bears barcode U0006902 as the holotype, therefore it ~~will not be~~ is un~~-~~necessary to ~~proceed to a lectotypification~~ ~~a~~propose a lectotype.

Distribution and habitat: *Christiana mennegae* was first known from Suriname and Brazil (Jansen-Jacobs & Westra, 1983; Kubitzki, 1995; Secco, 2000)~~(Jansen-Jacobs & Westra 1983; Kubitzki 1995; Secco 2000)~~ ~~but~~ and here we record it for the first time in

Comentado [A22]: Illustrated as cordate in the "c" item of line drawing

Comentado [A23]: "Rounded TO obtuse" in the supplementary material.

Comentado [A24]: Are authors sure about the morphology of the inflorescence of Christiana (formerly Tiliaceae)? The line drawing shows immature inflorescence only which does not appear to be paniculate, but a cimose one closely similar to the "bi-color unit" described by Bayer (1999 in Pl Syst Evol) for core Malvales. Bayer et al. (1999 in Bot J Linn Soc) indicated inflorescences of Brownlowioideae "as probably at least sometimes in many-flowered bicolor units". So I would suggest authors to double-check the inflorescence morphology of Christiana mennegae.

Formatado: Realce

Formatado: Realce

Comentado [A25]: You state bracts and bracteoles are triangular at the line just above.
I could not understand the difference between floral (linear) bracts and (triangular) bracteoles.

Formatado: Realce

Formatado: Realce

Comentado [A26]: Repeated information

Comentado [A27]: Line drawing shows both morphologically unisexual and bisexual flowers.

Formatado: Realce

Comentado [A28]: Better stated before flower length if these measurements are not included in the flower length.

Comentado [A29]: Are authors sure about staminodes? what are the evidence (including literature)?

Comentado [A30]: It is not a capsule! Carpels are free like in Christiana africana! Almost like in Sterculia! See Edlin (1935: p. 16): "The form of fhe fruit, however, is strongly suggestive of the tribe Sterculieae; it is of 3-5 free woody carpels, each of which contains a solitary seed;".See also Bayer et al. (1999: 287): "Some Brownlowioideae are described as apocarpous or have at least free carpels in fruit, which is also reminiscent of Sterculioideae" [i.e., secondary apocarpy]. See also Tschá et al. (2002: p. 5) in Hoehnea: "Fruto múltiplo...com 4-5 folículos, bivalves..")
Both Christiana africana and C. mennegae are morphologically similar.

Comentado [A31]: "Depressed-globose" in the supplementary material.

Comentado [A32]: According to Turland et al. (2018; Art. 7.10) the ICN requires that a type designation be effectively published. The mere publication of a photo of a specimen with no indication of "typus" or equivalent term does not warrant type designation (Art. 7.11).Therefore, in my view, it is necessary to effectively publish this (second-step) lecotypification.

Peru. This new record is a significant extension of its range, nearly 1,700 km from the ~~next~~ nearest recorded occurrence of the species (Fig. 1). This species can be found growing in island of evergreen seasonal forest in savannas in Suriname and ~~in~~ ombrophyllous rainforest at up to 600 m elevation in Brazil. In Peru, *C. mennegae* grows in wet forest in the central region, from~~at~~ 500 to~~–~~ 600 m elevation. According to Cruz & Taylor (2018), ~~this area~~the central region of Peru has a complex geology and its botany is yet little explored. The specimens listed by Secco (2000), deposited in IAN herbarium, unfortunately could not be found based on online searches??.

Phenology: The species apparently flowers at the onset of the wet season and has been collected with flowers in September, October and January and with fruits in July, September, November and January~~and fruits in September, November and January and only fruiting in July.~~

Comments: *Christiana mennegae* shares syncarpous fruits with the Neotropical species *C. eburnea* (Sprague) Kubitzki and *C. macrodon* Toledo~~ the syncarpous fruits,~~. A complete morphological comparison between the species of *Christiana* is ~~found~~presented in a table in the electronic supplementary material. ~~These species~~~~although they are~~ *Christiana mennegae* can be distinguished by the~~ir~~ narrowly elliptic, lanceolate or oblanceolate leaf-blades (*vs*. ovate or widely ovate to circular) with rounded or obtuse base (*vs*. truncate to subcordate or cordate). *C.~~.~~hristiana mennegae* also differs from *C. eburnea* by depressed-globose fruits (*vs*. subturbinate) and from *C. macrodon* by leaf-blades with entire or rarely slightly erose margins (*vs*. sparsely dentate). From *C. africana*, the most widespread species of the genus (Kubitzki, 1995), *C. mennegae* can be easily distinguished by the leaf-blades that are ~~(~~narrowly elliptic, lanceolate or oblanceolate, with rounded or obtuse base (*vs*. widely ovate with cordate base) ~~and fruits (syncarpous vs. apocarpous)~~ (Fig. 2). In addition, according to the results here obtained, *C. mennegae* is the only species to present glandular trichomes on the leaf-blades, a character that distinguishes it from the other four species of the genus. The presence of up to seven petals in *C. mennegae* was not recorded until the present analysis.

