A new species of *Thinouia* (Paullinieae, Sapindaceae) from the Amazon and its phylogenetic placement

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Abstract

*Thinouia* is a Neotropical genus of lianas with approximately 12 species and is the only genus in tribe Paullinieae with actinomorphic flowers. During a taxonomic revision of the genus and fieldwork in southwestern Amazonia, we found a new species that appears similar to *Thinouia trifoliata* (ex *Allosanthus*) because of its racemiform inflorescence. However, before describing the new species, we had to confirm that *Allosanthus* was congeneric with *Thinouia* so we could place the new species in the correct genus. The results of the phylogenetic analysis, based on molecular data (*trnL* intron and ITS sequences), show that *Allosanthus* should be included in *Thinouia*. Thus, the new taxon is described here as *Thinouia cazumbensis* sp. nov. The new species is described, illustrated and phylogenetic trees showing relationships within supertribe Paulliniodae and *Thinouia* and the congeneric *Allosanthus* are given.

Keywords

*Allosanthus*, Amazonia, Brazil, lianas, neotropical biodiversity, Paulliniodae, Paullinieae, Sapindales, Sapindaceae, taxonomy, *Thinouia*
Introduction

Thinouia is a neotropical genus of lianas that includes around 12 species, of which T. myriantha Planch. & Triana is widely distributed, including records from Mexico, Central America and northern South America (Ferrucci and Somner 2008; Acevedo-Rodríguez et al. 2011). The remaining species are distributed in Brazil, Bolivia, Paraguay and Peru, except for T. tomocarpa Standl. which is restricted to Mexico, Belize and Guatemala. Most Thinouia species occur in rainforest; a few species occur in savannah (BFG 2015).

Thinouia was proposed by Triana and Planchon (1862). It is characterised by the presence of umbelliform and racemiform thyrses, actinomorphic flowers with marginal or bifid petal appendages, an annular disc and schizocarpic fruits that split into three mericarps, each with a distal wing (Ferrucci and Somner 2008; Acevedo-Rodríguez et al. 2017).

Molecular phylogenetic studies show that Thinouia is a monophyletic group in Sapindaceae. In the most recent phylogenetic study, tribe Paullinieae (i.e. Cardiospermum, Lophostigma, Paullinia, Serjania, Thinouia and Urvillea) is a well-supported clade with Thinouia sister to the remaining genera (Acevedo-Rodríguez et al. 2017). In the same work, the monospecific genus Allosanthus (A. trifoliatus Radlk.) was maintained as a synonym of Thinouia, based on morphological characters. The only differentiating character (i.e. a racemiform inflorescence) was not considered worthy of generic recognition (Acevedo-Rodríguez et al. 2011, 2017).

During a taxonomic revision of the genus and fieldwork in south-western Amazonia, we found a new species of Thinouia that is similar to Thinouia trifoliata (Radlk.) Acev.-Rodr. & Ferrucci because of its racemiform inflorescence. Since we now have high-quality DNA material for the taxa previously assigned to Allosanthus, we re-analysed the placement of Allosanthus within Thinouia and further tested the monophyly of Thinouia s.l., which revealed the correct position of the new species.

Material and methods

Plant material

We collected the new species in Reserva Extrativista do Cazumbá-Iracema in Sena Madureira, Acre, Brazil. The collection was pressed and dried for vouchers, leaves were collected in silica gel for DNA extraction and reproductive structures were fixed in 70% alcohol for morphological analyses, which were performed using a stereomicroscope. The morphological structures were described using the terminology in Radford et al. (1974) and Weberling (1989). The herbarium abbreviations cited in the text follow Thiers (2020, cont. upd.).
Phylogenetic analysis

The phylogenetic analysis included the same taxa and molecular markers of Acevedo-Rodríguez et al. (2017), 93 taxa, plastid marker trnL intron and nuclear ribosomal internal transcribed spacer, ITS. Six samples (Allosanthus sp., Allosanthus trifoliatus, Thinouia mucronata, T. myriantha, T. obliqua and Thinouia sp.), including the new species, were added to the analysis, using the same molecular markers. For these additional taxa, approximately 60 mg of leaf tissue were pulverised with Tissuelyzer (Qiagen, Duesseldorf, Germany) for 3 min at 60 hz. The DNA extraction used the DNA NucleoSpin Plant II kit (Machery-Nagel, GmbH & Co. KG, Dueren, Germany) following the manufacturer’s protocol. Primers and the PCR amplification were used, as described in Acevedo-Rodríguez et al. (2017). Products were purified and sequenced by Macrogen (Seoul, South Korea). All sequences, vouchers and GenBank accession numbers are summarised in Appendix I.