Besides the morphological characters mentioned, *C~~.~~hristiana mennegae* can be separated geographically from the other species. While *C. mennegae* is currently known from Suriname, northern Brazil (Amazonas and Pará states~~, northern region~~) and Peru, *C. eburnea* is ~~found~~ known only ~~at~~from the type locality in Ecuador, and *C. macrodon* only ~~in~~from Southeastern Brazil (São Paulo state) (Sprague, 1908; Toledo, 1952; Kubitzki, 1995). *Christiana africana* ~~has the widest geographic distribution among *Christiana*~~

Comentado [A33]: “Evergreen seasonal forest in savannas” and “evergreen seasonal forest in omborphyllous rainf forest” sounds odd. To me evergreen and seasonal are oppositive. Also, “ombrophyllous rainforest” is redundant. In which bibliography is this based on? What the rationale?

Comentado [A34]: Based on online search? Or what?

Comentado [A35]: Fruits of C. africana and C. mennegae are apocarpous. Please compare them and compare with other apocarpous Malvaceae (e.g., Sterculia). Please check literature about Malvaceae (e.g., Edlin, 1935; Bayer et al. 1999; Tschá et al., 2002).
Also, information on morphology of the taxonomic group should appear in the introduction, not only here.

Formatado: Fonte: Itálico

Comentado [A36]: “to” in the table.

Comentado [A37]: Not in the description.

Comentado [A38]: “turbinate” in the table

Comentado [A39]: “to” in the table

Comentado [A40]: Somewhat cordate in the line drawing

Comentado [A41]: Fruits of both species are apocarpous!

Comentado [A42]: Is there evidence in the literature for the investigation of trichomes on leaves of the other four species?
Are authors convinced that there is no glandular trichomes on the leaves of the other four species?

If not, I would suggest modify this statement to something like “In addition, the presence of glandular trichomes on leaves is firstly reported for the genus and may help to distinguish C. mennegae from the other four species of Christiana known to date”.

~~species,~~ occurr~~sing~~ in Africa, Mexico, Central and South America (Brazil, Ecuador, Guianas and Venezuela~~ and Guyanas~~) and Madagascar (Kubitzki, 1995; Tschá et al., ~~Sales & Esteves,~~ 2002; ~~Door~~Dorr et al., ~~Jansen-Jacobs & Meijer,~~ 2007)~~, however no records of sympatry between it and *C. mennegae* have been found thus far~~. Several branches of the specimens were examined in the field to be sure that the species is functionally dioecious.

Conservation status: The last update of *Christiana mennegae* took place in 1998 and classified the species as vulnerable (World Conservation Monitoring Centre~~.~~, 2017). *Christiana mennegae* occurs in two protected areas in Brazil, in the Parque Estadual da Serra do Aracá in the Amazonas state and the Floresta Nacional de Carajás in the Pará state. In Suriname it occurs in the forest islands in savanna of the Sipaliwini Savanna Nature R~~Pr~~eserve. Despite appearing to be locally rare, this species spans throughout the Amazon basin, with ~~a~~an EOO of more than 1,000,000 km², so *C. mennegae* should be classified as Least Concern (LC) according to the IUCN (2012) criteria. It was not possible to estimate the size of the populations. However, with the ~~presente~~present data it has become possible to remove this species from the R~~r~~ed L~~l~~ist (World Conservation Monitoring Centre~~.~~, 2017).

Specimens examined: BRAZIL. Amazonas: margem de um igarapé que nasce na Serra de Aracá, 28/07/1977, fr., *N.A. Rosa & M.R. Cordeiro 1699* (RB00059283 [digital image]). Pará: Marabá, [Mun. Parauapebas], Serra dos Carajás, 29/11/1988, fr., *N.A. Rosa & F.C. Nascimento 5084* (K001214118 [digital image]); Mun. Parauapebas, FLONA de Carajás, imediações do Parque Zoobotânico, fl./fr., 06/09/2018, *D.C. Zappi et al. 4562* (MG); Parque Zoobotânico, fl., 05/10/2019, *R.G. Barbosa-Silva et al. 1424* (MG); Nova-Canaã dos Carajás [Canaã dos Carajás], 06/01/2001, fl./fr., *L.C.B. Lobato 2624* (MG163877); PERU. San Martin: Mariscal Cáceres, Tocache Nuevo, 15/11/1972, fr., *J. Schunke Vigo 5516* (F1780039 [digital image], MO, NY).

Micromorphology study

The five types of sessile trichomes observed in C~~.~~hristiana *mennegae* can be grouped under non-glandular and glandular types, as characteriz~~s~~ed by Theobald et al. (1979). The ~~only~~ glandular type is found both on petioles and sparsely distributed on the lower surface of the leaf-blade (Fig. 3 A–C). A detailed description of non-glandular trichomes can be found in electronic supplementary material, micromorphology

Comentado [A43]: This information was given in the paragraph above

Comentado [A44]: I have found evidence for sympatry between Christiana africana and C. mennegae: Tschá et al. (2002 in Hoehnea) cites five collections from different municipalities in the state of Pará, including Rio Vermelho which is c. 50 km east from Canaã dos Carajás.

Comentado [A45]: This can be better placed in Methods as "to be sure about the monoecy/dioecy of the species".

Comentado [A46]: Mss data and map do not show this species spans through the Amazon basin!! Please look at your map: at that scale, this species is known from only four localities (/five municipalities)!! And only seven collections!!

Comentado [A47]: What about AOO?? Why use EOO only??

Comentado [A48]: Please double-check the criteria carefully!!

Comentado [A49]: Which criteria? EOO only? What about number of locations sensu IUCN (2012) and number of collections/known (sub)populations??

Comentado [A50]: In favor of what?? This is a dioecious species known from only seven collections!! If we are not concerned about that, who will?!

study.~~There are four types of non-glandular trichomes, which can be either classed as unbranched (simple and two-armed trichomes) or fall into two different branched categories (stellate-rotate and stellate-multiangulate trichomes). Both simple and two-armed trichomes were observed only on petioles (Fig. 3 A), whereas stellate-rotate are observed in petiole and vascularization on abaxial face (Fig. 3 A–C, fig. 3 C). Stellate-multiangulate trichomes were observed only on reproductive structures, on the external surface of the calyx and fruit (Fig. 2 F, Fig 3 D-F). It is important to notice that while the trichomes are sparse in most parts of the plants, they are concentrated only in the reproductive structures (Fig. 3 A-F).~~

The endocarp of the fruit is smooth and lacked ~~had no~~ stomata (Fig. 3 E). Stomata were observed on the seed surface~~s~~, on both textures of testa surface (Fig. 3 G–H) and were sparsely distributed in the analyzed material.

Comentado [A51]: ????

Phylogenetic relationships

The tree topology with combined dataset (nuclear and plastid) in both ~~analyses~~ ~~analysis~~analyses showed that *C~~hristiana.~~* *mennegae* ~~appears~~ is ~~as a~~ sister to *C. africana*, although only the ML analysis resulted in a highly supported monophyletic branch (BS = 86; PP = 0.78)~~the only previously available species sampled for this genus~~ (Fig. 4). ~~However,~~As there are sequences available only for these two species of the genus, any further ~~the low statistical support values observed here (0.58 PP/ 72 BS) do not permit any further~~ conclusions on the relationships within *Christiana* are difficult to reach~~about the *Christiana* monophyly of *Christiana*~~. In addition, our results~~,~~ revealed *Christiana* in a strongly supported clade including the sampled species of *Berrya* and *Cardioptera* (BS = 78; PP = 1), although with no clear relationship patterns among the genera (Fig. 4)~~the genus emerged weakly supported as sister to *Carpodiptera* (0.85 PP/69 BS), thus constituting a clade sister to *Berrya* with strong support values (1 PP/ 68 BS)~~. Both ~~analyses~~ ~~analysis~~analyses recovered the same supported clades (see ~~maximum likelihood~~ML tree in *electronic supplementary material*).

Comentado [A52]: Term "Both" requires "analyses" (plural) not "analysis" (singular).

Comentado [A53]: No, you should not say that because you are not testing that!
Based on your sampling they ARE GOING TO BE sister groups in any analysis you perform as they belong in Christiana based on morphology and there is no other Christiana species in your tree!
So, you can say something like: "Christiana mennegae emerged in a clade with C. africana, the only other species of Christiana molecularly sampled to date, as we expected based on their morphology".