The alignments were performed using MAFFT (Katoh et al. 2002) using the default parameters implemented in Geneious 2020.0.5 (Kearse et al. 2012). Poorly-aligned regions were removed and adjusted manually. We used jModelTest 2.0 (Guindon et al. 2010; Darriba et al. 2012) and the Akaike Information Criterion (AIC) to select the best-fit model of nucleotide substitution for each dataset. The GTR+I+G was selected as the best model for the ITS dataset, whereas the GTR+G was selected as the best model for the trnL dataset. Bayesian Inference (BI) analyses were conducted using MrBayes 3.2.2 (Ronquist et al. 2012) in the online CIPRES Science Gateway interface (Miller et al. 2015) with four Markov Chain Monte Carlo (MCMC) runs using a random starting tree and 10 million generations, with a sampling frequency of one every 1000 generations. We used Tracer 1.7 (Rambaut et al. 2018) to check for convergence of the MCMC and to check for stationarity. We discarded 25% of the trees as burn-in.

Phylogenetic trees were plotted and built inside the R environment (R Core Team 2020), version 3.6.2, using the packages ggplot2 (Wickham et al. 2020), ggtree (Yu et al. 2017; Yu and Lam 2020) and cowplot (Wilke 2019).

Results

Phylogenetic results

The ITS dataset included 99 terminals and 876 bp, the trnL dataset included 99 terminals and 727 bp and the combined dataset included 99 terminals and 1604 bp. Phylogenetic trees from the analyses of the combined dataset showed high posterior probability values (PP > 0.8). Only the topology from the combined analysis is described here (Fig. 1). Separate analyses of each locus did not reveal any strong groupings that would indicate incongruences.
Figure 1. A bayesian 50% majority-rule consensus tree from a Bayesian analysis of the combined, two-marker dataset for Paulliniodae and outgroups B relationships of Thinouia and the congeneric Allosanthus [(= Thinouia trifoliata (Radlk.) Acev.-Rodr. & Ferrucci], including the newly-described Thinouia cazumbensis sp. nov. Bayesian posterior probability values are indicated above the branches.

Supertribe Paulliniodae is strongly supported as monophyletic (Fig. 1 A, PP = 1.0). The tribe Paullinieae is also strongly supported as monophyletic (PP = 1.0) and the genus Thinouia (including Allosanthus) is recovered as the clade, sister to the remaining genera of the tribe Paullineae (PP = 1.0). Thinouia species are grouped in two main clades that are in a polytomy with the new species Thinouia cazumbensis. The first clade (PP = 1.0) includes Thinouia obliqua, T. mucronata, T. restingae and T. cf. mucronata species. The second one (PP = 0.8) includes Thinouia sp., T. myriantha and T. trifoliata (= Allosanthus trifoliatus Radlk.) (Fig. 1).

Taxonomic treatment

Thinouia cazumbensis Medeiros, sp. nov.
urn:lsid:ipni.org:names:77212573-1

Figure 2

Diagnosis. The new species differs from Thinouia trifoliata by the 5-lobed floral disc, fruits with trichomes and basal petal appendages smaller than the petals, versus annular disc, glabrous fruits and marginal petal appendages larger than the petals.

Type. Brazil. Acre. Sena Madureira. Reserva Extrativista do Cazumbá-Iracema, Núcleo Cazumbá, castanhal coletivo, floresta ombrófila aberta com bambu, 9°8’30”S, 68°56’23”W, 20 Jul 2018, H. Medeiros, M. Silveira & E.M. Soares 3401, (holotype RB!; isotypes: INPA!, SPF!, UFACPZ!, US!).

Description. Tendrilled liana 6–8 m long; stem puberulent, with yellowish to whitish indumentum, lenticellate; cross section simple, cylindrical. Leaves trifoliol-
A new species of *Thinouia* (Sapindaceae) from the Amazon

Figure 2. *Thinouia cazumbensis* **A** fruiting branch **B** detail of leaf, abaxial view **C** racemiform inflorescence with a pair of basal tendrils **D** detail of inflorescence (cincinnus) **E** flower with removed petals showing a 5-lobed nectary disc **F** detail of fruit **G** infructescence (**A–G**) from H. Medeiros 3401 (RB). Photos by H. Medeiros.

ate; stipules ca. 2 mm long, hirsute-tomentose, linear triangular to lanceolate; petiole 2–8.5 cm long, canaliculate; petiolules of lateral leaflets 0.2–0.8 cm long; leaflets 7–14 × 3–9 cm, oblong to ovate-rhomboideal, apex acute, mucronate, margins entire to dentate-serrate, with 2–4 teeth reduced to inconspicuous glands, ciliate, base trun-
Figure 3. Geographic distribution of Thinouia cazumbensis.

cate, rounded to obtuse, sometimes cuneate on the distal leaflet, glabrous on both surfaces, domatia sometimes in the axils of abaxial secondary veins. Thyrses axillary, racemiform, ca. 8.5–16 cm long, peduncle 1.1–2.8 cm long, rachis of 7.5–16 cm long; numerous cincinnus, sessile. Flowers ca. 2 mm long, pedicel ca. 0.5 mm long; sepals 5, ca. 1 mm long, fused at the base, lobes ovate, acute, glabrous and with prominent veins on the internal surface, external surface villous; petals 5, ca. 1.5 mm long, obovate, obtuse, clawed, villous on the central part and margins, the rest glabrous; petal appendages rudimentary, bifid, smaller than the petals, basally adnate, villous; nectary disc glabrous, 5-lobed, lobes ca. 1 mm long; staminate flower: stamens 8, 1.5 mm long, filaments villous for more than half of their length, anthers glabrous, pistillode ca. 1.5 mm long; pistillate flower: staminodes ca. 1 mm long, pistil ca. 1.5 mm long, style 0.5 mm long, with 3 stigmas, ovary ca. 1 mm long. Fruits ovate, chartaceous, 5–5.5 × 2–2.3 cm; cocci slightly inflated, 1.2–1.4 × 1.1–1.4 cm, including the ca. 2–3 mm long stipe constricted at junction with wing; epicarp densely strigose (simple trichomes of same length) on cocci, strigose on wings; endocarp glabrous. Seeds trigonal ovoid, ca. 6 × 4 mm, basally attached, glabrous, mature embryo not observed.

Thinouia cazumbensis is differentiated from most species of Thinouia by the thyrses axillary, racemiform (Fig. 2A, C) and the 5-lobed nectary disc, a character that is unique and for the first time recorded in the genus (Fig. 2E). The lobed nectary disc
A new species of *Thinouia* (Sapindaceae) from the Amazon

Within *Thinouia* should be further investigated through morpho-anatomical studies to understand how nectaries evolved within the genus.

**Distribution and ecology.** *Thinouia cazumbensis* is known only from the Reserva Extrativista do Cazumbá-Iracema (Fig. 3) where it is an infrequent liana that reaches the canopy of the open rainforest with abundant bamboo (*Guadua* spp.) (Silveira 2005).

**Phenology.** Collected in flower and fruit during July.

**Etymology.** The epithet *cazumbensis* refers to Reserva Extrativista do Cazumbá-Iracema, where the species was collected. In the 1980s, local rubber tappers and extractivists fought against the area becoming a rural settlement and on 19 September 2002 succeeded in getting the area designated as a conservation unit (ICMBio 2007). Situated in the State of Acre between the municipalities of Sena Madureira and Manoel Urbano, the Reserva Extrativista do Cazumbá-Iracema covers an area of 750,794.70 hectares of the Western Amazon Corridor, one of the seven major ecological corridors proposed for Brazil (Ricardo and Lima 2004).

**Conservation status.** The species is only known from one locality in Acre and is categorised as Data Deficient (DD) according to IUCN (2019). Further field studies are needed to evaluate its conservation status more accurately.

**Discussion**

The broader relationships that we recovered within supertribe Paullinioidae largely agree with those in Acevedo-Rodríguez et al. (2017). Additionally, with the inclusion of new sequences of *Thinouia* in this study merged with sequence data from Acevedo-Rodríguez et al. (2017), our results recovered the same clades in tribe Paullinieae, where *Thinouia* forms a clade that is the earliest diverging lineage. Therefore, our phylogenetic results reinforce including *Allosanthus* in *Thinouia* as proposed by Acevedo-Rodriguez et al. (2011), based on morphological characters. The only differentiating morphological character (i.e. the racemiform inflorescence) was not considered worthy of generic recognition by Acevedo-Rodriguez et al. (2011, 2017) and the molecular data in the present study corroborate this conclusion. The position of the new species as a member of *Thinouia* is strongly supported albeit its relationship to other species is not fully resolved, perhaps because of our limited sampling of *Thinouia* or because only two markers have been sequenced.