Discussion

Jansen-Jacobs & Westra (~~Jansen-Jacobs & Westra,~~ 1983) described *Asterophorum mennegae* Jansen-Jacobs & Westra based on *Oldenburger et al.* 415 from Suriname. Later, Kubitzki (1995) expanded the circumscription of *Christiana* to include the ~~genus~~ genera *Asterophorum* (2 spp.) and *Tahitia* (1 sp.). Kubitzki (1995) recorded the occurrence of *A. mennegae* in Brazil for the first time. Although Kubitzki mentioned some vouchers from Amazonas and Pará states, ~~these materials~~the specimens have not been properly identified in ~~the~~ herbaria collections and were overlooked by Secco (2000), who reported the occurrence of *Asterophorum mennegae* as the first record of the species for the Brazilian Amazon~~,~~; ~~however~~ nevertheless, this publication was overlooked by ~~following~~ subsequent authors. Kubitzki's (1995) work is not easily accessible in Brazil and has not yet been made available in online libraries~~, therefore~~ and this could explain why Secco (2000) did not use Kubitzki's (1995) circumscription. Secco (2000) is not available in online repositories either. Coutinho (2020~~19~~) used online databases such as SpeciesLink and JABOT (http://rb.jbrj.gov.br/v2/consulta.php) and recorded only *C. africana* and *C. macrodon* in the Lista de Espécies da Flora do Brasil. In addition, the difficulty accessing ~~to access~~ Kubitzki (1995) and Secco (2000) maybe contributed to the deficient knowledge of *Christiana* until the present moment. The case of *C. mennegae* is an example of the taxa being overlooked that also highlights~~and~~ the collecting gaps in the Amazon. Similar floristic projects also ~~Other point collections also~~ reported dozens of new records and also species overlooked in the Amazon (Barbosa-Silva et al. 2016, Sierra et al. 2019).

S~~The s~~tomata on seed surfaces are relatively uncommon (Paiva~~, Lemos Filho & Oliveira~~et al., 2006), although ~~being~~ recorded for several unrelated plant families (Jernstedt & Clark, 1979), and the presence of such structures in *C. mennegae* is the first report for the ~~subfamily~~ Brownlowioideae. On the other hand, the presence of stomata in Malvaceae seeds has been reported in other subfamilies, such as Sterculioideae, Bombacoideae and Malvoideae (Corner, 1976). The variation on seed surface and indumentum in other groups of Brownlowioideae has already shown to be of taxonomic importance, such as presence of trichomes on the seeds in *Jarandersonia* (Chung et al., ~~Tan & Soepadmo,~~ 2012). ~~Besides being a stable character, t~~The presence of stomata in seeds may contribute to the taxonomy of the group~~, being previously~~ and was used in conjunction with other seed surface characters to recognize a new taxon in *Jarandersonia*~~,~~ ~~in~~from Borneo ~~(Asia)~~ (Tan et al.~~, Chung & Soepadmo~~, 2011). However, there is an example in the genus *Iris* L. (Iridaceae) in which the occurrence of seed stomata was not ~~an~~a useful taxonomic character (Wang & Hasenstein, 2016).

Five trichome types were found in *Christia~~n~~na mennegae* and among them, the glandular trichomes were seen only on the abaxial surface of the leaf-blades, as seen in

Comentado [A54]: Variation of what?

species of *Luehea* Willd., *Lueheopsis* Burret, *Vasivaea* Baill., *Triumfetta* L. and *Mollia* Mart. (Malvaceae-Grewioideae), as well as in *Heliocarpus popayenensis* H.B.K. (Malvaceae-Grewioideae) and *Christiana africana* (Westra, 1967), where the indument beneath the leaves is markedly different in comparison with that of the petioles and branches, as related here for *C. mennegae*. Westra (1967) performed a broad micromorphological study in *Apeiba* and provided valuable data about distribution and trichomes types for that genus~~in these species~~. Tan et al. (~~Tan, Chung & Soepadmo,~~ 2011) and Chung et al. (2012) ~~(Chung, Tan & Soepadmo, 2012)~~ used the trichome type on the leaf, fruit and seed, associated to other characters to distinguish between species of *Jarandersonia* (Malvaceae-Brownlowioideae). Nurhanim et al. (~~Nurhanim, Noraini & Chung,~~ 2010) studied the trichomes diversity in 18 species of *Pentace* Hassk. (Malvaceae-Brownlowioideae) and ~~stated that 15 trichomes types were present, and that the results evidenced their~~ demonstrated the systematic taxonomic value of the 15 trichomes types found.