**Conclusion**

*Thinouia cazumbensis* is supported as a distinct taxon, based on morphological and molecular sequence data. Its position within the genus is still undetermined, highlighting the need for in-depth taxonomic studies on this genus. Ongoing systematics studies, based on molecular and morphological analyses of *Thinouia*, should provide additional insights into the evolution and biogeographic history of this neotropical genus (H. Medeiros et al. in prep.).
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Appendix I

Voucher and GenBank information for the taxa included in the phylogenetic analyses. Listed as: taxon, collection, herbarium, place of origin and GenBank accession numbers (ITS, trnL intron). Herbarium acronyms follow Index Herbariorum (Thiers, continuously updated).

*Allophylastrum frutescens* Acev.-Rodr., Lima 812 (K), Brazil, KX584885, KX584982. *Allophylus abyssinicus* (Hochst.) Radlk., Desissa & Binggeli DD-318 (MO), Ethiopia, KX584886, KX584983. *Allophylus africanus* P. Beauv., Balkwill 4026 (MO), South Africa, KX584887, KX584984. *Allophylus arboreus* Choux, Wohlhauser & Stiefe 60072 (MO), Madagascar, KX584888, KX584985. *Allophylus bicrus* Radlk., Barthelat 828 (MO), Mayotte, KX584889, KX584986. *Allophylus bojerianus* (Cambess.) Blume, Ratovoson 961 (MO), Madagascar, KX584890, KX584987. *Allophylus chaunostachys* Gilg, Mwangoko 729 (MO), Tanzania, KX584891, KX584988. *Allophylus chirinensis* Baker f., Hizza 26 (MO), Tanzania, KX584892, KX584989. *Allophylus cominiana* Sw., Acevedo-Rodríguez 12216 (US), Mexico, KX584893, KX584990. *Allophylus crassinervis* Radlk., Acevedo-Rodríguez s.n. (no voucher), Puerto Rico, KX584894, KX584991. *Allophylus decipiens* (E. Mey.) Radlk., Phillipson 4194 (MO), South Africa, KX584895, KX584992. *Allophylus gardineri* Summerh., Pignal 1834 (MO), Mayotte, KX584897, KX584994. *Allophylus hirtellus* (Hook. f.) Radlk., Cheek 5059 (?), KX584898, KX584995. *Allophylus pervillei* Blume, Hoffmann 399 (MO), Mayotte, KX584899, KX584996. *Allophylus poungouensis* Pellegr., McPherson 16109 (MO), Gabon, KX584900, KX584997. *Allophylus puberulus* (Cambess.) Radlk., Somner 1069 (US), Brazil, KX584901, KX584998. *Allophylus racemosus* Sw., Acevedo-Rodríguez 12180 (US), Mexico, KX584902, KX584999. *Allophylus rubifolius* (A. Rich.) Engl., Kuchar 23357 (MO), Tanzania, KX584903, KX585000. *Allophylus sp.* Acevedo-Rodríguez 14847 (NY), Brazil, KX584904, KX585001. *Athyana weinmanniifolia* (Griseb.) Radlk., Pignal 11166 (US), Bolivia, KX584906, KX585003. *Balsas guerrerensis* Cruz Durán & K. Vega, Vega Flores 1318 (US), Mexico, KX584908, KX585005. *Bridgesia incisifolia* Cambess., Landrum 9824 (NY), Chile, KX584909, KX585006. *Cardiospermum corindum* L., Harder & Bringham 3495 (MO), Zambia, KX584912, KX585007. *Cardiospermum cuchujaquense* Ferrucci & Acev.-Rodr., Van Devender 92-1012 (ARIZ), Mexico, KX584914, KX585008. *Cardiospermum grandiflorum* Sw., ATBP 603 (MO), Uganda, KX584915, KX585009. *Cardiospermum grandiflorum* Sw., Gildenhuys H1 (?), Hawaii, KM062277, KM062362. *Cardiospermum heringeri* Ferrucci, Urdampilleta 437 (US), Brazil, KX584917, KX585010. *Cardiospermum urvilloides* (Radlk.) Ferrucci, Urdampilleta 425 (US), Brazil, KX584922, KX585013. *Chimborazoa lachnocarpa* (Radlk.) H.T. Beck, Wiggens 11060 (US), Ecuador, KX584923, KX585014. *Diatenoptyx sorbifolia* Radlk., Zardini 2928 (?), Paraguay, EU720534, EU721303. *Dicyoneura obtusa* Blume, Edwards KE142 (JCT), Australia, EU720428, EU721187. *Diploglottis campbellii* Cheel, Chase 2048 (K), Australia, EU720457, EU721224. *Guindilia dissecta* (Covas & Burkart) Hunz., Ferrucci 2928 (CTES), Argentina, KX584926, KX585017. *Guioa villosa* Radlk., McPherson
A new species of Thinouia (Sapindaceae) from the Amazon