Ultimately, it ~~is~~ was not possible to confirm ~~conclude that~~the monophyly of *Christiana* ~~is monophyletic from the analysis presented here~~ due to the low sampling of taxa and ~~low~~ conflicting statistical support obtained (BS = 86;~~0.56~~ PP = 0.78). On the other hand, our data supported the inclusion of *Christiana*, *Carpodiptera* and *Berrya*, which present ~~have~~ fused calyx-lobes (Whitehouse et al., 2001), in a strongly supported clade, as previously indicated by ~~however in the latter two genera the fruits are winged rather than capsular (Whitehouse et al., 2001). While *Berrya* and *Carpodiptera* appear as sister genera in phylogenetic studies (~~Alverson et al.~~,~~ (1999),~~;~~ Bayer et al. (1999), ~~)(~~Richardson et al.~~,~~ (2015) ~~, *Christiana* was associated with *Berrya* in the analysis by Bayer et al. (Bayer et al., 1999).~~and Hernández-Gutiérrez & Ma~~e~~gallón (2019)~~ sampled the three genera using plastid, mitochondrial and nuclear markers and observed a strongly supported clade (1 PP), although without a clear relationship among the three groups, that appear as a polytomy~~. Although ~~A~~ a close relationship between *Christiana* and *Carpodiptera* is reasonable, as they are the only Brownlowioideae genera with unisexual flowers (Bayer & Kubitzki, 2003),~~.~~ ~~The~~ increasing both the sampled species and the number of ~~inclusion of more~~ DNA regions ~~and an increase in sampled species~~ is crucial to clarify the relationships within this clade.~~ It is worth mentioning that the sequences of *C. africana* used here are from a voucher from Africa, thus the inclusion of another Neotropical specimen may be of great importance in forthcoming analyses.~~

Comentado [A55]: You did not state in the introduction or methods you intend to test monophyly of the genus. Why “discuss” that?

Comentado [A56]: This is the first time information on calyx fusion appears in the mss. I would suggest including such information on introduction and morphological description (see comments above).This discussion should be preceded by morphological information provided early in the mss.
I think “fused calyx-lobes” may not be the most appropriate term because Christiana seems to have a partially fused calyx with free short lobes. I suggest authors thinking better about that.

Comentado [A57]: Have this statement been published before? Or is an idea of the authors of this mss?

Comentado [A58]: This was the context before this study.

The availability of morphological and molecular data helps to~~and~~ accelerate~~s~~ the delimitation and identification of species (Ramalho et al. 2018), ~~and~~ thus allowing to~~better~~ rapidly quantify ~~the~~ plant diversity~~wealth~~ in~~of~~ the Amazon. Other A~~a~~mazonian species may~~be will~~ prove to have similar wide~~enormous~~ range expansions when more collecting~~on~~ effort~~s~~ is employed ~~occur~~ in remote areas ~~in the Amazon~~, more herbarium collections are digitized and published ~~photos of exsiccates from the Amazon are~~ online, and missing literature is eventually made available electronically ~~and old publications too~~.

Conclusions

The example of *C*hristiana~~.~~ *mennegae* highlights the need for continuous investment in the digitiz~~s~~ation of biological collections and the availability of online literature for the study of Amazonian biodiversity. The new occurrences recorded here ~~include samples~~ are represented by specimens collected many years ago but that were either overlooked or not identified, and therefore an already accepted species had escaped the radar of the Brazilian Flora until now. Our intention was to improve the knowledge to raise the profile of *C. mennegae* by adding data of its micromorphology and a preliminary phylogenetic perspective. It is possible that the discovery of the stomata in the seed surface in this subfamily may contribute towards the elucidation of Malvaceae systematics and evolution.

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Comentado [A59]: Alternatively, investment in training botanists and examining herbarium collections as claimed in literature (see Ahrends et al; 2011; Bebber et al. 2010) could help us when scarce financial support is available!

Comentado [A60]: I would be glad if authors had made literature like Kubitzki (1995) and Secco (2000) available to referees to facilitate our review.

Comentado [A61]: Seems to be unnecessary because information provided here was already presented above.

Comentado [A62]: This would not warrant good results if we do not have investment in training botanists in morphology and taxonomy.

Comentado [A63]: Maybe can be moved to discussion and discussed in the light of appropriate literature (including Bebber et al. 2010).

Comentado [A64]: I do not think the phylogenetic perspective presented is preliminary because phylogenetic placement of Christiana was already known before current study.
I think the use of few sequences for a single taxon do not justify the "preliminary" adjective

Comentado [A65]: In which way? It is not clear to me based on evidence presented here.

Comentado [A66]: Certainly were more than one analysis.

References

Comentado [A67]: I recommend to check Bayer (1999 as mentioned above), Edlin (1935 in New Phytologist), Tschá et al. (2002), etc., and include here.

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