A new species of Thinouia (Sapindaceae) from the Amazon

18040 (MO), New Caledonia, EU720544, EU721314. *Haploclœodium inoploœum* Radlk., Lap 117 (?), FJ514259, FJ514265. *Houssayanthus bitemnatus* (Weath.) Rzed. & Calderón, Catalán & Terán 837 (MO), Mexico, KX584927, KX585018. *Houssayanthus incanus* (Radlk.) Ferrucci, Ferrucci 2710 (CTES), Argentina, KX584928, KX585019. *Jagera ja-vanica* (Blume) Kalkman, Chase 2130 (K), Bogor, EU721236, EU720468. *Lepisanthes senegalensis* (Poir.) Leenh., Callmander 627 (MO), Madagascar, EU720492, KX585020.

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Rodríguez 12014 (US), Jamaica, KX584965, KX585052. Serjania mexicana (L.) Willd., Acevedo-Rodríguez 15080 (US), Mexico, KX584966, KX585053. Serjania paniculata Kunth, Acevedo-Rodríguez 15143 (US), Mexico, KX584967, KX585054. Serjania perulacea Radlk., Acevedo-Rodríguez 11134 (US), Bolivia, KX584968, KX585055. Serjania unguiculata Radlk., Acevedo-Rodríguez 15081 (US), Mexico, KX584969, KX585056. Serjania yucatanensis Standl., Acevedo-Rodríguez 12183 (US), Mexico, KX584970, KX585057. Talisia nervosa Radlk., Pennington 628 (MO), ~, EU720474, EU721244. Talisia obovata A.C. Sm., Lombello 13 (MO), Brazil, EU720485, EU721255. Thinouia cazumbensis sp. nov., Medeiros 3401 (RB) Brazil, MT853074, MT847016. Thinouia mucronata Radlk., Keller 6919 (US), Argentina, KX584971, KX585058. Thinouia cf. mucronata Radlk., Medeiros 3800 (RB) Brazil, MT853076, MT847018. Thinouia myriantha Radlk., Torke 2024 (HSTM), Brazil, MT853071, MT847013. Thinouia obliqua Radlk., Medeiros 3793 (RB) Brazil, MT853075, MT847017. Thinouia sp., Medeiros 2193 (RB), Brazil, MT853072, MT847014. Thinouia restingae Ferrucci & Somner, Somner 1074 (RBR), Brazil, KX584972, KX585060. Thinouia trifoliata (Radlk.) Acev.-Rodr. & Ferrucci, Medeiros 3331 (RB), Brazil, MT853073, MT847015. Thouinia acuminata S. Watson, Liston 633-2, —, EU720478, EU721249. Thouinia villosa DC., Hall 825 (US), Mexico, KX584975, KX585062. Tristiropsis acutangula Radlk., Chase 1358 (K), Bogor, EU720453, EU721220. Urvillea chacoensis Hunz., Acevedo-Rodríguez 11133 (US), Bolivia, KX584976, KX585063. Urvillea chacoensis Hunz., Keller 6834 (US), Argentina, KX584977, KX585064. Urvillea pterocarpa (Radlk.) Acer.-Rodr. & Ferrucci, Urdampilleta 321 (US), Brazil, KX585012, KX584921. Urvillea rufescens Cambess., Somner 1073 (RBR), Brazil, KX584978, KX585065. Urvillea ulmacea Kunth, Acevedo-Rodríguez 15145 (US), Mexico, KX584979, KX585066. Urvillea ulmacea Kunth, Reyes-García 5585 (MO), Mexico, KX584980, KX585067. Vouarana guianensis Aubl., Acevedo-Rodríguez 5031 (US), French Guiana, KX584981, KX585068.